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TABLE OF CONTENTS

MONDAY JULY 28, 2008
STREAM 1:
NEW THERAPEUTIC APPROACHES

1

Reduction of Blood Sugar and Fat by a Probiotic used as Feed Additive in Lambs

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Bioplus 2B is a probiotic used to increase growth and weight gain in some animals. However, the effects of this compound on other functions have not been recognized yet. This study aimed to examine the effects of bioplus 2B on total proteins, urea blood glucose, triglyceride and cholesterol in lamb. Thirty two lambs aged five to six months were randomly divided into four groups; they were feed with a standard feed and raised in the same conditions. The groups 1-3 were given 1000, 500 and 250 grams bioplus per ton of feed daily and the fourth group left as control. The lambs were raised for 3 months, and the blood samples were taken from each animal four times during the study. The amount of total proteins, urea glucose, triglyceride and cholesterol were determined in each lamb serum. Data were analyzed by ANOVA method and the p value less than 0.05 was considered significant. There were not significant changes in the levels of total proteins in the serum of different groups. However, significant changes were shown in the amount of glucose, triglyceride and cholesterol of treated groups in a dose-dependent manner. It was also shown that the amount of urea was significantly increased in treated lambs in some stages of sampling. It can be concluded that bioplus 2B can reduce the amount of blood sugar and fat, suggesting that it can be considered in human food to lower blood sugar and fat.

2

Study of *in vitro* Antibacterial Activity of Latex of Euphorbia Tirucalli

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The present study was undertaken to investigate *in vitro* antibacterial activity of latex of *Euphorbia tirucalli*, a plant found in various regions of Brazil and better known as aveloz or pencil tree, which is traditionally used for the treatment of tumors. Aerial parts of the plant were collected and deposited at Herbarium of UTFPR – Campo Mourão. The latex was extracted and kept in bottle sterilized under refrigeration until used for the biological tests. The antimicrobial assays were performed by diffusion disc method, using *Sthaphylococcus aureus*. All assays were carried out in triplicate. Whatman no. 1 sterile filter paper discs (5mm) were impregnated with latex solution (concentration of 0.2, 0.4 and 0.6% v/v). *In vitro* antibacterial activity was determined by using Baird-Parker Agar. 0.1mL of inoculum solution was uniformly spread on solidified agar. The discs were then applied and the plates were incubated at 37°C for 48h. The control assay was performed with discs without latex solution. The diluted solution of latex of the plant inhibited the growth of bacteria *Staphylococcus aureus*, featuring halos increasing bacterial inhibition, in accordance with the increase of concentration of the solution (0.2, 0.4 and 0.6% v / v), average by 1.33 mm, 2.17 mm and 2.33 mm, respectively for each concentration from the edge of the disk. The results confirmed the ability of inhibition of bacterial growth *in vitro* by the solution of latex of *Euphorbia tirucalli*.

3

Antiplasmodium falciparum Activity of some Medicinal Plants used in Treatment of Malaria in Uganda

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Malaria is a fatal disease caused by *Plasmodium* parasites that lives part of its life in humans and part in mosquitoes. It remains one of the major killers of humans worldwide, threatening the lives of more than one-third of the world's population especially in tropical and developing countries like Uganda. The emergence of resistance and inaccessibility to antimalarial drugs has lead to

increased mortality and morbidity especially among the vulnerable children and pregnant women. This has lead to search for alternative sources of antimalarial drugs especially from the medicinal plants which is cheap and accessible to majority of population. In this study the crude leaf extracts of ether and methanol from the *Aloe daweii (AD), Justicia betonica.L (JB)* and *Zanthroxylum chalybeum (ZC)* on *Plasmodium falciparum* were investigated using chloroquine as a positive control. The chemical tests on the extracts showed that *ZM* contain alkaloids, reducing sugars, mucilages and tannins. *AE* had anthraquinone aglycones, alkaloids and mucilages while *JE* had alkaloids and mucilages in the ether extracts respectively. In the methanol extract, *ZE* had anthraquinone aglycones, alkaloids and mucilages. The results showed that ether extracts of *JB* had IC50 of 11.40 ug/ml, *AD* had 13.16 ug/ml and *ZC* had 20.32 ug/ml. The methanol extract of *ZC* had IC50 of 11.72 ug/ml. The chloroquine had IC50 of 20.25 ug/ml. These results showed that these medicinal plants contain compounds with stonger *antiplasmodium falciparum* activity as compared to the chloroquine, which needs further investigation as a source for antimalarial drugs.

4

Inhibition of Hydrogen Sulfide Synthesis Attenuates Chemokine Production and Protects Mice against Acute Pancreatitis and Associated Lung Injury

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Hydrogen sulfide (H_2S), a novel gasotransmitter, has been recognized to play an important role in inflammation. Cystathionine-γ-lyase (CSE) is a major H_2S synthesizing enzyme in the cardiovascular system. The present study investigated whether chemokines are involved in H_2S associated pathogenesis of acute pancreatitis (AP) and associated lung injury. We examined the effect of DL-propargylglycine (PAG), a CSE inhibitor, on the synthesis of CC chemokine monocyte chemotactic protein (MCP)-1, regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) and macrophage inflammatory protein-1alpha (MIP-1α) as well as CXC chemokine MIP-2 in an *in vitro* and *in vivo* model of caerulein-induced AP and associated lung injury. In addition, the pancreatic acinar cells were treated with H_2S donor drug, sodium hydrosulfide. Expression of these chemokines in the pancreatic acini, pancreas and lungs was determined by quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry. Following treatment with PAG, RT-PCR and ELISA demonstrated down-regulation of caerulein-induced increase in MCP-1, MIP-1α and MIP-2 expression but had no apparent effect on RANTES expression. These results suggest that the pro-inflammatory effect of H_2S may be mediated by chemokines.

5

Chemopreventive Response of Aceclofenac in 1, 2-Dimethylhydrazine Induced Colon Carcinogenesis in Rats and Development of Colon Targeted Drug Delivery Systems

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Non-steroidal anti-inflammatory drugs (NSAIDs) currently focused for their chemopreventive response. These agents primarily inhibit the activity of the cyclooxygenase (COX) enzymes and thereby affect the synthesis of prostaglandin signaling molecules, which are involved in a wide range of physiological processes beyond inflammation. Aceclofenac was developed in order to provide a highly effective pain relieving therapy with a reduced side effect profile, especially GI events that are frequently experienced with NSAID therapy. The aim of the present study was to establish chemopreventive response of aceclofenac in experimental colon cancer induced by 1, 2-dimethylhydrazine (DMH) and to develop colon targeted drug delivery system thereof. The experiments were performed on Sprague Dawley male rats.

Thirty five weeks study was conducted during which the administration of DMH (subcutaneously) was continued for 18 weeks while NSAIDs (orally) treatment was continued for 35 weeks. All the animals were sacrificed in overnight fasted conditions after the end of treatments and studied for were thoroughly examined macroscopically for the presence of tumors. Different regions of colon were carefully examined under hand lens for counting the tumors. Chemopreventive response was assessed on the basis of tumor incidence, burden and multiplicity. The colonic segments containing tumors were dissected, fixed immediately in 10% formalin and processed routinely. The sections were stained with H&E staining and further examined under light microscope for the presence of carcinoma, hyperplasia and dysplastia, aberrant crypt focci associated with aggregates of lymphoid tissues and mucosal inflammation. In second part of our study tablets containing aceclofenac were prepared using direct compression method and were further coated by using pan coating method. Different coating proportions of the Eudragit S-100 and ethylcellulose with guar gum were studied. The tablets were coated to sufficient coat weight and studied for their in vitro release studies in absence and presence of rat caecal contents. The samples were analyzed using validated RP-HPLC method for determination of aceclofenac at 275 nm. The results demonstrate that there is a distinct occurrence of malignant tumors in DMH-induced colon, which were greatly inhibited by the treatment of aceclofenac. The in vitro release studies on ethylcellulose and guar gum coated tablets at 2%, 4% and 6% of coatings showed 5.68 ± 0.17, 5.29 ± 1.24 and 3.15 ± 0.46 % of drug release after 5h and was 60.85 ± 3.08, 53.80 ± 0.63 and 47.78 ± 0.31 after 24h. The release studies in presence of rat caecal showed enhanced drug release due to degradation of guar gum by microbial enzymes. It may be concluded that aceclofenac is effective in chemoprevention in the DMH-induced colon carcinogenesis and the developed drug delivery system will deliver the drug to the colon.

6

Diverse Classes of Antidepressant Activate Separate Neuroprotective Mechanisms in Cultured Human Neuroblastoma Cells

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The neurodegenerative hypothesis of depression postulates that depression is associated with impaired neuroplasticity and that the effectiveness of antidepressants may be partially attributed to neuroprotective properties. The current study investigated the effect of selected antidepressant drugs on cell viability and gene expression of pro- and anti-apoptotic pathways. Methods: The free fraction of plasma protein-bound drugs was determined in culture medium + 10% serum by means of HPLC analysis. Cultured SH-SY5Y cells were treated for 24 hours with and without 10 mM glutamate plus a pharmacological (lower) or higher free concentration of fluoxetine, mirtazapine, tianeptine, imipramine, *myo*-inositol, gabapentin or lithium. Thereafter mitochondrial activity was determined with the MTT cell viability assay, while the expression of genes encoding for pro-apoptotic factors (Bax, caspase-3 and caspase-8) and anti-apoptotic factors (BDNF, NF-kappa-beta, Akt, CREB and Bcl-2) was determined utilizing real-time PCR. Results: Free drug concentrations in culture medium + serum were determined. Pharmacological concentrations of lithium, *myo*-inositol, imipramine and gabapentin, but not of fluoxetine, mirtazapine or tianeptine, protected against glutamate-induced excitotoxicity (MTT assay). High concentration of lithium and gabapentin also significantly protected against excitotoxicity, while no protective drug effects were observed in the absence of glutamate. Pharmacological concentrations of fluoxetine, imipramine, tianeptine and *myo*-inositol significantly increase anti-apoptotic gene expression, while pro-apoptotic gene expression is significantly decreased with gabapentin, imipramine, lithium, mirtazapine, *myo*-inositol and tianeptine. In conclusion, the data suggest that pharmacological concentrations of diverse classes of antidepressant display different effects on cellular resilience pathways. Of these, some, but not others, involve protection against excitotoxicity.

7

Intestinal and Intraperitoneal Absorption, Bioavailability and Hepato-biliary Excretion after Unconjugated Bilirubin, Biliverdin and Bilirubin Ditaurate Administration in the Rat

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The bile pigments, bilirubin and biliverdin, possess antioxidant and anti-inflammatory properties and their exogenous administration protects against many models of tissue inflammation and damage. Despite the encouraging and versatile therapeutic potential of bile pigments, very little is known about their in vivo parenteral or enterologic absorption after exogenous administration. This study aimed to investigate the absorption and pharmacokinetics of bile pigment administration after intravenous, intraperitoneal and intraduodenal administration. Anaesthetized Wistar rats had their bile duct, jugular and portal veins cannulated. Sodium bilirubinate, bilirubin ditaurate and sodium biliverdinate were infused and the circulating bile pigment concentrations and their biliary excretion were measured over 180 minutes. When sodium bilirubinate, sodium biliverdinate and bilirubin ditaurate (1 mg; 2.7 mg/kg body weight) were administered intravenously, their plasma concentrations decreased exponentially over time and the native and metabolized compounds subsequently appeared in the bile. When administered intraperitoneally (1 mg; 2.7 mg/kg body weight), their absolute bioavailabilities equaled 17.1, 16.1 and 33.1%, respectively and correspondingly 40, 30 and 37% of the same bile pigment doses were excreted in the bile. Intraduodenal sodium bilirubinate and bilirubin ditaurate administration (10 mg; 27 mg/kg body weight) increased their portal and systemic concentrations and their systemic bioavailability equaled 1.0 and 2.3%, respectively. Correspondingly, 2.8 and 4.8% of the doses were excreted in the bile. Sodium biliverdinate was not absorbed. Bile pigments are significantly absorbed from the intestinal and peritoneal cavities, demonstrating potential routes of administration for the study of these compounds in models of inflammatory disorders.

8

Molecular Analysis of Parasite and Human Host Determinants in Sera from Individuals Susceptible or Immune to Malaria

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The mechanisms of protection in malaria host-pathogen interactions are still being elucidated. However there exist differential patterns of susceptibility/ resistance to the same pathogen by the natural hosts. In this study, we attempt a comparison of molecular and antigenic properties of sera taken from susceptible and immune individuals. The first phase involved the administration of a questionnaire and collection of blood. Fifty subjects eighteen years of age or older, who during the past twelve months have not suffered any malaria attacks "immune", versus another fifty susceptible individuals "non-immune" were selected from Bolifamba in the South West Province, Cameroon. In the second phase, solubilized proteins from *in vitro Plasmodium falciparum* cultures (laboratory strain, 3D7) were used for western blot analysis and immuno-precipitation with susceptible, immune sera and control serum from an un-exposed American. Immuno-fluorescence assay and flow cytometry were conducted to determine reactivity of sera with parasite antigens on the surface of infected or uninfected Red blood cells. These studies established that sera from both groups failed to react with surface of infected erythrocytes but strongly reacted with internal antigens. Further, sera from immune individual failed to recognize one or more specific antigens in western blots. We are currently analyzing whether immune and non immune groups can be distinguished by relative intensities of multiple antigens detected, cytokine levels and other host correlates of immunity.

9

AZD6140, the First Reversible Oral Platelet $P2Y_{12}$ Receptor Antagonist, has Linear Pharmacokinetics and Provides Near Complete Inhibition of Platelet Aggregation, with Reversibility of Effect in Healthy Subjects

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Introduction: AZD6140, the first reversible oral platelet $P2Y_{12}$ receptor antagonist, is in Phase III development for the reduction of clinical atherothrombotic events in patients with atherosclerosis. Three studies assessed pharmacokinetics, pharmacodynamics, and tolerability of single ascending doses of AZD6140 in healthy subjects. Methods: Subjects received single oral doses of AZD6140 0.1-100mg or placebo (Stud-1), 30-400mg (Study-2), or 900-1620mg (Study-3). Plasma concentrations of AZD6140 and its active metabolite AR-C124910XX were determined. Inhibition of ADP-induced platelet aggregation (IPA) (final and maximum extent) was measured at time points following the pharmacokinetic profile in Studies 1 and 2. Safety and tolerability were evaluated. Results: Absorption of AZD6140 was rapid (median T_{max} of 1.5-3 hrs across doses), as was formation of AR-C124910XX from AZD6140 (median T_{max} between 1.5-3 hrs). Mean $AUC_{0\text{-}inf}$ and C_{max} of AZD6140 and AR-C124910XX increased in an apparent dose-proportional manner, suggesting linear pharmacokinetics over the dose range studied. Mean $T_{1/2}$ for AZD6140 and AR-C124910XX were about 7.1-12 and 8.5-10.1 hrs, respectively. Two hours post-dose, AZD6140 100-400mg inhibited 20μM ADP-induced IPA by 88-95%. No safety or tolerability issues arose up to 900mg. At 1260mg, dose-limiting gastrointestinal adverse events were observed. Discussion: At 100-400mg doses, IPA reached a plateau of 88-100% and declined to 74-89% at 12 hr post-dose. Conclusion: AZD6140 had linear pharmacokinetics and was well tolerated up to single doses of 900mg. At doses ranging from 100-400mg, near complete IPA at 2 hrs post dose is evident. Offset of IPA effect follows declining plasma concentrations, indicating reversibility of effect.

10

Development of a Guinea Pig Model of Type II Diabetes for Testing QT-Prolonging Drugs

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Introduction: Drug-induced QT interval prolongation and cardiac proarrhythmia are problems for both clinicians and the pharmaceutical industry. Potassium channel (HERG)-blocking properties of new compounds are tested in vitro in the early pre-clinical phases. Although these assays are useful in detecting proarrhythmic risk, they do not consider concomitant in vivo QT-prolonging risk factors such as diabetes. Objective: Create a guinea pig model of type II diabetes to test QT-prolonging effects of drugs. Methods: Guinea pigs were fed ad libitum with either the Control (n=14), the High Fat High Sucrose (HFSD, n=8) or the High Fat High Fructose (HFFD, n=8) diet for 93 days. Blood samples were drawn each month and biochemical testing was performed to assess progressive metabolic changes in the animals. Results: After 93 days, mean weight was higher in the HFSD and in the HFFD, when compared to controls (928.25±44.84 and 939.17±89.94 g respectively, vs 821.00±63.74 g, both $p<0.02$). Both HFSD and HFFD had higher triglycerides than controls (1.54±0.40 and 1.28±0.58 mmol/L respectively, vs 0.62±0.19 mmol/L, both $p<0.005$) and higher total cholesterol (1.78±0.56 and 1.91±0.69 mmol/L respectively, vs 0.81±0.15 mmol/L, both $p<0.001$). Urea was also higher in both HFSD and HFFD than in controls (7.44±1.08 and 7.44±0.79 mmol/L respectively, vs 5.96±1.00 mmol/L, both $p<0.02$), indicating progressive kidney damage. Discussion and conclusion: It seems possible to generate a guinea pig model of type II diabetes using diabetogenic diets. Using wireless cardiac telemetry, this model could be used for assessing the combined deleterious effects of diabetes and QT-prolonging drugs in vivo.

11

Effects of *Asteracantha longifolia* Seeds on Sexual Behavior of Male Rats

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Asteracantha longifolia Nees (Acanthaceae) is a popular herb in traditional Indian medicine and constitute a group of herbs used as 'Rasayan' or adaptogen .The seeds of A. longifolia have been traditionally used as aphrodisiac. The study was therefore performed to evaluate ethanolic extract of seeds of A .longifolia at a dose equivalent to 100, 150 and 200 mg of per kg body weight in improving sexual behavior in male rats. The parameters taken into account were the effect of extract on body and organ weights, change in histoarchitecture of testis, fructose level in seminal vesicles and sexual behavior. The change in sexual behavior was assessed by determining parameters mount frequency, interomission latency, mount latency and post ejaculatory latency. Results were compared with the effect of testosterone administrated to standard group. Administration of ethanolic extract had pronounced anabolic and spermatogenic effect in treated animals as evidenced by weight gains in the body and reproductive organs. The histological examination of testis showed abundant spermatozoa in the lumen of the seminiferous tubules in rats fed with the extract when compared to the controls. The treatment also markedly affected sexual behavior of animals as reflected in reduction of mount latency, an increase in mount frequency and enhanced attractability towards female. The fructose content in seminal vesicles was significantly increases in treated groups. Thus it was concluded that drug was aphrodisiac and justifying the use in the traditional system of medicine as a Rasayana.

12

Experimental Study Regarding the Therapeutic Effect of the Polysaccharides Extract from *Plantago Sp.*

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The aim of the present study is the experimental research of the possible analgesic and sedative effects of the polysaccharides extract from *Plantago sp*. Data in the literature are inconsistent regarding the analgesic and sedative actions of various extracts from *Plantago sp*. After extraction of the polysaccharides fraction, studies of experimental pharmacology were "*in vivo*" conducted. Material and methods: The "writhing test" was used to study the analgesic effect and the "exploration test" was applied for the sedative effect evaluation. Results: The polysaccharide extract had a fast analgesic effect (at 15 minutes), which lasted shortly (max. 30 minutes). A dose-effect relationship can be expressed. The sedative effect was significant at 30 and 120 minutes after the test solution administration, but it was without significance at 60 minutes. It was also considered a dose-effect relationship. Conclusions: The polysaccharides fraction from *Plantago lanceolata* has a fast, short and dose-dependent analgesic effect. The same fraction has a slower and lasting dose-dependent sedative effect. Normally several compounds from the polysaccharides extract can present a sedative effect.

13

Effects of Icarrin on Learning-memory Ability and Development of Beta-amyloid in APP Transgenic Mice

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The pathological mechanism of Alzheimer's disease (AD) is not clear as yet and effects of treated drugs are not satisfied. Aim of the research is to investigate the protective effects of Icarrin, a component of Traditional Chinese Medicine–Epimedium on APP transgenic (Tg) mice of dementia. The mice of drug treated group were administered introgastrically by Icarrin (at doses 18 and 58 mg/kg/d) from 4 to 10 months old. The learning-memory ability was measured by Morris water maze (MWM) test. The expression of APP, beta-secretase, and alpha-synuclein, a precursor protein of non beta-amyloid component in brains were detected by immunohistochemistry and

western blot respectively. The content of beta-amyloid was measured by ELISA Assay. The amyloid plaques were detected by Congo red staining. The results showed that the escape latency and swimming distance of Tg mice in MWM prolonged. The expression of APP, beta-secretase, the content of beta-amyloid, the amount of amyloid plaques, and the expression of alpha-synuclein increased in brains of Tg mice. Icarrin decreased the latency and swimming distance of Tg mice, decreased the expression of APP, beta-secretase and the content of beta-amyloid, relieved amyloid plaques burden, and suppressed the expression of alpha-synuclein in brains of Tg mice. Icarrin could reduce the production and accumulation of amyloid by decreasing both expressions of APP, beta-secretase and alpha-synuclein, and thus enhance the learning-memory ability of Tg mice. These results suggest that Icarrin may have a promising application prospect in treatment of AD.

14

Nitric Oxide Release Combined with Nonsteroidal Antinflammatory Activity Prevents Muscular Dystrophy Pathology and Enhances Stem Cell Therapy

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Duchenne muscular dystrophy is a relatively common disease that affects skeletal muscle leading to progressive paralysis and death. There is currently no resolutive therapy although recent studies with systemic delivery of a specific stem cell, the mesoangioblast, have opened promising perspectives. We have developed a novel treatment, in which we combined the known beneficial effects of nitric oxide (NO) in muscle repair with non-steroidal anti-inflammatory activity using the NO-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) nitroibuprofen and nitroflurbiprofen. Here we report the results of long term (one-year) oral treatment with NO-NSAIDs of two murine models for limb girdle and Duchenne muscular dystrophies (alpha-sarcoglycan null and mdx mice). In both models, NO-NSAIDs significantly ameliorated the functional phenotype, assessed by measuring animal motility in vivo and muscle contractile properties in vitro, in the absence of secondary effects, efficiently slowing disease progression. Immunohistochemical analyses and Azan-Mallory staining of muscle biopsies showed that NO-NSAIDs reduced inflammation, prevented muscle damage and preserved the number and function of satellite cells. NO-NSAIDs were significantly more effective than the corticosteroid prednisolone analyzed in parallel. Furthermore, NO-NSAIDs increased fourfold the ability of mesoangioblasts to migrate into dystrophic muscles, to resist their apoptogenic environment and engraft into them, enhancing significantly their therapeutic efficacy.The new therapeutic strategy we propose is not selective for a subset of mutations, provides ground for immediate clinical experimentation with NO-NSAIDs alone, which are approved for use in humans, and may set the stage for combined therapies using donor or autologous, genetically-corrected stem cells.

15

Arterial Remodeling and Aortic Stiffness in Patients with Fabry Disease after 54 Months of Enzyme Replacement Therapy

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Fabry disease is a lysosomal deficiency of α-galactosidase A enzyme leading to accumulation of glycosphyngolipids (GSL) in tissues and premature mortality because of renal failure and cardiovascular diseases. We have already described accelerated vascular hypertrophy and elevated aortic stiffness. There is no yet evidence of enzyme replacement therapy (ERT) efficacy on arterial properties. Objectives: To evaluate

efficacy of ERT at usual dosage on arteries. Methods: 54±5 months follow-up (4 visits, first without ERT) in 36 patients (33±12yrs, population A) with serial measurements of a) radial and carotid intima-media thickness (IMT), local pulse pressure (PP) and distensibility obtained with echotracking device and b) aortic stiffness obtained through carotid to femoral pulse wave velocity (PWV) with tonometry. Among the 36 patients, 15 had more than 2 visits before treatment (-34±9 months, 6 visits, population B). Results: In population A, radial and carotid IMT increased during follow-up (+4±17 and +12±52µm/yr, respectively; P<0.05;RM-ANOVA) despite ERT, whereas PP and distensibility did not change. Results are confirmed by unchanged IMT progression rate before and after ERT in sub-population B. Aortic stiffness significantly decreased during follow-up in population A (-0.5±0.9m/s/yr,P<0.01). This was confirmed in population B (PWV:-0.5±0.5m/s/yr,P<0.01), in which the significant decrease in PWV after ERT contrasted with the lack of change before ERT (-0.2±0.9m/s/yr,NS). Conclusion: In our population, efficacy of ERT differed according to arterial site. The lack of effect of ERT on arterial wall hypertrophy contrasted with significant improvement in aortic stiffness. Further investigations are needed to assess relation between aortic stiffness and geometric properties.

16

Seaweeds as Sources of Natural Antioxidants: Chemical Evaluation and Antioxidant Activity of the Red Seaweed Bryothamnion Triquetrum (S.G.Gmelin) Howe

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In the past years there has been an increasing interest in the quest for antioxidants from natural sources for their application in oxidative stress related diseases. In this context, marine algae are a privileged reservoir of antioxidants with very low toxicity. In the present work we evaluated the chemical composition and antioxidant properties of the aqueous extract of the red seaweed Bryothamnion triquetrum. The extract had a high nutritional quality: proteins (9,5%), lipids (1,3%), carbohydrates (5,9%), fibers (10,2%) and ashes (43%). Phenolic content was 8.08 mg gallic acid equivalents/g of lyophilized. Cinamic (221.9 µg/g), p-coumaric (4187.3 µg/g) and ferulic acid (442.3 µg/g) were identified and quantified. The extract also contained ascorbic acid (0.1 mg/g) and carotenoids (0.04 mg/g). More than 20 minerals were evaluated obtaining very low/ not detectable levels of toxic metals and significant quantities of selenium. The antioxidant activity of the extract was determined: DPPH• radicals scavenging (38% / 4 mg of lyophilized); β-carotene-linoleic acid assay (12% / 4 mg); O2•– scavenging (IC50 = 0,36 mg/mL); OH• scavenging (IC50 = 2,11 mg/mL) and TBARS in spontaneous lipoperoxidation model (IC50 = 23.9 µg of lyophilized). Our results suggest that the antioxidant properties are related to the ability of the extract to scavenge radical entities and could be envisioned as the result of the additive and/or synergic effect between phenolic constituents and the other antioxidants, such as minerals, carotenes and ascorbic acid. B. triquetrum could be promising as a phytotherapeutical agent to modulate oxidative stress in different pathological conditions. Key words: seaweeds, Bryothamnion triquetrum, chemical composition, antioxidant activity.

17

Mesna for the Treatment of Hyperhomocysteinemia in Hemodialysis Patients

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Increased plasma total homocysteine (tHcy) is a graded, independent risk factor for the development of atherosclerosis and thrombosis. Over 90% of patients with end-stage renal disease (ESRD) have hyperhomocysteinemia (plasma tHcy $\geq$ 15 umol/L). Mesna, a thiol containing drug, can exchange with albumin-bound Hcy thereby enhancing dialytic clearance of plasma tHcy. We hypothesized that 12 mg/kg intravenous mesna administered predialysis for would cause a significant decrease in plasma tHcy compared to placebo. An initial, short, one-week placebo-controlled, double-blind, randomized, cross-over pilot study of mesna 12 mg/kg in subjects with end-stage renal disease yielded a significant lowering of tHcy. A second, longer study, of similar design was conducted with treatment arms of four weeks. Intravenous 12 mg/kg mesna or placebo was administered thrice weekly predialysis. The sum of spent dialysate was collected for analysis from three patients during the first dialytic session of each treatment arm. One week of 12 mg/kg IV mesna significantly decreased predialysis plasma tHcy by 12.8 ± 7.8% compared to placebo (placebo: 23.4 ± 8.0 umol/L vs. mesna: 20.5 ± 7.6 umol/L, P = 0.0044). Four weeks of treatment, on the other hand, yielded no significant decline in predialysis plasma tHcy (placebo: 18.3 ± 8.5 umol/L vs. mesna: 18.7 ± 6.3 umol/L, P = 0.41). However, mesna significantly increased total tHcy excretion during dialysis (placebo: 12.2 ± 6.4 mg vs. mesna: 14.7 ± 6.6 mg, P = 0.0068). Mesna significantly enhanced tHcy excretion, but no difference in predialysis plasma tHcy was observed following four weeks of treatment.

18

The Beneficial Effects of Endothelin-1 Converting Enzyme Inhibitor on Cardiac Expression of ET-A and ET-B Receptor in Myocardial Dysfunction Secondary to Pressure Everload by Aortic Banding

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ET-A receptor (ET-A) are prominently located in myocardium. ET-1 is inotropic effect in health ventricle, but is negative inotropic effect in failing heart. Previously, we reported chronic ascending aorta banding (AOB) could result in left ventricular dysfunction accompanied with secondary pulmonary hypertension. In the present study, we investigated whether the long term administration of endothelin-1 converting enzyme inhibitor (ECEI), CGS 26393, ameliorated cardiac myocardial remodeling, and altered the cardiac expression of ET-1, ET-A and ET-B receptor (ET-B) of left ventricle (LV) following AOB. The banded rats were randomized to receive saline from Day 1-28 (untreated group, n=9), CGS 26393 (30 mg/Kg, twice a day) from Day 1- 28 day (treated group, n=9). Subsequently, there was significant development of (1) increased perivascular fibrosis and (2) increased mean left atrial pressure and increased mean pulmonary arterial pressure in untreated group. Compared with untreated group, there were significant decrease in both mean pulmonary arterial pressure and mean left atrial pressure, and attenuation in perivascular fibrosis inLV in treated group. Also there was decreased plasma ET-1 level in treated group. Interestingly, the cardiac expression of ET-A mRNA in LV in untreated group was less than by in sham-operated group, but not different from in treated group. The cardiac expression of ET-B mRNA in LV in untreated group was not different from sham-operated group, but significantly less than in treated group. Conclusively, long term CGS 26393 could preventing cardiac dysfunction through inhibiting ET-1 system by decreasing ET-A and ET-1 generation in load-induced cardiac pathology.

19

***In Vivo* Selectivity of an Imidazole-based Heme Oxygenase Inhibitor**

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Introduction: Heme degradation is catalyzed through the action of heme oxygenases (HO) which are present in mammals as inducible (HO-1) and constitutive (HO-2) isoforms. This process results in the equimolar production of carbon monoxide, biliverdin/bilirubin and iron. To date we have identified a number of imidazole-based

compounds that inhibit HO *in vitro,* including several which have demonstrated selectivity for HO-1. Here we set out to investigate whether or not an imidazole dioxolane-derivative (I.D.) demonstrates selectivity *in vivo*. Methods: We used a non-invasive method of determining HO-activity *in vivo* by the measurement of exhaled CO. After baseline CO measurements were taken, male HO-1 knockout (n=10) and wildtype mice (n=10) were administered I.D. (0, 10, or 100micromol/kg) intraperitoneally and their CO excretion profiles were recorded over 5 hours. To maximize the detection of exhaled CO and identify inhibition of HO-activity, all animals were given intravenous injections of heme (30umol/kg), an endogenous substrate for HO. Results: 100micromol/kg I.D. resulted in significant inhibition of HO-activity in both the wildtype (62%) and HO-1 knockout (58%) mice. 10micromol/kg I.D. significantly inhibited HO-activity by 23% in the wildtype while only 14% in the HO-1 knockout, although the differences in inhibition between the groups were not statistically significant. Discussion/Conclusions: These results suggest that at these doses the I.D. does not appear to be as selective *in vivo* as it is *in vitro*. This will be important to consider when using this inhibitor as a research tool or when testing this compound for various therapeutic applications.

20

Therapeutic Targets for Protecting the Endothelium Against Hyperglycaemia-induced Dysfunction

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Introduction: Endothelial dysfunction is an early indicator of cardiovascular disease including that secondary to diabetes. Furthermore, diabetes-related microvascular disease correlates with hyperglycaemia and acute increases in blood glucose elicit endothelial dysfunction. Thus, the identification of cellular sites for glucose-induced endothelial dysfunction could provide targets for the development of new therapeutic approaches for the protection and/or treatment of endothelial dysfunction. Methods: Mouse microvascular endothelial cells [MMECs] were cultured in either low [LG, 5.5mM] or high [HG, 40mM] media to mimic the blood glucose observed in control or type 2 diabetic db/db mice. Westerns and RT-PCR data for COX-1 & COX-2, eNOS, Ser-1177 eNOS and O-linked glycosylated eNOS as well as SOD1, 2 and 3, NADPH oxidase subunits were obtained as well as measures of NO [DAF-2] and superoxide generation [DHE fluorescence] as markers of endothelial function and dysfunction. Results: HG elevated COX-2, eNOS, O-GlcNAAcylated eNOS, p22phox, depressed SOD1, 3 and Ser-1177 eNOS, enhanced superoxide generation and decreased NO generation. Inhibition of PKC, COX-2 as well as the provision of the tetrahydrobiopterin precursor, sepiapterin, prevented or restored the expression and functional changes. Discussion: These data, extending earlier studies, demonstrate the key role for COX-2, NADPH oxidase and oxidative stress in linking diabetes to endothelial dysfunction and indicate that changes in the expression and regulation of eNOS contribute to oxidative stress and endothelial dysfunction. Conclusion: The protection of eNOS and eNOS function via the maintenance of tetrahydrobiopterin is an important target for reducing the impact of diabetes-related vascular disease.

21

Opioid and Nonopioid Stress-induced Analgesia are Mediated By Spinal 5-HT7 Receptors via Activation of Descending Serotonergic Pathways

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Introduction: Acute stress suppresses pain by activating brain pathways that engage opioid or non-opioid mechanisms. In our recent study, we reported the involvement of spinal 5-HT7 receptors in the analgesic effects of systemic morphine and thus, we aimed to examine the role of spinal 5-HT and 5-HT7 receptors in the opioid dependent and independent endogenous analgesic systems activated by swim stress. Methods: The stress-induced analgesia of a 2-min swimming at 32 0C (opioid) and 20 0C (non-opioid) were

assessed using the radiant tail-flick test. The selective 5-HT7 receptor antagonist, SB-269970 (10 microgram) or saline were given intrathecally (i.th) 20 min prior to swimming. To further clarify the role of endogenous spinal 5-HT and descending serotonergic pathways, we investigated the effects of depleting spinal 5-HT, via i.th injection of 5,7di-hydroxytryptamine (5,7DHT) and dorsolateral funiculus (DLF) lesion. Results: Naloxone (5 mg/kg, s.c) pretreatment blocked analgesia induced by swimming at 32 0C, without altering analgesia induced by swimming at 20 0C. I.th administration of SB-269970 completely blocked the opioid and non-opioid form of swim stress induced analgesia. The opioid and non-opioid stress induced analgesia were significantly diminished in both spinal serotonin depleted and dorsolateral funiculus lesioned animals. Conclusion: These findings indicate that the involvement of spinal 5-HT7 receptors via descending serotonergic pathways in endogenous opioid and non-opioid analgesic systems activated by stress. These results indicate that spinal 5-HT7 receptors provide a promising new target for the development of analgesic drugs.

22

Characterization of Chitosan / Hyaluronic Acid Nanocarrier with Light-regulated DNA Release

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Introduction: Gene therapy, using a non-viral vector, presents an alternative method to deliver DNA into cells. However, a major pitfall of the method remains the low *in-vivo* transfection efficiency. We propose herein the use of a stable system obtained by complexing functionalized chitosan with hyaluronic acid. The novelty of the system relies on the use of chitosan coupled with gold nanoparticles pre-functionalized itself with a UV-cleavable bond. The DNA is tagged with Qdots which then is linked electrostatically to the UV-cleavable bond. The exposure of complex to UV-light allows then the monitoring of the DNA release through fluorescence resonance energy transfer. Materials: Chitosan (Ch) (Medipol, SA); Hyaluronic acid (HA) (Lifecore-Biomedical, USA); Low angle dynamic light scattering (DLS) (Malvern instruments, GB); Transmission electron microscopy (TEM)(JEOL2011, USA); Fluorescent microscope (Nikon; Ca); FACScalibur (BD Biosciences, CA); 500 MHz NMR spectrometer (Varian-INOVA, USA). Qdots are synthesized according the method described by Sandros M.G.*et al.*, 2007. Results: Our preliminary data show that DNA/Ch/HA nanoparticles have a higher transfection efficiency and smaller size then DNA/Ch alone. Molecular structure of UV-light responsive chitosan is analyzed by NMR and the properties are tested in-vitro. Discussion: Previous work showed that capacity of Qdots to track chitosan nanoparticles through tissue. With this system, we are able to follow the transfection of DNA/Ch/HA nanoparticles in cells or animal tissue through fluorescence resonance energy transfer. Conclusion: The proposed system seems a safe way to deliver DNA in targeted manner and offer the possibility to follow the internalization process either *in-vitro* or *in-vivo*.

23

A Novel Antibody-based Therapeutic for Benign Prostate Hyperplasia: Results of a Randomized Clinical Trial

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Benign prostate hyperplasia (BPH) is found in up to 60-80% of men over 60. Those with mild to moderate lower urinary tract symptoms (LUTS) are treated according watchful waiting strategy with use of symptomatic pharmacotherapy. A multicenter, 3-armed, placebo-controlled, randomized, parallel design study enrolled 241 outpatients (mean age 63.8±0.5) with moderate symptomatic BPH (IPSS score ≥10), who were randomized to oral ultra-low doses of antibodies to prostate specific antigen (afala, 132 pts, 2 tablets x 4 times a day for 16 weeks), placebo (55 pts, 2 tablets x 4 times a day for 4 weeks) or Serenoa Repens extract (S. repens, 54 pts, 320 mg once a day for 16 weeks). The outcome measures were International Prostate Symptom Score (IPSS), quality of life index from IPSS (QOL), maximum urinary flow rate (MFR), prostate volume, and serum PSA. By week 4, clinical efficacy of afala significantly surpassed placebo

($p<0.001$). By week 16 LUTS symptoms reduced by 43.7% in S. repens group (IPSS dropped from 14.2±0.4 to 8.0±0.4); by 44.2% in afala group (IPSS dropped from 14.7±0.3 to 8.2±0.4). QOL also equally improved in both groups. As a result of 16-week treatment MFR significantly increased by approximately 30% in both active treatment groups. No significant changes in prostate volume and serum free and total PSA were registered during the study. Adverse events rate were similar in 3 treatment arms. Thus, afala showed efficacy at least comparable to herbal medications, and can be considered as a promising and safe treatment option for BPH.

24

The PGFS Activity of AKR1B1 as a Marker and a New Target for Management of Human Pathologies

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Prostaglandins (PGs) are mediators of pain and inflammation also necessary for normal reproductive function. PGE2 and PGF2 are particularly important during menstruation and parturition. PGE2 and its biosynthesis by PGES have been studied extensively, but little is known on the production of PGF2 by PGFS. We have studied the expression of AKR1C3, the only PGFS identified in the human and AKR1B1 a new potentially highly functional alternate PGFS in human endometrial samples and endometrial cell lines. We have found that AKR1B1 (gene ID: 231) is expressed at a high level during the menstrual cycle and able to convert PGH2 into PGF2 in vitro. Transfection with AKR1B1 cDNA increases PGF2 production whereas its knockdown using siRNA reduced PGF2 production. Therefore the PGF synthase activity of AKR1B1 associated with diabetes complications may constitute a new drug target and provide a biological marker for human pathologies.

25

Development of Curcumin as a Mitochondrially – Targeted Antioxidant

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Increased production of reactive oxygen species and other free radicals occur during the pathological disease-processes. Therefore, a role for increased oxidative stress has been implicated in disease conditions including non-communicable diseases such as diabetes, cardiovascular diseases and cancer. In this context, exogenous supplementation of antioxidants is considered useful. Vitamin E is one of the most studied antioxidant, but still it lacks convincing beneficial effects as it is believed to work at the lipid bilayer membrane and not reaching intracellular levels. Since most of the oxidant-generating reactions occur at the site of mitochondria, there is an increased attention in the development of mitochondrially targeted antioxidants. There is some success in this area of research specialization. Curcumin, a natural product of turmeric has been claimed to work as an excellent antioxidant, but again, problem is its bioavailability. Therefore, this project, though ambitious, has two goals: 1) Development of curcumin as a mitochondrially targeted antioxidant 2) Testing it for its efficacy and beneficial actions using cell lines or animal model systems.

26

***In-vitro, In-vivo* and Rheological Evaluation of Gellan / Alginate Based In-situ Gel Forming Sparfloxacin Ocular Delivery Systems**

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Introduction: Poor bioavailability of ophthalmic solution caused by dilution and drainage can overcome by in-situ gelling ophthalmic delivery system from polymers that exhibit reversible ion-activated sol-gel transitions in cul-de-sac. Methods: The purpose of study was to develop and characterize a series of in-situ gelling gellan and sodium alginate-based solutions for the potential delivery of fluoroquinolone antibacterial, sparfloxacin for the treatment of acute and chronic

ocular infections. The rheology, gelling efficiency in simulated tear fluid, in-vitro release, ex-vivo corneal permeation from excised cornea, in-vivo ocular irritation and in-vivo pharmacodynamic anti-microbial efficacy were evaluated to determine the efficiency as ocular therapeutic system of the developed sterile formulations. Results and Discussion: The optimum polymer concentrations for instantaneous extended gelling was gellan (0.15 – 0.2%) alone, alginate (2%) alone, gellan-alginate (0.1 & 0.5%). The gels showed pseudo plastic thixotropic rheological behavior by Brooke-field rotational viscometer at the angular velocity from 0.5 to 100 rpm at a controlled ramp speed. The regression coefficient of in-vitro release and ex-vivo corneal permeation confirms the diffusion controlled Higuchi kinetic release of sparfloxacin from the formulations with the corneal permeability coefficient, P_{app} ($x10^{-6}$), alginate alone (4.06), gellan alone (3.67) and gellan-alginate (2.64). All formulations were non-irritant with zero average Drazie irritation score. The in-vivo antimicrobial efficacy studies in pre-inoculated rabbit eye using 0.5 McFarland suspension containing *S.aureus* and *P.aeruginosa*, instilled with selected formulations showed a 24 hour protection against the organisms when compared with conventional eye drops. Conclusion: The gellan-alginate based ion activated in-situ gelling formulation containing sparfloxacin was promised to be better once daily ocular therapeutic system than the gellan or alginate alone formulations and best than the eye drops to achieve a prolonged release with a increase in the pre-corneal residence time of the drug.

27

Chronic Administration of Chondroitin Sulfate does not Affect Cytochrome P450 and NADPH P450 Reductase in the Rabbit

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Chondroitin sulfate (CS) is a SYSADOA that elicits anti-inflammatory effects. Since patients take CS over long periods, it was of interest to assess whether CS modulates the activity of cytochrome P450 isoforms (P450). Two models were used, chronic intake of CS in control rabbits and in rabbits with a down-regulated P450 by an inflammatory reaction (IR). Six groups of 5 rabbits were used; three were used to assess the effect of CS on P450, one without CS and two receiving orally 20.5 mg/kg/day CS for 20 and 30 days; three groups received turpentine s.c. generating an aseptic IR (AIR) 48 h before their sacrifice, e.g. days -2, 18 and 28, exposed to CS for 0, 20 or 30 days, respectively. CYP3A6, CYP1A2 and NADPH P450 reductase (NADPH) activity, expression and RNAm were assessed in the hepatocytes. Compared with control rabbits, 20 and 30 days CS did not affect the activity of CYP3A6, e.g. 15582±1330, 13480±3052 and 14701±841, and of CYP1A2, e.g. 6532±1203, 11612±2403 and 7494±746, arbitrary units. The AIR increased seromucoids to 95.1±5.7 vs 8.4±1.6 mg/dl in controls ($p<0.05$). The AIR reduced CYP3A6 activity to 5972±464, 5415±541 and 2639±747, and CYP1A2 activity, 3026±113, 3856±1151, 3805±753 for control, 20 and 30 days of CS, respectively ($p<0.05$ compared without AIR). CS did not affect NADPH activity or expression. It is concluded that CS does not affect activity or expression of CYP3A6 and CYP1A2, nor prevents AIR-induced down-regulation. These results are in agreement with the absence of CS-drug interactions in humans.

28

T Regulatory Lymphocytes in Thyroid Malignancies

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Understanding the role of immune response in thyroid cancer is of great importance in order to elucidate its evolution and the possible therapeutic approaches. The action of T regulatory cells to suppress cytotoxic and effector T lymphocytes is the reason of dysfunction of immune response in patients with cancer. T regulatory lymphocytes (Tregs) are indispensable in the regulation of immune response and the control of immune homeostasis. Objective: Assessment of Tregs in malignant tissue of patients with thyroid cancer.

Patients and Methods: The subset of Tregs in peripheral blood and thyroid tissue of 10 patients (age18-46) with pappilary thyroid carcinoma was estimated by multicolor Flow Cytometry. As controls were used blood and thyroid tissue from patients with goiter (n=8), blood from healthy donors (n=53) and cord blood (n=55). Tregs were determined as T4 CD25bright and CD127dim/negative lymphocytes and expressed as % of T4. Results: Treg median values and 25-75 percentiles were as follows: normal blood 1.5(1.1-2.1), cord blood 3(2,2-3,9), thyroid cancer blood 2.2(1.6-2.9) and tissue 5.4(1.1-17.8), goiter blood 1.4(1-2.2) and tissue 2(0,8-3,5). Treg values are higher in tissue from thyroid cancer compared with goiter (t-test) 2) values in blood vs tissues differ in patients with cancer, but not in patients with goiter (paired t-test) 3) there is no significant difference among blood from patients with cancer, goiter and healthy donors (ANOVA) 4) cord blood has higher Treg values (ANOVA). Conclusions: Tregs were found significantly increased in tissue of patients with pappilary cancer but not in goiter. Blood values were within normal range

29

Effects of N(2-propylpentanoyl)urea on Hippocampal Amino Acids Neurotransmitters in the Pilocarpine Model of Temporal Lobe Epilepsy in Rats

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N(2-propylpentanoyl)urea(VPU) is a new valproic acid(VPA) analog with higher anticonvulsant activity than its parent compound in various animal models including seizure acutely induced by pilocarpine. Therefore we considered to investigate its effects on hippocampal amino acid neurotransmitters in a model of temporal lobe epilepsy in rats. Pilocarpine hydrochloride (380 mg/kg, i.p.) was used to induce status epilepticus (SE) which was terminated by diazepam (10 mg/kg, i.p.) at 30 min after the onset of SE. Animals were visually observed for 2 hr/day for an episode of spontaneously recurrent seizure (SRS) for 6 weeks. Microdialysis experiment was performed on those rats that developed SRS. Alteration of hippocampal amino acid neurotransmitters, determined by HPLC, was expressed as percentage of change from basal value before the administration of test substances. Among 38 rats used, 28 animals developed SRS with latency to the first occurrence of SRS at 11.48 ±0.46 days after SE. They were randomly assigned into control, VPU treated (50 and 100 mg/kg, i.p.) and VPA treated (300 and 600 mg/kg, i.p.) groups. Excitatory amino acid neurotransmitters, glutamate and aspartate, were significantly decreased by VPA whereas VPU, while persistently reduced glutamate level, exerted no effect on aspartate. Reduction of inhibitory amino acid neurotransmitters, comparatively to smaller extent than those observed on glutamate, was noted. VPU significantly depressed the level of both glycine and GABA whereas VPA exclusively reduced the level of glycine. The results obtained suggest that the anticonvulsant activity of VPU could be at least, in part, explained by the drastic decrease of hippocampal glutamate.

30

Preparation and Evaluation of a Floating Drug Delivery System for Calcium Acetate

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Introduction: Calcium acetate (CA), an effective phosphate binder, has been orally used in CRF patients to decrease hyperphosphatemia. Development of an oral floating drug delivery system (FDDS) of CA could provide a gradual release of calcium throughout the GIT and binding the phosphorous of food more effectively than conventional tablet. Methods: Different formulations of CA matrix tablets (450 mg) containing several synthetic and natural polymers, were prepared by wet granulation method. All tablets included an effervescent base. Tablets were evaluated for in vitro floating ability, bioadhessiveness, drug release and phosphate binding capacity. The drug release studies were carried out using a dissolution apparatus, Paddle method (50 rpm, 37° C) in 900 ml of distilled water. Withdrawn samples were assayed for amount of drug released by atomic absorption method at 422.8 nm. Results: The selected

formulation containing 15% effervescent base, 25% HPMC and 5% NaCMC ($H_{25}G_{15}C_5$) had floating lag time of 0.5 ± 0.1 min and duration of floating about 24 ± 2.5 h. The dissolution profile parameters for the selected tablet was as follows: Mean dissolution time (MDT) = 2.2 ± 0.4 h, Dissolution Efficiency (DE_8%) = 69.5 ± 1.0% and T_{50}% = 4.6 ± 0.15 h. This formulation had a phosphate binding capacity of about 50%. Discussion: The more HPMC content of the tablets, the more in the bioadhesive properties. Drug release data was fitted on Higuchi kinetic model. Increasing of calcium release causes increment of phosphorous binding. Conclusion: The selected formulation seems to be considered as FDDS for CA.

31

Effect of Insulin on the Streptozotocin and Glucose-treated Chick Embryos

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In an attempt to reduce the number of mammals used in drug research, we have been examining the use of chick embryos and found that they may be superior for predicting the effects of drugs. Streptozotocin (STZ) has been used as a model of insulin-dependent diabetes in animal. However, it was not known STZ brings about any changes in pancreas of chick embryos. At the present study, we determined the levels of blood glucose in chick embryo treated at the different doses of glucose and examined to produce a model of diabetes in chick embryo by treatment of STZ. Furthermore, we evaluated the effects of human insulin using this diabetes model. Fertilized eggs of White Leghorns were investigated. The different doses of glucose were injected into the eggs. STZ was injected into the each egg. Regular human insulin was injected into the air sac of STZ-treated eggs. The levels of blood glucose and serum insulin were determined. Levels of blood glucose and serum insulin in chick embryos increased with developing stages. Levels of blood glucose increased dose dependently. Blood glucose level of chick embryos treated with STZ was significantly higher than that in the control. Conversely, serum insulin level was lower than that in the control. In addition, the enhanced level of blood glucose in STZ-treated embryos reduced by injection of human insulin. In the present study, we proposed an experimental animal model with diabetes in chick embryos treated with STZ and the effects of human insulin using this model.

MONDAY JULY 28, 2008
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32

Effect of Acute Treatment with TGF-Beta1 Antisense Oligodeoxynucleotide on Renal TGF-Beta1 Expression and Renal Dysfunction in Experimental Diabetes

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Transforming growth factor (TGF)-Beta1 is implicated in the onset and progression of diabetic nephropathy. This study investigates the effect of TGF-Beta1 antisense oligodeoxynucleotide (ODN) on renal TGF-Beta1 mRNA expression and renal dysfunction in diabetes. Male Sprague-Dawley rats were made into 3 groups, diabetic, diabetic treated with TGF-Beta1 antisense ODN (D-ODN) and normal. Streptozotocin (60mg/kg i.p.) was used to induce diabetes in these animals. At 15, 30 and 45 days after diabetes induction, the animals were subjected to haemodynamic study. Two days before the haemodynamic study, the D-ODN group received TGF-Beta1 antisense ODN (2mg/kg). Renal TGF-Beta1 expression was measured by real-time polymerase chain reaction. Here we demonstrated that TGF-Beta1 mRNA was highly expressed in the kidney of diabetic rats ($p<0.01$). Parallel to this, renal dysfunction exhibited by severe proteinuria ($p<0.01$), hyper filtration ($p<0.01$), and increase fractional sodium and potassium excretion ($p<0.01$), was also present. Acute administration of TGF-Beta1 antisense ODN suppressed the expression of TGF-Beta1 mRNA in early ($p<0.01$) but not in late diabetes. Renal dysfunction was not improved.

However, our previous work demonstrated amelioration of renal dysfunction in diabetic animals upon chronic weekly administration of the ODN, suggesting that TGF-Beta1 antisense ODN is not capable of reversing renal dysfunction in diabetic animals but may prevent the progression of the disease. These data suggest that over expression of TGF-Beta1 in kidney is suppressed by the antisense ODN in early diabetes. Although the TGF-Beta1 expression is reduced with acute administration of the ODN in early diabetes, the renal dysfunction is not ameliorated.

33

Lack of Drug-drug Interactions with M118, a Novel, Rationally Engineered Low Molecular Weight Heparin, When Co-administered with Multiple Doses of Aspirin and Clopidogrel

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Background: M118, a rationally engineered LMWH administered both IV and SC, is being developed for usage in percutaneous coronary intervention and acute coronary syndromes. Since M118 may be administered with anti-platelet agents, the potential pharmacokinetic (PK) and pharmacodynamic (PD) interactions with aspirin (ASA) and clopidogrel were investigated. Method: This randomized, double-blind, placebo-controlled, crossover study was conducted in 15 healthy subjects receiving M118 (IV, 75 IU/kg) alone, ASA (325mg) and clopidogrel (300mg loading-dose + 75mg) alone, and the combination of all three drugs. Blood samples were collected for the determination of aXa and aIIa activities, ASA, Salicylic Acid (SA) and clopidogrel levels. PD and safety monitoring included ACT, bleeding time (BT) and platelet aggregation (PA). Results: The co-administration of M118 with ASA and clopidogrel did not affect the Cmax and AUCt values of SA and the Cmax values of clopidogrel, however AUCt of clopidogrel decreased by 24%. Additionally, there was no difference noted in BT and PA. No effects were observed on the bioavailability of M118 and on the maximum ACT value when co-administered with ASA and clopidogrel. Mean tmax values of aXa, aIIa, clopidogrel and SA remained unchanged when ASA and clopidogrel were administered alone or in combination with M118. No severe or serious adverse events occurred. Conclusion: This study showed no significant interactions on the reciprocal PK and PD effects of M118 in combination with aspirin and clopidogrel, although a slight decrease in AUCt for clopidogrel was noted. M118 administered in combination with aspirin and clopidogrel was well tolerated.

34

Differential Expression of HIV-1 Tat Protein by T Cells Confers Differential Sensitivity to Sulphamethoxazole-hydroxylamine

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Background: Pneumocystis pneumonia (PCP) is a major AIDS-defining disease with sulphamethoxazole (SMX) being the first line treatment. However, SMX is associated with hypersensitivity adverse drug reactions (ADR) with up to 50% incidence in HIV patients. Previous studies have shown that the HIV regulatory protein Tat together with a reactive metabolite of sulphamethoxazole, SMX-HA (sulphamethoxazole-hydroxylamine), is involved in the pathogenesis of these ADRs in HIV patients. This study was conducted to determine whether differential expression of Tat is associated with differential cellular sensitivity to SMX-HA. Method: Full length Tat (Tat101) as well as the fusion protein Tat101GFP were placed into a doxycycline-inducible vector where their expression can be differentially expressed. Next, the recombinant vectors were used to establish stably transfected Jurkat T cell lines. These cell lines were incubated with SMX-HA followed by an assessment of mitochondrial activity by MitoTracker Red, a dye that stains mitochondria in live cells where its accumulation is dependent on membrane potential. Results: Concentration-dependent toxicity was demonstrated with SMX-HA ($p<0.01$) in both the Tat101 and Tat101GFP cell lines. The Tat101 cell line showed a trend toward increased cellular toxicity as Tat101 expression increased. This trend was significant at

800 and 1000ng/ml doxycycline. The Tat101GFP cell line did not show any significant differences in toxicity with the differential expression of Tat101GFP. Conclusion: Increasing Tat101 expression leads to an increase in cellular toxicity when the cells are treated with SMX-HA. Fusion with GFP abrogates the effect of Tat in increasing the cellular toxicity to SMX-HA.

35

Short and Long-term Effects of Trazodone on Forced Swimming Test in Male Wistar Rat

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Trazodone is a clinically effective antidepressant that acts by inhibiting the uptake of serotonin. Commonly, high doses are used in clinical practice giving place to side effects in some patients, such as drowsiness, dizziness and dry mouth, among others. In basic research, trazodone has also been tested using relatively high doses and mostly in single dose, which is different from clinical regimens, in which a single administration has no effect, i.e., the clinical improvement occurs after weeks of treatment. The aim of this study was to determine a minimal effective dose in male Wistar rats and evaluate its effects in the short and long-term in the forced swim test (FST). The results indicated that 1 mg/kg by 21 days significantly increased the latency to the first immobility period (LFI) [$F_{(4, 51)}$= 3.0, p<0.03] and also significantly reduced the total time of immobility [$F_{(4, 51)}$=3.35, p<0.02] without changes in locomotor activity [$F_{(4, 51)}$= 1.79, p= 0.15, NS]. A lower (0.1 mg/kg) and higher doses (5 and 10 mg/kg) of trazodone had no effect. Treatment for 21 days with 1 mg/kg of trazodone significantly increased LFI as compared to 2-day and 14-day treatments [$F_{(2, 46)}$= 4.22, p<0.02]. In conclusion, trazodone had antiimmobility effects in the FST without affecting locomotion after three weeks of low-dose regimen, which supports the observation that it is necessary the instauration of slow-stepping plastic events to exert its antidepressant effect.

36

First, Second and Third Order Intervals of Neuronal Firing used as a Model for Exploring Order or Chaos

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The firing pattern study based on the analysis of the first, second and third order intervals may be used for exploring whether neuronal activity follows a random or an ordered sequence. We obtained the single unit extracellular recordings from basolateral amygdala, a structure involved in fear and anxiety, in three experimental groups. Control group of Wistar rats was devoid of any surgery. Since basolateral amygdala receives indirectly inputs coming from the accessory olfactory system, in other two groups, the vomeronasal organ was previously ablated and single unit recordings performed 2 hr or 14 days after surgery, respectively. Data were processed by specially designed software (Matlab) and the analysis included 25 action potentials for each recorded cell. One-way ANOVA illustrated that the first order intervals of firing significantly shortened after the acute vomeronasal surgery ($F(2,1568)=38.713$, $p<0.001$) but a recovery occurred given that the results from the group studied 14 days after lesion did not differ from control group. Similar results were obtained for the second ($F(2,1568)=67.936$, $p<0.001$) and third order intervals of firing ($F(2,1568)=87.158$, $p<0.001$) analysis. The interval histogram graphs showed a constant regularity in the distribution of intervals, suggesting that albeit vomeronasal organ ablation did modify the general firing rate, there is a constancy of intervals distribution. Therefore, we conclude that at least in basolateral amygdala the firing pattern follows a strict sequence, perhaps depending on the timing for membrane channels opening.

37

Studies on the Therapeutic Potential of Amoxicillin on Acetic Acid- induced Colitis in Rats

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This study was carried out to investigate the therapeutic potential of amoxicillin (AMX) and its possible mechanism of action on acetic acid induced colitis in rats. Animals were divided into six groups (n=10). Groups 1-3 were given twice daily doses of AMX (25, 50 and 100 mg / kg; p.o.); respectively, 48, 24, or 2 hr for two consecutive days before induction of colitis by acetic acid. Groups 4-6, however, received the same oral daily doses of amoxicillin for five consecutive days starting 6 hr after induction of colitis by acetic acid. In another set of animals (control), the same protocol was followed except that methyl cellulose was given instead of AMX to either saline or acetic acid treated animals. Induction of colitis was performed following the modification of the method described by Millar et al., 1996. Biochemical parameters such as reduced glutathione levels, myeloperoxidase (MPO) activity and extravasations of Evans blue were measured following standard assay procedures. AMX administered before or after induction of colitis attenuates both macroscopic and microscopic changes induced by acetic acid. Furthermore, the biochemical parameters were reversed and brought towards the control levels by both regimes. The results of this study suggest that the mechanisms of the beneficial effects of AMX against acetic acid- induced colitis may include prevention of depletion of reduced glutathione, reduction of MPO activity, decreased vascular permeability and inhibition of infiltration of inflammatory cells in the mucosa. These finding indicate that the beneficial effects of AMX may be partly due to its antioxidant and anti-inflammatory properties.

38

Influence of Age on Cisplatin Nephrotoxicity in Rats in Relation to their Oxidant Status

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This work examines the influence of age on some nephrotoxic signs of cisplatin (CP) in rats (aged 3, 7, 11 or 24 weeks) and its relation to the oxidant status in the kidneys and blood. At different ages, rats were injected intraperitoneally (i.p.) with either CP (6 mg/kg) or with 0.9% saline, and killed 6 days thereafter. Nephrotoxicity was evaluated histopathologically by light microscopy and biochemically by measuring the concentrations of creatinine and urea in serum, reduced glutathione (GSH) concentration, total antioxidant status (TOS) and superoxide dismutase (SOD) activity in renal cortex and serum, and by urinanalyses. In rats of different ages CP significantly increased the concentrations of urea and creatinine ($P < 0.05$) by about 111 to 131%, and 121 to 167%, respectively. CP treatment reduced cortical GSH concentration by about 24 to 38% ($P < 0.05$), TOS by 22 to 36% ($P < 0.05$) and the activity of SOD by about 23 to 29% ($P < 0.05$). CP treatment significantly increased urine volume and *N*-acetyl-beta-D-glucosaminidase (NAG) activity, and significantly decreased osmolality and protein concentrations. These effects were more expressed with age. Significant age-differences were found in most of the parameters used as indicators of nephrotoxicity in young and adult rats, with adverse renal effects being more severe with age. CP produced marked damage to the proximal renal tubules, and the induced morphological changes were also more pronounced with age. Cortical CP concentration in 3 week-old rats was about 66% of that in the 24 week-old rats. The present results indicate that age has a consistent effect on the antioxidant system in rats and that CP nephrotoxicity is increased with increases in age.

39

Pharmacogenetics of DHFR in Childhood ALL

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Methotrexate (MTX), a folic acid antagonist, is an important component of the treatment of acute lymphoblastic leukemia (ALL). Nevertheless, a subset of patients can develop drug resistance or drug-side effects, which may hamper the efficacy of treatment. A major mechanism of MTX action involves inhibition of the dihydrofolate reductase (DHFR). DHFR can play an important role in development of MTX resistance. Difference in DHFR expression can be in part due to the functional polymorphisms in the regulatory regions of DHFR gene. The aim of this study was to analyze the impact of DHFR polymorphisms located in the 555 bp region preceding major transcript initiation site, which has been shown to be a target of a regulatory non-coding interfering DHFR transcript. Fifteen SNPs were identified from dbSNP database and were genotyped in control population (n=43). Five SNPs were found polymorphic with minor allele frequency ranging from 8% to 36%. In addition, we discovered multi-allelic length polymorphism composed of 9bp sequence repeats. Five different alleles were detected in the population tested. Due to the linkage disequilibrium among identified variants, the polymorphisms can be organized in 9 haplotypes. Two SNPs and length polymorphism are identified as sufficient to define observed haplotypes. These tag polymorphisms will be genotyped in children diagnosed with ALL at Sainte-Justine hospital followed by the association studies between obtained genotypes/haplotypes and ALL outcome. The results, combined with others findings, may contribute to establish an individualize treatment for ALL patients.

40

Influence of GST Gene Polymorphisms on Busulfan Pharmacokinetics in Children

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Busulfan is a key compound in the conditioning regimens of children undergoing hematopoietic stem cell transplantation (HSCT). Inter-individual differences in busulfan levels may cause treatment failure and adverse drug reactions such as hepatic veno-occlusive disease and graft rejection. Since busulfan is mainly eliminated by glutathione S-transferase (GST)-catalyzed conjugation with glutathione, it is hypothesized that functional polymorphisms in GST genes may underlie variability in busulfan pharmacokinetics. We analyzed polymorphisms in GSTA1 (C-69T, A-513G, G-631T, G-1142C), GSTM1, (deletion-null genotype) and GSTP1 gene (A578G, C2293T). Genotyping was performed retrospectively in 28 children that underwent HSCT at CHU Ste-Justine with individualized dosing based on monitoring first dose pharmacokinetics following intravenous administration. Cumulative drug dose and pharmacokinetic data were correlated with genotypes. Haplotype analysis in GSTA1 gene revealed the existence of six haplotypes; five were previously defined as A1, *A2, *A3, *B1 and *B2, whereas newly defined *B1a differed from *B1 by the presence of G-513 allele. The carriers of the *B2 haplotype (11%) had higher cumulative busulfan doses (p=0.05), lower area under the curve (p_{AUC}=0.03), maximum plasma concentration (p_{Cmax}=0.03), and higher clearance (p=0.02). GSTM1 null individuals (50%) received lower cumulative doses (p=0.02), had higher drug plasma concentrations (p_{Cmax}=0.003 and p_{AUC}=0.008), and lower clearance (p<0.001). No association was found with GSTP1. GSTA1 and GSTM1 polymorphisms seem to have an impact on the busulfan pharmacokinetics in children following intravenous administration. A prospective study is ongoing to confirm these results and to evaluate if the optimal dose of busulfan can be determined according to the GST genotypes.

41

LC/MS/MS Method for the Analysis of Isosorbide Mononitrate in Human EDTA K_2 Plasma

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Purpose: Isosorbide mononitrate is a type of vasodilator. It relaxes blood vessels, increasing

the blood and oxygen supply to the heart. It is effective in the long-term treatment of angina associated with coronary artery disease. The purpose of this work was to develop and validate a specific and robust method for the determination of isosorbide mononitrate (IMN) in human EDTA K_2 plasma. Methods: IMN and its internal standard (isosorbide-5-mononitrate-6^{13}C) were extracted from human EDTA K_2 plasma by solid phase extraction using Oasis HLB 60 mg/3cc cartridges. Compounds were eluted with methanol. Analysis was performed on a MDS Sciex API 4000 tandem mass spectrometer with TurboIonSpray interface. Negative ions were measured with m/z $189.6 \rightarrow 61.9$ for IMN and $196.0 \rightarrow 62.0$ for IS. The chromatographic run time was 2.0 minutes on a Zorbax SB-C18 50 X 4.6 mm column. The mobile phase was a mixture of Milli-Q type water and methanol (80/20) with infusion of post-column alkalized methanol. Results: This assay was validated over a nominal range of 5 to 1250 ng/mL. Linearity over the calibration range was $\geq$ 0.9985. The between-run accuracy ranged from 98.53 to 100.67% with precision ranging from 2.53 to 3.18%. The within-run accuracy ranged from 1.73 to 7.22% with precision ranging from 1.73 to 7.22%. The recovery of IMN and its internal standard was greater than 74%. No matrix effect on quantitation was observed. IMN was found to be stable in human EDTA K_2 plasma after 73 hours at room temperature for short term stability, after 81 days at -20°C and -80°C for long term stability, after 77 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -20°C and -80°C. Dilution integrity and matrix selectivity were also demonstrated. Hemolysis effect was evaluated. Conclusions: This method is accurate, reproducible and was successfully applied for the analysis of clinical samples. Over 2000 study samples were analysed with accuracy ranging from 90.69 to 96.61% and precision ranging from 1.39 to 7.35%.

42

Effects of Polymorphic Variant of Endothelial Nitric Oxide Synthase on Hemorheological Variables in Healthy Nonsmoking Subjects

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Circulation is regulated by interaction between blood constituents and vasculature. Nitric oxide plays a major role in circulatory homeostasis. Endothelial nitric oxide synthase (eNOS) is expressed both by endothelium and blood cells, particularly by erythrocytes. Although vascular responses in individuals with eNOS genetic variants have largely been studied, there is limited knowledge about functional consequences of eNOS genetic variability in erythrocytes. The objective of this study was to investigate effects of 894G>T polymorphism on hemorheological variables, such as erythrocyte deformability, plasma viscosity and erythrocyte aggregation (rouleaux formation) in blood samples obtained from age-matched, nonsmoking, healthy volunteers with GG (n=25), GT (n=17) and TT (n=5) genotypes. Hemorheological variables were measured by means of the laser-assisted optical rotational cell analyzer. The parameters used for the assessment of erythrocyte aggregation were aggregation index (AI; directly proportional with the extent of aggregation) and the half-life for aggregation formation. There were no differences among genotype groups for erythrocyte deformability or plasma viscosity. However, the extent and the rate of erythrocyte aggregation were significantly decreased in individuals with TT genotype as compared to subjects with the wild-type allele (AI = 53.0±4.2% vs. 60.8.0±1.0%, respectively; P=0.022 and half-life = 3.7±0.5 vs. 2.5±0.1 seconds, respectively; P=0.017). These findings demonstrate that eNOS 894T allele is associated with inhibition of erythrocyte aggregation in nonsmoking healthy individuals and imply that eNOS genetic variants may contribute to pathogenesis of obstructive microvascular disorders by changing erythrocyte functions.

43

Effect of Atorvastatin on hs-CRP in Acute Coronary Syndrome

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Early introduction of statin therapy may improve the outcome in patients with acute coronary syndromes (ACS) by attenuation of inflammation as evidenced by reduction in highly sensitive C-reactive protein (hs-CRP). Atorvastatin in high dose is the most widely investigated drug for this purpose. Hence, this study was designed to evaluate the effect of low dose (20mg) atorvastatin on hs-CRP in patients with ACS. After measuring the baseline levels of hs-CRP and lipid fractions, Group A (n=50) patients received atorvastatin 20mg/day for 4 weeks in addition to usual antianginal treatment. Group B (n=50) patients received usual antianginal treatment for 4 weeks without atorvastatin. Following 4 weeks of treatment the same measurements were repeated. In group A, hs-CRP decreased by 82% from 2.32 to 0.57mg/dl ($p<0.001$) after 4 weeks of treatment. In group B, hs-CRP decreased by 54% from 1.91 to 1.04mg/dl ($p<0.001$). There was significant difference between mean percentage decreases in hs-CRP in patients of group A as compared to group B (82% vs 54%). The decrease in hs-CRP was also significantly more in the subgroups of smoking, hypertension and past history of cardiovascular disease in group A as compared to the group B. The study showed no significant difference in the secondary outcome measures between the two groups. The overall incidence of adverse effects was low. The use of low dose atorvastatin is safe, effective and offers an attractive approach for early treatment of ACS patients.

44

Two Allelic Variants of Aldo-keto Reductase *1A1* Exhibit Reduced *in vitro* Metabolism of Daunorubicin

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The anthracyclines doxorubicin (DOX) and daunorubicin (DAUN) are widely used in treatment of leukemias, lymphomas, sarcomas and carcinomas due to their success in increasing the life expectancy of cancer patients. However, the clinical use of these drugs has revealed interpatient variability in the development of dose-related chronic cardiotoxicity, which can often lead to the development of life-threatening complications such as congestive heart failure. One of the factors underlying this variability may be linked to the altered metabolism by enzymes that are non-synonymous single nucleotide polymorphisms (ns-SNPs) of aldo-keto reductase *1A1* (*AKR1A1*). In this study we looked at the wild-type human *AKR1A1* along with the two naturally-occurring ns-SNPs, *N52S* and *E55D*, for their ability to metabolize DOX and DAUN to their corresponding major metabolites, doxorubicinol (DOXol) and daunorubicinol (DAUNol). Using purified bacterially-expressed recombinant human enzymes, we show that both ns-SNPs are associated with a significantly reduced efficiency in metabolizing DAUN to DAUNol compared to the wild-type. DOX was metabolized by the wild-type and ns-SNPs, however, the levels of DOXol were below the limit of quantitation. A reduction in DOXol and DAUNol formation by these variant enzymes could result in an overproduction of semiquinone metabolites, which are hypothesized to be involved in the production of reactive oxygen species that can damage the heart. Therefore, *N52S* and *E55D* may be suitable genetic biomarkers to assess a cancer patient's risk of developing chronic cardiotoxicity prior to treatment.

45

Modelling the Kinetics of Transporter Mediated Uptake in Isolated Rat Hepatocytes

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There has been modest success when predicting transporter-mediated hepatic clearance from *in vitro* data. The integration of metabolic and active uptake intrinsic clearance with plasma protein binding often led to under predictions of hepatic clearance. We investigated the kinetic modelling of hepatocyte uptake experiments, and the influence of active uptake and protein binding kinetics. Active uptake into hepatocytes was investigated using hepatocyte uptake experiments in the presence and absence of albumin. Models, integrating combinations of active and passive

processes were developed in NONMEM. The models were fitted to the uptake data collected in the absence of albumin. A kinetic model of albumin binding using association and dissociation rate constants was compiled. This was added to the best model and this model was used to predict the observed hepatic accumulation data obtained in the presence of albumin. The hepatocyte uptake data obtained in the absence of albumin was fitted accurately by a model that included a saturable uptake process combined with bidirectional passive flux. The model also required an intracellular compartment to mimic canalicular vesicles. Predictions of protein binding effects made with current models, under predicted the albumin-influenced uptake data. The simulations performed, using the model with protein binding kinetics, accurately predicted the experimental data and identified influential processes. The plasma binding association rate constant determined the influence of plasma protein binding on disposition processes. The developed hepatocyte uptake model can be utilised within physiologically-based pharmacokinetic models for the accurate prediction of *in vivo* transporter-mediated disposition from *in vitro* data.

46

Erythrocytic and Plasma Antioxidant Properties of Statins in Hyperlipidemic Indian Patients – A Comparative Study for Pleiotropic Effects between Atorvastatin and Simvastatin

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Introduction: To assess the effects of hyperlipidemia on erythrocytic and plasma oxidative stress and its modulation by low dose simvastatin and atorvastatin (10 mg each) in Indian subset. Materials and methods: Forty statin naïve patients were evaluated for effects of hyperlipidemia on erythrocytic and plasma oxidative stress as assessed by measurement of TBARS & antioxidant enzyme levels. Patients were randomized into two groups of twenty patients each, receiving atorvastatin and simvastatin for eight weeks. Assessment was done at baseline and after eight weeks. Results: There was a significant increase in baseline erythrocytic and plasma oxidative stress in hyperlipidemic patients as compared to healthy controls (Erythrocytic TBARS levels were 294.1 ± 126.8 units in hyperlipidemics vs. 99.4 ± 22.50 units in normolipidemics, p= 0.002,). After eight weeks of therapy erythrocytic TBARS levels decreased 38.3% in atorvastatin group and 28.2 % in simvastatin group (p= 0.005). Erythrocytic superoxide dismutase, catalase and glutathione peroxidase levels increased by 32.1%, 90.9%, 19.8% (p= 0.01, 0.005, 0.005) respectively in atorvastatin group. The increase in simvastatin group was 32.9%, 12.6% and 36.9% (p= 0.01, 0.01, 0.05) respectively. Similar results were obtained for plasma oxidative and antioxidative parameters. The decrease in cholesterol levels was also significant in both the groups. There was no correlation between lipid lowering effects and antioxidative effects. Conclusion: The results of this preliminary study are highly favourable in demonstrating pleiotropic effects of low dose statins in Indian patients.

47

Fluorescent Ligands of the Bradykinin B1 Receptors: Investigative and Potential Diagnostic Tools

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Unlike the widely distributed and preformed B2 receptors, the bradykinin (BK) B1 receptors are highly regulated and induced under inflammatory conditions (e.g., in vascular cells, peripheral T cells during multiple sclerosis flares). To evaluate the potential usefulness of fluorescent B1 receptor probes applicable to live cell microscopy and cytofluorometry, combined chemical synthesis and pharmacologic evaluation have been conducted on novel 5(6)-carboxyfluorescein (5(6)CF)-containing peptides. Representative agents are the antagonist B-10376 (5(6)CF-EACA-Lys-Lys-[Hyp^3, CpG^5, D-Tic^7, CpG^8]des-Arg^9-BK) and the agonist B-10378 ((5(6)CF-EACA-Lys-des-Arg^9-BK). B-10376 is a surmountable antagonist in the rabbit aorta contractility assay (pA_2 7.67), has an IC_{50} of 62 nM to displace [^{3}H]Lys-des-Arg^9-BK from human

recombinant B1 receptors expressed in HEK 293 cells and can label with specificity human or rabbit B1 receptors expressed in the same cells (epifluorescence or confocal microscopy). Cytofluorometry shows the possibility to quantitatively identify the low endogenous population of B1 receptors in human vascular smooth muscle cells and its upregulation (interleukin-1 treatment). In all fluorescent applications, the specific labeling was prevented by an excess of a B1 receptor nonpeptide antagonist. B-10378 was a full agonist at the B1 receptor of the rabbit aorta, had a binding competition EC_{50} of 375 nM and is potentially useful to show the particular form of agonist-induced adaptation of this GPCR (translocation to caveolae-related rafts without internalization). A fluorophore has been introduced in B1 receptor agonist or antagonist peptides at the expense of a certain loss of affinity, but the resulting agents allow original applications (imaging in live cells, cytofluorometry).

48

Epigenetic Regulation of *UGT1A1* and *HNF1* Transcription Factor Gene Expression in Colon Cancer Cells: Potential Implications for Cancer Treatment

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UDP-glucuronosyltransferase 1A1 (UGT1A1) glucuronidates SN-38, the active metabolite of irinotecan commonly used to treat metastatic colorectal cancer. We previously showed that DNA hypermethylation represses *UGT1A1* expression in colon cancer cells. Our data suggest that *UGT1A1* is silenced as a result of CpG hypermethylation within a minimal region encompassing 260 bp upstream of the start codon. Our study aimed to determine if DNA methylation of the CpG -1 to -4 inhibits transcription factor(s) (TF)-mediated reporter activity and TF DNA-binding activity. The analysis of putative TF binding sites within this region reveals the presence of NF-Y, HNF1, CDX2 and USF sites. In gel retardation assay, the methylation of CpG-4 causes a significant decrease of the DNA-protein complexes stability. Furthermore, supershift assays support that HNF1alpha is part of a complex that likely binds a sequence in proximity of CpG-4, suggesting that CpG-4 methylation affects TFs binding. Luciferase assays further sustain a role for HNF1alpha in *UGT1A1* regulation in colon cancer cells, but not in HepG2 hepatic cells. Based on the differential expression profiles of *HNF1alpha* in cell lines, we also assessed if methylation affects its expression. In agreement with the presence of several CpG islands in the *HNF1alpha* promoter, DNA methyltransferase inhibitor treatment of *UGT1A1*-negative HCT-116 colon cells restored *HNF1alpha* gene expression, as observed for *UGT1A1*. We conclude that DNA methylation modulates *UGT1A1* and *HNF1alpha* expression in colon cancer cells. This mechanism may have implications for cancer treatment by potentially determining local inactivation of anticancer agents by glucuronidation and influencing tumoral response.

49

Bupivacaine plus Fentanyl was more Effective and Safer than Bupivacaine and Pethidine plus Haloperidol in Epidural Analgesia during Labour

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Spinal block for labour analgesia with high-dose of local anaesthetics has been related to adverse reactions (RAM) in the mother and the foetus-neonate. Our aim was to compare the efficacy and safety of analgesia with bupivacaine, bupivacaine plus fentanyl and pethidine plus haloperidol during labour. Parturients (aged 31.8±4.5 years, primiparous, mono-foetal gestation, at 37-42 weeks' gestation, cervical dilatation <4cm and rhythmic labour contractions) were randomized to

receive 12 ml/h of 0.25% bupivacaine (B, n=54, epidural), 0.125% bupivacaine plus 5 µg fentanyl (B+F, n=63, epidural), or combined 1mg/kg/1h pethidine plus 2.5 mg/kg/1h haloperidol (P+H, n=40, intravenous perfusion). In B+F and B groups, initial analgesia was followed by bolus of B+F on demand via patient-controlled epidural analgesia (PCEA). Epidemiological and clinical data was similar among groups. Maximal pain intensities (EVA-scale quantification, 30 min after analgesia beginning) were P+H 75±20 >B 72±15 >B+F 55±10. Delivery labour modes were (B/B+F/P+H): eutocic 24.1%/33.3%/25.4%, non eutocic 75.9%/66.7%/74.6%. B+F showed ($p<0.05$) i) shorter time between the analgesia induction and final delivery (7.3h) with respect to B (8.6h) and P+H (10.7h); ii) lesser requirements in complementary analgesia: intra-labour PCEA bolus number were B=8±1.2, B+F=3±0.5; intra-labour complementary analgesia use were P+H=47.8%, B=35.2%, B+F=25.4%; post-labour analgesia use in recovery room and maternity ward were: P+H= 45.6, B=36.6%, B+F=29.5%. RAM's incidence was (% in B/B+F/D+H): absence of RAM 59.3%/65.1%/43.8%; nausea-vomiting (14.8%/20.6%/25%), constipation (18.5%/19%/8.3%), cephalalgia (3.2%/1.9%/6.3%). No adverse neonatal outcomes were observed. Conclusion: Bupivacaine plus fentanyl was more effective and safer than bupivacaine and pethidine plus haloperidol in epidural analgesia during labour.

50

Metabolism of Deferiprone by Human UGT1A6: An *in vitro* Investigation of Genetic and Splice Variants

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Background: The hydroxypyridinone chelator deferiprone is used in the therapy of life-threatening iron-overload diseases. Deferiprone is known to be metabolized by UDP-glucuronosyltransferases (UGTs) to form deferiprone-glucuronide (DG), but a systematic evaluation of the contribution of individual human UGTs and impact of genetic variations has not been conducted. Methods: Sixteen human UGTs were studied for deferiprone glucuronidation, and clearances ($Cl_{int}=V_{max}/K_m$) were compared to human tissue samples. DG was measured by liquid chromatography coupled with mass spectrometry. Results: UGT1A6 is the main isoform involved, with minimal contribution from UGT1A7, 1A8, 1A9, 1A10, 2B7 and 2B15. A second metabolite, possibly a quaternary ammonium glucuronide, was formed in microsomes from human tissues and by UGT1A8, 1A9 and 1A10. UGT1A6 and liver and kidney microsomes had similar clearance rates (Cl_{int}= 1.4-3.0 uL/min/mg), but higher than the intestines (Cl_{int}= 0.04 uL/min/mg). The affinity of the allelic variants *2 ($A^7A^{181}S^{184}$), *3 (A^7), *4 (A^7S^{184}) and *5 (A^{181}) was in a range similar to *1 (K_m=7.4 to 8.2 mM), but their velocity was reduced (V_{max}=7.5-19 nmol/min/mg) compared to UGT1A6*1 (26 nmol/min/mg) ($p<0.05$). The UGT1A6 splice variant isoform 2 (i2), containing exon 5b instead of exon 5a and present in liver and kidney, demonstrates no glucuronidation activity for deferiprone. However, when co-expressed with the classical UGT1A6 isoform 1, a 2.5-fold lower Cl_{int} is observed ($p<0.05$). Conclusions: Deferiprone glucuronidation depends almost exclusively on UGT1A6. Genetic variations and interindividual variation in the expression of UGT1A6-i2 represent a potential source of variation in deferiprone metabolism.

51

Clinical Results of Intralesional Application of Epidermal Growth Factor in Diabetic Foot Ulcer

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Diabetic foot ulcers (DFU) are a significant healthcare problem affecting around 15% of people with diabetes mellitus in their lifetime. Epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation, and differentiation which can be useful to enhance wound healing. The aim of this work is to summarize the main clinical results and perspective of a novel formulation of recombinant, human EGF (rhEGF) for intralesional administration in DFU. A preliminary clinical study, where rhEGF (25 µg

thrice weekly for 5 weeks) was injected intralesionally in advanced DFU in 29 patients yielded encouraging positive results in terms of useful granulation tissue formation and prevention of major amputations in more than 50% of the treated patients. It was followed by a randomized, double-blind trial designed to test two dose levels (25 μg vs. 75 μg) of rhEGF in advanced DFU, through intralesional injections, 3 times per week for 5 to 8 weeks. A complete granulation response appeared after 5 weeks of treatment in more than 60% of the patients. Complete wound healing was reached in more than 50% of the patients after 20 weeks. These results were further supported in the national extension study for advanced DFU. A recently exploratory study has evidenced that the maintenance of therapy up to complete wound closure is safe and can produced better clinical result regarding the proportion of wound healing (80%). In conclusion, intralesional rhEGF significantly enhances advanced diabetic foot ulcer healing and reduces the risk of major amputation.

52

Comparison of Scopolamine-induced Deficits in Normal, Mature Monkeys and Normal Age-related Cognitive Impairments in Performance of Delayed Matching-to-Sample and Paired Associative Learning Tasks: Responses to the Cholinesterase Inhibitor Tacrine

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Due to its rich behavioral repertoire, the rhesus monkey provides an excellent animal model for assessing age-related cognitive processes and for preclinical testing of potential cognition-enhancing therapies. Although the paired associative learning task (PAL) is a sensitive measure for age-related cognitive dysfunction and a possible marker for preclinical Alzheimer's disease (AD), most non-human primate research has used working memory tasks (e.g. delayed matching to sample - DMTS) to study age-related cognitive decline. In this study, we compared performance of normal mature monkeys (n=4, 6-8 years) prior to and following scopolamine administration, modelling AD cholinergic-related memory deficits, on a DMTS and PAL tasks with performance of aged monkeys (n=4, 20-25 years). Scopolamine induced significant performance deficits on both tasks and both aged and scopolamine-treated mature animals exhibited similar types of performance deficits. On the DMTS task, both groups of animals performed long delay trials significantly worse than short delay trials. On the PAL task, both groups of animals had deficits in performing the most difficult levels of the task. The cholinesterase inhibitor tacrine (0.3 to 1 mg/kg, i.m., 30 minutes prior to testing) reversed the DMTS deficit in aged monkeys but failed to improve PAL performance in these animals while it improved performances in both tasks in scopolamine-induced deficits. These results support the continued use of these preclinical models but suggest that is important to assess the potential therapeutic value of new drugs by using more than 1 task in more than 1 model.

53

Novel Agonistic Properties of Arsenic Trioxide in Human Neutrophils: Role of Mitogen-Activated Protein Kinases (MAPK).

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Arsenic trioxide (ATO) has been shown to induce apoptosis or differentiation of acute promyelocytic leukemia cells. Also, this compound has been shown to activate all three members of the mitogen-activated protein kinases (MAPKs) in leukemia cells. We investigated the potential of ATO to induce signalling events in human neutrophils considering that promyelocytic cells are precursors of neutrophils. We hypothesized that ATO could enhance phosphorylation of stress-related kinases due to generation of hydrogen peroxide (H_2O_2) as we have reported previously. Here, we show that arsenic trioxide can effectively activate production of ROS and phosphorylation of p38 MAPK and *c-jun* NH_2-terminal MAPK (JNK) but not extracellular signal-regulated kinase 1 and 2 (ERK1/2), and this occurs by a H_2O_2-independent mechanism. We next demonstrated that ATO can enhance adhesion of neutrophils onto A549 cells. By using gelatin zymography, we also showed that ATO can stimulate the release of MMP-9

from neutrophils. The degranulation was shown to be restricted to secretory, specific and gelatinase, but not azurophilic granules as assessed by flow cytometry. Migration and phagocytosis of neutrophils were also enhanced by ATO treatment. Using pharmacological inhibitors of the three MAPKs, we found that all functions but apoptosis were dependent upon activation of JNK and/or p38. Activation of these kinases did not require elevation of intracellular calcium since no burst was seen, even at high concentrations of ATO. These observations demonstrate that ATO is a potent activator of neutrophils, acting through p38 and/or JNK signalling for functions other than apoptosis.

54

Regulation of Hepatic Drug Metabolism in a Mouse Model of Chronic Renal Failure

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Background: We have shown that chronic renal failure (CRF) downregulates cytochrome P450 (CYP450) isoforms in both intestine and liver in the rat. The mechanism remains poorly understood. The purposes of this study were a) to develop a model of CRF in the mouse and b) to study the effect of CRF on both CYP450 and Phase 2 enzymes in the mouse. Methods: Models of CRF were tested and sub-total nephrectomy (Nx 4/8) was selected because of the efficacy and reproducibility to induce CRF. Seric creatinine concentrations as well as BUN levels were used as CRF markers. Liver protein expression and mRNA levels of NAT2, CYP1A1, CYP2C29, CYP3A11, CYP2D and CYP2E1 were assessed by Western Blot analysis and qPCR, respectively. Results: Protein expression of CYP3A11, CYP2C29 and CYP2D was decreased in liver microsomes of CRF mice by 60%, 33% and 55%, respectively ($p<0.001$). Hepatic mRNA expression of CYP3A11 and CYP2C29 in CRF mice were downregulated by 36% and 39%, respectively ($p< 0.05$). Protein expression of NAT2 was decreased in liver cytosol by 47% in CRF mice ($p<0.001$). There's a correlation between CYP3A11 protein expression and BUN levels ($p<0.01$ $R^2 = 0,735$) Conclusions: This study demonstrates that protein expression of liver CYP3A11 and CYP2C29 are downregulated in CRF mice, secondary to reduced gene expression. This study also suggests that both Phase 1 and 2 enzymes are modified in CRF. This will allow the use of knock-out mice to precise the mechanism underlying CRF induced downregulation of CYP450 and Phase 2 enzymes.

55

Microvascular Endothelial Cells from Equilibrative Nucleoside Transporter 1 (ENT1)-Knockout Mice have Altered Purine Uptake and Metabolism Profiles

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Endothelial cells regulate levels of nucleosides and nucleobases in the vasculature via specific equilibrative transporters (ENTs) and intracellular metabolism. The predominant transporter is ENT1; therefore, the loss of ENT1 could have profound effects on vascular function, through adenosine receptor signaling or through changes in the production of reactive oxygen species (ROS). Microvascular endothelial cells (MVECs) were isolated from skeletal muscle of ENT1-knockout (KO) and wild-type (WT) mice and cultured in vitro. Reverse-transcriptase PCR confirmed the lack of ENT1 transcript and identified increases in mRNA levels for ENT3 and adenosine deaminase in KO MVECs. Transport of the nucleoside [3H]2-chloroadenosine, via ENT2, was significantly reduced in KO MVECs (Vmax = 0.19 ± 0.07 pmol/µl/s) compared to WT (Vmax = 1.2 ± 0.5 pmol/ul/s). Uptake of the nucleobase [3H] hypoxanthine was primarily mediated by equilibrative nucleobase transporter 1 (ENBT1) and was similar between KO and WT MVECs. However, in the presence of the ENT inhibitor dipyridamole, uptake was significantly increased in WT MVECs (Vmax = 17 ± 4 pmol/ul/s) compared to KO MVECs (Vmax = 12 ± 2 pmol/ul/s). To test for intracellular trapping of [3H] nucleoside metabolites, thin-layer

chromatography of [3H] hypoxanthine metabolism was performed and showed significantly higher levels of hypoxanthine and inosine in KO MVECs. More detailed examination of [3H] hypoxanthine uptake revealed differential effects of 2 hour mineral-oil induced hypoxia between KO and WT MVECs. These results show the loss of ENT1 in KO mice resulted in changes to purine transport and metabolic systems, likely to compensate for increased intracellular adenosine levels.

56

Enantioselectivity in Fluvastatin-lercanidipine Pharmacokinetics Interaction in Healthy Volunteers

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Hypertension and dislipidemia are independent risk factor to cardiovascular mortality and frequently are presented in same patient. Fluvastatin (FV), used to reduce cholesterol levels, and lercanidipine (LER), used to control blood pressure, are marketed as racemic mixtures. Therapeutic activities are 30-fold higher for the (+)-3R,5S-FV and 100-to 200-fold higher for S-LER in comparison to their respective antipodes. The present study describes the enantioselective LER-FV pharmacokinetics interaction in healthy volunteers. The crossed and randomized study was developed in three phases in 9 volunteers treated with single oral racemic dose of LER (20mg) or FV (40mg) or LER plus FV. Serial blood samples were collected from 0-24h. Plasma concentrations of LER and FV enantiomers were determined by LC-MS/MS, pharmacokinetic parameters were determined by WinNonlin software and Wilcoxon and Mann-Whitney tests ($P<0.05$) were used to analyze enantiomer ratios and to evaluate the pharmacokinetic drug interaction. Data were expressed as medians. In monotherapy, both the kinetic disposition of FV and LER were enantioselective; AUC values were significantly higher for (-)-3S,5R-FV than for (+)-3R,5S-FV (358.20 *vs* 279.68ng.h/mL) and for S-LER than R-LER (13.90 *vs* 11.88ng.h/mL). FV pharmacokinetic parameters were not enantioselective when associated with LER (AUC-)-3S,5R-FV – 325.21; (+)-3R,5S-FV – 316.44ng.h/mL). There was a significant reduction in S-LER (8.06 vs 13.90ng.h/mL) and in R-LER (6.76 *vs*11.88ng.h/mL) AUC values when FV was co-administrated. In conclusion, FV-LER interaction could be clinically relevant since AUC values of (+)-3R,5S-FV were increased with LER association and AUC values of both enantiomers of LER were reduced with FV association.

57

Milnacipran, a Serotonin-noradrenaline Reuptake Inhibitor, Enhances the Anticonvulsant Activity of Conventional Antiepileptic Drugs in The Maximal Electroshock-induced Seizures in Mice

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Influence of acute and chronic treatment with milnacipran (MLN) on the anticonvulsant action of conventional antiepileptic drugs (AEDs: valproate [VPA], carbamazepine [CBZ], phenytoin [PHT], and phenobarbital [PB]) were studied in the maximal electroshock (MES) test in mice. Electroconvulsions were produced by constant current (25mA, 0.2s) delivered via auricular electrodes by Hugo Sachs generator. The anticonvulsant effects of the AEDs administered alone and in combination with MLN were determined by calculating their median effective doses (ED_{50} values), protecting 50% of the animals against MES-induced seizures. Acute adverse effects of AEDs in combination with MNL were determined in the chimney and passive avoidance tests. MLN administered acutely at the dose of 10 mg/kg significantly increased the electroconvulsive threshold. The drug at 5 mg/kg enhanced the anticonvulsant activity of CBZ and PB. When applied at the dose of 10 mg/kg, MLN potentiated the protective action of all AEDs. Chronic treatment with MLN (5 and 10 mg/kg), significantly enhanced the electroconvulsive threshold and the protective action of VPA and CBZ. Acute and chronic treatment with MLN and its combinations with AEDs did not impair either motor coordination or long-term memory. In

conclusion, the present data support the hypothesis that increased synaptic serotonin and noradrenalin concentrations can lead to anticonvulsant effects and enhance the antiseizure action of AEDs. Since the anticonvulsant effect of MLN was observed even after a single injection and was not significantly modified after 14-day administration, it seems to be independent on receptor adaptive changes, necessary for the development of antidepressant action.

58

Analysis of Isotretinoin in Human EDTA K_2 Plasma using Liquid Chromatography Method with Tandem Mass Spectrometry

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Purpose: Isotretinoin is in a class of drugs called retinoids. It is used to treat severe acne that has not responded to oral or topical anti-infectives. The purpose of this work was to develop and validate a specific and robust method for the determination of isotretinoin in human EDTA K_2 plasma. Methods: Isotretinoin and its internal standard acitretin were extracted from human EDTA K_2 plasma (containing 5 % volume of L-ascorbic acid 20% w/v) by liquid-liquid extraction using methyl-ter-butyl ether. Analysis was performed on a MDS Sciex API 3000 tandem mass spectrometer with TurboIonSpray interface. Negative ions were measured with m/z $299.2 \rightarrow 255.1$ for isotretinoin and $325.2 \rightarrow 265.9$ for acitretin. The chromatographic run time was 9.0 minutes on a X-Terra RP-18 50 X 4.6 mm column. The mobile phase was a mixture of methanol and Milli-Q type water (55/45), 1 mM ammonium acetate, pH 10.50. Validation parameters were evaluated with both regular human plasma and charcoal stripped human plasma (endogenous levels of isotretinoin removed). Results: This assay was validated over a nominal range of 1 to 600 ng/mL. Linearity over the calibration range was $\geq$ 0.9979. The between-run accuracy ranged from 97.41 to 100.83% with precision ranging from 2.84 to 6.38%. The within-run accuracy ranged from 94.90 to 106.01% with precision ranging from 1.08 to 5.72%. The recovery of isotretinoin and acitretin was greater than 89%. No matrix effect on quantitation was observed. Isotretinoin was found to be stable in human EDTA K_2 (regular and charcoal stripped) plasma after 25 hours at 4°C for short term stability, after 7 days at -80°C for long term stability, after 136 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -80°C. Dilution integrity and matrix selectivity were also demonstrated. Conclusions: This method is accurate, reproducible and was successfully applied for the analysis of clinical samples. Over 3500 study samples were analysed with accuracy ranging from 99.67 to 104.62% and precision ranging from 4.56 to 10.73%.

59

Sex Differences in Methamphetamine Toxicity in Mice: Effect on Brain Dopamine Signaling Pathways

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Methamphetamine (MA) is a potent and addictive drug of abuse with increasing use. This drug acts on dopaminergic systems and can induce long-term effects related to motor and memory functions. The protein kinase B (Akt) and glycogen synthase kinase 3beta (GSK3beta) cascade are implicated in dopamine function and regulation of the dopamine transporter. The present experimentation investigated the molecular mechanisms involved in MA toxicity by the implication of Akt and GSK3beta in the striatum of mice. Intact male and female mice were treated with 20, 40 or 60 mg/kg of MA. Female mice treated with 60 mg/kg of MA did not survive the treatment. Striatal dopamine concentrations were decreased in male treated with 40 and 60 mg/kg of MA and in females treated with 40 mg/kg of MA. Administration of 40 and 60 mg/kg of MA decreased the dopamine transporter specific binding in the medial striatum of male mice. The lateral striatum was affected with treatment of 40 mg/kg in female and 20, 40 and 60 mg/kg of MA in male mice. MA reduced the levels of phosphorylated Akt only in male mice treated with 40 and 60 mg/kg as measured

by Western blots. The levels of GSK3beta were reduced with administration of 40 mg/kg of MA in female and in male treated with 20, 40 and 60 mg/kg. The results show that Akt/GSK3beta signaling pathways are modulated differently in response to MA between male and female mice and that dopamine depletion is related to changes observed in these molecules.

60

Sedentarity and Increased Visceral Adiposity in Adult Perinatally Iron-deficient Rats

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Background: Perinatal iron deficiency (PID) has adverse programming effects, which manifest as alterations in cardiometabolic function in adult offspring. Increased visceral adiposity is the proposed culprit for these sequelae, and may be potentiated by decreased physical activity. In this study we determined (i) the effect of PID on visceral adipose tissue (VAT) deposition and locomotor activity levels, and (ii) whether increased VAT altered the response of blood pressure to increased dietary sodium. Methods and results: Dams were fed a low iron diet (<10 mg/Kg Fe) prior to and throughout gestation. At delivery, dams were fed a normal iron diet (270 mg/Kg Fe). From 12 to 35 weeks of age, PID offspring were 25% less active than control offspring ($P<0.001$), as assessed by radiotelemetry. At 36 weeks of age, PID rats had 15% more VAT (normalized to body weight) than controls ($P<0.05$). Furthermore, the elevation of mean arterial pressure in response to increased sodium intake was approximately 2-fold greater in the PID group compared to controls ($P<0.05$). Conclusions: PID results in increased visceral adiposity, which is associated with enhanced blood pressure responsiveness to dietary salt, perhaps due to programmed sedentary behaviour.

61

Inhibition of Thrombin Generation in Plasma of Healthy Subjects Treated with Apixaban, An Oral, Direct Inhibitor of Factor Xa

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Apixaban is an oral, direct, highly selective, reversible factor Xa (Fxa) inhibitor in late stage clinical development for prevention and treatment of thromboembolic diseases. The aim of this study was to assess the effect of oral apixaban administration on ex vivo thrombin generation (TG). TG was triggered with tissue factor and measured using a calibrated automated thrombogram method in platelet-poor plasma samples from 32 healthy male subjects randomized to receive 2.5, 10, 25, and 50 mg apixaban or matching placebo in a double-blind, 4-period, intra-subject, single dose escalation study. Apixaban administration resulted in dose- and concentration-related changes in TG parameters. The time course of changes in TG parameters closely followed the apixaban plasma concentration-time profile, with maximal changes occurring around apixaban Tmax for lag time (64 to 169%), peak (-39 to -80%), time to peak (65 to 139%), propagation phase rate index (-51 to -87%) and endogenous thrombin potential (-13 to -45%). These changes represent the expected effects of direct factor Xa inhibition: prolonging the initiation phase and inhibiting the propagation phase of thrombin generation. Apixaban's effects remained evident at 12 hours post-dose for all dose panels. Oral administration of apixaban effectively inhibits plasma thrombin generation at doses as low as 2.5 mg, which is consistent with the demonstrated efficacy in recent Phase 2 clinical trials for the prevention and treatment of venous thromboembolism.

62

Conditioned Taste Aversion in Rats Following Prior Exposure to Repeated or Single Prolonged Stress

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It is well-known that prior exposure to stressful situations modulates memory and learning. As opposed to spatial and working memory, conditioned taste aversion (CTA) describes the ability to learn aversively motivated tasks and as such has direct bearing on posttraumatic stress disorder (PTSD). Spatial learning (explicit memory) is impaired following single prolonged stress (SPS) or SPS with restress (SRS), two

putative animal models of PTSD. However, we have earlier described that SPS and SRS evoke qualitatively different neurochemical and endocrine responses, suggesting that they activate a separate cascade of events. To test that prior SPS alters the consolidation of aversive memory, and whether these are modified by re-experience, we studied the effects of both stress paradigms on an associative aversive-conditioned response (viz. CTA) and its consolidation over time. Male Sprague-Dawley rats (150–170 g) were subjected to a series of three sequential severe stressors (SPS). On day 7 post SPS, the animals were exposed to a reminder (swim stress-SRS). The effects of SPS and SRS on aversive memory were determined 3 and 7 days post-stress in the CTA paradigm. Pre-exposure to SRS, caused a profound decrease in the ability to acquire and recall associational memory subsequent to trauma for at least 7 days post restress. SPS and SRS differ therefore with respect to their effects on aversive memory (CTA), in agreement with our earlier study on brain monoamines. These results have important implications in understanding the neurobiology of PTSD.

63

Modulation of Multiple Cytokine Profile during Lipopolysaccharide-induced Alteration of Methotrexate Pharmacokinetics in Rats

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Introduction: Administration of lipopolysaccharide (LPS) to rats results in cholestasis caused by alteration of hepatic transporters, which is partly attributable to cytokine mediated regulation. Functional consequences of the status for the kinetics of organic anions such as methotrexate, and more complex cytokine profiling are still lacking. Thus, we studied the effect of LPS and multiple cytokine production in relation to the elimination of methotrexate and expression of relevant transporters in the liver and kidney of rats. Methods: Pharmacokinetic parameters of MTX were calculated during steady-state of plasma concentrations. Expressions of transporters were determined by Western blotting and qRT-PCR. Cytokine profiling was performed by protein arrays. Results and Discussion*:* In LPS rats, biliary, renal and total clearances of MTX were decreased to 42%, 23% and 43%, respectively. Corresponding down-regulation was seen in hepatic Oatp2, Bcrp and Mrp2 transporters, while expression of reduced folate carrier (Rfc1) was preserved. Cytokine profiling revealed increased plasma levels of typical mediators of cholestasis IL-1beta, IL-6, IL-10 and TNF-alpha, but also other signaling molecules such as INF-gamma, IL-4, IL-1alpha, CINC-2, CINC-3, MIP-3a, LIX, and GM-CSF. In conclusion, endotoxemia produced profound impairment of hepatic and renal elimination of methotrexate in rats which was associated with increased concentration of multiple cytokines in systemic circulation.

64

Hematologic and Immunologic Parameters and Biomarkers of Smoke Exposure in the First Decade of Smoking

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To study initiation and progression of tobacco-related diseases, we evaluated clinical parameters and biomarkers in subjects with an average 10-year smoking history. A cross-sectional study of groups of 20 Never Smokers (NS), Moderate Smokers (MS) and Smokers (S) was performed. Subjects were recruited for specific cigarette tar yields and cigarettes smoked per day. In females, significant differences between the NS group and MS + S groups combined were seen for hemoglobin concentration, hematocrit, total leukocyte, neutrophil and lymphocyte counts. In males, only total leukocyte count was statistically significant. In serum/plasma biomarkers the only significant difference was that IgG showed a decreasing trend of NS > MS > S in both sexes (p for trend, $p < 0.01$) but no trend for IgM or IgA. LDL-cholesterol, HDL-cholesterol, Factor VII, triglycerides, cardiac troponin-I, interleukin-6, serum amyloid A and C-reactive protein were not different. Of the urinary biomarkers, only 11-

deydro-thromboxane B2, 2,3-dinor-thromboxane B2 and thymidine glycol were significantly elevated in either smoker group. Exhaled carbon monoxide and other smoke exposure biomarkers (nicotine plus metabolites) were significantly elevated in all smokers with a trend of NS < MS < S, also related to the number of cigarettes smoked per day (p for both trends, $p < 0.01$). Overall, statistically significant changes were seen between smokers and never smokers in parameters related to immune responsiveness, thromboxane turnover and oxidative stress; although parameters remained within the normal physiological range. These findings raise interesting questions in trying to establish early predictive biomarkers of risk for smoking-related diseases.

65

0.5% Bupivacaine Evokes Higher Values of Diastolic and Mean Arterial Pressure than 4% Articaine during Lower Third Molar Removal Requiring Osteotomy

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Local anesthetics represent one of the main therapeutical agents to provide intra and postoperative comfort in lower third molar removal. Objective: Comparison of the clinical efficacy of 4% articaine (A200) and 0.5% bupivacaine (B200), both with 1:200,000 epinephrine, for lower third molar removal. Methods: Fifty patients underwent removal of symmetrically positioned lower third molars, in two separate appointments, under local anesthesia either with A200 or B200, in a double-blind, randomized, and crossover manner. Systolic, diastolic and mean pressure, heart rate and oxygen saturation were evaluated. Results: In the surgeries with osteotomy, the comparison between A200 and B200 showed statistically significant differences in the diastolic (64.88 mmHg and 68.54 mmHg, respectively, P=0.001) and mean arterial pressure (86.25 mmHg and 89.33 mmHg, respectively, P=0.031) when data from all the surgical phases were pooled. The values concerning heart rate varied during the phases of the surgical procedures (P<.05), but were not influenced by the local anesthetic used. No statistically significant difference in relation to oxygen saturation was observed during the surgeries. Conclusions: B200 evoked higher values of diastolic and mean arterial pressure than A200 when osteotomy was necessary for lower third molar removal. Healthy patients can tolerate these increases, but patients with cardiovascular disease may not be able to.

66

Gender Differences in the Oral Pharmacokinetics of Losartan

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Losartan is an antihypertensive agent that is widely used in therapeutics. Establishment of dosage regimens requires the knowledge of the pharmacokinetics of the drug in the target population to be treated. It has been described that gender differences in the pharmacokinetics of drugs may exist, produced by changes in the metabolism and distribution of drugs between genders. However, in the case of losartan, limited information about differences between genders is available. The purpose of this study was to compare the oral pharmacokinetics of losartan between women and men. Fifty-two (26 women and 26 men) participated in this study that was carried out following the recommendations of the Declaration of Helsinki. All subjects were fit according to medical history, clinical examination and suitable laboratory tests and gave written informed consent for participation. After an overnight fast, volunteers received an oral dose of 50 mg losartan and blood samples were obtained at selected times for a period of 24 h after drug administration. Plasma was obtained and stored frozen at -80°C until analyzed by HPLC with fluorescence detection. It was observed that men reached twice the plasma levels than women, and that was reflected in increased Cmax and AUC. These results indicate the presence of gender differences in the oral pharmacokinetics of

losartan. This difference seems to be due to reduced clearance and volume of distribution. Although there are important differences in the oral pharmacokinetics of losartan between genders, therapeutic relevance of this difference remains to be elucidated.

67

Effect of Thyroid Hormone Pretreatment in the Acute Acetaminophen Administration

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Oxidative stress is highly related with cellular damage and cell death. However, recent studies have associated moderate levels of oxidative stress induce preconditioning effect reducing damage of a high dose of oxidative stress. We tested the hypothesis that pretreatment with L-3,3´,5 triiodotyronine (T3) before acetaminophen (APAP) administration induce a reduction of oxidative stress evaluated. Sprague-Dawley rats ip-treated with single dose of T3 (0,1mg/Kg) or vehicle 48 h before a high dose of APAP (500 mg/Kg) or vehicle. Serum levels of transaminases activities (AST and ALT) and IL-6 (ELISA), hepatic STAT-3 DNA-binding (EMSA) and protein carbonylation were assessed at 8 or 16 h after APAP administration. We also tested IL-6/STAT-3 pathway, as a mechanism associated with oxidative stress. Transaminases were reduced in pretreatment group vs. APAP without pretreatment. GSH content diminished in APAP group and it was partially reverse in pretreatment group. Also, IL-6 serum levels and STAT-3 DNA binding activity were reduced in T3 pretreatment group. It is concluded that high hepatotoxic doses of APAP trigger the IL-6/STAT-3 pathway, as a compensatory adaptative mechanism upregulating defense processes such as the acute-phase response, and T3 reduce the effect of APAP in the liver as a preconditioning mechanism.

68

Chronic Hepatitis B Viral Kinetics under Treatment of Antiviral Drug X

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Introduction: Antiviral drug therapy is a standard treatment of chronic hepatitis B. In this study, we aimed to model the pharmacodynamics (PD) of viral DNA counts which were followed up for one year in 102 HBV (+) patients. Method: Viral counts were measured at 0, 4, 8, 12, 20, 28, 36, 44 and 52th weeks after the initiation of drug therapy. Modeling was performed using NONMEM (ver.6.0). We assumed that log transformed DNA counts (V(t)) follow zero order synthesis (Syn) and first order loss (Loss x V(t)) and the antiviral drug inhibited the synthesis ($dV(t)/dt=Syn – Loss x(1-E)xV(t)$). To include the below quantification limit (< 300) data, we used Laplacian estimation method with F_FLAG variable. Result: In the final population PD model, the rate constant of viral elimination (Loss) was correlated with baseline viral count and Effect (E) included sex and the slope of early viral count change (first 8 weeks) as covariates. The new steady state (NSS) defined as a steady state of viral count after medication was below 5.8 and time to reach the NSS was predicted as 15~23weeks. Discussion: Although there are models explaining HBV kinetics of rather short-term treatment periods (< 1 month), our model may be applied to predict the effect in patients under long-term medication. Conclusion: Our model identified the influence of sex and the slope of early viral count change on antiviral drug's effect as well as the range of NSS and the time needed to achieve NSS.

69

Pharmacokinetic and the Effect of Capsaicin in *Capsicum frutescens* on Decreasing Plasma Glucose Level

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The active substance found in *Capsicum frutescens* (capsicum) that gives hot and spicy flavour was capsaicin which has many pharmacological effects. This research was conducted to study the effect of capsicum on plasma glucose level and find the tendency of its action correlated with the pharmacokinetic of capsaicin in capsicum. The study was performed in 12 healthy volunteers by having the OGTT as well as receiving the placebo and 5 grams of capsicum. Then the same volunteers were administered placebo and the same amount of capsicum to study the insulin secretion and measure the capsaicin level in plasma by using the HPLC method. The results show that the volunteers who received capsicum with OGTT have the tendency of the plasma glucose level lower than the placebo group with finding statistically significant different at 30 and 45 minutes ($P<0.05$). The group receiving the capsicum has the tendency of insulin level higher than the placebo group with also finding statistically significant different at 1 hr.,1 hr. 15 minutes,1 hr. 45 minutes and 2 hrs ($P<0.05$). The pharmacokinetic parameters of capsaicin shown as C_{max}, T_{max}, $AUC_{0-\infty}$, K_{el} , $T_{1/2}$ are 2.47 $\pm$ 0.46 ng/ml, 47.08 $\pm$ 6.89 min, 103.6 $\pm$ 38.99 ng.min/ml, 0.4 $\pm$ 0.4 min^{-1} and 24.87 $\pm$ 17.2min, respectively. The study found that 5 grams of capsicum has the effect to decrease plasma glucose level and stimulate insulin secretion. The result might come from capsaicin when absorbed into the body and has a stimulating effect on insulin secretion.

70

Upregulation of the Efflux Drug Transporter, P-glycoprotein (P-gp), by HIV Protease Inhibitors (Pis), in a Human Brain Microvessel Endothelial Cell Line

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Most antiretroviral agents, including protease inhibitors (Pis), exhibit low brain penetration which often results in HIV-encephalitis and antiretroviral drug resistance. One major reason for the low penetration of Pis into the brain is the functional expression of efflux drug transporters, i.e. P-glycoprotein (P-gp) at the blood-brain barrier (BBB). In addition of being known substrates and inhibitors of P-gp, Pis are also known to be inducers of this transporter. To date, limited information is available on the regulation of P-gp at BBB by nuclear receptors such as Pregnane X Receptor (PXR), and the effect of Pis on P-gp expression and function. By utilizing an immortalized human brain microvessel endothelial cell line, hCMEC/D3, representative of the human BBB, we investigated the effect of rifampicin and ritonavir (two known ligands of human PXR) as well as atazanavir, a novel PI, on the functional expression of P-gp. Using immunoblotting and immunocytochemistry at the electron microscope, we observed that P-gp and hPXR are expressed and localized in hCMEC/D3 cells. Treatment of hCMEC/D3 cells for 72H with atazanavir or ritonavir or rifampin (5-10uM) resulted in a 2-fold increase in P-gp expression and a 2-fold reduction in the cellular accumulation of rhodamine-6G, a fluorescent P-gp probe. These data suggest that exposure of hCMEC/D3 cells to atazanavir or ritonavir at clinical therapeutic concentrations result in an upregulation of P-gp expression and function in the *in vitro* human BBB model. Further work needs to be undertaken to confirm the role of the nuclear receptor, hPXR, in P-gp upregulation by Pis at the BBB.

71

Effect of Exendin-4 on Glucose Homeostasis in *Suncus Murinus* (House Musk Shrew)

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Glucagon-like peptide-1 (7-36) amide (GLP-1) is regarded as an incretin hormone in the periphery, and a neurotransmitter in the brain. GLP-1 neurons and GLP-1 receptor binding sites are densely expressed in the hypothalamus, which is known to be heavily involved in mechanisms controlling food intake and energy homeostasis. In the present studies, therefore, we examined the effect of central administration of a GLP-1 receptor agonist, exendin-4, on glucose

homeostasis. Female *Suncus murinus* were fasted 12 hour before experimentation. To elevate blood glucose levels, anaesthetized animals were administered glucose (5.55 mmol/kg, i.p.). 10 min later, they received an intracerebroventricular infusion of exendin-4 (0.3 – 3 nmol), or an intracerebral ventromedial (iVMH) or dorsomedial (iDMH) microinfusion of exendin-4 (3 – 30 pmol). In some other experiments, the GLP-1 receptor antagonist, exendin (9-39) amide (10 nmol, i.c.v.), was administered 10 min prior to exendin-4 (3 nmol, i.c.v.). Blood glucose levels were measured for 1 hour, sampling at 5-20 min intervals. Both the intracerebroventricular infusion and iVMH microinfusion of exendin-4 caused a dose-dependent reduction in blood glucose levels (P<0.01). Conversely, iDMH microinfusion of exendin-4 had no effect (P>0.05). Exendin (9-39) amide alone failed to modify blood glucose levels but it antagonized the glucose-lowering effect of exendin-4 (P<0.05). In conclusion, centrally infused exendin-4 probably reduces blood glucose in *S. murinus* via GLP-1 receptors. The glucose-lowering effect of exendin-4 involves the ventromedial hypothalamus.

72

Development of an Evidence-based Herbal Medicine for the Management of Vascular Dementia

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Introduction: There is currently no effective treatment available for vascular dementia (VaD), the second most common cause of dementia. Chinese herbal medicine has been used for dementia-like symptoms for centuries and could provide alternative and cost-effective treatments.
Methods: Based on the principles of Chinese medicine and conventional pharmacology, three herbs (*Ginkgo biloba*, *Panax ginseng*, *Crocus sativus*) were selected and combined in a formula, WNK for VaD. The dosage regimen and mechanism of action were determined in a series of preclinical studies using various PK and PD models. Acute and chronic toxicity of the formula was also assessed. A 16 weeks clinical trial was conducted in VaD patients to evaluate efficacy of WNK on cognitive function. Results: WNK significantly improved acquired dysmnesia caused by scopolamine, reserpine and sodium nitrate respectively in mice. The formula also reversed the biochemistry changes and memory and learning impairments induced by obstruction of the common carotid or right middle cerebral arteries in rats. The maximum-tolerated dose of WNK in mice is 1.71 g/kg. In the clinical trial, 62 VaD patients (32 receiving WNK, 30 receiving placebos) were recruited. The improvement in ADAS-cog, the primary efficacy parameter was significantly greater in patients receiving WNK than those receiving placebos although the baseline characteristics of ADAS-cog differed slightly (statistically insignificant) between the two groups. Discussion and conclusion: These studies have systematically constructed a body of evidence in support of WNK's therapeutic claim. The formula may improve cognitive function and seems safe in the animal models and the VaD patients.

73

Modeling of the Intravascular Mixing Phase of Neuromuscular Blocking Agents Following Intravenous Bolus Injection

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Introduction: The early plasma concentration vs time profile is crucial for an accurate estimation of the pharmacokinetic/pharmacodynamic relationship of drugs having a very rapid onset of action. The objective of this study was to develop a pharmacokinetic (PK) model that would adequately fit the data collected during the intravascular mixing (IVM) phase in four studies where NMBAs were administered as a 1.5 x ED95 bolus dose in anesthetized patients. Methods: The IVM phase was characterized by collecting arterial outflow during 10 sec intervals (doxacurium, vecuronium, atracurium or succinylcholine) for the first 2 min; samples were drawn at frequent times thereafter for at least 4 half-lives. The Inverse Gaussian Density (IGD) input function combined with compartmental models was implemented in NONMEM VI for PK

analysis of individual data sets. For each NMBA, this approach was compared with a non compartmental analysis. Results and Discussion**:** Satisfactory fit of data sets (including the IVM phase) was achieved with the IGD model for each NMBAs. A good agreement between pharmacokinetic parameters derived with both approaches was also observed. Time to peak concentrations (Tmax) did not differ significantly between NMBAs (P = 0.322), suggesting that circulatory factors do not have a major impact on Tmax in ASA I and II patients. For all NMBAs, mean estimates of central volume of distribution closely approximated the intravascular volume while apparent total body distribution did not exceed the extracellular space. Conclusion: The IGD model is flexible enough to provide a good simulation of the IVM phase of most NMBAs.

74

Human-gene Receptor Products-evidenced and Rat Pulmonary Artery Protein Expression Display Pulmonary Anti-hypertension by PDE5A and 5-HTT Inhibitor

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It has been noted that (1) cGMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca^{2+}-sensitization in vascular smooth muscle contraction; (2) action downstream of cGMP can reverse PKC-mediated Ca^{2+}-sensitization; and (3) cGMP-dependent protein kinase is regulated by Rho kinase (ROCK). KMUP-1, a cGMP-dependent type ROCK inhibitor, inhibits pulmonary artery hypertension and vascular contraction and enhances expression of endothelium nitric oxide synthase (eNOS), soluble guanylate cyclase (sGC) and protein kinase G (PKG) and opposing reduction of ROCK, phosphodiesterase 5A (PDE5A) and translocation of protein kinase Cα (PKCα) in isolated intact rat pulmonary artery. KMUP-1 relaxes phenylephrine-, thromboxane A_2 (TXA_2)-mimetic U46619- and 5-HT-induced vasocontraction and inhibits U46619-, hypoxia- and monocrotaline (MCT)-induced pulmonary artery hypertension (PAH), involving inhibition of PDE5A/PKCα/ROCK, 5-HTT and 5-HT-2B/2A receptors and activation of eNOS/sGC, more potently inhibiting U46619-induced PDE5A expression in rat pulmonary artery. KMUP-1 opens BK_{Ca}-channel in MCT-treated rats and inhibits 5-HT-induced Ca^{2+}-mobilization in smooth muscle cells. In hypoxic and MCT-treated rats, KMUP-1 reduces pulmonary vasocontraction, artery thickness and right ventricle hypertrophy, involving inhibition of 5-HTT expression. KMUP-1 inhibits VEGF in hypoxia and MCT-induced iNOS expression. Based on receptor binding ability, KMUP-1 selectively competes with agonist serotonin-induced receptor binding affinity in various human gene products of 5-HT receptor subtypes. In conclusion, KMUP-1 prevents from PAH via 1) eNOS/sGC activation and PDE5A/PKCα/ROCK inhibition, 2) 5-HTT inhibition and 5-HT2B/2A receptor blockade and 3) K^+-channel opening.

75

Studying N-acetylcysteine as an Antidote for Ifosfamide-induced Nephrotoxicity

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Background: Ifosfamide-induced nephrotoxicity is a serious adverse effect for children undergoing cancer chemotherapy. Our previous *in vitro* studies have shown that the antioxidant N-acetylcysteine (NAC), which is used extensively as an antidote for acetaminophen poisoning in children, protects renal tubular cells from ifosfamide-induced nephrotoxicity at a clinically relevant concentration. Our recent findings have further demonstrated that NAC can prevent ifosfamide-induced nephrotoxicity in a rat model. Furthermore, it is imperative to illustrate the lack of effect of NAC on the antitumour activity of ifosfamide. We hypothesized that NAC does not inhibit the antitumour activity of ifosfamide. Methods: We used cell lines from common pediatric tumours that are sensitive to ifosfamide. Human neuroblastoma SK-N-BE(2) and rhabdomyosarcoma RH4 cells were pretreated daily with 400 uM of NAC 4 hours prior to treatment of 2 and 4mM of ifosfamide concentrations for 5 days. Cellular viability was

assessed by Alamar Blue assay. Statistical differences were assessed by one-way ANOVA. Results: Ifosfamide at 2 and 4mM as a single agent therapy significantly decreased the growth of both cancer cell lines in a dose-dependent manner ($p<0.05$ and $p<0.001$, respectively). Four hundred uM NAC alone also showed a significant antitumour activity in such cells ($p<0.01$). A combined treatment of NAC and ifosfamide showed significant antitumour activity in a dose-dependent manner, similar to that of ifosfamide alone. Conclusion: These observations demonstrate that NAC may improve the risk/benefit ratio of ifosfamide by decreasing ifosfamide-induced nephrotoxicity without interfering with its antitumour effect in these cancer cells.

76

The Role of Carnosine in the CNS, Mediated by Histamine Pathway

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Previous studies show that the brain histamine seems to be involved in regulating seizure susceptibility and the progression of ischemia. However, histamine can not penetrate BBB and is inflammatory in the brain. Carnosine presents highly in the brain, serves as a reservoir for histidine. It is proposed that carnosine- histamine pathway exists in the brain and plays a role in the CNS. Few reports have demonstrated the relation between brain carnosine and histamine. And there is only limited information regarding effects of carnosine on kindled seizures and ischemia. Using amygdaloid kindled seizure model, we found that carnosine significantly decreased seizure stage, after-discharge duration and generalized seizure duration, and significantly prolonged generalized seizure latency in a dose-dependent and time-related manner. The effect of carnosine was completely antagonized by H1-antagonists pyrilamine and diphenhydramine, but not by H2-antagonist zolantidine. Alpha-Fluoromethylhistidine, a selective irreversible histidine decarboxylase inhibitor, only partially reversed the inhibition of kindled seizures induced by carnosine. In addition, carnosine significantly decreased glutamate contents in the brain. In vitro experiment in differentiated PC12 cells showed that carnosine suppressed excitotoxic neuronal injuries in time- and concentration-related manners. The effect of carnosine was antagonized by pyrilamine, but not by cimetidine. Carnosine had no effect on histidine decarboxylase, and increased both synthesis and release of histidine and histamine. Our studies provide the first evidence that the carnosine-histamine pathway exists in the CNS. Carnosine protects against amygdaloid kindled seizures and ischemia through carnosine-histamine pathway and H1-receptors. Carnosine might be an endogenous neuroprotective factor in the brain.

77

Efficacy of Single Dose of Tramadol: Altered Cholinergic System in Rat Brain without Nociception

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Available literature has been concentrated on the effects of analgesic drugs during the presence or induction of pain. No studies are available on their effects on biochemical profiles without pain. This investigation examines the cholinergic system in rat brain, plasma and erythrocytes under administration of the opioid drug tramadol without induction of pain. Single dose of tramadol was administrated subcutaneously to male adult Wistar rats weighing 150 ± 20 g, and the levels of acetylcholine (Ach) and activities of acetylcholinesterase (AchE) in different brain areas, plasma pseudocholinesterase (PchE) and erythrocyte cholinesterase (EchE) were estimated in synapotosomal fractions at 3, 6, 12 and 24 hours post-treatment. Increased Ach content and decreased AchE activity were observed in all the brain areas following tramadol treatment. However, PchE and EchE showed elevated activity. These changes peaked between 3 and 6 hours. The changes suggest that tramadol may exercise its effects through alterations in Ach levels and AchE activity as one of the facets of its analgesia. Opioid-induced depression of cholinergic transmission in the CNS may be an

important mechanism for the analgesic and side-effects of opioids. This work basically evinces curiosity as to what happens when an analgesic drug is taken without induction of pain. The observed changes in cholinergic system presumably to not cause any physiological lesion or harmful effects after a single dose, since the parameters examined reverted to control levels after the time limit of change. This observation indicates that tramadol can be administrated safely both under nociceptive and non-nociceptive conditions.

78

Bioequivalence Study of Two Brands of Nevirapine 200 mg Tablets in Healthy Thai Volunteers

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This study evaluated the bioequivalence of two brands of nevirapine 200 mg tablets. A randomized, two way, crossover study was conducted in 24 fasting healthy volunteers. Blood samples were collected throughout a 168-hr period after administration of reference product and test product. The plasma nevirapine concentration were determined via HPLC technique.The absorption of reference product were slightly higher than test product. The mean peak plasma concentration and area under the curve of both products were closely. Bioequivalence between the products was determined by calculating 90% confidence interval (90% CI) for the ratios of C_{max}, AUC_{0-t} and $AUC_{0-infinity}$ values for the test and reference products, using logarithmic transformed data. The 90% confidence interval for the ratios of C_{max} (91.83-106.41%), AUC_{0-t} (99.31-105.44%) and $AUC_{0-infinity}$ (93.45-107.00%) values for the test and reference products were within the 80-125% interval, in criteria of acceptance, proposed by Thai FDA. Two formulations were considered bioequivalent, in the rate and extent of absorption.
Key words: Bioequivalence, nevirapine, pharmacokinetics

79

Omeprazole Inhibits Preferentially the Metabolism of the (+)-(S)-Citalopram Eutomer in Healthy Volunteers

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Citalopram (CITA) is a selective inhibitor of serotonin (5-HT) reuptake used for the treatment of depression. (+)-(S)-CITA is twice as potent as the racemic mixture and 100 times as potent as (-)-I-CITA as an inhibitor of 5-HT reuptake. The study assess the influence of omeprazole on the kinetic disposition of the (+)-(S)-CITA and (-)-I-CITA in healthy volunteers. In a cross-over study, healthy volunteers (n=9) phenotyped as extensive metabolizers of CYP2C19 and CYP2D6 and with normal CYP3A activity received p.o. a single 20 mg dose of racemic CITA combined or not with omeprazole (20 mg/day for 18 days). Serial blood samples were collected up to 240 h after CITA administration. The enantiomers of CITA and of DCITA (demethylcitalopram) were analyzed by LC-MS/MS. Data are expressed as median and Wilcoxon and Mann-Whitney tests ($P<0.05$) were used to analyze enantiomer ratios and to evaluate the pharmacokinetic drug interaction. Kinetic disposition of CITA was enantioselective in the absence of treatment with omeprazole, with the observation of higher AUC values of (-)-I-CITA ($AUC_{S/R}$ ratio of 0.53 for CITA and 1.08 for DCITA). Omeprazole administration resulted in reduction in the oral clearance of (+)-(S)-CITA (0.56 *vs* 0.30 l/h/kg) showing a loss of enantioselectivity in the pharmacokinetics of CITA ($AUC_{S/R}$ ratio of 0.95 for CITA and of 0.95 for DCITA). In conclusion, the administration of multiple omeprazole doses to healthy volunteers inhibited in an enantioselective manner the metabolism of the (+)-(S)-CITA eutomer, with an increase of approximately 100% in plasma concentrations (AUC 487.50 *vs* 223.21 ng.h/ml).

80

Effect of Organic Nitrate Tolerance on Hepatic Esterase Activity

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Organic nitrates such as glyceryl trinitrate (GTN) are used to treat cardiovascular disease. However, prophylactic treatment with GTN can result in tolerance, limiting the drug's utility. GTN is considered to be a prodrug, requiring bioactivation for pharmacological activity, and it is proposed that the major cause of tolerance is inhibition of the enzyme responsible for GTN bioactivation. Aldehyde dehydrogenase 2 (ALDH2) has been proposed as the primary enzyme responsible for GTN bioactivation. ALDH2 activity is decreased in GTN-tolerant tissues and it has been suggested ALDH2 inactivation is the underlying cause of GTN tolerance. ALDH2 possesses dehydrogenase and esterase activity, and our objective was to examine the effects of GTN tolerance and known ALDH2 inhibitors on both of these activities. GTN tolerance was induced in rats using an *in vivo* model (0.4mg/hr GTN for 48hr), and dehydrogenase and esterase activity measured in liver mitochondrial and microsomal fractions in the presence or absence of GTN or the ALDH2 inhibitor, daidzin. There was no inhibition of mitochondrial or microsomal esterase activities by either inhibitor in control preparations, nor was esterase activity altered in preparations from GTN-tolerant animals. In contrast, both low K_m and high K_m dehydrogenase activity was inhibited in mitochondria and microsomes from tolerant animals, and by GTN in subcellular fractions from control animals. Daidzin was a relatively selective inhibitor of low K_m dehydrogenase activity. We conclude that GTN is a non-selective inhibitor of the dehydrogenase activity of ALDH and that the esterase activity of ALDH is not affected by acute or chronic exposure to GTN.

81

Simultaneous Determination in Human Plasma of Two Major Antihypertensive Drugs, Hydrochlorothiazide and Irbesartan, by HPLC-DAD

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Objectives: To develop and to validate a new very sensitive CLHP method able to detect and quantify in human plasma two usually prescribed anti-hypertensive agents: a diuretic, hydrochlorothiazide and an angiotensin II antagonist, irbesartan. Materials and methods: The extraction is of liquid-liquid type with ethyl acetate. After evaporation, the extract was injected in a C4 Symmetry® 300 Waters column (150 X 4.6 mm 5.0 µm). The mobile phases consist of phosphate buffer 0,01N pH 3,6 and acetonitrile. Separation is carried out with a gradient of elution at 1 ml/min. Detection is carried out using a DAD at 215 Nm. The analytical validation is led according to FDA recommendations. Results: The ranges of calibration were carried out around the values of the plasma levels met in therapeutic use (2.5 – 500 ng/ml and 20 – 4000 ng/ml for hydrochlorothiazide and irbesartan, respectively). Results showed good precision with good intra-day and inter-day exactitude values (ranges: -17.8% to 12.3% and -15.8% to 5.0 %, respectively) as well as an excellent linearity in therapeutic concentrations of HCTZ and irbesartan. Correlation coefficients were 0.9990± 0.0010 and 0.9976± 0.0029, respectively. LOQ were 2.1 and 8.9 ng/ml, respectively. The recoveries from plasma were 105.0 ±8.0% and 72.2 ±4.2%, respectively.Conclusion: HPLC equipment and mobile phases are cheap and widely common, allowing application of this assay easily in routine for the pharmacological therapeutic follow-up of patients with arterial hypertension. Monitoring hydrochlorothiazide and irbesartan concentration can help to optimize the effectiveness/tolerance ratio in patients with resistant hypertension.

82

Sensitive Analysis of Nebivolol in Human EDTA K_2 Plasma by LC/MS/MS using Automated SPE Extraction

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Purpose: Nebivolol is a long-acting, cardioselective beta-blocker currently licensed for the treatment of hypertension. It has mild vasodilating properties attributed to its interaction with the L-arginine/nitricoxide pathway, a property not shared by other beta-blockers. The purpose of this work was to develop and validate a sensitive and robust method for the determination of nebivolol in human EDTA K_2 plasma. Methods: Nebivolol and its internal standard nebivolol-d_4 were extracted from human EDTA K_2 plasma using Oasis MCX 10 mg extraction plates on MultiPROBE II EX HT system. Compounds were eluted with acetonitrile/ammonium hydroxide (95/5). Analysis was performed on a MDS Sciex API 5000 tandem mass spectrometer with TurboIonSpray interface. Positive ions were measured with m/z 406.3 $\rightarrow$ 151.2 for nebivolol and 411.3 $\rightarrow$ 151.2 for IS. The chromatographic run time was 2.1 minutes on a Zorbax SB-C18 50 X 4.6 mm column. The mobile phase was a mixture of methanol and Milli-Q type water (62.5/37.5) with 1mM ammonium formate and formic acid 0.1% (v/v). Results: This assay was validated over a nominal range of 20 to 2500 pg/mL. Linearity over the calibration range was $\geq$ 0.9992. The between-run accuracy ranged from 100.55 to 101.75% with precision ranging from 1.69 to 2.76%. The within-run accuracy ranged from 99.27 to 116.98% with precision ranging from 0.80 to 5.65%. The recovery of nebivolol and nebivolol-d_4 was greater than 71%. No matrix effect on quantitation was observed. Nebivolol was found to be stable in human EDTA K_2 plasma after 74 hours at room temperature for short term stability, after 76 days at -20°C for long term stability, after 100 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -20°C and -80°C. Dilution integrity and matrix selectivity were also demonstrated. Hemolysis effect was evaluated. Conclusions: This method is sensitive, accurate, and reproducible and was successfully applied for the analysis of clinical samples. Over 3400 clinical samples were analysed with accuracy ranging from 92.58 to 102.21% and precision ranging from 1.24 to 5.86%.

83

The Influence of Montelukast Sodium in Streptozotocin-induced Diabetes Mellitus in Rat

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Introduction: Synthesis of cysteinyl-leukotrienes (CysLTs) is increased in human and experimental diabetes mellitus. The role of these eicosanoids in the disease pathogeny is still unclear. This study investigated the effect of montelukast sodium (MK) (Cys-LT1 receptor antagonist) in streptozotocin (STZ)-induced diabetes mellitus in rat. Methods: Diabetes mellitus was induced by unique intraperitoneal injection of STZ (50 mg/kg). We worked on 4 groups of 10 Wistar male rats each which received as follows: group I (control) – saline; group II – MK 10 mg/Kg/day i.p. for 10 weeks; group III – STZ (50mg/kg), i.p., unique administration; group IV – STZ (50mg/kg), i.p., unique administration and MK 10mg/Kg/day i.p. for 10 weeks. Ten weeks after injection of STZ, MDA (malondialdehyde), CAT(catalase), GSH(reduced glutathione), SOD(superoxid dismutase) as well as GPx(glutathione peroxidase) were determined in blood and liver homogenate. A histopathological exam was performed. Results: Compared to group III, group IV exhibits statistical significant higher levels of GSH (0.32±0.04 versus 0.43±0.08 mmol/ml, $p<0.05$), SOD (4.28±0.64 versus 8.09±0.8 U/ml, $p<0.05$) in blood, SOD (16.86±1.19 versus 23.46±2.06 % inhibition, $p<0.05$) and CAT (1.37±0.2 versus 1.54±0.09 U/ml, $p<0.05$) in liver homogenate. Group III exhibited proliferation of the mesangial matrix and cells in the renal corpuscles associated with important thickness of glomerular basement and segmental glomerulosclerosis area. Group IV presented both normal and damaged renal corpuscles without mesangial hyperplasia maintaining only the basement membrane thickness. Discussion: Our data show that MK exerted differentiated influence on oxidative stress parameters. Conclusion: MK exhibits a partial protective effect on rats with STZ- induced diabetes mellitus.

84

Simultaneous Ciclosporine Pharmacokinetics Modeling in Whole Blood and in Peripheral Blood Mononuclear Cells (PBMCs)

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Introduction: Cyclosporine has a highly variable pharmacokinetics, a narrow therapeutic index and is subject to many interactions. Therefore, TDM is highly recommended for this drug. Moreover, its immunosuppressive action is due to its binding to cyclophylin (T-lymphocytes-cytoplasmic-receptor). Our aim was to evaluate if TDM in blood predicts well concentrations in target site.
Methods: Single oral dose of cyclosporine (2 mg/kg) was administrated to 19 healthy male volunteers previously genotyped for *ABCB1* G2677T/A-C3435T haplotypes (10 GG-CC and 9 TT-TT). Cyclosporine determination in PBMCs and whole blood was performed using LC-MS. NONMEM was used for pharmacokinetics modeling. Results: Two compartment model adequately described ciclosporine pharmacokinetics in whole blood. Since PK profiles in blood and in PBMCs were parallel, a proportionality factor was added to the model to fit PBMCs pharmacokinetics. No inter-individual correlation was observed in cyclosporine levels between PBMCs and whole blood. In contrast, intra-individual PBMCs and whole blood pharmacokinetics profiles didn't significantly differ with respect to t_{max} and $t_{1/2}$. Tested *ABCB1* genotypes had no influence on cyclosporine pharmacokinetics parameters in PBMCs and whole blood. Discussion: The observed large interindividual variability in PBMCs and whole blood cyclosporine pharmacokinetics and the absence of correlation between PBMCs and whole blood levels indicates that TDM based on whole blood concentrations is not a good predictor of the PBMCs drug levels. Conclusion: The selected population-pharmacokinetic model accurately characterised ciclosporine pharmacokinetics in whole blood and PBMCs. Since intra-individual PBMCs and blood concentrations were proportional, Intracellular measurement is a valuable alternative to the blood for TDM determination.

85

The Influence of Food on Lansoprazole Pharmacokinetics

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Background: Administration of the drugs together with food can significantly change activity of some drugs. Presence of food in gut has big influence on kinetic properties of the drugs applied orally. Study Design: The aim of the study was to examine whether high-calory breakfast, taken 30 min before, changes pharmacokinetics of lansoprazole in healthy volunteers. The study was designed as randomized, double-blind, cross-over clinical trial on 30 adult male volunteers. First, lansoprazole was applied orally, 30 mg on empty stomack, together with 200 mL of water, and after two weeks, in a some dose orally, after prearranged breakfast. The samples of the blood were taken during 48 hours time interval, and concentrations of lansoprazole were determined by HPLC method. Results: The C_{max} was significantly lower in fed volunteers (152.4 ± 111.3 versus 576.7 ±168.7 ng/mL). The $AUC_{0-\infty}$ in fed and fasted volunteers was 510.1 ± 398.9 and 1533.8 ± 682.5 ng x h/mL respectively. In fed volunteers the appearance of C_{max} was delayed (3.55 ± 1.16 versus 1.59 ± 0.65 hours). There was no difference in K_{el} of lansoprazole. The half-life of lansoprazole was slightly prolonged in fed volunteers, but difference is not ignificant. Conclusion: On the basis of the results, it is concluded that food significantly delays absorption of lansoprazole, decreases C_{max}, and slightly prolongs retention of the drug in the body.

86

Comparative Study of *CYP2C9 *2/*3* and *VKORC1* (-1639G>A) Genotyping with the Nanopshere Verigene System and the Third Wave Technologies Invader Assay

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Background: The FDA-approved package insert for warfarin now includes recommendations for *CYP2C9* *2/*3 and *VKORC1* –1639G>A alleles genotyping, along with various patient characteristics (e.g., age, body weight) to better estimate initial warfarin dosing. Current literature suggests polymorphisms of *CYP2C9* and *VKORC1* genes account for 17% and 15% variability in initial warfarin dosing, respectively. When combined with clinical factors, more than 50% of the variability in response to warfarin dosing is now explainable. In this study we compared results of CYP2C9*2/*3 and VKORC1 –1639G>A genotyping with two different platforms and technologies. Methods: 24 anonymized human DNA samples were genotyped for *CYP2C9/VKORC1* using the Nanosphere Verigene platform and subsequently genotyped using the Invader *CYP2C9/VKORC1* assay according to the manufacturer's instructions by two operators who were blinded to the initial genotyping results. Genotypes were confirmed by DNA sequencing. Results: Call rates of 95.8% and 100% were obtained by the two operators using the Invader assay. These results were similar to those obtained using the FDA cleared Nanosphere Verigene assay (95.8%). There was 100% concordance between the Verigene and Invader genotyping results. Invader assay is performed preferably in batches up to 29 samples in 4 hours, in contrast Verigene processor can be used on demand and it takes 90 minutes. Conclusion: Our data suggest that genotyping for *CYP2C9*2/*3* and *VKORC1* –1639G>A using the Nanosphere Verigene and the Third Wave Invader assays are comparable and may be used in clinical therapeutic decision making.

87

Antinociceptive Effect of Solanum Melongena Hydro-alcoholic Extract on Acute and Chronic Pain Models in Male Mice

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Introduction: patients who are suffering pain usually seeking medicinal recommendations about their nutritional regime. One of the most contraindicated vegetative regarding pain modulation is Solanum Melongena. In this study we conducted to evaluate the antinociceptive effect of Solanum Melongena hydro-alcoholic extract on acute and chronic pain models in male mice. Materials and Methods: This study was conducted on 70 male mice divided into 14 groups. Formalin test was used for chronic and tail flick test for acute pain assessments. In each category 3 test groups were administered 3 different doses of extract (10, 100 and 1000 mg/kg), 3 positive control groups received 3 different doses of morphine sulfate (1, 2 and 4 mg/kg). Distilled water was injected to animals in the remaining negative control group in each category. Results: In tail flick test the analgesia index in test groups was dose dependently greater than the negative control group and the differences were significant at 45 to 60 minutes after the drug administration ($p< 0.05$). The analgesia index was the same as 4 mg/kg morphine sulfate ($p> 0.05$). In chronic pain assessment The analgesic effect of 1000mg/kg solanum extract was the same as 2 and 4 mg/kg morphine sulfate($p> 0.05$) but greater than 1 mg/kg morphine sulfate($p< 0.05$).Conclusion: According to our findings solanum extract in a dose dependent manner leads to relieve both acute and chronic pain in mice.

88

Open Labeled, Uncontrolled Pharmacokinetic Study of a Single Intra-muscular hCG Dose in Healthy Male Volunteers

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This study compared blood and cerebrospinal fluid (CSF) pharmacokinetic characteristics of two forms of human chorionic gonadotropin (hCG): Pregnyl, derived from human urine, and Ovitrelle a recombinant form, to yield background CSF PK in advance of a novel neuro-regenerative therapy for ischemic stroke. Two separate groups, each with six older male human subjects, were dosed with either form of the drug at 10,000 IU intramuscularly (IM), and followed over a 36-hour period. No significant difference was observed when plasma levels of hCG were observed for either preparation of hCG (Peak plasma conc.: 316±53 vs 270±60 @12 hours, 311±38 vs 321±60 IU/L @ 24 hours; AUC: 10053±1268 vs 8793±1768, Pregnyl and Ovitrelle, mean ± SD, respectively). Additionally both forms of circulating hCG distributed to the central nervous system (CNS) as manifest by an increased number of subjects whose CSF samples showed detectable levels of hCG in their CSF over a 36-hour period. Similarly, there was no significant difference between the two forms when distribution to the CSF was compared at 36 hours (2.0 and 1.2 IU/L; range 1.9-2.1 and 1-1.4 IU/L for Pregnyl and Ovitrelle, resp.). This preliminary study in normal human volunteers suggests that the two forms of hCG tested, Ovitrelle™ and Pregnyl™, when administered IM, distribute in a similar fashion into the circulation and CSF. Consequently, we conclude that these two drugs likely demonstrate bioequivalent pharmacokinetics with respect to the CSF.

89

Identification of Key Domains that Confer Human OATP1B1 and OATP1B3 Transporter Substrate Specificity

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Organic Anion Transporting Polypeptide transporters OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) are highly homologous (80% identical), liver-enriched transporters known for their ability to facilitate the hepatic uptake of numerous endogenous and xenobiotic compounds including clinically important drugs such as the HMG-CoA reductase inhibitor (statin) class of drugs. Despite their remarkable sequence similarity, there are some notable differences in their substrate specificity. In particular, the gastrointestinal peptide hormone cholecystokinin-8 (CCK-8) is transported by OATP1B3 but not OATP1B1, suggesting there are key regional or amino acid differences that confer such substrate specificity. Accordingly, we utilized homologous recombination of linear plasmid DNA by *E. coli* to generate a library of monomer length OATP1B1-1B3 and OATP1B3-1B1 cDNA chimeras in a tandem head-to-tail configuration. Characterization of [3H]-CCK-8 transport by these chimeric transporters in HeLa cells indicated that discrete regions in OATP1B3 confer CCK-8 transport activity. Specifically, we noted a loss of CCK-8 transport activity in OATP1B1-1B3 chimeras when the chimeric junction was near the predicted transmembrane helix 1, while a gain of CCK-8 transport activity was noted in OATP1B3-1B1 chimeras at similar junctions. Thus our findings indicate that amino acid residues close to the extracellular boundary of predicted transmembrane helix 1 of OATP1B3 are essential to CCK-8 substrate recognition. In summary, our data reveal important new insight into regions in OATP1B3 and OATP1B1 that are likely to participate in substrate recognition, and more focused mutagenesis studies are planned to further elucidate the molecular determinants governing the structure-activity relationship of these clinically important hepatic drug uptake transporters.

90

Influence of the CYP2D6 -1584C>G Promoter Polymorphism on the Phenotype of Debrisoquine in Healthy Volunteers

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Introduction: Ultrarapid drug metabolism (UM) mediated by CYP2D6 is associated with inheritance of alleles with duplicated or amplified functional *CYP2D6* genes. Genotyping for duplicated *CYP2D6* alleles only explains a fraction (10-30%) of the UM phenotypes observed

in Caucasian populations. The genetic and/or biochemical basis for the UM phenotype in individuals lacking duplication alleles remain so far unexplained. The *-1584C>G* polymorphism is possibly associated with the UM phenotype and was identified as another major factor for expression and function with the mutant *-1584G* promoter type being consistently associated with significantly higher expression than *-1584C*. Aims: To analyse the relationship between the presence of the *CYP2D6 -1584C>G* polymorphism and the metabolic ratio of debrisoquine (MR) in a previously phenotyped population of healthy volunteers. Methods: Two hundred and forty-four unrelated healthy individuals living in Cuba were studied to *CYP2D6 -1584C>G* polymorphism, 123 were Cuban-Caucasians (CCs) and 121 Cuban-Mestizos (CMs). *The -1584C>G* polymorphism was analysed by a new PCR and PCR-RFLP technique in these individuals.Results and Conclusions: The frequency of *CYP2D6 -1584C>G* polymorphism was higher in CCs than CMS (0.23 *vs*. 0.14 respectively $p<0.05$). The MR (mean±SD) of individuals with one or two *CYP2D6* active genes and *-1584G* allele (0.7±0.5 and 0.6±1.1, respectively) was lower ($p<0.05$) than that of individuals with one or two *CYP2D6* active genes with the *-1584C* allele (2.7±3.9 and 1.1±2.6, respectively).

91

Decreased Vascular ALDH2 Expression in Response to Nitrate Tolerance

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Organic nitrates such as glyceryl trinitrate (GTN) are commonly used to treat myocardial ischemia and congestive heart failure. GTN is proposed to act as a prodrug that requires bioactivation for pharmacological activity. However, continuous administration results in tolerance development, limiting its clinical usefulness. Aldehyde dehydrogenase 2 (ALDH2) has been proposed to be the primary enzyme responsible for GTN bioactivation, and ALDH2 inactivation has been proposed as the sole basis of nitrate tolerance. In the present study, we utilized our *in vivo* GTN tolerance model to investigate the role of ALDH2 in GTN bioactivation and tolerance. We assessed changes in ALDH2 protein, mRNA and activity levels in rat blood vessels during chronic GTN exposure (0.4 mg/hr for 6, 12, 24 and 48 hr) in relation to changes in vasodilator responses to GTN. A time-dependent decrease in both ALDH2 expression and activity occurred (80% in tolerant veins and 30% in tolerant arteries after 48 hrs exposure to GTN), concomitant with decreased vasodilator responses to GTN. However, after a 24 hr drug-free period following 48 hr GTN exposure, the vasodilator responses to GTN had returned to control values, whereas ALDH2 expression and activity were still markedly depressed. The dissociation between reduced ALDH2 activity and expression, and the duration of the impaired vasodilator responses to GTN in nitrate-tolerant blood vessels, suggest factors other than changes in ALDH2-mediated GTN bioactivation contribute to nitrate tolerance.

92

A Study Comparing the Efficacy of Ciprofloxacin with Rosiglitazone and the Combination of these Two Drugs in the Treatment of TNBS Colitis in the Rats

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In this study we aimed to investigate the effect of the antibacterial agent ciprofloxacin in the treatment of TNBS colitis of the rats in comparison to the PPAR-(ligand rosiglitazone. We also studied the efficacy of ciprofloxacin in comparison to the combination of these two drugs. Methods: Study was carried out in 2 groups of Spraque Dawley rats, namely the control (intracolonic saline) and the colitis (intracolonic TNBS) groups. Each group was divided into 4 treatment subgroups; and methyl cellulose (carrier), ciprofloxacin, rosiglitazone or ciprofloxacin + rosiglitazone were administered orally for 7 days. At the end of the treatment period rats were decapitated and the macroscopic score of the colon was determined. Colon and ileum tissue samples were collected for determination of MDA, GSH, MPO, luminol, and lusigenin levels as well as histologic and histochemical studies (TNF-alpha and NF-kappa B). Results: Colitis increased the macroscopic and

microscopic scores of the colon and these were decreased significantly by all treatments. Luminol, lusigenin, and MDA levels increased and GSH levels decreased significantly in colitis, demonstrating oxidative damage. These parameters were reversed after all treatments. The significant increase in MPO levels (demonstrating neutrophil infiltration), as well as TNF-alpha and NF-kappa B levels were also decreased in treated groups, indicating an anti-inflammatory effect. Conclusion: These findings suggest that antibacterial agents such as ciprofloxacin would be effective in the treatment of inflammatory bowel diseases, and the combined therapy with the PPAR-γ ligand rosiglitazone does not offer any advantage over treatment with ciprofloxacine.

93

An Aseptic Inflammatory Reaction Reduces NADPH P450 Reductase Activity and Expression in the Rabbit

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The inflammatory reaction (IR) produces an early decrease in cytochrome P450 (P450) activity due to changes in P450 isoforms activity, and a late decrease in P450 secondary to P450 isoforms down-regulation. This study aimed to document whether an IR affects NADPH P450 reductase (NADPH) activity to explain the early reduction in activity. Rabbits received s.c. turpentine to produce an aseptic IR and 48 hours later, control and animals with IR were sacrificed and the hepatocytes harvested and incubated for 48 h. Compared with control hepatocytes, in IR hepatocytes CYP3A6 activity, expression and mRNA decreased by 60, 40 and 38%, respectively ($p<0.05$), NADPH activity, expression and mRNA decreased by 25, 22, 33% ($p<0.05$). To assess potential serum mediators responsible for NADPH activity and expression reduction, control hepatocytes were incubated with pro-inflammatory cytokines for 48 h; IL-6 and IL-1β reduced NADPH and CYP3A6 activity and expression ($p<0.05$), but not IFN-γ and TNF-α. To understand the signal transduction pathways implicated in IL-6-induced decrease in NADPH activity and expression, control hepatocytes were incubated with inhibitors of several kinases 30 min prior the addition of IL-6. Inhibition of p38MAPK, ERK1/2, JNK, PI3K and STAT3 did not prevent IL-6-induced decrease in NADPH activity. On the other hand, in absence of IL-6, inhibition of ERK1/2, STAT3 and JNK reduced basal NADPH activity ($p<0.05$). It is concluded that an AIR and pro-inflammatory cytokines reduce activity and expression of NADPH and that may contribute to the IR-induced decrease in P450 activity.

94

ABCB1 *and* Cytochromes P450 Genotypes and Phenotypes: Influence on Clozapine Plasma Concentrations

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Introduction: The in vivo implication of various cytochrome P450 isoforms and of P-glycoprotein on clozapine kinetics is unclear. We aimed to thoroughly examine the genetic factors influencing clozapine kinetics. Methods: 75 patients treated with clozapine were genotyped for CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, and ABCB1. In addition, patients were phenotyped with caffeine and midazolam to assess CYP1A2 and CYP3A4/5 activity, respectively. The steady-state trough plasma concentrations of clozapine and norclozapine were measured. Results: A strong correlation was measured between CYP1A2 activity measured by the caffeine test and plasma concentrations of clozapine ($r=-.606$; $p<0.001$), and norclozapine ($r=-.480$; $p<0.001$) but CYP1A2*1F genotypes did not influence either clozapine or norclozapine plasma concentrations ($p>0.5$). In addition, a correlation was found between CYP3A4/5 activity and clozapine/norclozapine plasma concentrations but only at high clozapine dosages. CYP2C19 genotypes significantly influences clozapine plasma concentrations, with the CYP2C19 poor metabolizers (*2/*2 genotypes) having 2 ($p=0.034$) and 1.3 (N.S) fold, respectively, higher clozapine and norclozapine plasma concentrations

than the extensive metabolizers (*1/*1, *1/*2). Smokers had lower clozapine and norclozapine concentrations than non-smokers. Patients treated with fluvoxamine (25–300 mg/day), an antidepressant and a strong CYP1A2 inhibitor, had a mean 4 and 3 fold increased clozapine and norclozapine concentration, respectively, as compared to patients with clozapine only. The CYP2B6, CYP2C9, CYP2D6, CYP3A5, and ABCB1 genotypes did not influence clozapine plasma concentrations.Discussion and conclusion: In vivo, CYP1A2 is the main CYP isoform involved in clozapine metabolism, with CYP2C19 contributing moderately, while the contribution of CYP3A4 being significant only at high clozapine plasma concentrations.

95

Genetic Polymorphisms of Glutathione S-transferase M1 and T1 in Hausa, Ibo and Yoruba Populations of Nigeria

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Introduction: Genetic polymorphisms of drug metabolising enzymes are important determinants of interethnic and inter-individual variability in the pharmacological response to drugs. While the molecular basis of such variations is known and well documented for many populations, such data are nonexistent on Nigerian populations. The glutathione-S-transferases (GSTs) provide a major biochemical defence against environmental toxins and their polymorphisms have been implicated in some diseases. Methods: Three hundred healthy volunteers from three major Nigerian ethnic groups: Hausa (N = 98), Ibo (N = 101) and Yoruba (N = 101) were genotyped for *GSTM1* and *GSTT1* using PCR techniques, as approved by the ethics committee of the Obafemi Awolowo University. Results: The *GSTM1*0* genotype frequencies were found at 37% in Hausa, 23% in Ibo and 31% in Yoruba. Also, the *GSTT1*0* genotype frequencies were 42%, 36%, and 35% for the Hausa, Ibo and Yoruba respectively. Discussion: The *GSTM1*0* genotype frequency is lower in Ibo compared to the Hausa and Yoruba but the average frequency of 30% in Nigerians is comparable to 27% and 28% observed in Southern Africans and African-Americans respectively. While the average frequency of 38% of *GSTT1*0* is higher than the 24% and 22% observed in southern Africans and African-Americans respectively. The values were however, lower than the 64% for Orientals but higher than the 10-24% reported for Caucasians.
Conclusion: The genotype status of *GSTM1* and *GSTT1* shows that the Nigerian population may be prone to reactive metabolites than other Africans and Caucasians but may be better protected than Asian populations.

96

Phenotyping of CYP2C19 and CYP3A4 by Using Omeprazole as Probe Drug and Genotyping of CYP2C19 for Common Allelic Variant in Ethiopians

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The present study attempted phenotyping of CYP2C19 and CYP3A4 enzymes using omeprazole as a probe and genotyping CYP2C19 for the two common mutants alleles, *CYP2C19*2 and CYP2C19*3*. 150 healthy Ethiopian volunteers were recruited and given 20 mg omeprazole orally. Plasma concentrations of omeprazole, 5-OH-omeprazole & omeprazole sulfone were determined with HPLC. Logarithm transformed plasma concentration metabolic ratio was used to evaluate activity of CYP2C19 and CYP3A4. Genotyping was performed with Taqman® allele discrimination for *CYP2C19*2* and *CYP2C19*3* alleles. The prevalence of Ems and PMs was found to be 6% and 94%, respectively when CYP2C19 activity was assessed using a cut off value of 0.8. There was significant difference between PMs (1.12 ± 0.22) and Ems (0.12 ± 0.27) ($P < 0.0001$). Genotypic analysis revealed that 1.33% were genotyped as

PMs with *CYP2C19*2/*2* and 98.67% were genotyped as Ems, of which 76% were homozygous for *CYP2C19*1/*1*, 22.67% heterozygous for *CYP2C19*1/*2 and CYP2C19*1/*3*. Allelic frequency of *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* were 87%, 11% and 2%, respectively. Correlation of phenotyping with genotyping revealed that whilst seven of genotype Ems were PMs on phenotyping, all genotype PMs were PMs on phenotyping. Assessment of CYP3A4 activity showed normal distribution with two outlier subjects of lower and higher activity. There were neither sex-dependent differences nor any effect of CYP2C19 on CYP3A4 or vice versa. These findings collectively indicate that omeprazole could be used as a probe drug in simultaneous phenotyping of CYP2C19 and CYP3A4, and the analyses points to the fact that consideration of dose adjustment is imperative in usage of particularly CYP2C19 substrate drugs.

97

Preliminary Identification of Specific Polymorphisms in *UGT* and *ABCC2* Genetic Determinants of MPA Disposition in Thoracic Transplant Recipients

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Background: Mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), is known for wide inter-patient variability in its pharmacokinetics. The metabolism of MPA to the phenolic (MPAG) and acyl (AcMPAG) glucuronides is mediated by UGT enzymes, whereas multidrug resistance-associated protein 2 (MRP2/ABCC2) is involved in disposition of MPA and its metabolites. This study aimed to investigate contributions of *UGT* and *ABCC2* genetic polymorphisms to the pharmacokinetic variability of MPA in thoracic transplant recipients on steady-state MMF therapy. Methods: Blood samples were collected over a 12-hour period from 52 thoracic transplant recipients on a steady-state MMF regimen and followed by quantitation of MPA and metabolites (via high-performance liquid chromatography with ultraviolet detection), and genotyping of *UGT* and *ABCC2* (via direct sequencing of polymerase chain reactions). Results: Multivariate analyses showed that MPA pharmacokinetics and impact of *UGT* and *ABCC2* polymorphisms differ between lung and heart transplant recipients. For lung transplant recipients, co-medication, *UGT2B7* -79, *UGT1A9* -440/-331 and *UGT1A1* -3279 polymorphisms impact significantly on MPA, free MPA and MPAG exposure; conversely, age, gender, *UGT1A8* codon 237, *UGT1A1*28*, and *UGT2B7*2* variants impact on MPA, free MPA, MPAG and AcMPAG exposure in heart transplant recipients. In addition, *ABCC2* codon 417 (Val^{417}Ile) is associated with lower MPA C_{max} in lung transplant recipients. Conclusions: Clinical pharmacogenetic studies of MPA are scarce in the thoracic transplant population. Specific polymorphisms in *UGT* and *ABCC2* genes, and possibly interactions of polymorphisms, are involved in determining MPA disposition. Larger clinical pharmacogenetic studies in heart and lung transplant subpopulations are warranted.

98

P-Glycoprotein Expression in Human Sigmoidal and Rectal Biopsies in Patients with Ulcerative Colitis

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P-Glycoprotein (P-gp) is suspected to have a role in etiology, pathogenesis and treatment of ulcerative colitis; however, in humans data of P-gp expression in the colon is rare. We investigated 111 rectum and sigmoid biopsies of patients, being participants of the phase III clinical study SAS-6/UCA (1 x 1g or 3 x 0.5g daily mesalazine suppositories).with acute ulcerative proctitis for P-gp expression at baseline and *ABCB1* genotype. For histochemistry 2µm slides from paraffin-embedded tissue were incubated with the P-gp-specific antibody JSB1 and stained with the fluorescent antibody FITC. Using 40 fold

magnification, regions of interest with columnar epithelium were digitally photographed and stored in a database. As a marker of P-gp expression and to exclude contingent differences in brightness, the ratio of 11 fluorescent signals in the brush border membrane and 11 in the cytoplasm of enterocytes were calculated. *ABCB1* genotyping of 3435C>T and 2677G>T/A was performed using PCR/RFLP. The geometric means ± SEM of fluorescence ratios (n=111) were lower in sigma (1.24±0.05) than in rectum (1.57±0.07; $p<0.01$). The ratios in sigma and rectum were 1.34±0.12 and 1.49±0.17 in 3435CC (n=22), 1.2±0.07 and 1.55±0.08 in CT (n=58), and 1.27±0.08 and 1.65±0.15 in TT carriers (n=31) respectively, indicating no statistical significant difference in P-gp expression according to *ABCB1* genotype. In summary, we present a new reliable and sensitive method for determination of P-gp expression in human intestinal biopsy specimens. P-gp expression at baseline was higher in inflammatory tissue, independent of *ABCB1* genotype.

99

Nitrofurantoin Transport by Placental Choriocarcinoma JAR Cells: Involvement of BCRP, OATP2B1 and other MDR Transporters.

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The breast cancer resistance protein (BCRP/ABCG2) is a member of ABC transporter superfamily. It is expressed abundantly in the placental syncytiotrophoblast. Recently, nitrofurantoin (NF) was found to be a specific substrate of BCRP, and it was suggested that BCRP interacts with OATP2B1 in the placental trophoblast. Involvement of P-gp and MRPs in NF transport has not been studied yet. The aim of this study was to determine the role of BCRP in NF transport in JAR cells (a human choriocarcinoma cell line), and the possible involvement of OATP2B1, P-gp and MRPs to this transport. JAR cells were grown in enriched DMEM/F-12 medium to confluency. Cells were pre-incubated with transfer buffer or with BCRP, P-gp, MRPs and OATP2B1 inhibitors for 15 min., followed by incubation for 30 min. with NF, or with NF and the inhibitors mentioned earlier. NF levels were analyzed by HPLC.Incubation with specific inhibitors of BCRP (FTC, Chrysin and Novobiocin), showed a significant increase in NF accumulation in the cells. Inhibitors of OATP2B1 (EGCG and BSP) had no effect on NF accumulation. Specific inhibitors of P-gp and MRPs (Verapamil and Indomethacin respectively) also had no effect on NF accumulation in JAR cells. We concluded that NF is a specific substrate of BCRP, and BCRP has an active role in NF transport in JAR cells. It is shown for that P-gp, MRPs, and the SLC superfamily member – OATP2B1, probably have a negligible contribution to NF transport in JAR cells.

100

Analysis of Gene Expression Profiling from Human Blood Cells Reveals Potential Biomarkers of Growth Hormone Action in Adults with Growth Hormone Deficiency

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Growth hormone deficiency in adults (AGHD) results in a well defined clinical syndrome, characterized by a disturbed body composition (e.g., reduction in lean mass and an increased fat mass). These abnormalities are reverted by GH administration, and GH replacement therapy is at present definitely admitted for treatment of AGHD. We focused on blood cells from AGHD to explore the hypothesis that gene expression profiling of peripheral blood cells may not only provide new insights into the study of GH actions, but also permit to identify surrogate biomarkers of its activity. Oligonucleotide microarrays were used to analyze global gene expression profiling in blood samples to identify distinct patterns of gene expression that distinguish AGHD-untreated from AGHD-treated patients that might serve as surrogate biomarkers of GH activity. The blood samples were harvested pre-treatment and at the end of the first 4-weeks from AGHD patients receiving GH therapy. Results from matched pairs

of blood samples from 10 patients were queried for expression changes that consistently correlated with GH administration. 163 transcripts met this selection criterion. In addition, discriminant analysis showed that expression profiles of 27 transcripts (e.g., LCAT, ASB6) could be used to classify patients by treatment arm in a predictive fashion. These results demonstrates for the first time that using whole blood as a pharmacogenomic surrogate, changes in gene expression profiles can be detected that are reflective of GH treatment in adults and establish a foundation for the further exploration of peripheral blood cells as a surrogate for biomarker analyses in clinical endocrinology studies.

101

Evaluation of Possible Gender Differences in the Oral Pharmacokinetics of Enteric-coated Pantoprazole

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Pantoprazole is a H+ pump inhibitor that is widely used in the treatment of gastrointestinal disorders. In order to establish rational dosage regimens, it is necessary to know the pharmacokinetics of the population to be treated. Previously, it has been described that gender differences in the pharmacokinetics of drugs may exist, produced by changes in the metabolism and distribution of drugs between genders. However, in the case of pantoprazole, limited information about differences between genders is available. The purpose of this study was to compare the oral pharmacokinetics of pantoprazole between women and men. Fifty-two (26 women and 26 men) participated in this study that was carried out following the recommendations of the Declaration of Helsinki. All subjects were fit according to medical history, clinical examination and suitable laboratory tests and gave written informed consent for participation. After an overnight fast, volunteers received an oral dose of 40 mg pantoprazole in an enteric-coated formulation and blood samples were obtained at selected times for a period of 8 h after drug administration. Plasma was obtained and stored frozen at -80°C until analyzed by HPLC. It was observed that women Cmax, Tmax and T1/2 were similar between genders, however, increased values of AUC were observed in women. However, when this value was normalized by the dose administered based on the weight of the subject, differences dissapear. These results allow us to conclude that there is no important differences in the oral pharmacokinetics of pantoprazole between genders.

102

Food does not Affect the Pharmacokinetics of Apixaban, an Oral Factor Xa Inhibitor

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Apixaban is an oral, selective, direct-acting, reversible inhibitor of coagulation factor Xa in clinical development for prevention and treatment of thromboembolic diseases. Pharmacokinetics (PK) and pharmacodynamics of some oral anticoagulants are influenced by food, leading to difficulty in maintaining optimal anticoagulation. Effect of food on apixaban PK was determined in this open-label, randomized, crossover study. Twenty-four healthy male subjects received a single oral apixaban 10 mg dose after a 10-hour fast and following a high-fat meal. Serial blood samples were collected for apixaban plasma concentration and clotting time (CT) determination. Absence of effect was concluded if 90% confidence intervals (CI) for ratios of fed to fasted geometric means for maximum plasma concentration (Cmax) and area under the concentration time curve to infinity (AUC(INF)) were contained within the 80% to 125% no effect interval. Apixaban was safe and well tolerated in both treatments. Three subjects discontinued due to minor adverse events; 21 were included in the final analysis. There was no difference in apixaban exposure following fed and fasted administration; Cmax, AUC(INF), and half-life were comparable under both conditions. Cmax and AUC(INF) point estimates and 90% Cis were 1.10 (1.004, 1.197) and 1.04 (1.004, 1.086). Time to Cmax was delayed by 1 hour following the meal. CT changes from baseline were similar between treatments. In conclusion, consumption of a high-fat meal does not affect apixaban PK.

Therefore, apixaban can be administered without regard to meals and still provide a consistent effect. This represents a significant advance compared to current oral anticoagulants.

103

Evaluation of the Impact of CYP2C19 and CYP2D6 Genotype in the Pharmacokinetics of Citalopram

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Introduction: In vitro studies have shown that citalopram (CT) is metabolized by multiple isoforms, including CYP2C19, CYP3A4, and CYP2D6. However, in vivo studies have not confirmed the contribution of CYP2D6 to CT metabolism. Material and Methods: An evaluation of genotypic differences in the disposition of CT was carried out during a bioequivalence study including thirty-five volunteers (19 males; 16 females). They received in each period a single 20 mg oral dose of one of the two formulations of CT. Both formulations were found bioequivalent. Subjects were also genotyped for the main variants of CYP2C9 (*2 and *3), and CYP2D6 (*4). A linear mixed model, for log-transformed and adjusted data to doses and weight, was performed considering formulation, period, sequence and genotype as fixed effects, and individual nested sequence*genotype as random one. Results: We found statistically significant intersubject variability in pharmacokinetics of CT according to the CYP2C19 genotype ($p<0.012$) and to the CYP2D6 genotype ($p<0.047$). Mean of AUCt adjusted for dose/Kg administered–(ng*h/ml)/(mg/kg)- was 2333.8 in volunteers with *1/*1 genotype, 2589.6 in *1/*2 and 3580.5 in *2/*2. Apparent CT clearance (ng/ml/h/kg) was 7.1, 6.4 and 4.7 respectively. On the other hand, individuals with CYP2D6*1/*4 showed a mean adjusted AUCt and CT clearance of 2794 and 6 and those with *1/*1 genotype 2345.5 and 7.1. Conclussion: Our study confirms CYP2C19 contribution to CT metabolism in vivo. Also, our results suggest that CYP2D6 genotype influence in vivo CT metabolism.

104

The Effects of Amiodarone on Rhodamine-123 Transport in Rats

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Amiodarone is known to produce drug-drug interactions via inhibition of drug excretion in the liver. P-glycoprotein has been suggested as the potential site of these interactions. Therefore, the present study investigated the influence of amiodarone on the pharmacokinetics of rhodamine-123 (Rho-123), a P-glycoprotein substrate, in rats. Effects of amiodarone (a single i.v. bolus of 25 mg/kg or pretreatment of 25 mg/kg orally for 4, 7, and 14 days) on Rho-123 kinetics were tested in rats during steady state of Rho-123 plasma concentrations and *in vitro* during accumulation study of Rho-123 in rat primary hepatocytes. The single bolus of amiodarone reduced biliary excretion and clearance of Rho-123 to 56% and 58%, respectively. After any pretreatment duration, amiodarone did not change the kinetics of Rho-123. Interestingly, unlike after intravenous administration, amiodarone was undetectable in plasma after the pretreatment, but its bile concentrations were similar in all animal groups, suggesting identical conditions for canalicular transport blockade. The *in vitro* transport study revealed concentration dependent inhibition of Rho-123 uptake into hepatocytes. In conclusion, our data indicate that amiodarone-mediated inhibition of Rho-123 transport in the liver does not occur at the canalicular P-glycoprotein transporter but rather at the basolateral membrane of hepatocytes.

105

Population Modeling of Inter-occasion Variability of Mycophenolic Acid in Pediatric Kidney Transplant Recipients

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Mycophenolate acid (MPA) is an inhibitor of inosine monophosphate dehydrogenase (IMPDH) contributing to overall immunosuppression. In an ongoing PK/PD study in pediatric kidney transplant patients while monitoring MPA concentrations and IMPDH activity over time we observed large within patient PK variability as a function of time after transplant. This suggests the importance of early aggressive monitoring of MPA and IMPDH inhibition to optimize drug exposure and outcome. The purpose of present study was to develop a population model in a Bayesian algorithm that would account for time after transplantation differences. Patients (n=22) were de novo pediatric kidney transplant recipients participating in the PK/PD study while on MMF (450mg/m2), in combination with tacrolimus and steroids. MPA concentrations (438 samples) were measured by validated HPLC assay at 3 different occasions: Early post-transplant (day 1-3), at discharge (day 4-9) and during stable treatment (3-6 months). PK data were analyzed with NONMEM VI using a 2-compartemet model with first-order absorption and lag time. Covariate analysis included demographic and laboratory data. Time after transplant was modeled as between occasion variability. Weight, but not age, as a covariate for volume of distribution significantly improved the PK model (ΔOBJ=26, $p<0.001$). Introducing between occasion variability for clearance allowed the simultaneous modeling of all data across occasions. In the final model mean CL/F at discharge was estimated as 17.0 L/hr (CV=30.3%), which was decreased by 38% at stable treatment. This study suggests that between occasion variability is an important factor to be considered when modeling MPA concentration time data.

106

Interactions among Antiepileptic Drugs and Dosage Adjusting for Clinical use in Japanese Epileptic Patients

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We clarified the correlation between the daily dose (D) and a steady-state serum concentration (Ct), and analyzed the influences of the concomitant use among antiepileptic drugs such as valproic acid (VPA), carbamazepine (CBZ), zonisamide (ZNS), phenobarbital (PB), and phenytoin (PHT). Ideal body weight or extracellular water volume as a transforming factor, led the level/dose (L/D) ratio to be independent of the patient's age and gender in monotherapy of VPA or CBZ, ZNS, PB, and PHT, respectively. And each Ct was revealed to be dependent on only one variable regarding the transformed daily dose. Models based on the assumption that each value of an alteration ratio was independent from one other and multiplicative for VPA, CBZ and ZNS, and that coadministered drug inhibited drug-metabolizing enzyme competitively for PB, was adopted. Michaeris-menten model was adopted for PHT. The analysis clarified that CBZ, PB, and PHT significantly lowered ($p < 0.05$) Ct to 0.81, 0.88, and 0.83 the value of VPA alone, and that PB and PHT significantly lowered Ct to 0.77 and 0.71 the value of CBZ alone, and that VPA, CBZ, PB and PHT significantly lowered the L/D ratio of ZNS alone to 0.87, 0.85, 0.85, and 0.80, respectively. VPA, CBZ, and PHT significantly increased ($p < 0.05$) the L/D ratio of PB to 1.47, 1.18, and 1.19, respectively. Daily PHT dose was decreased to 0.89, 0.91, 0.90, and 0.84 the dose of PHT alone to maintain Ct in therapeutic range when VPA, CBZ, ZNS, and PB were coadominestered, respectively. Key words: antiepileptic drug; transforming factor; level/dose ratio; concomitant therapy; alteration ratio

107

Fluoxetine Disposition in Patients Suffering from Chronic Hepatitis C Treated with Interferon Alpha.

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Introduction: Combination therapy with interferon (IFN) and ribavirin is currently considered the

gold standard for chronic hepatitis C treatment. However IFN may induce severe depression. Fluoxetine (FLU) has been successfully used to prevent or treat depression. Nevertheless IFN has been suggested to be able to modify Cyp 2D6 activity. Therefore we decided to study the effects of IFN on FLU disposition in candidates for the combination antiviral chemotherapy. Methods: After approval by the Ethical Committee and after obtaining informed consent, a group of adult patients (13 males, 7 females; aged 54.10 ± 1.09 years) were submitted to phenothyping by means of the dextromethorphan test and to the evaluation of the principal FLU kinetic constants (FLU and nor-FLU AUC; FLU C_{max}, T_{max} and $t_{½}$beta) before and after two months of IFN administration. FLU and nor-FLU serum concentrations were determined by means of an LC/MS/MS technique. Results: No statistically significant differences were seen on the mean values of dextromethorphan metabolic ratios and of kinetic parameters studied. However we identified a group of 12 patients presenting a statistically significant difference ($p< 0.05$) of the post-treatment period $t_{½}$beta values in comparison to the pre-treatment ones. Discussion: Generally speaking these data seem to indicate that IFN is not able to modify FLU disposition. However at the same time they seem to suggest that in some patients IFN could ameliorate it. Conclusion: We suggest that INF, instead of inhibiting biotransformation of FLU, might even increase it, probably by reducing the viral hepatic damage.

108

CYP2C19 Genotype is a Useful Marker of Optimal Dose of a Proton Pump Inhibitor in the Maintenance Therapy of Symptomatic Gastroesophageal Reflux Diseases

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Backgrounds/Aims: A maintenance therapy with a proton pump inhibitor (PPI) is performed in patients with gastroesophageal reflux diseases (GERD). The acid inhibitory effect of a PPI depends on CYP2C19 genotype status. Then, we investigated whether CYP2C19 genotype status was associated with the symptomatic recurrence of GERD during maintenance therapy with a PPI. Methods: 124 patients with GERD were continuously treated with lansoprazole 30 mg/D after cure of mucosal breaks by lansoprazole 30 mg/D for 8 weeks. When reflux symptoms disappeared, the dose of lansoprazole was decreased to 15 mg/D. The reflux symptoms were checked by interview every 2–4 weeks. When reflux symptoms recurred, the dose of lansoprazole was restored to 30 mg/D. Patients' CYP2C19 genotypes were classified into rapid metabolizer (RM: *1/*1), intermediate metabolizer (IM: *1/*X) and poor metabolizer (PM: *X/*X) (*X = *2 or *3). Results: Patients consisted of 54 RMs, 56 Ims and 14 PMs. Of 124 patients, 18 RMs, 28 Ims and 8 PMs were treated with lansopraozle 15 mg, however, 88.9% (16/18) of RMs, 78.6%(22/28) of Ims and 50% (4/8) of PMs (50%) experienced symptomatic recurrence. The hazard ratio of symptomatic recurrence of GERD in RMs in comparison with PMs was 4.82 (95%C.I.:1.36 – 17.02, P = 0.015). Finally, 96.3% of RMs, 85.7% of Ims and 71.4% of PMs were continuously treated with lansopraozle 30 mg/D as the maintenance therapy (P = 0.018). Conclusion: GERD patients with the RM genotype of CYP2C19 are at higher risk of symptomatic recurrence when treated with low dose of a PPI. The CYP2C19 genotyping test seems a useful marker to determine the optimal dose of a PPI in the maintenance therapy of GERD.

109

Alternative Methods for Covariate Selection: Application in Epilepsy

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Introduction: In population pharmacokinetics (PK) analysis, the covariate modelling establishes the relationship between model parameters and patients characteristics. The covariates can explain the between subjects variability and provide a rational for dose adjustment. With antiepileptic drugs (AEDs), patients are often treated with several AEDs simultaneously and one consequence of poly-therapy is the occurrence of PK drug-drug-interactions in patients. Method:

Two methods were evaluated using NONMEM VI to identify the best model of combination of covariates. Using the Likehood ratio test, the stepwise forward selection method evaluates the contribution of each covariate pre-selected to the model. The covariates are added one at the time to the model until no remaining variable produces a significant decrease of the objective function. The Wald approximation method (WAM) starts with evaluating all potential covariates. Using the Wald's approximation, the resulting full model is being screened until ten potential final models are selected. The analyst chooses the best combination using predefined criteria. Results: The final models obtained using these two procedures are compared, qualified and discussed; Relationships between the retained covariates are evaluated and finally the analysis time is measured. Conclusion: The WAM method allows testing all possible combinations of covariates, although covariate model (linear, power…) need to be pre-defined in the full model and can't be changed. The advantage of the stepwise forward selection is that a full model is not need but it is time-consuming and may result in competing models.

110

Relationship between Genetically-related Differences at the Serotonergic System and Psychopathological Symptomatology in Anorexia Nervosa

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Anorexia nervosa (AN) is a complex psychiatric disorder influenced by both genetic and environmental factors. The serotonergic system is involved in the regulation of eating behaviour. Accordingly, disturbances of serotonergic neurotransmission have been implicated in the pathogenesis of AN. The serotonin transporter linked-polymorphic region (5HTTLPR) and the 1438 A/G polymorphism in the 5HT2 receptor gene were analyzed in 112 women, 36 patients of AN who met DSM-IV criteria and 76 healthy subjects, in order to test the hypothesis that these genetic variants confer susceptibility to AN. We also analyzed their possible associations with a variety of clinical symptoms and personality traits. The 5HT2 -1438 A allele was significantly more frequent in patients than in controls (56 vs. 41%; chi square p=0.0025). No differences were observed for the 5HTTLPR allelic variants. The -1438 A/G polymorphism displayed a gene-dose effect on the global severity index (GSI) of the SCL-90-R test (mean GSI ± SD: 1.08±0.59, 1.49±0.61 and 2.29±0.68 for AA, AG and GG carriers, respectively; AA vs. GG: p= 0.004, ANOVA/Tukey test). No association of 5-HTTLPR with either physiological or psychological signs was observed in the AN patients. Our preliminary results suggest that the A allele of the -1438 A/G polymorphism confers higher susceptibility to AN, and that this polymorphism can be linked to the severity of the syndrome.

111

***In Vitro* Investigation of the Biological Activity of Etoposide Catechol and the Factors Contributing to its Activity**

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Etoposide catechol is the main etoposide metabolite. A thorough investigation of the biological activity of etoposide catechol may help in understanding the inter-patient variability in response to etoposide treatment. We have examined the biological effect of etoposide catechol in comparison to etoposide in two colorectal cell lines SW620 and HCT116. Etoposide catechol was cytotoxic; with an EC50 concentration of approximately 26 microM. Etoposide was three times more potent than etoposide catechol. This relative potency was further confirmed by cell cycle distribution and mitochondrial membrane potential studies. Both etoposide and the catechol induced an approximate doubling in GSH concentration in the two cell lines; but this was greatest in HCT116 cell line. Depletion of GSH using DL-Buthionine-(S,R)-sulfoximine resulted in a doubling in

etoposide and etoposide catechol intracellular concentrations, and a two-fold increase in the cytotoxic activity of both compounds. A similar increase in intracellular concentration of etoposide and etoposide catechol and their cytotoxicity resulted from the inhibition of P-gp using verapamil. Etoposide, but not etoposide catechol, was found to induce CYP3A activity in the SW620 cell line. In vitro etoposide catechol binding to human plasma proteins was concentration dependent and ranged from 70.1 to 88.2%. In conclusion, etoposide catechol is an active metabolite but is less potent than the parent drug. The biological activity of etoposide catechol is markedly affected by changes in GSH concentration and P-gp activity. However, the catechol metabolite is unlikely to contribute significantly to the in vivo effects of etoposide.

112

Allelic Imbalance in the Expression of Human CYP1A2 Gene

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Introduction: Human CYP1A2 gene is genetically polymorphic and displays wide inter individual and inter ethnic variations in it expression and activity. Several SNPs has been identified although coding SNPs causing amino acid changes are rare. Apparently, genetic polymorphisms in the CYP1A2 gene do not explain the observed variations in enzyme activity to a major extent. Objective of the study was to investigate whether the expression of CYP1A2 in humans is regulated by cis- and/or trans-regulatory polymorphisms to display allelic imbalance (AI) by measuring the relative allelic expression levels in human livers using a marker SNP. Methods: The genotyping and detection of possible AI in CYP1A2 gene expression was performed by mini-sequencing using tag arrays on 68 human liver samples. Results: Out of 68 individuals genotyped for the coding marker SNP (rs2470890 C/T), 32 were (48.5%) heterozygous. In 23 heterozygous livers, we measured the relative C/T signal ratio from cDNA and compared it with the ratio from the respective genomic DNA as a control. Ten out of 23 (43.5%), individuals showed significant allelic imbalance in *CYP1A2* gene expression. In conclusion, the preliminary result indicates the presence of variation in allelic expression in CYP1A2 gene influenced by cis and/or trans-regulatory polymorphisms, giving a new insight in the genetic regulation of CYP1A2. In addition to other genetic and environmental factors, allelic imbalance in gene expression of CYP1A2 could be of importance to explain the phenotypic variability in the pharmacodynamic and pharmacokinetic outcomes of drug therapy.

113

Model of Metformin (MET) Absorption-Pharmacodynamic Implications

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Introduction: MET is a first choice drug for the treatment of Type-2 Diabetes Mellitus and Metabolic Syndrome. Some evidences suggest that MET is fully effective only *per os*. Thus, absorption is a main feature of MET action. Methods: We have simulated MET absorption from previous data in order to clarify some aspects. Data from unpublished 500 mg bioequivalence study was analyzed using MS-Excel-2002 (Microsoft Corp) and WinNonLin5.1 (Pharsight Corp) following an unicompartmental model. MET absorption fraction (fa) was theoretically estimated and MET permeability (p) was calculated from absorption kinetic constant (k_{abs}). Results: Model AUC_{inf}; Cmax; tmax; Cl_T; Vd_{area} and $t½_{elim}$ were: 7.4 mg/L*h; 1.05 mg/L; 3 hr; 31.1 L/h; 168 L and 3.75 hr respectively; k_{abs} was 0,9 h^{-1}. We estimated a fa of 0.48 and a p of 0.0002 seg^{-1}. These data are in agreement with fa-p relationship curve published by Amidon and colleagues and they could correspond to a drug that is absorbed without transports. Discussion and conclusions: MET is a high solubility-low permeability drug, so the last property could be the limiting step in its absorption. We propose that MET passes through paracellular way disolved in water, despite the presence of apical cation

transports in enterocytes. Then it is incorporated and concentrated inside cells from blood by basolateral OCT1 cation transport. Inside enterocytes, MET inhibits respiratory chain and ATP levels fall. In this scenario glucose uptake from intestinal cells is reduced (therapeutic effect) and in some patients, gastrointestinal side effects may appear (adverse reactions).

114

Blood-Brain Penetration of the Enantiomers of Venlafaxine and its Metabolites in Mice Lacking the abcb1a/b Genes

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Introduction: Mice lacking the abcb1a/b genes were used to study the pharmacokinetics and penetration of the enantiomers of venlafaxine (VEN) and its three metabolites O-desmethylvenlafaxine (ODV), N-desmethylvenlafaxine (NDV) and N,O-didesmethylvenlafaxine (DDV) into the brain in absence of the P-glycoprotein (P-gp) at the blood-brain barrier. Method: P-gp knockout and wildtype mice received an i.p. injection of 10mg/kg body weight of racemic VEN. The mice were decapitated after 1, 3, 6 and 9 hours and the drug concentrations in brain and serum were determined using LC/MS/MS. Results: The brain concentrations of the S- and R-enantiomers of VEN, ODV and NDV were higher in knockout mice than in wildtype mice. This indicates that both of the enantiomers of these metabolites are substrates of P-gp and that P-gp at the blood-brain barrier reduces the penetration of these metabolites into the brain. No major group difference was found in brain S/R ratios of ODV and NDV, indicating that the enantiomers have similar affinity for P-gp. In contrast, a minor difference in S/R enantiomer ratio of VEN was detected, indicating a possible enantiomeric difference in P-gp affinity. Conclusion: The present study confirms that P-gp has an active role in exporting CNS drugs out of the brain and that the enantiomers of VEN and its metabolites are substrates of P-gp.

115

Population Pharmacokinetics of Teicoplanin in Febrile Neutropenic Korean Patients

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Introduction: Teicoplanin's activity is known to be time-dependent and the therapeutic trough concentration (Cmin) greater than 10 mg/L. To evaluate the adequacy of the dosage regimen of teicoplanin in febrile neutropenic patients who have underlying hematologic malignancies, we performed a population PK study. Methods: One hundred forty seven plasma teicoplanin concentrations obtained from 49 patients were used. After loading with 400 mg (6 mg/kg) i.v. teicoplanin, 200mg (3 mg/kg) i.v. was given once daily. Peripheral venous blood samples per patients were taken within 4 hours after dosing. Using prior information from previous reports, mixed effect analysis was performed (NONMEM, Ver.6). Two compartment basic model was fitted to our data. On the basis of the estimated PK parameters we simulated data for 200mg, 300mg, 400mg, 500mg maintenance dosing scheme and defined the optimal loading, maintenance dose in Korean patients. Results: Typical values of V1 (4.97 L) , V2 (45.4 L), Q (10.83 L/h) and CL (1.5 L/h) of the final two-compartment PK model were significantly greater than those reported in healthy subjects. Body weight and creatinine clearance were determined as significant covariates. Between subject variability of V1, V2, Q and CL were 32%, 50%, 41% and 28%, respectively. Discussion and Conclusion: Simulation based upon the final population PK model suggested that 400 mg/day was recommended to assure therapeutic concentrations in the neutropenic patients.

116

Estrogen Prevents Caffeine-induced Diuresis in Rats

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Studies have linked caffeine consumption to a reduced risk of developing Parkinson's disease in men, but not in women. In the kidney, caffeine is known to induce diuresis in rat and man. The present study investigates whether the renal effect of caffeine in rats is influenced by gender. Male and female Wistar rats were volume-expanded, by gavage, with 0.9% NaCl (4% of body weight) in the absence (control) and in the presence of caffeine (10 - 100 mg/kg). To evaluate the role of estrogen in caffeine-induced diuresis, rats were ovariectomized and treated with exogenous estrogen (50 µg/kg/day, subcutaneously, for 4 days) before caffeine. Caffeine induced significant increase in diuresis in male rats. Urine volume was elevated by caffeine (100 mg/kg, n=5) from 4.37 ± 0.60 ml (control, n=9) to 8.02 ± 0.75 ml ($p<0.05$). Caffeine also increased glomerular filtration rate in male. On the other hand, caffeine (100 mg/kg, n=5) had no effect on both diuresis (4. 88 ± 0.77 ml *versus* 4.23 ± 0.91 ml, control, $p>0.05$) and glomerular filtration rate in female rats. Nevertheless, caffeine (100 mg/kg, n=6) increased urine production in ovariectomized rats (7.60 ± 0.80 ml *versus* 3.80 ± 0.90 ml, n=5, control, $p<0.05$). This caffeine effect was prevented by estrogen replacement in ovariectomized rats (6.92 ± 0.97 ml, *versus* 5.88 ± 0.74 ml, control, n=5, $p>0.05$). Altogether, our data indicate that caffeine increased diuresis in male, but not in female rats. This lack of caffeine effect in female may be due to the estrogen hormone.

117

Population Pharmacokinetic Analyses of TRITON-TIMI 38 (TRITON) to Evaluate the Influence of Intrinsic and Extrinsic Factors on Prasugrel Active Metabolite Exposures

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Introduction: TRITON-TIMI 38 compared prasugrel to clopidogrel in a randomized, double-blind, multicenter study of 13,608 subjects with acute coronary syndromes planned for percutaneous coronary intervention. The prasugrel dosing regimen was a 60-mg loading dose followed by daily 10-mg maintenance doses. Pharmacokinetic sampling in approximately 10% of the study population was undertaken. Methods: A multi-linear regression model was used to quantitatively predict prasugrel's active metabolite (AM) concentrations from measurement of its 2 downstream inactive metabolites. Population-based methods were then applied to pharmacokinetic data from 1159 subjects to characterize AM pharmacokinetics. The potential influence of body weight (WT), body mass index (BMI), age, sex, renal function, diabetes, tobacco use, and other disease status on Bayesian estimates of prasugrel-AM exposures were assessed. Results: Baseline characteristics were representative of the overall study population. Prasugrel-AM mean exposure in subjects weighing <60 kg was 30% (90% confidence interval [CI]: 1.16-1.45) higher compared to subjects ≥60 kg or 42% (90% CI: 1.27-1.58) higher compared to those ≥85 kg (approximate median WT in TRITON). Mean Prasugrel-AM exposures for subjects ≥75 years of age were 19% (90%CI: 1.11-1.28) higher compared to subjects <75 years or 25% (90%CI: 1.16-1.34) higher than those <60 years (approximate median age in TRITON). There was no significant influence of BMI, sex, diabetes, tobacco use, renal impairment, or other disease status. Conclusions: The pharmacokinetics of prasugrel-AM was adequately described by a multi-compartmental model and consistent with results from previous studies. The exposure of prasugrel-AM was higher in subjects <60 kg or ≥75 years of age.

MONDAY JULY 28, 2008
STREAM 3:
MEDICINES AND SOCIETY

118

Caffeine Fatalities – Intentional Intoxications are a Problem Despite Sales Restrictions

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Caffeine is widely available in beverages and in different OTC-products, including tablets containing 100 mg. In 2004 the amount of caffeine tablets that could be bought over the counter were restricted to 30 in Sweden. The objective was to study the effect of this decision on fatal caffeine intoxications.*In Sweden all cases undergoing forensic autopsy are screened for a number of drugs including caffeine. All cases from 2004 and onwards with a caffeine concentration over 80 microgram/g blood were recorded.*We present 10 cases, most of them clear suicides where caffeine has contributed to the fatal outcome, seven of them after the restriction of the amount sold was introduced.The conclusion is that caffeine is not as harmless as one might believe. An overdose of caffeine alone, intentional or not, might be deadly. Despite restrictions of the number of tablets sold over the counter fatal intoxications are at least as common as before in Sweden. Restrictions of the amount sold over the counter seem not to be effective in preventing suicide with caffeine.

119

Beyond the Writing: An Evaluation of Doctors' Prescriptions in Benin City, Nigeria

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Introduction: Prescription writing is influenced by several factors, and inadequate training in clinical pharmacology results in gaps in knowledge and prescribing skills. Methods: Prescriptions from General Outpatient encounters in all (8) public and 9 randomly selected private hospitals in Benin City, Nigeria were surveyed; each doctor provided information on age, gender, academic background, drug information sources, access to the National Essential Drugs List (EDL), and what they considered the strongest influence(s) on their prescribing. The WHO prescribing indicators were calculated. Data were analysed using SPSS version 15.0. Results: Eight hundred and thirty three prescriptions from 28 doctors (response rate 70%) were assessed: age and working experience 38.8 ± 8.1 and 13.4 ± 8.4 years respectively. Pharmaceutical details were the most popular sources of drug information and promotional activities by pharmaceutical companies influenced prescribing more than undergraduate training. Influences during internship contributed most to current prescribing practices. Doctors in private hospitals prescribed more medicines (average number of drugs per encounter 3.96; $p = 0.009$) and injections (% encounters with injections 38.51; $p = 0.11$). Those >40 years prescribed more antibiotics (% encounters with antibiotics 52.63; $p = 0.029$). Discussion: Undergraduate training in clinical pharmacology is not a strong influence on doctors' prescribing, and doctors appear to lack skills for rational prescribing and appropriate use of drug information sources. Conclusion: Polypharmacy and overuse of antibiotics and injections are problems. Interventions to improve prescribing in Nigeria should include strengthening undergraduate training, structured training during internship and measures to regulate promotional activities by industry.

120

Use of Antipsychotic Agents in the Elderly Population of Manitoba between 1996 and 2006

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Introduction: Antipsychotic medications have been used in the elderly to control psychotic symptoms in a variety of diagnoses. Concerns have been raised in recent years about increased prescribing of the second-generation agents (SGA; risperidone, olanzapine, quetiapine) and their safety. The utilization of antipsychotics in the elderly population of Manitoba over a decade was evaluated in this study. Methods: Administrative health data was collected from the Manitoba Population Health Research Data

Repository covering all residents of Manitoba aged 65 and over who had at least one prescription for an antipsychotic during the period from April 1, 1995 to March 31, 2006. Demographics, diagnoses, numbers of prescriptions and hospitalizations were determined for patients living in the community or in personal care homes (PCH). Results: Prevalence of antipsychotic users increased from 3.4% in 1996 to 5.3% in 2006. The male to female ratio was consistently at 0.5. Total number of antipsychotic prescriptions dispensed to the elderly increased from 23,673 in 1996 to 96,911 in 2006 (with more than 87,000 for SGA). The elderly population living in PCH showed the highest prevalence of antipsychotic use (21% in 1996 to 46% in 2004-2006). The most prevalent diagnosis was dementia and the most prescribed agent was risperidone. Hospitalizations/patient showed a steady decrease in the community-dwelling population of antipsychotic users between 4.5 in 2001to 2.3 in 2006. Conclusion: SGA are extensively used in the elderly population of Manitoba, particularly in those living in PCH. Further analysis is warranted to determine the benefits of such use.

121

Clopidogrel and Aspirin: How Long is Long Enough in Medical Management of Non-St Elevation Acute Coronary Syndromes?

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Introduction: The optimal duration of therapy with clopidogrel plus aspirin in non-ST elevation acute coronary syndromes (NSTEACS) is uncertain. Current guidelines are based on the CURE trial which showed a statistically significant benefit with dual therapy compared to aspirin alone when administered for up to 12 months (median 9 months). However, cumulative hazard plots of the CURE data indicate that most of the benefit from dual antiplatelet therapy may have occurred in the first 3 months. Methods: From Figure 1 in the primary CURE publication we measured the cumulative hazard for the primary outcome (cardiovascular related death, nonfatal myocardial infarction, or stroke) on each treatment arm at t=3 and 12 months. Results: For the time period 0 to 3 months the cumulative incidence was: aspirin alone 7.9%, aspirin + clopidogrel 6.0%; absolute risk reduction 1.9%; relative risk reduction 24% (95% CI: 13% to 33%; P<0.001). From 3 to 12 months the cumulative incidence was: aspirin alone 4.9%, ASA + clopidogrel 4.5%; absolute risk reduction 0.4%; relative risk reduction 8% (95% CI: -11% to 25%; P=0.38). Discussion: Because of expense and increased risk of bleeding it would seem to be prudent to use combination antiplatelet therapy for as short a time as clinically necessary. Conclusion: Based on measurements taken from cumulative hazard plots, our analysis indicates that most of the benefit from dual antiplatelet therapy in NSTEACS occurs within the first 3 months. A prospective randomized trial is needed to confirm these findings.

122

Medication Reviews with Computerized Expert Support - An Evaluation of a Method to Improve the Quality of Drug Utilization in the Elderly

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Introduction: Medication reviews with pharmacological expertise is a way to secure the quality of drug treatment. The medication review may contribute to the detection of drug-related problems (DRP) and to increase awareness of the problems. The aim was to examine if a computerized system for medication reviews could support doctor's decisions and improve the quality in drug treatment. Methods: A descriptive intervention study. The study included 275 patients living in community settings and shelter homes, using five or more drugs. Patient data were analyzed using a software programme and scrutinized by a clinical pharmacologist. Pharmaco-therapeutic advice was sent to the

doctor as a guide for the medication review. Main outcome measure was initiation and discontinuation of drugs, changes of doses and rates of identified DRP´s. Result: Expert opinions were given for 275 patients, mean age 85; 70% female. An average of 3.3 remarks per opinion was given concerning unsuitable drugs, dosing when decreased kidneys function, drug-drug interactions and improvement according to quality indicators. On average 1.5 DRP was attended to. The most common action taken was withdrawal of a drug (n=208). The drug use decreased from 10.4 to 9.5 drugs per patient, and several quality indicators were met. Conclusion: Medication reviews with computerized, expert support by a clinical pharmacologist at a distance resulted in detection of several DRP´s and to decisions on changes in the drug use. In most cases the patient's clinical state was unchanged. The average drug use decreased and the overall quality and safety was improved.

123

Role-playing Study Program of the Informed Consent

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Clinical research coordinators (CRCs) are required to have high communication ability in presenting the Informed Consent(IC) process to Patients. We think that Informed Consent training is an advanced version of the program to develop communication skills necessary for health care workers. So we tried to develop Role- Playing Study Program of the Informed Consent (IC). At first we prepared five disease scenarios, background of the patients and evaluation sheets of each role (CRCs, Patient, Observer). We showed these scenarios for Pharmacy students who had practical training at the clinical trial coordinating office, and they divided three roles (CRCs, Patient, Observer). After role-playing exercises of the Informed Consent (IC) process, they checked the evaluation sheets and gave feedback to CRCs. Those role-playing exercises were greatly appreciated by trainees for providing the opportunity to learn how important an attitude toward patients and practical communications were, and has proved to be useful to pharmacy students. We will develop higher quality programs and prepare audio-visual aids, and also apply them to communication education for new medical personnel and other professionals.

124

Treatment Choice of Chronic Pelvic Pain Syndrome

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Introduction: The cause of chronic pelvic pain syndrome is frequently unknown. Biologic agent succeeds to isolate only in 5 – 10 % of cases (Weidner i sar., 1991). The disease is caused by Escherichia coli, Staphylococcus aureus or Haemophilus influenzae in the most cases. Treatment consists of antibiotic uses (Naber, 1993), non-steroidal anti-inflammatory remedies (Canale et al., 1993) or blockaders of alpha-adrenergic receptors (Barbalis et al., 1998). Chronic pelvic pain syndrome is named "nonbacterial" prostatitis. The aim of this study was to estimate efficiency of antibiotic uses, non-steroidal anti-inflammatory remedies or blockaders of alpha-adrenergic receptors in treatment of inflammatory chronic pelvic painful syndrome. Methods: Prospective, open controlled clinic study has been carried out. One hundred and fifty mail patients had been included who had discovered inflammatory chronic pelvic painful syndrome. All patients had been divided in 3 groups according to the type of treatment: Group one: patient treated with fluoroquinolone antibiotics (Ciprofloxacin) (n=50) Group two: patient treated with nonsteroid anti-inflammatory medicines (Indometacin®) (n=50); Group three: patient treated with blockaders of alpha-adrenergic receptors (Tonocardin®) (n=50); Treatment lasted for 6 weeks. Results: All the patients filled in NIH chronic prostatitis symptom index questionnaires (Litwin et al., 1999) before the treatment, and treatment success is defined as sum reducing NIH prostatitis questionnaires for at least 4 points. In first group positive effect was in

24 patients (48 %). NIH chronic prostatitis symptom index was 16,4 ± 6,2 before the treatment and 12,4 ± 5,3 after the treatment. There was significant difference ($p < 0,05$). In second group results were 16,6 ± 6,2 before the treatment and 16,2 ± 7,5 after the treatment ($p > 0,05$). And in third group results were 17,8 ± 7,3 before the treatment and 12,2 ± 6,7 ± 7,5 after the treatment ($p < 0,001$). Discussion: In the last five years reports of chronic prostatitis efficiency studding are based on prostatitis index. Many studies showed successful use of treatment with blockaders of alpha-adrenergic receptors in treatment patients with chronic prostatitis pain syndrome (Rosette and all., 1992; Evliyaoglu and all., 2003; Cheah and all., 2003; Cheah and all., 2004). Conclusion: Use of blockaders of alpha-adrenergic receptors remedies are "first choice drugs" in treatment of chronic pelvic pain syndrome. Use of blockaders of alpha-adrenergic receptors remedies has an advantage over antibiotics and nonsteroid anti-inflammatory medicines use because it reduces unjustified using of antibiotics and according to that, it reduces appearance of antibiotic resistance.

125

Assessment of Problem Based Pharmacology Teaching for Medical Housestaff during a Respirology Rotation

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Medications play a major role in the management of respiratory disease. The challenges of providing an experiential educational program to cover the required content involving diagnosis and management decisions while maintaining a busy and ever more complex patient load, is substantial. Traditional medical school therapeutics provides basic pharmacologic information but physicians may have difficulty in application of this knowledge especially to those patients with substantial comorbidities. Furthermore while medication safety has become a focus in contemporary healthcare there is little formalized teaching at many academic centres regarding patient and medication safety. We developed a problem based case featuring several illustrative therapeutic and safety issues which was delivered by a pharmacist in a small group session to medical housestaff at our facility. Assessment of the program was performed using a pre–post multiple choice questionnaire specifically focused on dealing with the patient in the presented case. The test, completed prior to and immediately following the case discussion, was comprised of 6 questions which dealt with the indications for drug therapy, dosing of medications, precautions regarding drug therapy and risk-benefit assessment of the chosen therapies. Sixteen medical housestaff attended, participated and completed both multiple choice tests. The mean score prior to the problem based discussion was 2.13+/- 0.78 which improved to 3.88+/-1.72 ($p= 3.94 \times 10^{-11}$). Scores on a multiple choice test of therapeutics and safety were improved in medical housestaff using this format. Conclusion: Patients with type 2 diabetes that are being treated with insulin appear to follow more of the recommended management protocols but are less likely to feel their diabetes is adequately controlled when compared to non-insulin users.

126

The Assessing Cardiovascular Targets (ACT '07) Program: Results from a Practice Reflective Assessment Across Canada

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Objective: To assess whether treatment targets in Canadian clinical guidelines (hypertension – 2007, dyslipidemia – 2006, diabetes – 2003, metabolic syndrome – 2006) are met, and characterize patients with cardiovascular risk in community based clinical practice. Methods: A convenience sample of 400 general practitioners across Canada participated between September and December 2007. Patient case reports were completed during normally scheduled office visits. Current results were compared to a similar survey of 450 general practitioners and 17,188 patients conducted in January to April 2006 using 2003 dyslipidemia, 2003 diabetes, & 2005 hypertension guideline targets. Results: 14,923 patients analyzed, 80% were taking lipid-lowering drugs. Demographics: 58% male, 35% 65 years or

older, 57% 45-64 years. CV risk factors identified: 62% hypertension, 40% diabetes, 23% family history premature CAD, 21% previous history of MI, stroke, or PAD, 21% evidence of atherosclerosis, 21% current or recent smoker. 46% of cohort had three or more risk factors. Physician assessed CV risk level: 57% high, 25% moderate, 18% low. Patients NOT at guideline targets 2007 survey vs. 2006 survey: hypertension 22% vs. 26%, LDL-C 40% vs. 34%, TC:HDL-C 28% vs. 31%, triglycerides 40% vs. 51%, FBG > 6.2 mmol. 34% vs. 44%, waist circumference 52% vs. 55%. Conclusions: Aggregate data shows an overall improvement in some parameters vs. previous results however, despite drug treatment many patients are still not at lipid or blood pressure target levels. Community practice physicians in this survey prescribe lipid-lowering drugs to predominantly high (57%) and moderate (25%) CV risk patients.

127

Economic Evaluation of Long-acting Insulin Analogues for the Treatment of Patients with Type 1 and Type 2 Diabetes Mellitus in Canada

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Introduction: In 2005, $181 million was spent on insulin products, up 17.5% from 2004. Conversion from non-analogue to analogue insulin has increased expenditures. Policy makers require clinical and economic evidence for reimbursement decisions. Methods: Cost-utility analysis from the perspective of a third-party provincial payer was conducted using the CORE Diabetes Model. Clinical outcomes were derived from recent meta-analyses. Costs and utilities, both discounted at 5%, were obtained from published sources. Sensitivity analyses were performed to test robustness of results.Results: Type 1 Diabetes mellitus (T1DM) – the incremental cost-utility ratio (ICUR) for insulin glargine relative to neutral protamine hagedorn (NPH) was $87,932 per QALY gained (incremental cost, $3,423; incremental QALYs, 0.039). The ICUR for insulin detemir compared to NPH, was $387,729 per QALY gained (incremental cost, $4,344; incremental QALYs, 0.011). Type 2 Diabetes mellitus (T2DM) – the ICUR for insulin glargine relative to NPH, was $642,994 per QALY gained (incremental cost, $4,945; incremental QALYs, 0.008). Insulin detemir was more costly ($6,521 per patient) and less effective (-0.034 QALYs) than NPH. Results were sensitive to variations of parameters in sensitivity analyses. Conclusions: Compared with NPH, the use of long-acting insulin analogues for the treatment of diabetes mellitus is associated with relatively high incremental cost-utility ratios.

128

Adaptation of the GRADE Method for Developing Recommendations on the use of Insulin Analogues for the Management of Diabetes Mellitus

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Introduction: This poster presentation demonstrates how the Canadian Agency for Drugs and Technologies in Health through its Canadian Optimal Medication Prescribing and Utilization Service has adapted the GRADE approach for developing recommendations on the use of insulin analogues in the management of diabetes mellitus (DM). Methods:Outcomes relevant to prescribing decisions for treatment of DM were identified and ranked by an expert committee. Unique GRADE Evidence Profiles were developed for each treatment comparison and specific patient population. Each Profile presented meta-analytic estimates of effect size for identified outcomes and, where data permitted, the results of pharmacoeconomic analyses. Quality and internal and external validity of the evidence was assessed. Recommendations were developed, and the strength of each was determined. Results: Thirty-two outcomes were identified as relevant to making prescribing decisions. Estimates of effect size for each outcome, by treatment comparison, were derived from 94 meta-analyses. Thirty-three Profiles were produced from which twenty-seven recommendations were generated. Discussion: Benefits of applying GRADE include the creation of a list of outcomes that are important or critical to consider when making recommendations and the development of a format to present the entire body of evidence in a systematic and detailed manner. Challenges include the necessity of

subjective scientific judgment, incorporation of evidence from single studies, and integration of resource utilization data. Conclusion: GRADE provides a systematic process to identify, analyze and present a large body of evidence and a transparent methodological approach for the development of evidence-based optimal therapy recommendations.

129

Quality Indicators of Academic Clinical Trials in Denmark 1993-2005

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Introduction: The number of academic clinical trials in Denmark has declined steadily from 1993 to 2005 without interference of the European Clinical Trials Directive implemented in 2004. To understand the scientific impact of this decline, the question remains: Has the quality of academic drug trials changed during this period? Method: 386 approved applications for academic clinical drug trials submitted to the Danish Medicines Agency 1993-2005 were reviewed for 13 methodological characteristics, e.g. randomization, blinding, use of control group, GCP monitoring, statement of primary endpoint, method for generation of allocation sequence and publication. Results: Rates of randomization, statement of a primary endpoint, GCP monitoring, medicine compliance and publication increased significantly during the period. Remaining characteristics did not change significantly. Discussion: The quality of clinical trials is not well defined. This study represents a fairly new approach using trial protocols instead of publications, which eliminates the lag time from conduct to publication. According to the rates of GCP monitoring and medicine compliance, implementation of GCP in academia in Denmark seems succesfull. Adherence to GCP does not per se ensure the quality of a trial. However, it provides quality in the sense of controlling and securing the validity of the collected data. Conclusion: The results indicate an increase in the quality of academic drug research. However, it is surprising that methodologically important characteristics such as blinding, use of a control group and method used for generation of allocation sequence did not improve significantly during the period.

130

Docetaxel Plus Cyclophosphamide is Cost-effective versus Doxorubicin Plus Cyclophosphamide in the Adjuvant Treatment of Operable Breast Cancer: A Canadian Economic Analysis of US Oncology Adjuvant Trial 9735

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Introduction: The objective of the study was to determine the lifetime cost-effectiveness of docetaxel plus cyclophosphamide (TC) compared to doxorubicin plus cyclophosphamide (AC) in the adjuvant treatment of operable invasive breast cancer. The analysis utilized 7-year follow-up data from the US Oncology Adjuvant Trial 9735 (Trial 9735) and was conducted from the Canadian (Ontario) government payer perspective.Methods: Overall survival (OS) and disease-free survival (DFS) from Trial 9735 were used to estimate 7-year survival and disease recurrence. Survival was extrapolated to lifetime using estimates of Canadian general population life expectancy. Canadian resource utilization and unit costs were applied to estimate the costs of chemotherapy (including drug and administration costs), chemotherapy-related toxicities and disease recurrence. Quality of life weights used in the calculation of quality-adjusted life years (QALYs) were derived from the literature.Results: Under base case assumptions, cost per life year gained (TC vs. AC) was $6,842 and cost per QALY gained was $8,251, discounting costs and outcomes at 5% per annum. In a sensitivity analysis conducted with a 7-year time horizon,

cost per life year gained was $36,120 and cost per QALY gained was $43,248. The base case results were robust with respect to all other one-way sensitivity analyses. Conclusion: TC provides gains in terms of life years and QALYs compared to AC and results in very favourable lifetime and 7-year cost-effectiveness ratios. TC is a clinically and economically attractive alternative to AC for patients receiving adjuvant chemotherapy for operable breast cancer in Canada.

131

Role of Adverse Drug Events in Admission to Intensive Care Unit

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Background: adverse drug events (ADE) represent more than 6% of hospitalisations and can be life threatening. Data about ADE leading to admission to intensive care unit (ICU) are missing. Aim: to assess the role of ADE in admission to ICU and to determine their preventability. Method: prospective observational pilot study. Admissions to the ICU of the Geneva University Hospitals for a month were analysed. Demographic and medical data as well as drug history were systematically collected. A clinical pharmacologist decided on drug imputability according to WHO criteria: She also determined preventability. Results: 34 of the174 admissions (20%) were due to an ADE. Imputability was classified as certain in 3 cases (9%), probable in 7 cases (20%) and possible in 18 cases (70%). 3 of the 7 probable ADE (40%) were judged preventable. Most of the ADE were cardiovascular or haemorrhagic events. The most often imputed drugs were beta blockers, platelet antiaggregants and glucocorticoids. Factors associated with admission to ICU because of an ADE were polymedication (>3 concomitant drugs), renal failure (creatinine clearance <50ml/min) and being a woman. Conclusion: This pilot study suggests that ADE, many of which are preventable, play a significant role in ICU admission. Considering the human and the financial burden of an admission to ICU, a larger study is warranted to confirm these results.

132

Preventing Deep Vein Thrombosis (DVT): How Does Canada Fare?

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Recent research in the United Kingdom and the United States has shown that medical patients are not receiving appropriate thromboprophylaxis in acute care hospitals, with two-thirds of discharged patients not receiving prophylaxis in accordance with guidelines (Amin et. al, 2007). In the U.K., a recent parliamentary report found that more than 10,000 patients have died from deep vein thrombosis (DVT) because hospitals are not following guidelines. Venous Thromboembolism (VTE) is in fact the third most common cardiovascular disease, after heart attack and stroke, and can be prevented through the appropriate use of medications if guidelines are followed. Do Canadian physicians treat DVT appropriately? Do Canadian patients comply with appropriate treatment regimens? The purpose of this study is to both determine how Canada compares to the US and the UK in adherence to treatment guidelines for the prevention of DVT, as well as discover how many Canadian deaths could have been averted if proper treatment had been provided. The US study used hospital discharge information from the Premier Perspective inpatient database; however this type of data is not available in Canada. Therefore the authors will conduct an extrapolation of the US study data regarding the burden of illness of DVT.

133

Initial Treatment Effectiveness of Acute Sinusitis in a Representative National Cohort

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In 2001, the French Health Ministry developed a national plan to optimize antibiotic prescription.

In this context the effectiveness of the initial treatment for acute sinusitis in current medical practice in France was assessed. A prospective observational cohort study of patients with acute sinusitis was carried out by a representative sample of general practitioners (GPs) and ear-nose-throat specialists (ENTs). Patients diagnosed with acute sinusitis were included in two 4-week periods (March and September 2005). Effectiveness of the initial treatment was evaluated by the absence of failure 10 days after inclusion, and the lack of a relapse/recurrence between the 11th and 60th day. Cox proportional hazard model was used to identify prognostic factors of failure. 1174 GPs and 120 ENTs included 5693 patients. Sinusitis was maxillary (47.7%), ethmoido-frontal (28.5%), pansinusitis (22.8%) and sphenoid (0.7%). Antibiotics, analgesics, corticosteroids, NSAIDs and others symptomatic medicines were prescribed to 92.4%, 50.1%, 48.8%, 18.0% and 44.7% of the patients respectively. Failure at 10 days varied from 4.2% to 8.2% for antibiotics and was 13.6% without antibiotic. Relapse/recurrence at 2 months varied from 9.6% to 14.4% for antibiotics and was 19.2% without antibiotic. Compared to patients without antibiotics, the relative risk of failure varied from 0.28 to 0.58 for the various antibiotics. For patients without failure, the risk of relapse/recurrence did not differ between patients prescribed antibiotics or not. Initial antibiotic prescription for the treatment of acute sinusitis reduced the risk of failure at 10 days but had no effect on the risk of relapse/recurrence at 2 months.

134

Teaching Clinical Pharmacology to Medical Students in Africa

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Introduction: Textbooks and course material for teaching clinical pharmacology to medical students is almost exclusively directed at developed countries. South Africa is an emerging middle income country. The public health care services are inadequately funded and insufficiently staffed. The burden of disease includes a rapidly increasing prevalence of lifestyle diseases due to ignificant and change in diet, but infectious diseases, notably HIV and tuberculosis, still predominate. Public sector health policy includes the adoption of the essential drugs programme (EDP), as recommended by the WHO. Methods: Our medical school has recently undergone major undergraduate curriculum reform with implementation of problem-based learning methods. This involves the facilitation of small groups of students, including bedside tutorials. Didactic lectures have been minimised. The core curriculum is linked to the burden of disease in the country and therapeutics is limited to drugs on the national EDP and standard treatment guidelines. The objective is to enable students to develop the skills required to use essential medicines rationally in the management of common conditions. Clinical pharmacology teaching is integrated in all the 6 years of training, but most teaching occurs in the later years. Results: Student assessments of clinical pharmacology have been very positive. The national council that audits medical training has identified our teaching programme as a model for the country. Conclusions: Teaching clinical pharmacology in developing countries must be appropriate to the burden of disease and the limited drug formulary. Integrating teaching into a problem-based curriculum has necessitated teaching in all the undergraduate years.

135

Role of Pharmacometrics in Modern Clinical Pharmacology: An Analysis of Two Journals

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The purpose of this study was to evaluate the weight of pharmacometric studies in modern Clinical Pharmacology through an analysis of the research articles published in 2005 and 2006 in Clinical Pharmacology and Therapeutics (CPT) and the British Journal of Clinical Pharmacology (BJCP). European authors in all research studies were the largest group in both journals (55% of 509 papers), although European dominance was greater in BJCP. CPT and BJCP devoted similar space to pharmacometrics: 25 and 20% of the

articles, respectively, but focus on pharmacometrics was greater among European authors. The second place was occupied by authors from Asian countries in CPT and by authors from Australia and New Zealand in BJCP. American authors were third in both cases. Healthy volunteers were the most common type of subjects included in pharmacometric studies in both journals, but the ratio of healthy volunteers/patients was lower in the BJCP. Only about 20% of the articles from both journals describing pharmacometrics included dose/response relationships. There was a marked difference in the number of articles reporting time course of effects: They were rare in CPT (14%) but appeared in 40% of the BJCP articles. "Cardiovascular" and "CNS, PNS & Pain" articles were dominant in both journals and the richest in technology. Although pharmacometric studies are by no means absent from two of the most important journals of Clinical Pharmacology, it is evident that its role seems to have reached a secondary status. It is difficult to avoid the feeling that the discipline may suffer from this dilution of its core activity.

136

Long Term Outcomes of Children with Prenatal Exposure to Valproic Acid and Carbamazepine: Meta-analysis

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Background: Studies investigating long–term adverse effects of antiepileptics on cognitive functioning are limited or conflicting. Objective: To estimate intellectual disability of children with prenatal exposure to valproic acid and carbamazepine, by measuring IQ scores in a systematic meta-analytic review. Methods: A literature search using Pubmed and Medline was performed to identify all original articles that investigated cognitive functioning following *in utero* exposure to antiepileptic drugs. Eleven studies met the inclusion criteria. Eight studies; 3 valproic acid and 5 carbamazepine evaluated IQ testing as a measure of cognitive development and were included in the final analysis. IQ was measured by the Wechsler, Bayley or McCarthy intelligences scales depending on age. Results: **A** total 67 children were exposed to valproic acid and 151 children to carbamazepine. The mean Full Scale IQ (FSIQ), Verbal IQ (VIQ) and Performance IQ (PIQ) was significantly lower in the valproic acid group compared to the unexposed group ($p<0.001$, $p<0.001$, $p<0.007$).Full Scale IQ (FSIQ) and Verbal (VIQ) of children exposed to carbamazepine was not statistically different when compared with the unexposed group ($p<0.095$, $p<0.097$). In a sub analysis of carbamazepine exposure in three studies using Wechsler intelligence scale to assess IQ, PIQ was significantly different between the two groups ($p<0.002$). Conclusions: Exposure to valproic acid in pregnancy is associated with significantly reduced intelligence in children whose mothers were treated for epilepsy. Exposure to carbamazepine in pregnancy does not appear to be associated with reduced Full and Verbal intelligence in children.

137

The Impact of Psychiatrist Volume on Drug Cost for Schizophrenia Patients: A Population-based Study

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Since drug cost represents a major proportion of healthcare expenditure in the case of schizophrenia, the need to pursue improvements in the cost-effectiveness of treatment has become critical. The volume of healthcare services provided by physicians has been investigated under different surgical procedures to determine their relationship with treatment costs. However, the economics of scale issues have not been examined in drug cost for schizophrenia treatments. Therefore, association between psychiatrist volume and drug cost for hospitalized schizophrenia patients is explored in this study. We use the National Health Insurance Research

Database for 2005, identifying the study subjects from the database by ICD-9-CM principal diagnosis code 295.There are 134,752 admissions in our sample which are divided equally into three psychiatrist volume groups. A multiple regression was performed to determine the impact of psychiatrist volume on drug cost after controlling for psychiatrist, patient and hospital characteristics. The results showed that average drug cost associated with hospitalized schizophrenia patients were inversely related to psychiatrist volume. The drug cost of patient treated by high-volume psychiatrist and medium-volume psychiatrist were significantly lower than those of low-volume psychiatrist. The potential cost savings from drugs could be as much as 20% for low-volume psychiatrist. Keywords: Drug cost, Psychiatrist Volume, Schizophrenia

138

Epidemiologic Characteristics of Drug Consumption and Self-medication in Adolescents from the Timis County Romania

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Among the unique health problems that adolescents are facing is the abuse of substances. The geographic position of the Timis County that is at the border with the Western world, is another factor that encourages the abuse of substances while the receptiveness of the young population is a reality. A survey has been carried out on a significant sample of adolescents in the schools all around the Timis County. The sample consisted of 2908 pupils with ages between 15-19, 1498 girls and 1410 boys in the period 2005-2007. The method of research was epidemiological descriptive retrospective investigation based on the questionnaire CORT 2004 for the investigation of risk behaviour and personality profile for adolescents. A percentage of 5,3 of the teenagers are drug consumers: 3,4% experimental consumers, 1,1% occasional consumers, 0,2 drug addicts. The drugs that are most commonly used by consumers rank as follows: marijuana – 89,2%, Ecstasy – 10,5%, LSD – 8,6%, cocaine – 7,9%, heroin – 6,5%. The addiction to drugs can be characterised as follows: 16% of the adolescent drug users have consumed drugs in the last 30 days, whereby 16,3% have consumed marijuana, 2,4% cocaine, 1,7% Ecstasy, 1,2% heroin. A percentage of 0,3 of the adolescents resort to medicines without having any medical recommendation at least once a week. This phenomenon is more obvious in girls 12,9% than in boys 9,7 %. The results of this survey are useful in the process of rethinking prevention methods by developing strategies of information and formation towards a healthy lifestyle.

139

The Impact of Benefit Plan Design on Cost and Health Outcomes

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Introduction: When private payers implement benefit plan changes to control costs, the longer term consequences may not be considered. The objective was to identify scientific studies that examined the impact of changes in private drug plan formulary design on the health of private plan beneficiaries. Methods: A search of the medical literature was conducted using the PubMed search engine. Search terms included combinations of reimbursement, formulary, plan, payer, restriction, cost, and adherence. The 'related articles' that were featured in PubMed were also used to identify relevant papers. Results: While no published studies of Canadian employer-sponsored drug plans were identified, there were fifteen North American studies that focused on the effects of changes in drug plan design. This body of research demonstrated three key points. Aggressive cost-control measures reduced the use of prescribed medications, which lead to increased events related to uncontrolled disease and the use of other more expensive health services. Higher levels of adherence to drug therapy, which increased drug costs, were associated with lower overall health costs. Employee satisfaction was decreased when significant cost-sharing changes were introduced, when access was more restrictive, or when claims were denied. Conclusions: A short-term focus on

controlling drug costs is likely to have negative consequences on the health, productivity and satisfaction of plan members. Employers need a longer term framework to guide and support health plan decision-making that avoids sudden or drastic changes to health benefits. Careful consideration of drug plan design and cost-sharing can improve medication adherence, health outcomes, employee satisfaction, and costs.

140

The Willingness to Pay to Minimize Chronic Pain

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The objective of the study was to identify chronic pain patients' preferences for levels of improvement in pain related morbidity (PRM) by measuring their willingness to pay (WTP) for completely minimizing their pain intensity and pain related morbidity (PRD). The study was a cross sectional non-randomized design. Patients were recruited from a multidisciplinary pain centre in Edmonton, Alberta, Canada. A computer administered discrete choice experiment was used to explore patients' WTP for various levels of improvement to pain related morbidity. Patients chose between two varying combination of treatments which differed in terms of their level of improvement to pain intensity, level of improvement to PRD and out of pocket monthly cost. Results indicate that persons with chronic pain are willing to allocate between 19% and 52% of their gross family income to minimize the morbidity caused by chronic pain. For every dollar an individual was WTP to improve their disability to the lowest severity (mild), he/she was WTP approximately $2 to reduce pain intensity to moderate and $3 to reduce pain intensity to mild. Treatment and management strategies that focus on reducing pain intensity would have the greatest impact on improving health related quality of life. Furthermore, there is major potential for significant returns on investment from investing in pain management centers.

141

Challenges to Evaluate the Practice of Traditional Medicines

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Background: Traditional medicines are used by an increasing number of subjects in Canada. Traditional Chinese medicine (TCM) is regulated in several Canadian provinces. In a CIHR funded trial aimed at assessing the effects of TCM to improve the QoL of lung cancer patients, the TCM doctors could use 58 herbs that could be combined according to TCM diagnosis. We report in this abstract the experience of interacting with Ethics committees, Natural Health Product Directorate (NHPD) and clinicians in this context. Results: Ethics committees had difficulties with the use of raw herbs (instead of standard finished products) whose combination (hence effects) may change from one patient to the other. The potential drug-herb interaction was also a concern. NHPD's guidelines have been developed to assess quality and safety of finished products. Raw herbs that are used in various combinations, changing in time do not fit with this model. Issue of toxicity and absence of documented information was another issue that led to withdrawal of several herbs. Clinicians were not convinced of the TCM efficacy and some of them mentioned the "absence of equipoise" for refusing to participate. Recruitment of patients to participate in TCM trial (to improve quality of life) versus other drug trials (to improve disease free survival) was considered unacceptable by some oncologists. Conclusion: Overall, the experience suggests that our system is not yet well adapted to conduct this type of evaluation; hence, unfortunately, the need to wait longer for valid and relevant data regarding TCM efficacy and safety.

142

Empirical Bayesian Data Mining for Discovering Safety Signals in Military Electronic Health Records

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Adverse drug events (ADEs) constitute a major public health problem and post-marketing drug surveillance has become increasingly important. Current practices to date have relied on measures of disproportionality techniques used on spontaneous reporting databases to identify ADEs. When considering the frequency and severity of adverse events, it is clear that new databases, such as claims data and electronic health records need to be evaluated for adverse event discovery and validation. Our objective was to apply a Baysiean data mining algorithm to the Department of Defense's (DoD) claims database that was enriched with electronic medical records to detect drug-specific adverse event effects. Examples of drug-adverse drug events were chosen from recent published Food and Drug Administration's (FDA) safety alerts for drugs. Generated signal scores from the DoD database were compared with scores from the FDA's Adverse Event Recporting System database using the Baysian Multi-Item Gamma Poisson Shrinker (MGPS) algorithm. Case Series were generated from the DoD database and they were evaluated to validate the generated signals. The chosen examples show that the MGPS data-mining approach can identify drug-specific effects. The case series generated from MGPS provided supporting medical data that was used to validate the safety signals. The MGPS data mining algorithm can be used to identify drug-specific effects and case series can be generated to improve signal validation.

143

Reversible Cardiogenic Shock and Amiodarone in Perfusion

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Introduction: Amiodarone (Cordarone*), having a narrow therapeutic margin requires a rigorous manipulation; especially when it is administered in continuous perfusion. We report 5 cases of supervention of reversible cardiogenic shock after perfusion of the product. Objective: Our work studies the relation between continuous perfusion of Amiodarone and reversible cardiogenic shock.
Clinic history: 5 patients aged over 60 years, treated in the cardiology department for Ventricular Fibrillation and Complete Arythmy through Auricular Fibrillation. Cordarone* was administrated at 300mg for 30 minutes. Each one of these patients showed a pathology associated outside the cardiovascular sphere (bronchopneumopathy, pulmonary pathology, intoxication to asbestos, blood pressure, obesity).
Results: After the perfusion, a cardiogenic shock appeared 30 minutes later, made of acute lung edema (ALO) and severe hypotension. The echoheart revealed a very severe deterioration of true left ventricular contractile function (LV) lowered 60%. Immediately, it was decided to take reanimation measures. The evolution was favourable for the 3 patients and death for the two others. Discussion: The cardiogenic shock induced by intravenous Amiodarone is reported in samonographiy. However, the reversibility of the deterioration of LV wasn't described. This makes of this clinic history a genuine one. The intrinsic imputability using the French method shows a likely cause-effect relation. Conclusion: The supervention of cardiogenic shock after Amiodarone perfusion should be considered with the clinical doctor so as to react within the shortest delay possible to improve the charge of this serious adverse effect. *N.B.: The details of this clinic history will be reported in the final communication of the congress.*

144

Economic Analysis of Febrile Neutropenia Primary Prophylaxis Strategy with Pegfilgrastim in Women with Breast Cancer – A Canadian Perspective

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Febrile neutropenia (FN) is a major toxicity associated with chemotherapy that often limits the provision of adequate chemotherapy doses on an optimal schedule, resulting in poor clinical outcomes and increased costs. The objective is to evaluate the cost-effectiveness of primary prophylaxis with pegfilgrastim with 6-day and 11-day filgrastim prophylaxis among Stage-II breast cancer patients in Canada from a health service payer perspective. The clinical data came from a comprehensive literature review and were supplemented with modified Delphi Panel expert opinion. Canadian costs were applied within a life-time Markov model where the study population included women 30-80 years of age with breast cancer receiving 4-8 cycles of chemotherapy with approximately 20% FN risk. Key aspects of the model included FN risk, FN-related case fatality, and chemotherapy dose intensity and its relationship to long-term survival. Primary prophylaxis with pegfilgrastim increased quality-adjusted life expectancy by 18 days while lowering costs ($1,725) when compared with primary prophylaxis with 11-day filgrastim. A comparison of pegfilgrastim with 6-day filgrastim prophylaxis demonstrated that pegfilgrastim costs approximately $2,953 more while increasing life expectancy by 35 days. This resulted in an ICER of $31,415/QALY gained. In summary, pegfilgrastim primary prophylaxis is a cost-effective option compared with 6-day filgrastim and is a dominant strategy (i.e., less costly and more effective) when evaluated against primary prophylaxis with 11-day filgrastim. Furthermore, pegfilgrastim primary prophylaxis has a very attractive cost-effectiveness profile when compared against other supportive care agents in oncology. These results were robust to various deterministic as well as probabilistic sensitivity analyses.

145

Provision of Drug Information Service in North India

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In order to promote the judicious use of medicines in North India, a Drug Information Centre (DIC) under the joint aegis of WHO India Office and Karnataka State Pharmacy Council was established at Sirsa (Haryana) in January 2007.The first centre of its kind in North India, which is fully equipped with trained manpower, infrastructure and information sources and registered with IRDIS, an International Register of Drug Information Services. The sole objective of DIC is to provide most authentic and latest information about the drugs to healthcare professionals, patients and consumers free of cost. Since its inception, till date out of a total of 253 queries attended to, 58% were patient-related to medication counseling followed by 21% from community and hospital pharmacists and 12% from various physicians for better patient care and update their knowledge. Seventy-two percent of the queries were replied verbally, 23% through both verbal and written and 11% written mode only. Almost all (72%) the queries were attended to within few minutes and remaining was answered within a day. Queries from healthcare professionals were mostly pertaining to drug profile, indication and administration /dosage. Most of the queries and answers remained text books based. Only 26% of them were complex where other sources of information including secondary (Micromedex), primary sources and Internet etc. were used. Most of the queries were made by direct access or through telephone. The feedback analysis reveals that the DIC rendered a yeoman service to the society as a whole.

146

Attitude of Physicians towards Adverse Drug Reaction Reporting in North India

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To strengthen the Pharmacovigilance Programme, a study was conducted to assess the attitude and behavior of physicians in Northern states of India, regarding reporting of adverse drug reactions (ADR). A self-administered questionnaire comprising 27 questions was delivered to a stratified random sample of 880 registered physicians exploring the physician's attitude towards reporting and the factors affecting either positively or negatively their attitude. Of the total response of 60%, the study revealed that 97% of them considered ADR reporting as an integral part of their professional duties and acknowledged the importance of reporting, whereas only 11% were aware about ADR reporting program. However none of them admitted that they have ever submitted any ADR report to any pharmacovigilance centre. Although 6% of them claimed that they submitted ADR report to the concerned pharmaceutical company through their representatives. The study further revealed various bottlenecks that prevented physicians from reporting ADR including unknown address (88%) of ADR reporting centers, reporting forms not available (82%), ignorance about reporting process (72%) and uncertainty concerning causal relationship between ADR and the drug (78%). A large number of them (84%) asserted that a feedback from the program would encourage them to report and 83% expressed that publication of ADR bulletin will go a long way in exhorting them to report such cases. In all, 92% suggested that training and educating the physicians would certainly give a fillip to the programme. The encouraging results of the study however, made it clear that assiduous efforts were needed to overcome the barriers identified to make the program a success.

147

Medications for Restless Legs Syndrome in Pregnancy

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Introduction: Evidence from epidemiologic studies document that pregnant women have at least two or three times a higher risk of experiencing restless legs syndrome (RLS) than the general population. However, there is little information regarding treatment of RLS during pregnancy. Methods: A literature search using PubMed and MEDLINE from 1960 to December 2007 was performed to identify publications regarding medications for RLS and their safety in pregnancy. Key search words included: pregnancy; restless legs syndrome; dopaminergics; opioids; antiepileptics; benzodiazepines; congenital malformations. Results: Four classes of medications have been used for patients with RLS, however pregnancy represents a therapeutic concern. Although dopamine agonists, ropinirole and pramipexole, have been approved by the FDA for the treatment of RLS and currently are the first-line treatment for daily symptoms, there is very little information on the teratogenic risks of these new medications. Therefore, they are not currently recommended during pregnancy. Medications with a more extensive safety record in pregnancy include: opioids, antiepileptics such as carbamazepine and gabapentin, and some benzodiazepines. Ruling out iron deficiency should be an essential part of a treatment plan for RLS in pregnancy. Conclusion: Opioids are currently the drugs with the largest safety record available to treat RLS in pregnancy. Carbamazepine, gabapentin and clonazepam may be useful alternatives, given their apparent fetal safety and experience with use in pregnancy.

148

Efficacy and Safety of Antidepressants for Treatment of Depression in Coronary Artery Disease: A Meta-analysis

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Introduction: Depression occurs in 18%-45% of patients with coronary artery disease (CAD) and is associated with an increased risk of acute coronary events and mortality. Our objective was to quantitatively summarize the data on the efficacy and safety of antidepressant treatment for depression in CAD. Methods: We performed a

metaanalysis of randomized, double-blind, placebo-controlled trials with a database search of the English literature (up to 2008) and a manual search of references. Results: 5 studies with antidepressants (ADs) (mirtazapine, citalopram, fluoxetine, sertraline; 9 to 24 weeks duration) involving 1165 subjects (586 ADs, and 579 placebo) with documented coronary artery disease and meeting standardized criteria for depression (DSM-IV) were included. ADs were superior to placebo for decreasing Hamilton Depression Rating Scale (HAM-D 21) scores (weighted mean difference [95% confidence interval]: 1.18 [0.49, 1.87], p=0.0008) and Beck Depression Inventory (BDI) scores (n=373 ADs/ n= 369 placebo) (weighted mean difference: 2.27 [0.60, 3.94], p=0.008). Proportion of patients (n=216 ADs/ n=213 placebo) who responded (≥50% reduction in HAM-D) (odds ratio [OR]: 1.72[1.17, 2.54], p=0.0005) and remission rate (HAM-D≤8) (OR: 1.80 [1.18, 2.74], p=0.0005) were also significantly higher with AD versus placebo with no significant differences between the 2 groups for overall dropouts (OR: 0.84 [0.42, 1.68], p=0.0005) or dropout due to adverse events (OR: 1.30 [0.75, 2.25], p=0.0005). Studies combined did not show significant heterogeneity (all p>.05). Conclusion: Treatment with antidepressants for depression in coronary artery disease results in significant therapeutic effects without substantially increased rates of discontinuation.

149

Impact of Adherence to Antihypertensive Agents on Health Care Costs for Patients in Primary Prevention

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Aims: Cardiovascular diseases (CVD) are a heavy economic burden on individuals, society and health services. Antihypertensive agents (AH) have been shown to reduce the risk of major cardiovascular events. Few data has been provided on the impact of adherence to AH on health care costs. Objective: To evaluate the impact of low adherence to AH on health care use and their related direct medical costs. Methods: A cohort of 96715 patients with essential hypertension was reconstructed from the Régie de l'assurance maladie du Québec and Med-Echo databases. Subjects included were between 45 and 85 years old, initially free of CVD, and newly treated with AH agents, between 1999 and 2004. Pharmacy record were used to assess the adherence to AH drugs measured in terms of the proportion of days' supply of medication dispensed over a 3-year period, and categorized as ≥80% or <80%. Mean all medical costs were estimated. A two-part model was used to predict hospital costs among patients who were hospitalized, and among those with and without hospitalization. Results: The mean age was 65 years, 38% were male, 8.5% had diabetes, and 19% had dyslipidemia. Low adherence to AH increased the risk of being hospitalized by 11% (OR: 1.11: 1.06-1.16) compared to highest one. Mean all medical costs were at $6,826. Low adherence yielded an excess of predicted costs of $3,300 per patient among those who were hospitalized; and an excess of $1,200 among those with and without hospitalization. Conclusion: Low adherence to antihypertensive therapy is correlated with health care costs mainly related to hospitalization.

150

An Evaluation of Spontaneous and Therapeutic Abortions Following use of Antidepressants in Pregnancy

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Background: it has been suggested that of use of antidepressants in pregnancy increases the risk for both spontaneous (SA) and therapeutic abortions (TA). Our objective was to ascertain how many women in this cohort reported a SA or TA and compare the results with a non-exposed comparison group. Methods: At The Motherisk Program, we analyzed pregnancy outcomes of 1243 women in our data base exposed to antidepressants during their pregnancy, as well as a comparison group of 1243 women who were not exposed (non-teratogen group). To evaluate the rates of SA, we matched for time of call, to rule out women who may have had a SA early on and subsequently did not contact us. Results: 937 women who fit the criteria, were matched to 937

in the comparison group.131 (14%) suffered a SA in the exposed group and 83(8.8%) in the non-teratogen group, OR 1.6 (95% CI 1.2-2.2). There were 26 (2.4%.) women who reported a TA in the exposed group compared to 8(0.7%)in the comparison group. OR 3.3 (95% CI 1.4-7.3) Conclusions: The use of antidepressants in early pregnancy appears to be associated with a small but significant increase rate of both SA and TA. Without a comparison group of depressed, non-medicated women, it is impossible to say if the higher rate of SA's is due to the antidepressant or the underlying disease. The higher rate of TA's may be fear of teratogenic effects, due to controversy surrounding antidepressant use in pregnancy.

151

Systematic Review of Different *in vitro* Tests in the Diagnosis of Anticonvulsant Hypersensitivity Syndrome (AHS): *In vitro* Lymphocyte Tests

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Anticonvulsant hypersensitivity syndrome (AHS) is a rare but potentially fatal host-dependent disorder occurs in susceptible patients upon exposure to certain drugs. Misdiagnosis of this disease is quite common due to its close resemblance to other clinical conditions and hard to define clinical picture. The *in vitro* tests which utilize isolated peripheral blood lymphocytes, i.e., lymphocyte transformation test (LTT) and lymphocyte toxicity assay (LTA), have offered a promising advantages of being relatively reproducible and bear no potential harm to patients. However, the sensitivity and specificity of both tests in the diagnosis of AHS are yet to be determined and their negative and positive predictive values are still unknown. We systematically reviewed the available English literature between 1964 and 2007 for publications on the use of LTT and LTA as diagnostic tests for AHS. The review discussed the numerous factors that appear to affect the final outcome of the tests. These factors include timing of the tests from reaction onset, type of the drug tested, clinical picture of the reaction, and simultaneous administration of other drugs. It is quite clear that the results of the tests are determined by these factors which explain the variability of the results in the analyzed data. In many studies, in which the positivity of LTT ranged between 20% to 90%, the results seem also to be affected by the use of unstandardized methodology and ill-defined inclusion criteria of the cases. Optimizing the test conditions and performance as well as further understanding of the disease pathophysiology will contribute positively to our ability to predict and diagnose this illness.

152

Delayed Antiarrhythmic Effect of Nitroglycerin in Anesthetized Rats: Involving of CGRP, PKC and mK$_{ATP}$ Channels

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Introduction: Delayed anti-infarct and anti-stunning effects of nitroglycerin (NTG) have well been established in some animal models. The main goals of this study in anesthetized rats were to determine whether NTG has a delayed antiarrhythmic effect and if so, whether calcitonin-gene related peptide (CGRP), protein kinase C (PKC) and mitochondrial K_{ATP} channels (mK_{ATP}) involve in triggering of this response. Methods: On day 0, male Wistar rats received NTG (120 μg/kg, iv) with or without pre-administration of PKC inhibitor chelerythrine

(CHE), capsaicin (CAP) to deplete CGRP from sensory nerves or mK_{ATP} channel blocker 5–Hydroxydecaonic acid (5HD). On day 1, their hearts were subjected to 30 min ischemia and 120 min reperfusion. Results: In rats pretreated with NTG, the incidence of ventricular tachycardia and ventricular fibrillation and the mortality rate significantly reduced (from 100%, 61% and 18.1 % in control group to 45.4%, 10% and 0 % in NTG group, respectively). Infarct size also reduced from 58 ± 4.7% in control group to 31 ± 3.7% in NTG group. These effects were abolished by CHE, CAP and 5HD, which none of them alone had any effect on infarct size or the incidence of myocardial arrhythmias. Conclusion: These results show that a low dose of NTG has a delayed anti-arrhythmic effect and this effect may share a common mechanism with anti-infarct effects of this drug, involving CGRP release and PKC and mK_{ATP} activation.

153

Study on the Knowledge, Attitude and Practice of Adverse Drug Reaction (ADR) Reporting Among Physicians in a Teaching Hospital in Northern Nigeria

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Aim: Adverse drug reactions (ADRs) contribute to excessive health care costs through increased patient morbidity and mortality. Thus, there is an urgent need to create awareness among physicians towards ADR monitoring. This study was carried out to investigate the knowledge and attitudes of doctors to adverse drug reactions (ADR) in Aminu Kano Teaching Hospital, a tertiary health facility in Kano, northern Nigeria and to identify the reasons for under-reporting. Methods: A structured questionnaire was distributed to junior medical doctors (house-officers and residents) and the data was analyzed using SPSS version 12. Results: A total of 42 medical doctors filled and returned the questionnaires. Twenty-five (59.5%) and 17(40.5%) of the respondents were house-officers and residents respectively. An overwhelming majority (92.9%) indicated that ADR is an important medical problem while only 40.5% were aware of procedures guiding reporting of ADRs. Eighty-six (86%) have observed ADR at least one occasion but only 28.6% of them reported these cases. Eighty-eight percent (88%) of the respondents think that only serious ADRs (eg. Steven-Johnsons syndrome) need to be reported. Ignorance of the rules of reporting (66.7%), lack of knowledge of the forms for reporting (66.7%) and ignorance of the type of reaction to be reported (42.9%) were the main factors responsible for not reporting suspected ADRs. Only fourteen (33.3%) of the medical doctors were aware of the presence of a pharmacovigilance unit in the hospital. Conclusion: Our study shows that many physicians are lacking in knowledge of ADR reporting. Continuous medical education in the area of pharmacovigilance will contribute positively in reversing this pattern.

154

Public Health Hazards Related to the Antibacterial Agents Usage in Iran

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Introduction: Antibacterial agents are used frequently for treating animal diseases. When given to animals during the lactation period, they are excreted partially in the milk and produce problems of public health and dairy processing significance. Also, if use of antibacterial agents is necessary, as in treatment of poultry diseases, a withholding period must be observed until the residues are negligible or can no longer be detected. The presence of antibacterial agent residues in animal and poultry products is considered to be a potential problem, because of the possibility of: a) inducing bacterial drug resistance or enhancing pathogenicity in human intestinal flora, 2) potential production of allergic hypersensitivity reactions or other toxic effects in human from ingestion of these drugs via meat, milk or poultry products. Allergic reactions are dose-dependent and a sensitized individual can react to an infinitesimally small amount of the allergen. Materials and Methods: The study was to survey on antibacterial agents' usage in dairy cattle and poultry farms in Iran. The broiler farms (240 farms) and dairy cattle farms (220 farms) were randomly selected in different regions of provinces. Results and Discussion: According to

the performed studies, the percentage of consumption rate of antibacterial agents was high in some regions of provinces. The reason for this increasing rate might be due to more populated dairy cattle and poultry farms and unhygienic condition and the improper climate condition in these regions of provinces.

155

Benzodiazepines Prescription in a Developing Countries in the Private Sector: Survey with General Practitioners

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Introduction: Benzodiazepines are well tolerated drugs but they can induce abuse and addiction when people use its for long time with high doses. In developed countries, there are clear rules on the prescription and the use of these drugs. These rules were edited after a lot of studies on these drugs. However, in developing countries, few studies are done on these drugs. That is why we undertook this study with the aim to evaluate benzodiazepines prescription with general practitioners, in the private sector, in Dakar, the Senegalese's capital, in order to make recommendations for rational use of these medicines. Methods: We use a questionnaire to do the survey with a representative sample of 55 general practitioners. The questions focused on the prescribed benzodiazepine in first intention against a given pathology, the knowledge on the pharmacological proprieties and the rules of prescription of benzodiazepines. Results: Results showed that general practitioners don't use the correct product against insomnia because the use in first intention prazepam which has a long half life. In addition, they don't know very well the pharmacological proprieties and the rules of prescription of these drugs. Conclusion: In conclusion, our study showed training problems in benzodiazepines for general practitioners of the private sector in Dakar and the necessity to do continuous training for these doctors in this domain.

156

Abstract of the Impact of Biologic Drugs on the Psoriasis Market: Quantifying Emerging Trends

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Introduction: According to the International Federation of Psoriasis Associations, an estimated 125 million people worldwide are afflicted with Psoriasis. Of these, 600,000 are Canadians. In recent years the introduction of biologic drugs has revolutionized this market both in terms of treatment options and in terms of the cost associated with those treatment options. The objective of this study is to accurately quantify the cost impact of biologic drugs on the Canadian Psoriasis market.Methods: The patient cohort was comprised of Canadians who, through their treatment history, were identified as having received Psoriasis-specific medications between 2002 and 2007. For each of these patients, claims level data was extracted from the Brogan Inc. Drug Plan Databases and a cost analysis conducted. Biologic drugs considered for this study included Amevive, Raptiva, Humira, Enbrel and Remicade. Results: Preliminary results show total prescription drug expenditure for Psoriasis treatment has doubled since 2002. This is primarily due to an average annual increase of 26% in the private sector and can be associated directly with the introduction and expense of new biologic drugs which have remained unlisted by the public plans for the treatment of Psoriasis. Discussion and Conclusions: The cost burden of new Psoriasis treatments in Canada changed significantly following the introduction of biologic drugs as treatment options. Though this study provides a Canadian context, these products are expected to have a significant impact on the global Psoriasis market. Given trends identified here, cost growth in this market is expected to continue into the future.

157

Cost-effectiveness of Statins in Moderate Risk Patients in Canada

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Introduction: Canadian guidelines' LDL-C target is 3.5 mmol/L in patients at moderate cardiovascular risk. We modeled long-term cost-effectiveness for titrating simvastatin, atorvastatin, and rosuvastatin to meet this Canadian LDL-C target.Method: Patients' baseline characteristics were based on a Canadian observational lipid study's moderate risk patients (CALIPSO, Can J Cardiol 2005). Efficacy data from the STELLAR trial was used to model post-treatment lipid levels with those not achieving the LDL-C goal titrated over one year at quarterly intervals. The resulting modified TC/HDL-C ratios were entered into a probabilistic Markov model in which Framingham risk equations predict fatal and nonfatal events over 20 years. Costs were drawn from published Canadian cost-effectiveness analyses and utilities from the USA Medical Expenditure Panel Survey. Results: An estimated 88% of rosuvastatin patients achieved goal at the initial 10-mg dose compared with 76% and 60% for atorvastatin 10mg and simvastatin 10mg, respectively. Simvastatin provided 9.21 life years per patient, with atorvastatin and rosuvastatin providing an additional 0.21 and 0.31 life years, respectively. Rosuvastatin generated 7.40 QALYs (Quality Adjusted Life Years), an additional 0.14 and 0.08 compared with simvastatin and atorvastatin. Rosuvastatin dominated (i.e., was more effective and cost efficient than) atorvastatin, and had cost per QALY of Can$2,237 compared with simvastatin. Discussion: Rosuvastatin is clearly cost-effective against any reasonable cost-effectivess threshold compared with generic simvastatin. Rosuvastatin dominated atorvastatin in cost-effectiveness. Conclusion: Rosuvastatin appears the optimal statin in terms of clinical and cost effectiveness with which to titrate moderate risk patients to Canadian LDL-C target.

158

Physician-pharmacist Collaboration in Cardiovascular Disease Prevention: A Qualitative Study Nested within a Cluster Randomized Controlled Trial (RCT) in Primary Care

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Background: Studies suggest that dyslipidemia treatment is suboptimal in primary care. TEAM study, a cluster RCT, evaluates the effectiveness of a pharmacist-physician collaborative care (CC) to dyslipidemia patients. The physician is responsible for the diagnosis and prescription of pharmacotherapy, while pharmacist initiates treatment, monitors effectiveness, safety and adherence to treatment and adjusts medication dosage accordingly. Objective: Explore the perception of physicians, pharmacists and patients of the CC. Methods: At the end of study, focus groups with patients (2 groups, 12 patients) and pharmacists (2 groups, 12 pharmacists) and individual interviews with physicians (7) were conducted. Results: All reported that CC was more structured and systematic. Patients felt they received better follow-up and admitted being reassured, well informed about their condition and the need for treatment, which make them more incline to take better care of themselves. These positive impacts were largely attributed to the accessibility of pharmacists as well as their ability to communicate easily. Patients reported almost no reserve regarding the CC. Considering the shortage of physicians and the increased prevalence of chronic diseases, physicians perceive interprofessional collaboration as almost inevitable. However, they are afraid that it may alter their privileged relationship with patients and may constitute a threat against their overall medical follow-up of patients Pharmacists consider that CC is time-consuming and may not be applicable to all patients.Discussion: Patients highly appreciated the CC and professionals perceived it as beneficial for patients. Conclusion: The real-life applicability of CC in primary care remains to be defined.

159

The Cost-effectiveness of Pregabalin in Patients with Central Neuropathic Pain

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Introduction: Patients with chronic central neuropathic pain (CNeP) typically report considerable pain that requires frequent healthcare resources (HR) utilisation. The purpose of this study was to estimate the cost-effectiveness of pregabalin for the management of CNeP in a Canadian practice setting from a Ministry of Health perspective. Methods: A stochastic simulation model was used to determine the effect of adding pregabalin to current treatment on daily pain and associated costs in a hypothetical cohort of 1000 patients with chronic CNeP. The model was based on data from a randomized, double blind, placebo-controlled, parallel-group, multicentre clinical trial. Modeled outcomes of interest included quality-adjusted life-years (QALYs) and mean number of days with no/mild pain over the trial duration of 12 weeks. HR utilisation (including drug costs) was assessed from a survey conducted with a group of 149 Canadian physicians. Corresponding costs were obtained from Ontario Drug Benefit, London Health Sciences Centre, and the *Régie de l'Assurance Maladie du Québec*, and are expressed in 2007 Canadian dollars. Sensitivity analyses were conducted on model's assumptions. Results: Compared with no additional treatment, treatment with pregabalin yielded a cost-utility ratio of $9,648/QALY, and a cost-effectiveness ratio of $10/day with no/mild pain. Sensitivity analyses suggested that the resulting ratios were very robust. The most prominent variation reported was for the extension of the time horizon up to 52 weeks ($23,087/QALY). Discussion & Conclusions: Model simulations demonstrate that adding pregabalin to the current pharmacotherapy received by CNeP patients, compared to no additional treatment, is a cost-effective treatment strategy.

160

Pregnancy and Abortion Rates Among Women Obtaining Emergency Contraceptives from Pharmacists in British Columbia

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Introduction: Hormonal emergency contraceptives (Ecs) can reduce the risk of pregnancy after unprotected intercourse. Pooled estimates of the Yuzpe and levonorgestrel EC regimens suggest that the pregnancy rate following EC in clinical trials is ~2%. In British Columbia, women have been able to access Ecs prescribed by pharmacists since 2000. Using health databases, a cohort of pharmacist-provided EC users was studied for rates of pregnancy and abortion after EC. Methods: Women (~13,000) requesting Ecs from pharmacists in 2001-2002 following unprotected intercourse (index event) provided written consent for treatment that included reproductive health information. Anonymized consents were matched to the provincial prescription database and linked to medical billing data. Pregnancies possibly resulting from the index event were included in the analysis. Results: A total of 9432 consent forms were matched. The mean age of EC users was 25.6 +/- 7.7 years, and the estimated risk of pregnancy was 4.1%. The mean time to receive EC after unprotected intercourse was 25.4 +/- 19.0 hours. The Yuzpe and levonorgestrel EC regimens were provided 39.2% and 60.8% of the time, respectively. The mean cycle day of unprotected intercourse was 16.3 +/- 7.5. Preliminary analysis indicated that 240 (2.5%) and 121 (1.3%) EC prescriptions were associated with time-compatible total pregnancy- and abortion-related events, respectively. Discussion: The initial estimated pregnancy rate among women seeking Ecs from pharmacists suggests that Ecs are ~40% effective in the routine practice setting. Multivariable modeling will be performed. Conclusion: Approximately 50% of pregnancies following EC use end in abortion.

161

Incidence and Impact of Adverse Drug Reactions in Canadian Forces Members

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Introduction: Reporting of adverse drug reactions (ADRs) forms an integral part of post-marketing surveillance. The Canadian Forces (CF) drug benefit program has two programs designed to monitor and detect ADRs. To complement these programs, additional information was sought to gain insight into the occurrence rate and impact of ADRs. Methods: Two questions regarding ADRs were included in a general health survey, one regarding ADRs experienced in the preceeding year and one regarding whether these ADRs caused reduced function. This survey was administered to all full-time, actively serving CF members from April to November 2004. Medications implicated in ADRs were categorized according to the WHO Anatomical Therapeutic Chemical Classification System. Results: From 2913 responses, a total of 337 ADRs were reported among 269 members. The most commonly implicated drug categories were agents affecting the nervous system (31.5%), respiratory (22.5%) and musculoskeletal system (13.4%). Most reported ADRs were associated with prescription drugs (71%). Thirteen ADRs caused the member to be less productive, and 12 caused them to miss work completely.Discussion: Prescription drugs and medications affecting the nervous system, respiratory tract and musculoskeletal system were major sources of ADRs. Almost 1 in 10 respondents who experienced an adverse drug reaction noted a negative impact on their work performance. Conclusions: Although less common than in hospitalized patients, ADRs remain significant, even among ambulatory, generally healthy members of the Canadian Forces. ADRs may thus be contributing significantly to reduced work productivity and absenteeism in the general adult population.

162

Implementation in Catalonia of a Register of Healthy Volunteers who Participate In Phase I Clinical Trials

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In 2007 the Department of Health of Catalonia created a centralized register of healthy volunteers who participated in phase I clinical trials. This pioneering initiative was one of the first of its kind in Spain and one of the first worldwide. Internet-based central register *Registre de Voluntaris Clínics* (RVC), which is run by the Generalitat de Catalunya, is a supportive tool to prevent trial subjects from taking part in too many trials, and it is currently used by all Catalan Phase I units. All of these units are physically located within a hospital in Barcelona metropolitan area: Hospital Universitari de Bellvitge (L'Hospitalet de Llobregat); Hospital de la Santa Creu I Sant Pau (Barcelona); Institut Municipal d'Investigació Mèdica-Hospital del Mar (Barcelona); Hospital Germans Trias I Pujol (Badalona), Coorporació Sanitària Clínic (Barcelona) and Laboratoris Dr. Echevarne-Hospital Dos de Maig (Barcelona). The RVC goal is to guarantee the protection of health, rights and welfare of the subjects who take part in this type of clinical trials, as well as to assure the scientific and technical quality of the activities of clinical research by preventing concomitant exposure (overexposure) to IMP and giving an assurance of the wash-out period between trials. If volunteers do not follow this wash-out period, some adverse effects are observed (carry-over effects, excessive blood drawn in short term, etc.) Subjects are informed that participation within a trial involves as well the inclusion of some sensitive personal data (such as name, gender, date of birth, passport number, and date of last IMP) in the RVC. By signing an RVC consent form, subjects agree such data to be collected and processed, but only for purposes related with the RVC. This form is signed per subject, clinical trail and phase I unit. Only authorized users who belong to phase I units in Catalonia have access to this register and it allows to identify volunteers, so as to know if they have taken part in a clinical trial, in which unit,

when they have been administered the last dose of a IMP, and the end of the wash-out or rest period. The new register is integrated into the Applications Gateway of the Ministry of Health, which guarantees the suitable safe environment, as well as the application of all the measures of protection of the personal data established in current regulations in Spain.During year 2007, 147 healthy volunteers have been registered, yet it is expected a significant increase during 2008. The starting of this register has allowed to detect and prohibit 3 of these volunteers to participate in a clinical trial due to the fact they had not passed the corresponding wash-out period.

163

Evaluation of Adverse Effects in Medical Reports of Hypertensive Outpatients Treated in Ambulatório Araújo Lima (HUGV) Justifying the Importance of Pharmacogenomics

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Introduction: Frequently, the hypertensive patient has some difficulty in decreasing the pressure levels or presents adverse effects. This response's difference varies among people, and can be explained by variations in the coded proteins in the genome, which justifies the Pharmacogenomics. Methods: the purpose was to present a panorama of the interindividual variability in hypertensive outpatients, providing evidence of the importance of Pharmacogenomics. The work consisted of a Longitudinal Observational Descriptive Study of data collected from medical reports of outpatients from Ambulatório Araújo Lima subscribed in the Hypertension Program from January 2002 to December 2004. It was filled in a questionnaire with data like prescribed medication, changes in dose and drugs, decrease of pressure levels, and presence of reported adverse events. Results: from 54 patients included in the study, 74% had at least one change of dose during the treatment and 70% had at least one new drug introduced. 28% (15 patients) presented adverse effects, the majority (12 of the 15 patients) related to an excess of the anti-hypertensive action (somnolence, dizziness, hypotension). Discussion: some genetic variants probably propitiate an excess of the reduction of blood pressure, like the SNPs 1817 G/A and 278 G/T of the alfa-2 adrenergic receptor and the SNPs Met 235 Thr and 6 G/A of the angiotensinogen. Conclusion: data evidence the difficulty of adjusting the treatment of Systemic Hypertension to each patient and the presence of adverse effects in a great proportion of the medical reports included. An approach of Pharmacogenomics could minimize this difficulty.

164

Incidence of Hospitalizations for Serious Hepatic Events Associated with NSAIDs use

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Background: Serious hepatic events are a rare consequence of treatment with non-steroidal anti-inflammatory drugs (NSAIDs). As there is a lack of clinical data, we evaluated the incidence of serious hepatic events associated with NSAIDs exposure, compared to that of known hepatotoxic and non-hepatotoxic agents in a defined population. Methods: A random sample of patients who received NSAIDs or acetaminophen was taken from RAMQ. Patients had to be free of pre-existing liver conditions and covered by RAMQ during the study period 1999–2000. An event was defined as a hospitalization for a serious hepatic event. Four exposure groups were defined: 1) NSAIDs, 2) acetaminophen, 3) lorazepam, 4) non-exposed (no NSAIDs, acetaminophen, and lorazepam). A rate of event per 100,000 person-years was calculated for the 4 groups of exposure. Rate ratios (RR) were calculated to compare the NSAIDs, acetaminophen, and lorazepam groups to the non-exposed group. Results: A total of 515,637 patients were included and 629 events were identified. The incidence of hospitalization for serious hepatic event was 78.1/100,000 patient-years' exposure for NSAIDs compared with 101.7/100,000 patient-years' exposure for acetaminophen, 75.1/100,000 patient-years for

lorazepam, and 53.8/100,000 patient-years for the non-exposed group. Compared to non-exposed group, a RR of 1.45, 1.89, and 1.40 was seen for NSAIDs, acetaminophen and lorazepam, respectively. Conclusion: The risk of serious hepatic events with NSAIDs was similar to lorazepam, a drug not known to be hepatotoxic. In this patient population, exposure to prescribed acetaminophen was associated with the highest frequency of serious hepatic events.

165

The Early Clinical and Economic Benefits of Atorvastatin in a Canadian Setting

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Introduction: Recent analyses from RCT indicate that, compared to simvastatin, atorvastatin treatment results in a reduction of cardiovascular (CV) events during the first year of treatment. This study was conducted to estimate the early clinical and economic consequences of initiating atorvastatin *versus* simvastatin from a Canadian societal perspective. Methods: A cost-consequence model was developed to estimate CV events and costs over the first two years of treatment associated with initiating atorvastatin or simvastatin in a hypothetical cohort of 100,000 new patients with risk factors. RCT data were used to estimate the CV event rate for each statin. CV events included myocardial infarction, stroke, and revascularization procedures. Corresponding direct costs were obtained from the Ontario Drug Benefit and Case Costing Initiative. Indirect costs were obtained from Statistics Canada. All costs were expressed in 2007 Canadian dollars. Univariate/Multivariate sensitivity analyses (Monte-Carlo simulation) were conducted on model assumptions. Results: Within two years of treatment initiation, the use of atorvastatin is predicted to prevent 1648 CV events (95%CI: 1343-1956) per 100,000 new patients with risk factors compared with simvastatin. Similarly, the cost of CV events was reduced by $50.8 million (95%CI: $41.9-$59.8). The incremental cost associated with atorvastatin treatment was $31.3 million. This resulted in a net saving of $19.5 million (95%CI: $10.7-$28.7). Results were sensitive to assumptions regarding simvastatin efficacy and levels of persistence. Discussion & Conclusions: Based on this model, atorvastatin use is predicted to result in cost savings to the Canadian society over simvastatin use within two years of therapy initiation.

166

TNM for Toxicology: The DOODP/LM (Death, Organ Failure, Organ Damage, Discomfort, Pregnancy/Lactation, Therapeutic Monitoring) System

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Comparison of Toxicity of treatments is often difficult. The safety of a drug is linked to effectiveness and indication i.e the disease for which it is used. For rare diseases effectiveness information is often lacking. For these diseases the clinician chooses treatment based on perceived effectiveness and the drug's known safety. When studies are not available, comparison is often done by clinicians based on lists of events without frequencies. We propose a system to help clinicians to compare the toxicity of different drugs in a simple and standardized way. The system is organized similarly to the TNM systems to stage tumors. It is organized into 6 categories of drug related adverse events. They are: 1. death, 2. organ failure, 3. organ damage, 4. discomfort, 5. pregnancy/lactation and 6. frequency of monitoring. Each category is populated by a number. The number reflects the frequency of the most common adverse event per 1000 patient treatment years. Tables with data on frequency and other events are linked to each category. Where this data is missing or of low quality, this is noted. The frequency of death is often unknown, thus death is coded as reported or not reported. Lactation and pregnancy are coded according to FDA terminology. Suggested

monitoring is coded in terms of frequency and duration. Due to its simplicity this system is necessarily imperfect but it allows a standardised comparison of drugs. It would also be more compelling for pharmaceutical companies to supply necessary data, since it makes areas of limited knowledge transparent.

167

Experiences with Contraception use among Youth in Northern British Columbia

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Introduction: Despite public health efforts, pregnancy rates among teens in northern British Columbia are 60% higher than the provincial average. Determining barriers to the use of contraception among teens at risk of unwanted pregnancy has important health policy implications. This study evaluated the impact of gender, place and culture on the experiences of northern youth in accessing and using contraception effectively. Methods: Ethnographic fieldwork was employed in the northern community of Fort St. James (population 2,000). During 8 weeks of participant observation, in-depth interviews were conducted with 20 female and 20 male English-speaking youth (ages 15 – 19) with varied socio-cultural and economic backgrounds and diverse contraceptive experiences, using a purposive sampling strategy. Interviews were also conducted with 10 health care and social service youth sexual health providers. Results: Findings suggest that lack of timely and appropriate information on contraception, myths regarding side effects of contraception, lack of convenient clinic appointment times, scarcity of physicians for prescribing contraception, lack of interpersonal skills related to negotiating contraception use, high cost of contraception and geographic barriers to health service access in the northern community were negatively impacting contraception use among northern youth. Discussion and Conclusion: Despite the known safety and effectiveness of contraception, public health strategies that have focused on individual knowledge, attitudes and decision-making have had limited success. Utilization of our strategic partnership of multidisciplinary researchers, health decision-makers, Aboriginal representatives, community stakeholders and clinicians will facilitate the timely launching of targeted interventions designed to improve youth contraception services in northern settings.

168

Cost-effectiveness of Post-prostatectomy Treatment Selection by Risk Prediction Tools

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Introduction: We examined the economics of managing patients with prostate cancer after prostatectomy. Tools that predict risk of biochemical recurrence can guide the decision of whether to undergo adjuvant treatment and may improve upon current clinical practice. These tools are the Kattan postoperative nomogram, which uses clinicopathological features, and the Prostate Px® test, which employs additional morphometric and immunofluorescence features of the prostate specimen. Methods: Cost-effectiveness of the Prostate Px test, the nomogram and current practice was compared using a Markov model. The modeled treatment for low risk patients was watchful waiting. The modeled treatments for high risk patients were local radiation, hormonal therapy and watchful waiting. Costs, utilities, and transition probabilities were obtained from literature. Cost and effectiveness were estimated by Monte Carlo simulation. The impact of uncertainty in the parameter values was assessed by sensitivity analysis. Results: The expected quality-adjusted life years for Prostate Px test, nomogram, and current practice were 8.11, 7.39, and 6.47. The expected costs were $17,549, $14,162, and $14,104. Discusssion: The superior performance of both Px test and nomogram over current practice resulted from identifying high risk patients likely to benefit from adjuvant treatment, while sparing the remaining patients the added cost and toxicity of treatment. The Prostate Px test was cost-effective compared to both the nomogram and current practice. The nomogram was cost-effective compared to current practice.

Conclusions: Incorporation of risk prediction tools in the initial management of patients after prostatectomy resulted in increased quality-adjusted life years at an acceptable increase in cost.

MONDAY JULY 28, 2008
STREAM 4:
CLINICAL PHARMACOLOGY IN SPECIAL POPULATIONS

169

Frequency and Risk Factors of Drug Induced Liver Injury of Anti-tuberculous Drugs in Egyptian Patients

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Background: Around 8.9 millions new tuberculosis 'T.B' cases were estimated at 2004. Hepatotoxicity is reported with patients using drugs as first-line anti-tuberculous regimens, among them 6-12% die. Identifying the risk factors for developing hepatotoxicity would probably reduce morbidities and mortalities associated with T.B management. Objectives: To study the frequency and risk factors of hepatotoxic reactions in patients receiving anti-tuberculous drugs, and their relative value in early detection. Design: Prospective cohort study. Setting: Suez Chest Hospital Subjects: 77 patients consecutively presenting to Suez Chest Hospital with active T.B diseases (WHO criteria), who are eligible for anti-tuberculous regimens (WHO guidelines) were included. Outcome Measures: Rate of hepatotoxic reactions according to the diagnostic criteria, and rates of fast and slow acetylator phenotypes. Results: Hepatotoxic reactions have been diagnosed in seven (9.1%) patients. By univariate analysis, age over 60 years (p=0.02), alcoholism (p =0.02), extra-pulmonary tuberculosis (p=0.02), and severe forms of tuberculosis (p=0.03) were statistically significant risk factors. Fifty eight (75.3%) of the study sample were slow acetylators, while 8 (10.4%) were fast acetylators. Three patients (37.5%) of fast acetylators and only (6.9%) of the slow acetylators developed hepatotoxicity (p=0.03). Logistic regression models showed that fast acetylator phenotype was the only significant (p=0.04) risk factor for early hepatotoxicity. Alcoholism (p=0.01) was a significant risk factor for late hepatotoxicity. Conclusions: Hepatotoxic reactions among patients receiving anti-tuberculous drugs remain a considerable problem. Two patterns of liver injury can be observed. The first occurs earlier and is associated with fast acetylator phenotype. The second occurs later and is associated with alcoholism.

170

Comparative Cardiac Effects of Halofantrine With or Without Concomitant Administration of Kolanut or Fluconazole in Healthy Volunteers

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Background: Objectives: To study the electrocardiographic changes of concomitant administration of kolanut (a caffeine containing nut) or fluconazole with halofantrine. To compare the effects on the QTc interval of fed single oral doses halofantrine, and halofantrine with kolanut or fluconazole Methods: Eighteen healthy male volunteers received a single oral dose of halofantrine (500mg), halofantrine with kolanut (12.5mg) or halofantrine with fluconazole (25mg) at random order, during a crossover study, with a wash out period of 6 weeks between treatments. Twelve lead electrocardiography (ECG) was performed to measure the PR and QT interval (QTc). Blood tests were performed to determine the plasma concentration of halofantrine and N-desbutyl-halofantrine. Results: While the PR interval were shortened by halofantrine alone and halofantrine with kolanut (169.29±28.67 to 165.29±28.007 and 172.73±29.843 to 163.00±18.336ms), it was prolonged by halofantrine with fluconazole (177.70±27.394 to 186.59±44.434ms). Compared with the pretreatment QTc, there was prolongation of QTc (384.76±21.727 to 394.12±21.525; 381.36±22.29

to 388.30±17.26 and 382.35±20.08 to 390.84±21.97) in all the three treatment groups, at 6 hours. These were not statistically significant (p>0.5) and none exceeded 440ms. Concomitant intake of kolanut with halofantrine was found to significantly decrease Cmax and AUC of both halofantrine and the metabolite desbutylhalofantrine. Conclusion: The pharmacokinetic profile observed from the plasma concentrations of halofantrine and the active metabolite desbutylhalofantrine, though appeared consistent with the PR interval reduction, it was not consistent with the QTc prolongation. Discordance between cardiac events and drug levels may occur.

171

Protein Binding of Cefazolin in Plasma and Amniotic Fluid during Pregnancy

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Aim: Document cefazolin (CFZ) plasma protein binding variability during pregnancy and compare observations with non-pregnant adults. Determine co-variates of CFZ concentration in amniotic fluid (AF). Methods: CFZ plasma and AF samples were collected in mothers in whom CFZ was used for prophylaxis during in utero surgery. The unbound CFZ fraction was reported by median and range and compared with reported observations in non-pregnant adults. Correlation and multiple regression were used to search for co-variates of the plasma unbound CFZ fraction and the CFZ-AF concentration respectively. Results: 130 plasma and 43 AF samples were collected during 30 interventions. Median plasma unbound CFZ fraction was 0.25 (range 0.14 – 0.41). Significant correlations between the plasma unbound CFZ fraction and total CFZ plasma concentration (0.46), time (-0.38) and albumin (-0.39) were documented. Median unbound CFZ fraction was higher in pregnant compared to non pregnant adults (0.25 vs 0.19, $p < 0.001$). Total plasma CFZ concentration and albumin were co-variates of the plasma unbound CFZ fraction. Median CFZ-AF was 0.7 mg/L, median unbound CFZ-AF fraction was 0.78. A significant correlation between CFZ-AF and time (0.42) or gestational age (0.48) was observed. Median CFZ-AF was significantly lower in cases with polyhydramnios (0.39 vs 0.96 mg/L, $p < 0.001$). In a multiple regression model, polyhydramnios was the only independent co-variate. Conclusions: The concept of saturability of CFZ in plasma is confirmed, but CFZ binding capacity is lower during pregnancy. CFZ-AF is mainly unbound and CFZ-AF concentration in part depends on clinical (gestational age, polyhydramnios) characteristics.

172

Gentamicin use in Paediatric Intensive Care: A Review of Practice

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Introduction: Although extended dosing intervals for gentamicin are used in PICU, data to support optimal doses are lacking. In practice, doses have been extrapolated from adult and neonatal populations. The aim of this study was to assess whether a dose of 8mg/kg every 24h leads to efficacious (peak> 10mg/L) and safe plasma concentrations (trough <2mg/l). Methods: 6 month prospective observational study. Plasma concentration data analysed using population PK modelling software (NONMEM).Covariates analysed for inclusion into the PK model were; age, weight, serum creatinine, urea, renal replacement therapy. Monte Carlo simulations (n=1000) of peak concentrations at the end of infusion (t=30mins) were performed using the final covariate model. The target bactericidal exposure was defined as a peak: MIC ratio of 8. Results: Data from 50 children (median (IQ range) weight and age: 4.8 (3.4-12.3) Kg and 14 (4-7) months respectively) were analysed. 238 plasma concentrations were included in the PK analysis. A 2-compartment i.v. infusion model with log-normal between subject variability and combined additive/proportional residual error was developed. Weight and age were the only significant covariates included in the model. Discussion: For organisms with a gentamicin MIC of 2mg/L, a dose of 8mg/kg provides >90% probability of achieving the target ratio for all age/weight groups. However, this leads to >25% of neonates with trough levels > 2mg/L 24 hours post dose. Conclusion: A dose of 8mg/kg achieves therapeutic plasma concentrations to treat common infections for the majority of PICU patients

however the dosing interval needs to be extended for the neonatal group.

173

Therapy Interval between Betamethasone Antenatal Administration (BET) and Delivery on the Incidence of Hyaline Membrane Disease (HMD) in 24- 28th Weeks Premature Infants

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In a recent meta-analysis, treatment with antenatal corticosteroids was confirmed to produce an overall reduction in neonatal RDS but underlined that further information is required about optimal dose to delivery interval (Obstet Gynecol. 2007 Jan;109(1):1,89-90. The prevalence and odds ratios of HMD according to therapy interval was evaluated from data collected retrospectively in 333 dossiers of infants born from 24th to 28th weeks. Newborns were exposed to a either 12 mg betamethasone (BET) twice at 24-h interval (1994 to 1997, or 12 mg BET at 12-h interval (1-12-24 hours), 1989-1994). Regression analysis, adjusted for confounding factors, including ritodrine (n=150) (1.04 (0.57 – 1.90)) and the year of treatment (1.14 (1.01 – 1.29)) have been done with SAS, The study was approved by the ethic comity. Amongst newborns included, 296 (89 %) were exposed to BET. A greater reduction was observed in adjusted odds ratio (0.15 (0.04-0.54)) among subjects exposed during intervals ≥ 48 hours and < 7 days between BET administration and delivery (HMD=52,9%) than to those exposed to interval lower than 24h (0.33 (0.09 – 1.29)) , to 24 to 48 hours (0.33,(0.07-1.58) or to non exposed (Oradj=1). This study shows that the incidence of HMD is clearly dependant on time-lag run after the initiation of betamethasone treatment and suggests that interval from administration to delivery should be ideally delayed for 48 hours to reduce significantly the incidence of HMD among very premature infants.

174

Tobramycin Drug Monitoring in Children with Cystic Fibrosis

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Introduction: Intravenous tobramycin is a widely recommended treatment for respiratory exacerbations in cystic fibrosis patients. Once daily dosing (ODD) of tobramycin is now widely recommended, due to the fact that it is potentially less nephrotoxic than multiple daily dosing. Debate continues however as to what is the most appropriate method of monitoring. We report here our experience with ODD dosing of Tobramycin and therapeutic drug monitoring (TDM) using an area under the curve model. Methods: Our institution has been using ODD of tobramycin in children with CF since 2003. The initial does has been 10- 12mg/kg and then adjusted according to drug levels. The first level is taken at 60 minuets and 4 to 6 hours after the end of the infusion, with the target AUC being 100mg h/L. Results: From September 2003 until September of 2007 we collected 106 data sets form 23 patients. One patient accounted for 32 data sets. The age range at time of sampling was 2 to 18 years. The does per kilogram ranged form 5.9 to 19.9 mg/kg. With the ACU varied form 49 to 129 mg h/L. Discussion: As the average life expectancy of patients with Cystic fibrosis continues to increase (currently it is into the fourth decade) their exposure to this potentially toxic antibiotic will increase. Therefore it is important to minimize the potential toxicity through the best possible TDM. Conclusion: These results highlight the need for individualised dosing of Tobramycin in children with CF, through TDM.

175

The Influence of Tobacco Smoke on Plasma Non-enzymatic Antioxidant Concentration in Healthy Tunisians

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Introduction: Tobacco smoke contains many reactive oxygen species (ROS) that cause oxidative stress. Crucial role in defending the organism against ROS play vitamins E and A. The aim of the study was to investigate the influence of tobacco smoke on concentration of these vitamins. Methods: Eighty nine healthy males between the age of 19 and 53 years were enrolled in the study divided into 2 groups: group I = 43

smokers and group II consisted of 46 non-smokers. High performance liquid chromatography (HPLC) was used to estimate the plasma concentration of vitamin E and vitamin A. Results: Plasma vitamin E levels were significantly lower in the group of smokers than those non-smokers (group I: 11.30 ± 1.70 mg/l versus group II: 12.82 ± 1.79 mg/l, $p < 0.000$). Practically no difference in concentration of vitamin A was found between the two studied groups (group I: 1.06 ± 0.20 mg/l, group II: 1.10 ± 0.16 mg/l, $p = 0.27$). Discussion: The obtained results suggest that tobacco smoke weakens the organism's antioxidant barrier by decreasing the concentration of plasma vitamin E, while no influencing significantly the plasma concentration of vitamin A. Conclusions: The low level of plasma vitamin E might result in reduced activity of the non-enzymatic antioxidant defense system and might be responsible for increased oxidative stress occurring in smokers healthy Tunisian men.

176

Differences in Neonatal Symptomatology between Antidepressants and Opiates Exposed Neonates: Issues in Clinical Assessment

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Neonates that are suspected to suffer from withdrawal symptoms are generally assessed with a Neonatal Abstinence Syndrome (NAS) score. The aim of the study was to compare type and prevalence of symptoms present in neonates exposed to antidepressants in late pregnancy with opiates exposed neonates used to design a NAS scoring system and to evaluate if that score is appropriate to assess antidepressants exposed neonates. Exposure to antidepressants in late pregnancy was studied in 73 neonates. Data on antidepressants exposed neonates were abstracted retrospectively from maternal and neonatal hospital charts. Prevalence and type of symptoms were compared with symptoms in opiates exposed newborns reported in the literature (n=85). The most prevalent symptoms in antidepressants exposed newborns were tachypnea (42%) and tremors (30%). The most prevalent symptoms in opiates exposed newborns were tremors (90%), restlessness (85%) and hyperactive reflexes (51%). Many symptoms, found in both antidepressants and opiates exposed neonates, were found in very different proportions in each cohort. Symptoms quite prevalent in the opiates exposed neonates like sneezing (31%), hyperactive reflexes (51%) and frantic suckling (25%) were far less common in antidepressants exposed neonates. Lower arousal symptoms, very prevalent in antidepressants exposed neonates, are not assessed in the NAS score. Decreased reactivity was found in 25 % and decreased tonus in 21% and these are significantly associated with antidepressants exposure ($p<.001$). Conclusion: Opiates and antidepressants do not produce the same pattern of symptoms in neonates and consequently each requires a specific tool to evaluate the gravity and nature of symptoms.

177

Effectiveness of Ezetimibe 10mg/day Co-administered with Statins versus Statin Dose Doubling in Patients with Coronary Artery Disease (CAD) who are not at Target LDL-C on Statin Monotherapy: The EZE (STAT)^2 Trial

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Introduction: Patients with Coronary Artery Disease (CAD) who are not at recommended LDL-C target levels require effective and aggressive lipid lowering treatment. For these patients, coadministration of ezetimibe with a statin may be more effective in the management of hyperlipidemia when compared to doubling of the statin dose. Methods: Patients with or at high risk for CAD with above target LDL-C while on statin monotherapy were randomized to ezetimibe 10 mg/day co-administered with a statin (EZE+Statin) or statin dose doubling (STAT2). Results: Of the 936 patients enrolled, 398 (EZE+Statin: 262. STAT2: 129) had confirmed CAD. Mean (SD) age was 65.0 (11.0) years and 302 (69.6%) were male. The mean percent reduction in LDL-C was -29.3% (95% CI -31.2%

to -27.3%) in the EZE+Statin group and -14.7% (95% CI -17.8% to -11.7%) in the STAT2 group. The mean percent reduction in the EZE+Statin group was significantly higher compared to STAT2 (P<0.001). In the EZE+Statin group, 76.4% and 37.4% of patients achieved target LDL-C <2.5 mmol/L and <2.0 mmol/L compared to 45.5% and 17.1% in STAT2 patients respectively (P<0.001). The odds ratio of achieving target LDL-C was 3.9 (95% CI 2.5 to 6.2) for LDL-C <2.5 mmol/L and 2.9 (95% CI 1.7 to 5.0) for LDL-C <2.0 mmol/L in favor of the EZE+Statin group. Conclusion: For patients with CAD who are not at target LDL-C with statin monotherapy, ezetimibe coadministered with a statin is significantly more effective in reducing LDL-C and achieving target LDL-C levels when compared to doubling the statin dose.

178

Levels of Ranitidine Determined by the Gestational Age in Neonates Subject to Fasting

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We included 50 infants, which were classified according to their gestational age, who entered the Neonatal Intensive Care Unit General Hospital Durango, Mexico, and requiring treatment with ranitidine, for the prevention of bleeding in the digestive tract, at a dose of 3 mg/kg/day. We measured the level of ranitidine by the method of High Performance Liquid Chromatography (HPLC). Objectives: To determine the concentrations reached ranitidine after treatment, in order to assess the risk of toxicity, in a population of neonates undergoing fasting. Results: The plasma concentrations of ranitidine reached in infants studied after administration of a single intravenous dose, found that 50 of 34 children studied showed levels of ranitidine ≥ 400 ng/mL (risk of toxicity) corresponding at a ratio of 0.68, and the therapeutic range seen between 100 and 400 ng/mL (therapeutic range) of the 50 children studied 11 children had levels within this range, corresponding to a ratio of 0.22, while in levels subtherapeutic with concentrations of less than 400 ng/mL, only 5 children showed concentrations in these values with a ratio of 0.1. Obviously, the higher proportion of children were at risk of poisoning, and only a proportion of 0.22 corresponded to children who reached levels within the therapeutic range, and 0.1 of children showed levels subtherapeutic. The results will contribute to improved therapeutic management of ranitidine in patients neonates considering pharmacokinetic parameters.

179

Effects of the Angiotensin II Type 1 Receptor Antagonist Valsartan on the Expression of Superoxide Dismutase in Hypertensive Patients

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The role of oxidative stress in the pathogenesis of vascular diseases such as hypertension has been well recognized. Angiotensin (Ang) II is regarded as a pro-oxidant because it can stimulate the production of reactive oxygen species. The purpose of this study was to evaluate whether treatment with the Ang II type 1 (AT_1) receptor antagonist valsartan has an antioxidant effect in patients with mild to moderate hypertension. A randomized, double-blind, placebo-controlled study was conducted in 48 stage I and II hypertensive subjects. Patients were followed every 4 weeks for 12 weeks after randomization to valsartan titrated to 80-160 mg once or twice daily or matching placebo. The erythrocyte superoxide dismutase (SOD) activity and expression of SOD–mRNA in polymorphonuclear leukocytes were measured before and after treatment. Valsartan showed concentration-dependent inhibition of reactive oxygen species generation in polymorphonuclear leukocytes from hypertensive patients. The erythrocyte superoxide dismutase activity before treatment was more than two times higher in hypertensive subjects compared to normal controls. Superoxide dismutase activity decreased significantly after 12-weeks of treatment with valsartan but did not change with placebo. The amount of SOD-mRNA in the polymorphonuclear leukocytes decreased progressively over 3 months in the hypertensive subjects receiving valsartan treatment but did not change in the placebo group. The production of reactive oxygen species is increased in

hypertension, and superoxide dismutase activity is increased, presumably as a compensatory mechanism. Treatment with valsartan but not placebo resulted in a progressive down-regulation of SOD-mRNA expression and a reduction in superoxide dismutase activity, suggesting antioxidant activity and a reduction of reactive oxygen species generation. These findings imply that AT_1 receptor antagonists may provide benefits to hypertensive patients beyond blood pressure reduction.

180

Male Sex Hormones Regulate the Participation of Endogenous Thromboxane A_2 in the Vasomotor Response of Rat Mesenteric Artery. Role of Neuronal Nitric Oxide

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It is known that male sex hormones influence cardiovascular function by modulating the release and response of nitric oxide (NO) and prostanoids. Since thromboxane A2 (TXA2), one of the most important vasoconstrictor prostranoid, participates in vascular tone regulation, the aim of this study is to investigate whether male sex hormones affects the participation of endogenous TXA2 in the responses induced by electrical field stimulation (EFS), as well as the mechanism involved on it. For this purpose, endothelium-denuded mesenteric arteries from control and orchidectomized male Spagre Dawley rats (6 months old) were used to analyze: (i) the production of TXA2, (ii) the effect of the TXA2 synthase inhibitor, furegrelate, in the EFS – induced response, and (iii) the effect of furegrelate in the EFS-induced NO release and response. The TXA2 release was increased in arteries from orchidectomized rats. In arteries from control rats, furegrelate decreased the EFS-induced response, while it increased the EFS-induced NO release and the vasodilator response induced by the NO donor, sodium nitroprusside (SNP). In arteries from orchidectomized rats, furegrelate did not modify the EFS-induced response, NO release or the response to SNP. These results indicate a protective action of endogenous male sex hormones on the vasculature since the TXA2 release was increased in arteries from orchidectomized rats; the effect of endogenous TXA2 on the EFS response is under male sex hormone regulation and involves neuronal NO release.

181

Felodipine Bioavailability as a Function of Enteric CYP3A4 Expression in Patients with Celiac Disease

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Background: Patients with active celiac disease have reduced expression of enteric CYP3A4. The mechanism, functional relevance, and clinical impact of these changes has not been established. Methods: Consenting patients with active and inactive celiac disease who required diagnostic esophagogastroduodenoscopy were invited to contribute enteric biopsy samples and participate in a single dose pharmacokinetic (PK) study of felodipine ER 10 mg. CYP3A4 content was determined by immunohistochemical (IHC) staining graded by two independent investigators and by semiquantitative western blots. TNF alpha and IL6 mRNA levels were determined by RT-PCR. Felodipine plasma concentrations over 8 hours were measured using gas chromatography and PK variables calculated. Results: Forty-five patients enrolled in the study, 10 were defined as active and 35 as inactive. Serum tissue transglutaminase (TTG) levels ranged from 0.4 to 807 U/ml. By IHC, CYP3A4 expression was graded 0-4. Felodipine AUC ranged from 0.5 to 84 ng/ml*h. There were no significant correlations between TTG level, IHC grading, AUC or Cmax of felodipine. Conclusions: Felodipine PK variables following oral absorption of 10 mg dose do not correlate with CYP3A4 content by IHC assessment in patients with celiac disease. This may relate to greater variability in bioavailability of felodipine in these patients because the increase in bioavailability associated with a loss of CYP3A4 may be antagonized by the loss of absorptive surface area associated with enteric villus atrophy. Inflammation may mediate changes in enterocyte structure and CYP3A4 content. This hypothesis is currently being examined.

182

Effects of Inhaled Corticosteroids Exposure on Bone Mineral Density in Children and Adolescents

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Objective: To determine whether asthmatic children or adolescents who are exposed to inhaled corticosteroids (i.e., budesonide, fluticasone propionate) are at a greater risk of bone mineral disturbances and impaired ossification when compared with nonexposed individuals. Material and methods: We studied serum activity levels of total alkaline phosphatase (TAP) of a randomized trial of 55 subjects aged 5-23 years (study group), receiving anticonvulsant monotherapy for at least one year, and the results were compared to 55 age-matched healthy subjects (control group). The epileptic patients were classified in three subgroups, accordingly with their anticonvulsant monotherapy: carbamazepine (n = 23), valproic acid (n = 22), and respectively new anticonvulsants, as oxcarbazepine, lamotrigine and topiramate (n = 10). The serum activity levels of total alkaline phosphatase were determined by the kinetic-colorimetric method, with paranitrophenylphosphate. Results and Discussions: According to One-way ANOVA's results, as compared to the control group, significant differences within each subgroup were pointed out. No significant differences between the three subgroups with anticonvulsant monotherapies were revealed (p = 0.411). Conclusions: Chronic exposure to inhaled corticosteroids revealed a low bone mineral density in children and adolescents with asthma. Key words: inhaled corticosteroids, asthma, children, adolescents, bone mineral density Abbreviations: BMD - bone mineral density; FP - fluticasone propionate; BUD – budesonide.

183

Efficacy of Metronidazole + Miconazole Combination (Neo-Penotran®) in the Treatment of Vaginal Infections

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Vaginal infections are among the most common medical problems seen in general practice. There are 3 common types of vaginitis: vulvo-vaginal candidiasis, bacterial vaginosis and trichomonal vaginitis. Vaginitis due to simultaneous infection with at least 2 pathogens is also highly prevalent and makes up approximately 30% of all cases. In practice, as laboratory results take some time to obtain, the diagnosis of the underlying cause of vaginitis is usually made on the clinical features. In such cases, where the cause of the vaginitis is unconfirmed and may be of mixed origin, a single form of medication capable of 3 types of vaginitis effectively should provide a valuable form of therapy. Further, a comparison of dosage forms for the treatment of vaginitis showed that physicians prefer vaginal ovules over vaginal tablets. The combination of Metronidazole 500 mg + Miconazole 100 mg as vaginal ovule (Neo-Penotran®, Embil Pharmaceutical, Turkey) is designed to fulfil the above requirements. An in vitro study showed that the vaginal ovule was superior to a generic vaginal tablet (Nidazol M, I.E.Ulagay) with regards to melting times and desintegration test, carried out according to EP 5 and BP / EP 2002. 7 clinical studies with the participation of 636 patients reveal that the vaginal ovule is highly efficacious in treating single and mixed vaginal infections with clinical and microbiological cure rates of 79-100% when applied twice daily for 7 or 14 days. The ovule combination provides a valuable treatment for vaginitis with excellent tolerability and acceptability.

184

Clinical Pharmacology Outpatient Clinic: Consultations on Drugs in Pregnancy

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Introduction: The estimated prevalence of birth defects is 1-3%. Teratogenic exposures include radiation, dietary exposures and medications. More than 90% pregnant women receive at least 1 drug prescribed during pregnancy. Methods: We reviewed records from our outpatient clinic on consultations on drugs in pregnancy (2002-2006) with the aim to evaluate the patterns of medication use using the FDA pregnancy risk classification. Patients were contacted for information on the pregnancy outcome. Results: In the mentioned period 484 pregnant women aged 17-46 years (mean age 29.8 years) were referred for consultation on 1.001 drugs and 10 radiological diagnostic procedures. More than 95% women were referred during the first trimester of pregnancy for consultations on drugs already taken (88%), and 11% were referred for therapy recommendation during pregnancy. In more than one third of patients taking drugs for a chronic condition, an adjustment of therapy was suggested. The mean number of drugs taken was 2.1 (range 1-11). The prevalence of FDA category C, D and X drugs was 37%, 19% and 7%, respectively. Follow-up for 222 patients (46%) showed that 84% of women delivered a healthy child, 8 % had a spontaneous abortion, 7% had an artificial abortion (4/15 patients were assessed as having high risk for fetal malformation) and in 2 pregnancies birth defects occurred. Discussion: High prevalence of consultations for drug exposure to high-risk drugs in pregnancy (FDA pregnancy category D/X drugs) was registered. Conclusion: Consultation by a clinical pharmacologist contributes to the safe use of drugs during pregnancy.

185

Endogenous Female Sex Hormones Regulate the Participation of ATP-dependent Potassium Channels in the Acetylcholine-induced Response

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Vascular tone is regulated by ATP-dependent potassium (KATP) channels by modulating endothelial nitric oxide (NO) release and function. Vascular wall structure and function are influenced by ovarian steroids hormones through NO action. The aim of this study was to determine whether endogenous female sex hormones influence the involvement of KATP potassium channels in the endothelial NO release and function. For this purpose, aortas from estro and ovariectomized female Sprague-Dawley rats (6 months old) were used to analyze: (i) the expression of endothelial NO synthase (eNOS); (ii) the endothelial NO release; (iii) the Ach-induced vasodilator response; and (iv) the effect of the blocker of KATP channels, glybenclamide, on the Ach-induced NO release and on the Ach-induced response. The expression of eNOS was similar in arteries from estro and ovariectomized rats. The Ach-induced NO release was similar in arteries from both groups; glybenclamide decreased the NO release only in aortas from estro rats. The Ach-induced relaxation was similar in aortas from both groups of rats; glybenclamide diminished the Ach-induced response in aortas from estro rats, while did not modify that response in aortas from ovariectomized rats. These results show that ovariectomy: (i) did not alter the eNOS expression or endothelial NO release, (ii) maintanined the Ach-induced NO release when the KATP channels are blocked, and (iii) abolished the participation of KATP channels in the Ach-induced response. Despite these alterations the Ach-induced vasodilator response is maintained.

186

Antenatal use of Selective Serotonin Reuptake Inhibitors and QTc Interval Prolongation in Newborns

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Introduction: Prolongation of the QT interval is a risk factor for sudden death. SSRI antidepressants can prolong the QT interval, and are widely used by pregnant women. Whether antenatal exposure to SSRI causes QT prolongation in offspring is unknown. We determined the effect of maternal use of selective serotonin reuptake inhibitor (SSRI) antidepressants during pregnancy on the QTc interval of the newborns. Methods: Between

January 2000 and December 2005, we collected data on all newborns born at a single tertiary care hospital. The electrocardiograms of those exposed to SSRI antidepressants immediately prior to delivery were compared to those of healthy control newborns matched on gestational age. The tracings were interpreted by a pediatric cardiologist unaware of drug exposure. Results: We identified 52 newborns exposed to SSRI antidepressants in the immediate ante-partum period and 52 matched controls. The mean QTc was significantly longer in the group of newborns exposed to antidepressants as compared to controls (409 ± 42 milliseconds vs. 392 ± 29 milliseconds; p = 0.02). Five newborns (10%) exposed to SSRI antidepressants had a markedly prolonged QTc interval (> 460 milliseconds), compared with none of the unexposed newborns. The longest QTc interval observed among exposed newborns was 543 milliseconds. All drug-associated repolarization abnormalities normalized shortly after delivery. Discussion/Conclusion**:** Antepartum exposure to SSRI antidepressants is associated with transient, yet significant risk of QTc interval prolongation in neonates. Additional research is needed to determine if exposure to SSRI antidepressants in late pregnancy is associated with arrhythmias or early neonatal death.

187

Fetal Exposure to Isotretinoin - An International Problem

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Background: Isotretinoin is a known teratogen. Pregnancy prevention programs aimed at minimizing isotretinoin exposure in pregnancy have been implemented in North America with limited success. Information on isotretinoin exposure in pregnancy in countries other than the US is limited. Objective: To compare the management of fetal risk of isotretinoin in three countries, including information given to women, implementation of contraceptive methods, and pregnancy outcomes. Methods: Pregnant women exposed to isotretinoin who called teratogen information services (TIS) in Israel, Italy and Canada between July 1998 and October 2006 were interviewed at the time of initial consultation and after the expected day of delivery. Results: Fifty-three pregnant women exposed to isotretinoin contacted the TIS. Only 41% reported using a birth control method. Just one patient (2%) reported using two different forms of contraception, as required for prescription of the drug. Forty-five percent of exposed pregnancies were terminated before delivery and 22% delivered healthy babies. Two babies were born with malformations, both compatible with isotretinoin teratogenicity. Conclusions: Since isotretinoin-exposed pregnancies still occur, there is a need for more effective prevention strategies, which should take into account cultural differences among countries.

188

Comparative Evaluation of Ispaghula Husk (Psyllium or Plantago ovata) and Glipzide on Glycemic Control & Safety in Newly Diagnosed Patients of Type 2 Diabetes Mellitus

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Introduction: Antidiabetic effect of Ispaghula husk has been reported in western population. So, objective of present study was to evaluate the safety and efficacy of Ispaghula husk as an antidiabetic agent in Indian patients and to compare its effects with glipizide. Method: A randomized, open standard- control parallel study was conducted in eighty patients of newly diagnosed diabetes mellitus of age group 25-65 years. The patients were taken from outdoor of Medicine department, Medical College, Amritsar, India. Patients were randomly divided into two groups of 40 each. Group I was treated with Ispaghula husk 3.5 gm twice daily; Group II with glipizide 5 mg once daily. The treatment was

continued for 12 weeks in both the groups. Fasting blood glucose levels (FBG) and monitoring of adverse events were done in both groups at 0,2,4,8 and 12 weeks; post-prandial blood glucose (PPBG) concentration and HbA1c were evaluated at 0 and 12th week. Results – At the end of 12 weeks, mean levels of FBG, PPBG, and HbA1c were decreased by 23.06% (P> 0.05); 62.23%(P> 0.05) and 16.46%(P> 0.05) respectively with Ispaghula husk whereas, mean levels of FBG, PPBG, and HbA1c were decreased by 30.68%;(P> 0.05); 41.69%(P> 0.05); and 18.58% (P> 0.05) respectively with glipizide. Mild gastrointestinal disturbances were observed in 20% patients with ispaghula husk whereas nausea, hypoglycaemia & malaise in 10% each were observed with glipizide. Conclusion: – Ispaghula husk is recommended as a dietary adjunct in patients with type 2 diabetes mellitus before initiating conventional drug therapy

189

Self-reported versus Meconium-screened Rate of Prenatal Alcohol Exposure amongst Newborns in a High Risk Obstetric Unit

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Introduction: Confirmation of prenatal alcohol exposure (PAE) is often required to diagnose fetal alcohol spectrum disorder (FASD). Fatty acid ethyl esters (FAEE) in meconium is a sensitive and specific biomarker for PAE in the final two semesters of pregnancy. We recently reported a 2.5% rate of FAEE positive meconium in infants born in Grey-Bruce, Ontario. High-risk maternal-fetal conditions are transferred to a tertiary healthcare facility in London, Ontario; as such not all residents deliver within the Grey-Bruce region. The objectives of the present study were three-fold: to determine the prevalence of FAEE positive meconium in high-risk unit infants; compare the rate of FAEE positive meconium in high-risk setting versus the general population; and compare the rate of FAEE positive meconium with maternal self-report of PAE. Methods: Infants born to Grey-Bruce residents delivering at St. Joseph's Health Care, London, Ontario were screened and informed of an anonymous prevalence study. They were provided specimen bags and instructions on meconium collection. FAEE were quantified using gas chromatography-mass spectrometry. Charts were abstracted at year-end to determine the rate of self-reported PAE. Results: Forty-six meconium specimens were collected from August 1, 2006 – July 31, 2007. Twelve specimens (26%) tested positive for FAEE. This translated to a 10-fold higher rate than babies born in primary healthcare settings (RR=10.46, 95%CI=5.32-20.57, p<0.0001). Only one mother self-reported PAE. Discussion: Referral to high-risk tertiary unit confers a 10-fold risk for in utero alcohol exposure. Our study demonstrates that meconium screening is more effective at detecting PAE versus maternal self-report.

TUESDAY JULY 29, 2008
STREAM 1:
NEW THERAPEUTIC APPORACHES

190

Analysis of N-acetyltransferase 2 (NAT2) Polymorphisms as a Risk Factor to Isoniazid-induced Hepatitis on Tuberculosis Treatment in Amazonian Population

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Introduction: The efficiency of tuberculosis treatment is explained by polychemotherapy and by the prolonged time of treatment with presence of Adverse Drug Reactions (ADRs). Objectives: To determine the prevalence of patients with signals of hepatic lesion and the slow and rapid acetylator NAT2 genotypes within the enrolled patients. Methods: The work consisted of a descriptive observational study of tuberculosis patients treated in the Ambulatório Araújo Lima in Manaus, with pyrazinamide, rifampicin and

isoniazid. Patients had their transaminases and bilirrubin levels monitored during treatment and the analysis of the NAT2 genotypes using PCR-RFLP was performed. Results: from 2006 to 2007 15 patients were enrolled in the research with patients' 20-30-years-old. It was studied the NAT2 genotype in 13 of the 15 included patients. 9 patients (69,2%) were genotyped as *5/*5, a slow acetylator genotype. 6 of these (66,7%) presented some signal of hepatic lesion. Out of the 9 patients with signals of hepatic lesion, 5 (55,5%) were genotyped as *5/*5. Discussion: This ADR has been attributed to the deficiency of the hepatic enzyme N-acetyltransferase 2 (NAT2), which has its catalytic activity genetically determined. Nowadays, thanks to Pharmacogenomics, it is well reconignized that poor metabolizers (slow acetylators) of this enzyme are prone to develop hepatitis when using isoniazid.Conclusion: There was a high prevalence of patients genotyped as slow acetylators (69,2%) suggesting a more thoroughly study of the prevalence of NAT2 genotypes in the Amazonian population; 66,7% of the patients genotyped as slow acetylators presented some signal of hepatic lesion, and 55,5% of the patients with some signal of hepatic lesion were genotyped as slow acetylators.

191

EP 80317, a Selective CD36 Ligand, Exerts Cardioprotective Effects through the Early Activation of the PI3K-Akt Pathway

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Ischemic heart disease is ubiquitous worldwide, and is likely to prevail over the next decades as it is strongly associated with risk factors such as obesity and diabetes. FAT/CD36 has been identified as the major long-chain fatty acid transporter in the heart. Yet, the role of CD36 in the context of myocardial ischemia/reperfusion (I/R) remains controversial. In the present study, we addressed this role by the use of EP 80317, a selective CD36 ligand. C57BL/6 wild type mice were pretreated for 2 weeks with EP 80317 (300 μg/kg/d) s.c. Mice were then either euthanized and the hearts subjected to global ischemia (30 min) and reperfusion (90 min) for the measurement of myocardial infarction area, or alternatively, underwent transient left coronary artery ligation (30 min) and reperfusion (6 hours). Our results show a 75% ($p < 0.001$) reduction in myocardial necrosis in WT mice. This effect was not observed in CD36-deficient mice. Following transient myocardial ischemia, the ratio of phosphorylated to total Akt, an index of akt activation, increased by 44% ($p < 0.05$) in a CD36-dependent manner. These observations were associated with reduced circulating NEFA levels, from 0.48 ± 0.04 mmol/L in vehicle (non fasted mice) to 0.35 ± 0.05 in EP 80317-treated mice. We conclude that EP 80317 may exert cardioprotection through its metabolic effect mediated by CD36, involving activation of the PI3K-Akt signaling pathway.

192

Pharmacokinetics-Pharmacodynamic Modeling of Biomarker Response and Tumor Growth Inhibition in Xenograft Mouse Models with GDC-0879, A Potent Selective B-Raf Kinase Inhibitor

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The Raf family of protein kinases are involved in cellular responses relevant to tumorgenesis, including cell proliferation, invasion, survival and angiogenesis. Frequent activating mutations in B-RAF have been observed in several tumor types, including malignant melanoma and colorectal carcinoma. The majority of these mutations are in exon 15 which results in a val^{600} Glu (V600E) amino acid substitution, leading to constitutive kinase activation. GDC-0879 is a novel oxime-containing molecule that is a potent and selective B-Raf inhibitor. The objective of this study was to characterize the relationship of GDC-0879 plasma concentrations of orally-dosed GDC-0879 to pMEK1 inhibition in A375 (melanoma) xenograft tumors and tumor growth inhibition in A375 and colo205 (colon) xenograft models (both V600E mutants). Using indirect response models to characterize the relationship between plasma

concentration of GDC-0879 and both pMEK1 inhibition and tumor growth inhibition, the IC_{50} value for pMEK1 inhibition in A375 tumors by GDC-0879 was estimated to be 3.06 uM and the IC_{50} values for tumor growth inhibition in A375 and colo205 were estimated to be 8.80 and 20.0 uM, respectively. ED_{50} estimates in A375 and colo250 models were estimated to be 28 and 32 mg/kg, respectively. Overall, the *in vivo* potency of GDC-0879 was similar in both A375 and colo205 xenografts. These results are useful in estimating the target concentrations/doses required in the clinic and to determine appropriate clinical dosing regimens.

193

Cuban Traditional Medicine with Natural Products: From the Ethnomedicine to the Clinical Pharmacological Evaluation. *Mangifera indica* L Stem Bark Extract (Vimang) Like an Example with High Therapeutic Impact

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The rich cuban plant biodiversity, with very high index of endemic flora, offers to public health important therapeutic alternatives. Many Cuban research projects in different center of investigations focused to obtain natural health product (NHP) as the main goal. These investigations should concluded in the controlled clinical trials, and pharmacoepidemiological studies, in order to known the real effectively of medicinal plant as part of therapeutic police of the Ministry of Health. Many examples show the utilization of NHP from tropical plants in Cuba. In particular, we present the result of investigations with an aqueous extract from stem bark of *Mangifera indica* L (VIMANG) that has been used in Cuba during several years in ethnomedical practices. Phytochemical characterization of the extract has led to the isolation of different phenolic constituents, with the glucosylxanthone mangiferin as the majority component. The extract has demonstrated antioxidant activity as the main pharmacological property. Others studies have shown that the extract also possesses others pharmacological activities, such as: anti-inflammatory, antiallergic, analgesic and immunomodulator, with very complex and multifactorial mechanisms of involved action. Different clinical studies have been developed, demonstrating the therapeutic effectiveness of Vimang as antioxidant supplement in pathologies where oxidative stress is related with their etiology. This is only an example that illustrates the impact of the Cuban ethnomedicine in the national politics of health, on the base of the rational employment and with a deep scientific support, of the medicinal plants as true therapeutic alternatives.References: Pharmacol Res 2007;55: 351-358.

194

***In Vivo* Cervical Delivery of Gemcitabine for Radiosensitization**

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Along with surgical resection, chemoradiation is the mainstay of therapy for the treatment of advanced cervical cancer. However, administration of intravenous chemotherapy concomitantly with abdominal radiation is not without systemic side effects. Localized delivery of radiosensitizing chemotherapeutics (e.g., gemcitabine) may lead to higher drug targeting of the affected tissue while limiting potential systemic exposure and toxicity. Cervical application of gemcitabine was accomplished via a cervical delivery device (CerviPrep™). Gemcitabine was compounded as a gel and applied to the cervix in seventeen women undergoing hysterectomy for the treatment or staging of gynecological cancer. Uterine vein samples were collected at 30 minutes post-application and samples from the peripheral circulation were also collected at 30, 60, and 90 minutes post-application. Samples were analyzed (HPLC/UV detection) for gemcitabine and 2'2-difluorodeoxyuridine concentrations. Formulation preparation protocol was not followed in four subjects, and thus data from these subjects was not included. In an additional two subjects, samples hemolyzed and could not be reliably analyzed for gemcitabine. Of the remaining 11

subjects, clinically appropriate concentrations of gemcitabine (4.4-26.6 microM) were measured in five subjects. Interestingly, of the six subjects in whom gemcitabine concentrations could not be measured, four had cervical cancer, suggesting altered gemcitabine uptake mechanisms in these patients. Gemcitabine concentrations in peripheral plasma were not measurable (<2 microM) in any subject. These results suggest that targeted drug delivery of radiosensitizing chemotherapeutics to the cervix is possible and thus, may be useful in limiting systemic exposure and side effects as compared to intravenous administration.

195

Chromatin and CREB Signaling during Corticosteroid and Theophylline Therapy of COPD

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Chronic obstructive pulmonary disease (COPD) is characterized by acute exacerbations and progressive lung function decline. It is well established that pro-inflammatory genes are activated in asthma and COPD. However corticosteroids (ICS), which effectively switch off pro-inflammatory genes in asthma seem to be ineffective in COPD patients. We assessed the effect of treatment with ICS, with and without theophylline, on pro-inflammatory signaling pathways in COPD. Thirty seven patients with stable disease received one of three different courses of therapy: Formoterol alone (F), Formoterol/Budesonide (F/ICS), and Formoterol/ICS/Theophylline (F/ICS/Th) b.i.d. for 4 weeks. Lung function was measured before and after treatment. Cytosol, nuclear extracts and acid extracted histones isolated from induced sputum leucocytes were evaluated for the expression of CREB and phosphorylated CREB (CREB-P), HDAC-2, and acetylated histones H3 and H4 before and after treatment. We found that F/ICS increased the expression of ac-H3 by 31% ($p<0.001$) but decreased both ac-H4 and HDAC-2 expression by 22 and 23% respectively ($p<0.01$, $p<001$). However, F/ICS/Th decreased ac-H3 by 53% ($p<001$) in comparison to baseline, and further decreased expression of ac-H4. Expression of CREB was increased in both cytosolic and nuclear fractions by 40 and 24% respectively ($p<0.001$, $p<0.01$), while CREB-P increased by 50 and 51% ($p<0.01$) in both cellular compartments after F/ICS and F/ICS/Th. These findings suggest that ICS/Th treatment may decrease inflammatory molecular signaling pathways in COPD. Conversely, activated CREB–related signaling and activation of proinflammatory genes may result in poor response to ICS therapy.

196

Insulinotropic Properties Of Beta-glucans Produced by Submerged Mycelial Culture of a Medicinal Mushroom *Laetiporus Sulphureus* var. *Miniatus*

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In the present study, optimum culture conditions for the production of extracellular polysaccharides (EPS) in submerged culture of an edible mushroom, Laetiporus sulphureus var. miniatus and their stimulatory effects on insulinoma cell (RINm5F) proliferation and insulin secretion were investigated. The maximum mycelial growth (4.1 g/l) and EPS production (0.6 g/l) in submerged flask culture were achieved in a medium containing 30 g/l maltose, 2 g/l soy peptone, and 2 mM MnSO4·5H2O at an initial pH 2.0 and temperature 25°. In the stirred-tank fermenter under optimized medium, the concentrations of mycelial biomass and EPS reached a maximum level of 8.1 g/l and 3.9 g/l, respectively. Interestingly, supplementation of deep sea water (DSW) into the culture medium significantly increased both mycelial biomass and EPS production by 4 and 6.7 fold at 70% (v/v) DSW medium, respectively. The EPS were proved to be glucose-rich polysaccharides and were able to increase proliferation and insulin secretary function of rat insulinoma RINm5F cells, in a dose-dependent manner. In addition, EPS also strikingly reduced the streptozotocin-induced apoptosis in RINm5F cells indicating the mode of the cytoprotective role of EPS on RINm5F cells.

197

Induction of Apoptosis by 'COXIBs' Involve Intracellular pH and Calcium-ion Concentration Changes during Experimental Colon Carcinogenesis

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The role cyclo-oxygenase-2 inhibitors (COXIBs) Aspirin, Celecoxib and Etoricoxib as a chemopreventive agents was studied in experimental colon cancer induced by 1,2-dimethylhydrazine(DMH). Rats were injected subcutaneously with DMH 30mg/kg body weight per week for 6 weeks. The animals were simultaneously treated with COXIBs per oral at the dose of, aspirin- 60mg/kg body weight, celecoxib- 6mg/kg body weight and etoricoxib- 0.6mg/kg body weight. The animals were sacrificed after 6 weeks of treatments. Colonic mucosal changes and early precancerous lesions/ carcinoma in DMH alone and DMH+COXIBs treated animals were established in the different regions of the colon by morphological and histopathological examinations which were greatly regressed by the simultaneous treatment of the three COXIBs. The results demonstrate that, there is a distinct occurrence of pre-malignant alterations in DMH induced colon in the form of mucosal plaque lesions and carcinomas which were greatly reduced by the COXIBs used. In the isolated colonic epithelial cells (CEC), the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were measured using the Fura2-AM as a Ca^{2+} binding fluorophore. The percentage of $[Ca^{2+}]_I$ was found to be increased in DMH+COXIBs treated group when compared with the DMH group indicating the mediatory role of Ca^{2+} in the regression of cancer by COX-2 inhibitors. The intracellular pH (pH_i) in the CEC were also measured which was found to be decreased following DMH while elevation of pH_i was seen in the DMH+COXIBs treated animals, possibly leading to the increased apoptosis as also, revealed by acridine orange and ethidium bromide staining of CEC when examined under fluorescence microscope. To further establish the molecular mechanism of COXIBs induced apoptosis, the caspase 1 and 3 activity was measured using fluoregenic substrates Ac-YVAD-AMC and Ac-DEVD-AMC respectively. The specific activity of each caspase was found to be decreased following DMH administration while the enhanced activity was observed in DMH+COXIBs treated animals, while expression of cyclo-oxygenase-2 and caspase were also analyzed by Western immunoblotting. The findings of the present investigation indicate the chemopreventive modalities of the COXIBs, which involve the alteration of intracellular pH along with participation of signaling molecules like Ca^{2+} in the apoptotic process involving both caspase1 and 3.

198

AKL-0707 GHRH Super Analogue in Wasting Syndrome Associated Chronic Kidney Disease: A Phase 2 Randomized, Double-blind, Placebo-controlled Trial

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Introduction: AKL-0707, growth-hormone-releasing-hormone (GHRH) analogue, may be more physiological and safer than pharmacological GH as it stimulates endogenous GH secretion while sustaining its natural pulsatile rhythm. The objective was to determine GHRH efficacy and safety in treating wasting syndrome in patients with chronic kidney disease (CKD). Methods: 28 malnourished patients with CKD have been randomly assigned to 2mg b.i.d. s.c. GHRH (n=12) or placebo (n=14) for 28 days. Endpoints included endocrine testing, subjective global assessment (SGA), and nutritional status. Results: Administration of GHRH induced 393%

increase (p<0.000 GHRH vs. placebo) in GH secretion rate, without disrupting endogenous GH pulsatile rhythm. IGF1 levels increased from 344.98 ± 156.92 to 702.11 ± 179.48ng/ml with no changes in placebo. Body composition changes have been noted in GHRH with fat-free mass gain (DEXA: +1.6 ± 1.11kg p<0.0216; BIA: +3.3 ± 1.24kg p<0.0146) and fat mass loss (DEXA: -0.4 ± 0.89kg p<0.0292; BIA: -1.8 ± 1.07kg p<0.0091). 6/9 patients in GHRH group previously rated mildly/severely malnourished in SGA appeared well nourished after 28 day treatment, with no changes in placebo. Treatment was well tolerated. Discussion: The positive changes in body composition and SGA indicate that AKL 0707 may reverse wasting syndrome in CKD patients treated with GHRH. Although several beneficial trends in the improvement of kidney function have been observed in GHRH-treated patients, none were statistically significant. Conclusion: GHRH analogue administration induced marked improvement of the nutritional status of patients with CKD. Longer duration studies are needed to determine the beneficial effect on renal function.

199

Effect of Ectoine in an Experimental Model of Colonic Inflammation

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Ectoine is a compatible solute found in moderately halophilic bacteria conferring resistance towards stress. It protects skin from dryness and premature aging by stabilizing cell membranes and influencing different downstream signaling pathways involving various mediators, such as ICAM-1. Since Inflammatory Bowel disease (IBD) was also found to involve ICAM-1, it was of interest to study whether ectoine might benefit this condition. An *in vivo* experimental model for IBD has been established in male Wistar rats by giving them intra-colonically 10 mg/kg trinitrobenzene sulfonic acid (TNBS) in 50% ethanol under light ether anaesthesia. This induced the development of lesions, which were then examined macroscopically 4 days later. Ectoine was given orally at different dose levels (30 – 300 mg/kg) for 1 week before TNBS and for the following 4 days. Ectoine reduced the area of lesions and colonic and spleen mass indices, showing a U-shaped dose response relationship, the best effect being observed with 100 mg/kg. Biochemically, it prevented changes in myeloperoxidase activity and reduced glutathione levels in the colon. Ectoine also protected against changes in the level of various mediators, including ICAM-1, TNFα, IL-1β, IL-10, LTB_4, and PGE_2 in both blood and colonic tissue. The effect was comparable to that of sulfasalazine (300 mg/kg), which was used as reference drug. The protective value of ectoine was further confirmed by histopathological examination of the colon. The findings point to a potential therapeutic usefulness of ectoine in inflammatory conditions of the lower gastrointestinal tract.

200

Vitamin K has Preventive Effect against Ethanol-induced Hepatic Stellate Cell Activation

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Introduction: Fibrosis is a key histologic feature that characterizes the progression of alcohol-induced hepatic injury in chronic alcohol abusers. Hepatic stellate cells (HSC) are central to the fibrotic response to liver injury as these cells undergo activation with an increase in extracellular matrix deposition during fibrogenesis. Reactive oxygen species (ROS) have emerged as important stimuli to collagen synthesis in HSC. Therefore, suppression of production of ROS has been proposed as therapeutic strategies for prevention of liver fibrosis. In this study, we investigated the effect of vitamin K on ethanol-induced activation of HSC. Methods: HSC were isolated from male Wistar rats by pronase/collagenase perfusion. HSC were incubated with 100 mM ethanol (EtOH) or 500 microM acetaldehyde (AcCHO). Type I collagen expression, intracellular H_2O_2 levels and lipid peroxidation were measured. Vitamin K (50 microM) were dissolved in DMSO and added with EtOH or AcCHO simultaneously. Results: EtOH or AcCHO was increase of the expression of type I collagen. 4-Methylpyrazole, the inhibitor

of alcohol dehydrogenase, was decreased and the production of ROS and lipid peroxidation. The expression of type I collagen with EtOH or AcCHO was decreased with the addition of vitamin K1 or K2. Furthermore, the production of ROS and lipid peroxidation was suppressed by the addition of vitamin K1 and K2. Conclusion: These results suggest that vitamin K has protective effect against ethanol-induced HSC activation. Vitamin K may be a promising therapeutic means for the management of alcoholic liver diseases.

201

Influence of Anti-IgE Antibody Omalizumab on Airway Remodeling and the Expression of Interleukins in Asthma

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To study the relation between interleukin-4 (IL-4), IL-5, IL-13, transforming growth factor-beta(2) (TGF-beta(2)) and airway remodeling and to investigate the effects of omalizumab on airway inflammation and airway remodeling of asthma. Methods: Thirty five female BALB/c mice were randomly divided into a remodeling group and a treatment group (omalizumab group), with 10 BALB/c mice in each group. The mice were sensitized by ovalbumin (OVA), and only the omalizumab group was treated with omalizumab. The number of total cells and eosinophils in bronchoalveolar lavage fluid (BALF) were counted. Light and electronic microscope were used to detect the pathologic histology and morphologic change. In situ hybridization and reverse transcription-polymerase chain reaction (RT-PCR) were used to measure IL-4, L-5, IL-13, and TGF-beta(2) mRNAs in the lung. Results: The numbers of total cells and eosinophils in BALF of the remodeling group were (5.7±1.3) x 10(5)/ml and 2.43±0.18, while those of the treatment group were (4.1±1.4) x 10(5)/ml and 1.67±0.23, respectively, the difference being significant ($P<0.05$). Histological and electronic microscopic examination showed extensive airway inflammation, notably accumulation of significant numbers of eosinophils and lymphocytes in the remodeling group. In the treatment group, the inflammation was significantly decreased, with decreased production of mucus, decreased collagen and granule of mucus around airway, less proliferation of airway epithelium, smooth muscle hypertrophy and airway spasm. In situ hybridization showed that the expression of IL-13 mRNA and TGF-beta(2) mRNA in the lung of the remodeling group were 22±9 and 18±3 respectively, while those of the treatment group were 16±5 and 9±4. Conclusions: Omalizumab could effectively inhibit airway remodeling and decrease in the expression of IL-13 mRNA and TGF-beta(2) mRNA as well as IL-4 mRNA and IL-5 mRNA in the lung in asthma.

202

Tocotrienol Suppresses NFkB Signaling Pathway & Apoptosis in the Experimental Model of Diabetic Neuropathy

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Diabetic neuropathic pain, an important microvascular complication in diabetes mellitus is recognised as one of the most difficult types of pain to treat. The development of tolerance, inadequate relief and potential toxicity of classical antinociceptives warrant the investigation of the newer agents to relieve this pain. Reactive oxygen species and cytokine induced activation of nuclear factor kappa-B signaling pathway and apoptosis in sciatic nerve are implicated in the pathogenesis of diabetic neuropathy. The aim of the present study was to explore the effect of tocotrienol on nerve functions, oxidative-nitrosative stress, inflammation and apoptosis in streptozotocin induced experimental diabetes. Streptozotocin-diabetic rats developed neuropathy which was evident from significant reduction in motor nerve conduction velocity, nerve blood flow, increased thermal hyperalgesia associated with enhanced oxidative-nitrosative stress, release of inflammatory mediators (TNF, IL-1beta, NFkB) and caspase 3. Chronic treatment with tocotrienol (25, 50 and 100 mg/kg body weight; p.o.) for 4 weeks starting from the 4th week of streptozotocin injection significantly suppressed behavioral, biochemical and molecular changes associated with diabetes. Tocotrienol offered better protection than tocopherol in this experimental

model of diabetic neuropathy. Moreover, diabetic rats treated with insulin-tocotrienol combination produced robust effect on molecular parameters as compared to their per se groups. Taken together, the data reveal that suppression of NFkB signaling pathway and caspase-3 by tocotrienol prevent diabetic neuropathy and point towards the therapeutic potential of tocotrienol in diabetic neuropathic pain.

203

A Novel Designer Natriuretic Peptide, CU-NP, in Human Aortic Endothelial Cells: Evidence for NPR-B Involvement in Cyclic GMP Response

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Introduction: CU-NP is a novel synthetic natriuretic peptide (NP) which consists of the 17-amino-acid ring of human C-type NP (CNP), a NP of endothelial cell origin and an agonist of NP receptor (NPR)-B, and the N- and C- termini of human urodilatin, a NPR-A agonist, which is potently natriuretic unlike CNP which lacks renal actions. The rationale for its design was to minimize hypotension, a dose-limiting side effect of urodilatin, by replacing the ring of urodilatin with that of CNP. We hypothesized that CU-NP would nonetheless retain the ability to activate cGMP via NPR-B. Methods: CU-NP 14.14 pmol/kg/min was infused i.v. in dogs to evaluate cGMP-activating, cardiorenal and neurohumoral effects. In human aortic endothelial cells (HAEC), cGMP response (quantified by radioimmunoassay) was determined by incubating CU-NP for 10 min with or without an antibody to the ligand-binding domain of NPR-B. Results: In anesthetized dogs, CU-NP ($P<0.05$) activated cGMP, enhanced natriuresis and diuresis, lowered PCWP, and inhibited the renin-angiotensin-aldosterone system (RAAS) without inducing hypotension. In HAEC, CU-NP and CNP (10^{-6} M) increased cGMP (mean±SEM, pmol/mL) to 0.30±0.02 and 0.17±0.04, respectively ($P<0.01$, CU-NP *vs* CNP; $P<0.001$, CU-NP *vs* control; $P<0.001$, CNP *vs* control). In the presence of the NPR-B antibody (1:100), the cGMP responses were attenuated: 0.19±0.02 and 0.08±0.01, respectively ($P<0.01$ CU-NP and $P<0.05$ CNP *vs* no antibody). Discussion and conclusion: CU-NP activates cGMP *in vivo* and *in vitro*, the latter involving NPR-B in HAEC. These favorable cardiorenal and RAAS-inhibiting actions linked to cGMP activation support a potential therapeutic role for CU-NP.

204

Protective Effects of Mildronate in Experimental Model of Type 2 Diabetic Goto-Kakizaki Rats

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Mildronate [3-(2,2,trimethylhydrazinium)propionate] is an anti-ischemic and cardioprotective drug which mechanism of action is based on its regulatory effect on pathways of carnitine biosynthesis and uptake mechanisms. It has been shown that under ischemic conditions mildronate treatment shifts the myocardial energy metabolism from fatty acid oxidation to the more favorable glucose oxidation. This study was carried out to investigate whether the long-term mildronate treatment could influence the glucose level and prevent diabetic complications in experimental model of type 2 diabetic Goto-Kakizaki (G-K) rats. G-K rats were perorally treated with mildronate at a dose of 200mg/kg daily for 4-weeks. Mildronate treatment significantly decreased the carnitine concentration in rat plasma and gradually decreased the blood glucose concentration from 13 to 9.2 mM. Mildronate strongly inhibited fructosamine accumulation and loss of pain sensitivity, as observed in tail-flick latency test. Mildronate treatment ameliorated the enhanced contractile responsiveness of G-K rat aortic rings to phenylephrine. In addition, the anti-ischemic action of mildronate was confirmed in isolated G-K rat heart ischemia-reperfusion model where in mildronate-treated hearts the necrosis zone was significantly decreased for 31%. These results demonstrate for the first time that mildronate treatment possesses cardioprotective effect, decreases blood glucose concentration and prevents loss of pain sensitivity in experimental model of type 2 diabetic G-K rats. This finding

indicates that the mildronate treatment could be beneficial in diabetes patients with cardiovascular problems.

205

TERTAV: Efficacy of Recombinant Streptokinase Administration on Thrombosis of Hemodialysis Access Fistula. Multicenter, Open Study

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Objective: To know the effect of recombinant streptokinase (rSK) in reestablishing adequate blood flow rates through thrombosed arte riovenous shunt. Site: Hemodialysis services from 7 hospitals of Cuba. Intervention: rSK (Heberkinasa, Heber-Biotec, Havana) in a one-hour infusion through the distal portion of vascular access until its patency or a total dose of 1, 000,000 IU in 100 mL saline. Results: 22 consecutive chronic hemodialysis patients were included. Recovery of the vascular access was achieved, with signs of patency in 16 patients (72.7%), 9 of them (40.9%) totally. Treatment was unsuccessful in 6 patients (27.2%), two of them did not receive the infusion due to adverse events. rSK was effective in establishing blood flow rates ≥200 mL/min after one month in 13 cases (59.1%) since 3 of the responders had restock. Major hemorrhagic events were observed in one patient (4.5%) and minor bleeding in 5 (22.7%). rSK appears to be effective for reestablishing adequate blood flow rates through hemodialysis fistula that are thrombosed or have low blood flow rates.

206

Disease Progression in MRL/lpr Lupus-prone Mice is Reduced by a Specific PDE4 inhibitor

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Cyclic nucleotide phosphodiesterase isozymes (PDE1 to PDE11), which specifically hydrolyse the second messengers cAMP and cGMP, play a major role in the control of normal and pathological cell responses. The PDE4 family, cAMP-specific, is mainly present in the cells of immune system and is implicated in inflammation and oxidative stress. For pharmaceutical companies, PDE4 represents a new therapeutic target in inflammation. PDE4 might thus contribute to the development of autoimmune diseases such as systemic lupus erythematosus. Therefore we investigated the effect of NCS613, a specific PDE4 inhibitor we conceived and characterized as an anti-inflammatory compound, in comparison with the anti-inflammatory compound, pentoxifylline, on disease progression in MRL/lpr lupus-prone mice.Three groups of 5 week-old female MRL/lpr mice (n=10) were evaluated: Control, NCS613 (30µg/100µL) and pentoxifylline (100µg/100µL), injected i.v. at weeks 5, 7, 9 and 13 of age. Mice were maintained and observed until death. In contrast to pentoxifylline, which did not significantly overcome lupus disease, NCS613 increased survival rate of mice ($P<0.0052$), significantly decreased proteinuria (-23%) and anti-dsDNA antibody (-55%) levels even 10 weeks after the last injection. TNFalpha-secretion by MRL/lpr PBMCs measured at 11 and 14 weeks in response to LPS was decreased after NCS613 and pentoxifylline treatments. Pentoxifylline (IC50=135µM) was without PDE4 specificity and 3200 less potent than NCS613 (IC50=0.042µM). This study shows that, contrary to pentoxifylline, the PDE4 inhibitor, NCS613, is effective against lupus progression and suggests that NCS613 could be helpful for lupus treatment, opening thus a new therapeutic way.

207

Antinociceptive Activity of Methanolic Extract of *Epilobium hirsutum*

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Antinociceptive activity of methanolic extract of aerial parts of *Epilobium hirsutum (EH)* was determined in the Hot plate and writhing tests in mice. Nearly all extracts showed a dose dependent and marked analgesic activity in mice in the thermal and chemical models of analgesia when compared to the control. Methanol extract at dose of 500 mg/kg showed higher activity (97.7 % writhing inhibition) than diclofenac 50 mg/ kg i.p. (77.8 %, $p < 0.05$) and morphine 5 mg/kg i.p. (91.2 %, $p < 0.05$). Methanol extract, in all tested doses (200-500 mg /kg) significantly increased the pain threshold in hot plate test ($p < 0.05$). *EH* extract at 200 mg/ kg showed a similar effect to morphine at 5 mg/ kg. These finding indicate the potential therapeutic use of methanolic extract of aerial parts of *EH* as a potent antinociceptive agent. LD_{50} was obtained 1.5 ± 0.1 g/ kg. *EH* extract did not induce locomotor impairment in mice at any tested doses.

208

Protective Effect of the Extract of *Eclonia Cava* on Ethanol-induced Hepatic Injury

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Introduction: Alcohol-induced liver disease is the most common hepatic disease in western countries. Ethanol affects the antioxidant balance of hepatocytes. Ethanol-induced oxidative stress is the consequence of the combined effect of an increased production of reactive oxygen species (ROS) by the mitochondria and the alcohol-inducible cytochrome P-4502E1 and the impairment of antioxidant defenses. *Eclonia cava* is classified under the Laminaria family of brown algae. Several algal species have been reported to prevent oxidative damage by scavenging free radicals and active oxygen and hence able to prevent the occurrence of cancer cell formation. In this study, we investigated the cytoprotective effect of the extract of *Eclonia cava* (EEC) on ethanol-induced hepatic injury. Methods: Hepatocytes were isolated from male Wistar rats by collagenase perfusion. The cells were cultured with or without ethanol (100 mM) and EEC. EEC (0~50 microgram/ml) was added to culture medium with ethanol simultaneously. Cell viability, lipid peroxidation and intracellular glutathione (GSH) were measured. Results: Ethanol significantly decreased the cell viability of rat hepatocytes. However, the addition of EEC (12.5-50 microgram/ml) reversed the cell viability to control level. Ethanol increased the production of lipid peroxidation in time-dependent manner. Intracellular GSH levels in ethanol-treated hepatocytes were significantly decreased. On the other hand, EEC decreased the production of lipid peroxidation significantly. Furthermore, intracellular GSH levels in EEC-treated hepatocytes were increased to the control levels. Conclusion: EEC is a good food component for the prevention from ethanol-related hepatic injury.

209

Differential Involvement of COX Isoforms on Neutrophil Chemotaxis in Experimental Models of Inflammation

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It was shown previously that pretreatment using celecoxib, a selective cyclooxygenase (COX-2) inhibitor or indomethacin, a non-selective (COX) inhibitor, reduced LPS-induced leukocyte migration to the rat peritoneal cavity. Here, we investigated whether celecoxib (12mg/kg, Cx) or indomethacin (2 mg/kg, Indo) would reduce neutrophil migration induced by fMLP in a classic chemotactic assay (Boyden chamber). Cx and Indo (Controls = 26.6±1.45, Cx =12.8±3.04, Indo= 6.26±2.19 cells/field) inhibited chemotaxis induced by fMLP. A mouse cremaster preparation was used to assess, via intravital microscopy, the microvasculature to further investigate which step of cell recruitment was affected by drugs. Cx and Indo also inhibited leukocyte migration induced by 50 µg/Kg LPS injected in this model. However, Cx effect was associated with reduced rolling and adhesion of the cells, whereas Indo was only effective to inhibit cell adhesion. Furthermore, pretreatment using SC 560, in either normal or LPS-challenged scrotal cavities, did not alter leukocyte migration or cell adhesion, but it did enhance the rolling activity of the leukocytes in both cases. Taken together, these results indicate that: 1) the activity of COX-1 is mainly related with traffic of leukocyte under physiological conditions, and 2) COX-2 activity is

mainly related with traffic of cells under inflammatory conditions in vascular beds. Our data also suggested a possible effect of selective COX-2 inhibitor on the expression of adhesion molecules.

210

Trace Amine-associated Receptor 1 is a Potential Target for Addiction and Psychiatric Therapeutics

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In brain, biogenic amines activate both postsynaptic and presynaptic receptors prior to reuptake by dopamine, norepinephrine and serotonin transporters (DAT, NET and SERT). Newly discovered trace amine-associated receptor 1 (TAAR1) is expressed in brain monoaminergic areas and is activated by a spectrum of biogenic amines and amphetamines. Our research has revealed that TAAR1 activation leads to cyclic AMP accumulation, resulting in cellular phosphorylation cascades and changes in monoamine transporter kinetic function. In transfected cells, TAAR1 activation elevates cyclic AMP and inhibits uptake of [^{3}H]dopamine, [^{3}H]norepinephrine and [^{3}H]serotonin by DAT, NET and SERT, respectively, whereas activation of monoamine autoreceptors (D2s, alpha2A and 5HT1B) inhibits cyclic AMP and enhances uptake by DAT, NET and SERT. Our studies in brain synaptosomes found that the biogenic amines co-activate monoamine autoreceptors and TAAR1 in a balanced manner, whereas the psychostimulant methamphetamine activates TAAR1 but not autoreceptors, resulting in inhibition of [3H] monoamine uptake in synaptosomes of rhesus monkeys and wild type mice. However, in synaptosomes prepared from TAAR1 knockout mice, this inhibition does not occur, demonstrating that TAAR1 mediates methamphetamine effects in brain. Together, these results provide evidence that TAAR1 functions in brain as a receptor that regulates presynaptic monoamine transporter function. In this regard, novel compounds which target TAAR1 may be efficacious modulators of monoamine transporters, with properties distinct from classic monoamine transporter inhibitors (e.g., antidepressant SSRIs, the anti-hyperactivity medication methylphenidate). Also, our findings suggest a potential target for development of novel therapeutics for psychostimulant addiction.

211

Inhibitory Effects of Adiponectin on Cell Migration Induced by Insulin-like Growth Factor-1 in Vascular Smooth Muscle Cells

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Insulin-like growth factor-1 (IGF-1) is known to cause vascular smooth muscle cell (VSMC) migration. It is implied that IGF-1-induced VSMC migration is involved in atherosclerosis. On the other hand, adiponectin (APN) which was secreated from adipose tissue has been identified as one of the adipocytokines. It was suggested that APN suppresed insulin-resistance and atherosclerosis. In the present study, we investigated that the effects of adiponectin on IGF-1-induced cell migration and its intracellular signaling pathway in rat aortic smooth muscle cells (RASMCs). Cell migration was evaluated by a modified Boyden chamber assay, and intracellular signaling was determined by Western blotting analysis. It was observed that IGF-1 stimulated RASMC migration in concentration-dependent manner (from 1 to 100 ng/ml). IGF-1-induced RASMC migration was inhibited by 1 and 10 μg/ml of APN by 18 ± 5% and 28 ± 7%, respectively. IGF-1 also activated extracellular signal-regulation kinase ½ (ERK1/2) and Akt in a concentration-dependent manner (from 1 to 100 ng/ml) in RASMCs. APN significantly attenuated IGF-1-induced ERK1/2 activation, but not Akt. IGF-1-induced RASMC migration was suppressed by PD98059, a MEK inhibitor. In addition, APN induced the activation of AMP-activated protein kinase (AMPK) in RASMCs. Moreover IGF-1-induced ERK1/2 activation was inhibited by AICAR, an AMPK activator in RASMCs. These results suggested that the inhibitory effects of APN on IGF-1-induced VSMC migration were caused by ERK1/2 inhibition via AMPK activation.

212

Chronic Inhibition of Inducible Nitric Oxide Syntase (iNOS) Improves Cardiovascular Function in Streptozotocin (STZ) Induced Diabetes

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Experimental and clinical studies have indicated a direct link between chronic hyperglycemia and depressed cardiovascular function. Studies in the STZ diabetic rat model of Type 1 diabetes have demonstrated the development of cardiovascular abnormalities such as depressed heart function, mean arterial blood pressure (MABP) and heart rate (HR), endothelial dysfunction and attenuated pressor responses to vasoactive agents. We hypothesized that iNOS causes cardiovascular abnormalities in STZ diabetic rats, which can be corrected by inhibiting iNOS. Control and diabetic rats (STZ, 60mg/kg i.v.) were treated orally with L-NIL, a specific inhibitor of iNOS (3 mg/kg) for 8 weeks. At termination MABP, HR and pressor responses to methoxamine (100-300 nmol/kg) were measured in freely moving conscious rats. Further, cardiac performance was evaluated in isolated working hearts. Heart, aorta and mesenteric arteries were collected for western blotting and immunohistochemical localization of iNOS, eNOS and nitrotyrosine. Diabetic rats showed depressed cardiac performance (left ventricular pressure, -dP/dT, +dP/dT), MABP and HR, and attenuated pressor responses to methoxamine that were all improved by treatment with L-NIL. Further, decreased eNOS and increased iNOS expression (and activity) were associated with elevated nitrotyrosine in superior mesenteric arteries and hearts of untreated diabetic rats. L-NIL treatment reduced the expression and activity of iNOS, and nitrotyrosine levels and significantly improved eNOS expression in the heart and superior mesenteric arteries. The results suggest that iNOS plays a critical role in the pathophysiology of cardiovascular abnormalities in rats following diabetes.

213

Differential Arterial Adaptation to Simulated Microgravity is Associated with Changes in eNOS Activity

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Orthostatic intolerance (OI) is observed following exposure to microgravity, and may occur in the absence of hypotension. Based on previous *in vivo* studies, differential adaptation of the control of vascular tone by nitric oxide (NO) in the cephalic and caudal vasculature has been proposed as a mechanism underlying this phenomenon. The objective of this study was to investigate differential changes in the L-arginine/NO pathway using a model of simulated microgravity (14 day head down tilt; HDT). NO production from carotid and iliac arteries from control and HDT rats was measured in response to stimulation with acetylcholine in the presence and absence of L-NAME and the iNOS-selective inhibitor 1400W. Expression levels of eNOS and iNOS were also compared using western blotting and immunohistochemistry. NO production was similar in iliac arteries, but decreased in carotid arteries from HDT rats compared with controls. L-NAME decreased NO production similarly in both groups, while 1400W had no effect. Neither eNOS nor iNOS expression was different between groups. It was concluded that differential adaptation of the L-arginine/NO pathway in response to simulated microgravity likely reflects changes in eNOS activity. A decreased contribution of eNOS to cephalic vascular tone may compromise cerebral autoregulatory vasodilation during orthostatic challenge, and represents a potential mechanism underlying OI following microgravity exposure.

214

Effect of an Antioxidant on Diet-induced Obesity in Mice

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Metabolic syndrome is defined as a cluster of development of type2 diabetes, heart disease, and hypertension. Obesity is of central importance in

pathophysiology and clinical diagnosis of metabolic syndrome. However, the molecular mechanisms that regulate the formation of adipose tissue are not fully understood. Growing evidence suggests that reactive oxygen species, such as superoxide anions and hydrogen peroxide, act as important secondary messengers in intracellular signal transduction pathways for several functions, such as proliferation and differentiation. In the present study, we investigated whether reactive oxygen species (ROS) is involved in adipocyte formation both in vitro and in vivo. An antioxidant N-acetyl-L-cysteine (NAC) was found to block adipocyte differentiation in mesenchymal cell line C3H10T1/2 cells. DCF assay revealed that differentiation-inducing agents induced ROS generation in the cells. We next extend our observations in vitro to an in vivo animal model. A high-fat diet (HFD) is known to lead to obesity and the development of the metabolic syndrome. We tested whether NAC affect amount of adipose tissue in HFD-fed mice. Administration of NAC in drinking water reduced body weight and amount of adipose in mice fed HFD. In contrast, NAC did not reduce the body weight in mice fed a normal fat diet. The level of food intake was not significantly changed among the groups of mice. These results strongly suggest that ROS work as a regulator of the adipogenesis and obesity. Our findings will provide new insight into the pathogenesis and drug development for metabolic syndrome.

TUESDAY, JULY 29, 2008
STREAM 2:
FROM FUNDAMENTAL TO CLINICAL PHARMACOLOGY

215

Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of M118, a Novel Rationally Engineered Low Molecular Weight Heparin Given as a Subcutaneous Injection in a Rising Dose Cohort Regimen in Healthy Subjects

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Introduction: M118 is a novel, rationally engineered LMWH with attributes of monitorability, reversibility and potent anti-Xa (aXa) and anti-IIa (aIIa) activities in development as adjunctive pharmacotherapy for Acute Coronary Syndromes (ACS). This study evaluated the pharmacokinetic (PK) and pharmacodynamic (PD) properties of M118 following a single subcutaneous (SC) administration in normal volunteers. Methodology: 36 healthy males received escalating SC doses of M118 (25, 50, 75, 100, 125 or 150 aXa IU/kg) in cohorts of 6 subjects, 4 active/2 placebo (saline). Blood samples for determination of ACT, aPTT, INR, aXa and aIIa activities, and other safety labs were collected for 36 hours. Results: M118 kinetics were best described by *flip-flop* characteristics. A_{max} values for aXa and aIIa ranged from 0.08 – 0.98 and 0.09 – 0.48 IU/mL, respectively. Plasma aXa and aIIa activities were highly correlated ($R^2 \geq 96\%$). aXa and aIIa activities were correlated ($R^2 \geq 76\%$) to the ACT levels at doses ≥75 IU/kg. Maximum APTT and ACT change from baseline were different from placebo from 75 to 150 IU/kg and 125 to 150 IU/kg, respectively. INR remained within normal range at all doses. No severe or serious AE's occurred. Injection site erythema was the most frequently reported AE. Conclusion: Single-dose SC injection of M118 was safe and well tolerated up to 150 IU/kg. PD effects were correlated to PK at dose levels ≥75 IU/kg supporting the use of PD markers as a surrogate to monitor plasma levels of M118. These results support further clinical development of M118 in ACS.

216

In Silico Formulation Development: Accelerating the Path to Combination Products

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Recent efforts in the pharmaceutical and biotech industry have focused on pairing two drugs in a single controlled release capsule or tablet as a means of improving patient compliance or increasing therapeutic benefit. The development of a fixed-dose combination product, however, must overcome a variety of manufacturing and formulation challenges. In some cases, maximal therapeutic and commercial benefit is only

obtained by combining two drugs with very different pharmacokinetic and physical chemical properties. To both accelerate and reduce the risk of developing these 'hard to formulate' combination drugs, we utilize in silico modeling techniques to explore the impact of different formulation strategies prior to initiating laboratory work. The goal of the current study was to determine if a single formulation could deliver a BCS Class I (highly soluble; highly permeable) and a BCS Class III (highly soluble, poorly permeable) compound as a once a day dose. Individual absorption models were generated based on the physiochemical and permeability properties and a range of in vitro dissolution profiles were input into the model. The simulation predictions were then evaluated and optimized through iterative modeling to isolate a single release profile that could generate the desired systemic exposure levels for the two drugs in combination.

217

Population Pharmacokinetic Analysis of Ziprasidone in Korean with Sparsely-sampled Data Using "Prior" Subroutine of the NONMEM

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Introduction The aim of this study is to find steady-state AUC (24h) of ziprasidone, an atypical antipsychotic agent, with one-point blood sampling to estimate the exposure-response relationship in patients. Methods The study population consisted of 59 Korean outpatients without somatic disorder who have been given ziprasidone orally for more than a month. A population pharmacokinetic analysis was performed using the NONMEM (Ver. 6.0). A one compartment linear model with first-order absorption was adopted to find a base model. A generalized additive modeling was done for elucidating the relationship between pharmacokinetic parameters and covariates. Then, the "Prior" subroutine was applied to the analysis. The parameters used as prior data were obtained from a literature. Clearance, volume of distribution and absorption rate constant were estimated. Results A total of 59 ziprasidone concentrations were available for population pharmacokinetic analysis. Additive error model and FOCE-I estimation method provided the best base model. There was no significant covariate that had correlation with pharmacokinetic parameters. The model improved as the "Prior" was applied. The final estimate of clearance was 45.5L/hr, volume of distribution was 256L, and absorption rate constant was 0.59hr-1. Discussion The newly introduced "Prior" subroutine appeared to be a powerful tool for analyzing sparsely sampled data. Although the analysis was performed with only one point of plasma concentration per patient, NONMEM successfully estimated the parameters. The AUC calculated hereafter is to be used to estimate the exposure-response relationship. Conclusion Pharmacokinetic parameters were stably estimated by using "Prior" subroutine although the data were sparse.

218

Changes in Serum Testosterone Levels after the Administration of Testosterone Enanthate in Healthy Volunteers

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Background: In our previous study, healthy volunteers were given 500 mg testosterone enanthate, and the urinary excretion of testosterone glucuronide was monitored for 15 days. It was noted that the profile of testosterone could be divided in three groups, "slow, fast and ultrafast" rising groups, depending on the slope of the excretion curves. Moreover the peak was 5 times higher in the ultrafast rising group than the slow rising group. Here we have investigated the serum pattern of testosterone and its metabolites prior to testosterone injection (day 0) and two days after administration. Hypothesis: Individuals belonging to the ultrafast rising group display altered levels of serum testosterone after testosterone administration. Methods: Blood testosterone and dihydrotestosterone concentrations were measured by GCMS and the glucuronidated androgen metabolites (androstanediol-17-

glucuronide, androsterone-glucuronide, and etioG) were analysed by LCMSMS. Results: On day 0 there was no difference in serum testosterone or metabolites between the three groups, whereas on day 2 significant differences were observed. Individuals belonging to the slow group increased their testosterone levels from 4.7 ng/mL to 7.5 ng/mL. The ultrafast group increased their testosterone levels to 25 ng/mL. All testosterone metabolites measured were also higher in the ultrafast group. Discussion: The reason for this discrepancy in the bioavailability of testosterone is not known but the distinct division into three groups suggests a genetic explanation. One may speculate that the 3-fold higher levels of systemic testosterone in the ultrafast group may be associated with altered anabolic, androgenic effects or adverse reactions. Conclusion: There is large inter-individual variation in serum testosterone levels after testosterone enanthate administration.

219

Acetylation and Oxidation Phenotypes in Patients with Bronchial Asthma

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Acetylation and oxidation phenotypes in patients with bronchial asthma may be related to asthma phenotypes and asthma severity. Also they can predict possible drugs interaction, effectivenes and toxicity. Metabolic ratio for sulfadimesine (substrate of N-acetyltransferase 2) and amidopirine (substrate of CYP2B, 2C and 3A) with subsequent determination of acetylation and oxidation phenotypes was studied in 253 patients with bronchial asthma and 268 healthy individuals. Patients with bronchial asthma demonstrated bimodal distribution of acetylation phenotypes similar to healthy population with poor : extensive acetylators ratio 52.6% : 47.4% and trimodal distrtibution of amidopirine oxidation with poor: intermediate: extensive metabolizers ratio – 63.0%: 31.9%: 5,1% in asthma patients and 48.8% :47.3% :3.9% in healthy group. Prevalence of poor metabolizers was significally higher among asthma patients ($p<0.05$). We did not find strong correlation between phenotypes of metabolism and asthma severity but in poor acetylators asthma manifested earlier and had longer duration. Patients with moderate-to-severe asthma tended to be poor amidopirine metabolizers. In poor acetylators asthma attacks were more often triggered by respiratory infections ($p<0.001$) and food allergy ($p<0.02$) compared with extensive acetylators when asthma attacks were more often triggered by metheorological factors ($p<0.05$). Among poor amidopirine metabolizers asthma attacks were more often triggered by metheorological factors ($p<0.01$) compared with moderate and extensive metabolizers. Respiratory infections, irritants and metheorological factors triggered asthma attacks in all patients which had poor acetylation / poor oxidation phenotypes. Generally patients with poor acetylation / poor oxidation phenotypes admitted the maximum of triggers, which suggested poor asthma control. Phenotypes of metabolism (acetylation and oxidation) associated with severity of bronchial asthma and different factors triggered asthma exacerbation. Prevalence of poor amidopirine metabolizer among patient with bronchial asthma should be considered when prescribing drugs with similar metabolic pathways.

220

CRO/CPU–Cardiac Safety Core Lab Collaborations – from the Individual Project to a Formal Collaboration – Keys to Success

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Session attendees will understand the key factors that lead to effective collaboration across a project (contracting to study execution and data submission) as well as the cornerstone of a formal collaboration. As an industry, we require the integration of many services in the conduct of clinical research studies. The foundation for successful collaborations whether for an individual project or a formal relationship are based on clear lines of communication, expectations, defined process, shared ownership of the success of the project. From the individual project the goals are more immediate, while for the formal collaboration, there needs to be a strategy to reach the shared goals, whether they are financial, operational or a combination of both. With the formal collaboration, it is not enough to say or send out a press release

regarding a partnership. There needs to be a formal implementation and/or business plan in place in order to achieve a shared success. Both groups, independent of the size of the organization must be willing to commit resources in order to meet the shared goals. This is also experienced on project by project basis and in fact may evolve organically into an informal partnership. The end goal is a project that runs well and meets the expectations of the sponsor and can be flexible to meet the changing research environment whether on a project specific or formal service relationship agreement.

221

Cardiac Expression of P-gp and octn Transporters in Doxorubicin Treated Mice

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Background: The efflux transporter P-glycoprotein (P-gp) has been identified in a variety of tissues in man and mouse and shown to transport a multitude of drugs, among them doxorubicin, which therapeutic use is limited by cardiotoxic side effects (cardiomyopathy). In this context, cardiac expression of octn1 (slc22a1), octn2 (slc22a2) and octn3 (slc22a21) may be of interest too, because of their function in cellular uptake of antioxidants like carnitine or ergothioneine. We therefore investigated the cardiac expression of these transporters in doxorubicin treated mice. Methods/Results*:* C57Bl/10 mice were treated with doxorubicin (20 mg/kg body weight, intraperitoneally). Hearts of these mice as well as of untreated control animals were investigated after one, three and five days of doxorubicin administration. Gene expression was measured using real-time PCR technology. Here, we could demonstrate a significant increase of P-gp (mdr1a) after doxorubicin administration with the most pronounced effect after 3d (1d: 139%, 3d: 161%, 5d: 80% of control). While all octn transporters could be detected in murine heart, only octn1 was significantly regulated (1d: 193%, 3d: 164%, 5d: 112% of control). Additional *in vitro* experiments using the murine cardiomyocyte cell line HL1 confirmed the effect of doxorubicin on P-gp expression both at the expressional and functional level. Conclusion: The mRNA-expression of P-gp, octn1, octn2 and octn3 could be demonstrated in normal and doxorubicin treated murine heart samples. We observed an enhanced mRNA expression for P-gp (mdr1a) and octn1 after doxorubicin exposure. These findings may be important for intracardiac concentrations of doxorubicin and antioxidants like ergothioneine.

222

Evaluating the Influence of Herbal Medicines on Human Cytochrome P450 Activities by a Cocktail Approach: Angelicae tenuissima, Angelicae dahuricae, Scutellariae baicalensis

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Introduction: We investigated the effect of three herbal medicines (*Angelicae tenuissima, Angelicae dahuricae and Scutellariae baicalensis*) on human cytochrome P450 activities. Methods: Twenty-four healthy male subjects were assigned to one of three treatment groups (*A. tenuissima, A. dahuricae or S. baicalensis*), each consisting of 8 subjects. A cocktail of probe drugs for CYP enzymes was orally administered before and after multiple administrations of herbal medicines, three times a day for 13 days. Probe drugs were caffeine 100 mg (CYP1A2), losartan 50 mg (CYP2C9), omeprazole 40 mg (CYP2C19), dextromethorphan 30 mg (CYP2D6), chlorzoxazone 400 mg (CYP2E1) and midazolam 7.5 mg (CYP3A4). The probe drugs and their metabolites were quantified in plasma or urine. Changes in CYP activities were evaluated by metabolic ratios of the probe drugs at reference time points following the herbal medication period, compared to the baseline values. Results: *A. dahuricae* significantly decreased CYP1A2 activity to 10% of baseline (95% confidence interval; P-value, 0.05-0.21; P<0.001). Compared to baseline values, the metabolic activities were decreased to 71% (0.54-0.94; P=0.024) for losartan (CYP2C9) and 63% (0.40-0.99; P=0.046)

for midazolam (CYP3A4) after administration of *S. baicalensis*. In addition, *S. baicalensis* showed a 1.42-fold (1.03-1.97; P=0.039) increase in chlorzoxazone metabolic activity (CYP2E1*). A. tenuissima* did not significantly affect CYP activities. Conclusion: Changes in certain CYP activities were observed after the administration of *S. baicalensis and A. dahuricae* in healthy volunteers. Therefore, herbal medicines containing *S. baicalensis* or *A. dahuricae* are candidates for further evaluation of CYP-mediated herb-drug interactions in humans.

223

Repeated Administration of ET_B Receptor Agonist, IRL-1620, Produces Tachyphylaxis to Only its Hypotensive Effect

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IRL-1620 a highly selective ET_B receptor agonist, selectively and transiently increases tumor blood flow and has been shown to enhance tumor delivery and efficacy of anticancer drugs. An IND application for the use of IRL-1620 in patients with recurrent or progressive carcinoma has been recently cleared by the USFDA for a phase I clinical trial. The effect of acute repeated administration of IRL-1620 on blood pressure, heart rate and blood flow (renal and cerebral) has not been studied. The present study was conducted in urethane anesthetized rats to determine the cardiovascular effects of acute repeated intravenous administration of IRL-1620 in cumulative doses of 1.6 µg/kg, 5.0 µg/kg and 15.0 µg/kg at 60 min intervals. It was found that IRL-1620 did not affect heart rate (352 ± 12 beats/min at baseline and 356 ± 9 beats/min following IRL-1620 treatment). IRL-1620 produces a transient fall in blood pressure. A fall in mean blood pressure of 20.46% with 1.6 µg/kg, 23.43% with 5.0 µg/kg and 2.69% with 15.0 µg/kg dose of IRL-1620 was observed. IRL-1620 produced a decrease in renal blood flow of 28.37%, 17.22% and 22.22% with 1.6, 5.0 and 15.0 µg/kg dose, respectively. On the other hand IRL-1620 produced an increase in cerebral blood flow of 23.64%, 16.29% and 24.58% with 1.6, 5.0 and 15.0 µg/kg dose, respectively. The findings indicate development of tachyphylaxis to IRL-1620 only to the fall in blood pressure (mediated through NO), while decrease in renal and increase in cerebral blood flow were not affected. It is concluded that acute repeated administration of IRL-1620 can be carried out without affecting blood flow.

224

Role of Nitric Oxide (NO) during Stress and its Potential as a Target Molecule for Drug Development

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Stress is any emotional/environmental stimulus that is capable of disrupting the physiological homeostasis, and adaptogens help to cope with pathophysiological states precipitated by such aversive situations. Nitric oxide (NO) has been recognized as a neuroimmunomodulator and the present study investigated the possible role of NO as a modulator of stress-induced behavioral, neuroendocrinal and immunological responses in rats. Restraint stress (RS, for 1 or 6h) induced (a) neurobehavioral suppression in the elevated plus maze test, (b) elevations in plasma corticosterone, and (c) modulated levels of pro- (TNF-alpha, IL-6 and IL-1 beta) and anti-inflammatory (IL-4) cytokines, in a stressor intensity dependent manner. These changes were associated with alterations in plasma and brain NO metabolites (Nox) and oxidative stress markers (MDA and GSH). Pretreatment of rats with the NO mimetics, L-arginine and isosorbide dinitrate, attenuated most of stress responses, whereas the NO synthase inhibitors, L-NAME and 7-nitroindazole tended to aggravate them. Repeated exposure to RS induced differential degrees of adaptation to the stressor, which was more apparent during the RS (1h) as compared to the RS (6h) paradigm. Pharmacological modulations of NO levels by drugs induced predictable changes in stress markers and these were supported by brain Nox, MDA and GSH data. The results of the study suggest that NO may act as a complex regulatory molecule during stress and could be a potential target for drug development for the treatment of stress and stress related disorders.

225

ABCB1 Polymorphisms Influence Steady State Plasma Levels of Risperidone Active Moiety

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Risperidone, a widely used antipsychotic drug, is metabolized to its active metabolite, 9-hydroxyrisperidone, mainly by the cytochrome P450 enzymes (CYP) 2D6 and 3A4/5. The antipsychotic effect is assumed to be related to the concentration of the sum of risperidone and 9-hydroxyrisperidone, the active moiety. Both risperidone and 9-hydroxyrisperidone are substrates of P-glycoprotein, a transport protein involved in drug absorption, distribution and elimination. We studied the influence of polymorphisms in genes encoding CYP3A4/5 and P-glycoprotein (*ABCB1)* in a patient population previously evaluated for the influence of CYP2D6 polymorphisms on the steady state plasma levels of risperidone, 9-hydroxy-risperidone and the active moiety. Forty-six schizophrenic patients treated with risperidone for 4- 6 weeks were genotyped and their plasma levels of risperidone and 9-hydroxyrisperidone were measured. Dose corrected plasma levels (C/D) of risperidone, 9-hydroxy-risperidone and active moiety showed up to 68-, 9-, and 10-fold variation, respectively. Patients with the *3435T/T, 2677T/T, 1236T/T ABCB1* genotype had significantly lower levels of 9-hydroxyrisperidone C/D and active moiety C/D than patients with other *ABCB1* genotypes (p=0.026 and 0.028, respectively). None of the patients carried *CYP3A4* variant alleles while six patients had *CYP3A5*1*3* genotype. The *CYP3A5* genotype did not influence risperidone C/D, 9-hydroxyrisperidone C/D or active moiety C/D levels. The *CYP2D6* genotype associated with risperidone C/D (p=0.0012) but not with 9-hydroxyrisperidone C/D (p=0.5) or active moiety C/D (p=0.8). The *CYP2D6* genotype has a significant effect on steady state plasma levels of risperidone, while *ABCB1* polymorphisms have an effect on steady state plasma levels of 9-hydroxy-risperidone and the active moiety.

226

Effects of Glyceryl Trinitrate on Vascular Nitrite Accumulation and Expression of the ATP-binding Cassette Transporter, ABCA3

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Glyceryl trinitrate (GTN) has been used therapeutically for over 100 years to treat heart conditions such as angina pectoris and heart failure. A major product of GTN biotransformation is inorganic nitrite anion, the intracellular oxidation of which may lead to the formation of tyrosine-nitrated proteins and cellular damage. Nitrite transport in bacteria is mediated by ATP-binding cassette (ABC) transporters, and immunohistochemical studies indicate the presence of ABCA3 in rat aortic smooth muscle and endothelial cells. Our objective was to assess whether changes in ABCA3 mRNA expression or nitrite transport occur during chronic exposure to GTN. Accordingly, male Sprague-Dawley rats were exposed to 0.4 mg/hr GTN for 48 hours to induce GTN tolerance, and the aortas removed. ABCA3 mRNA levels were assessed by real-time or semi-quantitative RT-PCR; nitrite transport was assessed by measuring nitrite efflux during a 30 minute incubation of aortic segments in Krebs' solution. In control aorta, endothelium removal resulted in a 25% decrease in ABCA3 mRNA levels, indicating that the transporter is highly expressed in endothelial cells. Furthermore, mRNA levels were decreased by 50% in aortas from GTN tolerant animals, and this was associated with both an increase in nitrite accumulation and a decrease in nitrite efflux from tolerant aorta. These findings indicate that chronic GTN exposure results in altered expression of ABCA3, and that this is associated with impaired nitrite transport in blood vessels from tolerant animals.

227

Prediction of Myelosuppression and Nephrotoxicity of Anti-tumor Platinum Derivative in Rats and Human

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Introduction: The platinum antitumor drugs, cisplatin, carboplatin, nedaplatin and oxaliplatin, differ in their toxicities. The relationships between pharmacokinetics of these platinum drugs and developed parameters for predicting their nephrotoxicity and myelosuppression were investigated. Methods: The drugs were administered to male Wistar rats by intravenous bolus injection or infusion, and total clearance and the apparent ratio of tissue concentrations of unchanged drug to plasma concentration (Kp_{app}) at steady state were determined. Apparent hydrolysis rates of each drug were determined by *in vitro condition*. Nephrotoxicity and myelosuppression were estimated by blood urea nitrogen (BUN) and platelet count, respectively. Tissue exposure to platinum was estimated as the product of the area under the plasma concentration–time curve for unchanged drug (AUC_p), Kp_{app} and the apparent hydrolysis rate constant ($k_{hydrolysis}$), and toxicity factor is defined by the product of Kp_{app} x $k_{hydrolysis}$ as an intrinsic parameter of drugs. Results and Discussion: The relationship between AUCp x toxicity factor and BUN fitted well to an E_{max} model. In bone marrow, this function also correlated with platelet count. In summary, the product of AUCp x toxicity factor is determining factor of pharmacokinetic basis for these platinum drug-induced nephrotoxicity and myelosuppression in rats, and this toxicity factor may be a useful parameter for predicting the degree of toxicity of platinum antitumor compounds. We also tried to extrapolate these toxicity factors determined in this study to predict nephrotoxicity and myelosuppression of human data.

228

Regulation of Brain Cytochrome P450 Enzymes in a Rat Model of Chronic Renal Failure

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Background: It has been shown that chronic renal failure (CRF) is associated with a downregulation of liver and intestinal cytochrome P450 (CYP450) in the rat. The present study aimed to investigate the repercussions of CRF on Brain. CYP450 isoenzymes mRNA and protein expression, as well as activity in different brain regions (cortex, cerebellum, hippocampus, and rest of brain parenchyma), have been studied in order to determine the effects of CRF on cerebral drug metabolism by CYP450. Methods: The entire brain of CRF rats (induced by 5/6th nephrectomy) and control rats (sham laparotomy) was dissected into 4 parts (cortex, cerebellum, hippocampus, and rest of brain parenchyma). Protein and mRNA expression of CYP1A, CYP2C and CYP3A were assessed by Western Blot assay and Real Time PCR, respectively. CYP3A activity was assessed using DFB metabolism into DFH in brain microsomal preparation. Results: In CRF rats, mRNA levels of CYP1A and CYP3A were decreased significantly by at least 40% ($p< 0.05$) in the cerebral cortex and hippocampus. Protein expression of these isoforms was decreased by at least 50% ($p< 0.05$) in all structures. A significant decrease in CYP3A activity was observed in some compartments. Moreover, a significant 60% decrease ($p< 0.05$) in CYP2C11 protein expression was observed in all the structures studied. Conclusions: CRF is associated with a decrease in some major drug-metabolizing enzymes, which could explain an increase in bioavailability of drugs in the brain.

229

Effects of Short-term ACE Inhibitor Treatment on the Peripheral Insulin Sensitivity in Non-obese Hypertensive Patients

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Introduction: To compare the effects of effects of a short-term treatment with two structurally different ACE inhibitors on peripheral insulin sensitivity and haemodynamic parameters in non-obese hypertensive patients with normal glucose tolerance. Methods: The study included 20 patients treated with ramipril and 24 treated with cilazapril over 12 weeks, with mild-to-moderate

essential hypertension, aged 18 – 65 years and BMI < 27 kg/m2. Bergman´s Minimal Model (MINMOD) test was used for the assessment of peripheral insulin sensitivity (Si). Minimal model test, oGTT and 24-hour AMBP were performed at 0th and 12th week of the study. In addition, measurement of office blood pressure was performed every two weeks. Results: The patients were treated with 3.75±0.21 mg ramipril and 3.90±0.33 mg cilazapril. After 12 weeks cilazapril therapy we could note a significant increase in the value of the Si which was within the normal range for the referential model (1.59±1.13 vs. 3.01±1.17 x 10-4 min/microU/ml, p<0.05). After the short-term ramipril treatment, an improvement of Si was not statistical significant (1.22±0.66 vs. 1.78±0.80 x 10-4 min/microU/ml, p>0.05). Both ACE inhibitors resulted in a significant reduction of both SBP (ramipril: 158±12 vs. 135±11 mmHg; cilazapril: 164±11 vs. 145±9.9 mmHg, p<0.01) and DBP (ramipril: 96±7.0 vs. 79±4.6 mmHg; cilazapril: 101±6.9 vs. 82±4.7 mmHg, p<0.01). Conclusion: In our study cilazapril induced better improvement in insulin sensitivity than ramipril whereas ramipril pointed at better hypotensive effect. These results support the estimation that different metabolic and hypotensive effects of structurally different ACE inhibitors are probably dose-dependent.

230

A Thorough QT/QTc Study of Moxifloxacin in Healthy Adult Filipino Subjects: Lack of Ethnic Differences in Pharmacokinetics and QTc Prolongation

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Since limited thorough QT/QTc (TQT) data is currently available for Asian populations, we performed a TQT in-house validation study using a 400mg single oral dose of moxifloxacin, a standard positive control substance, in 69 (35 males, 34 females) healthy adult Filipino subjects. We evaluated its effects on the pharmacokinetics and QT/QTc interval of Filipinos, and examined ethnic factors using previously published Japanese (n=40) and Caucasian (n=61) documents. Blood was sampled for pharmacokinetic analysis in parallel with digital ECG collections at -1, 0.5, 1, 2, 3, 4, 6, 8 and 23 hours, and time-matched blood and ECG collections were also conducted on the days before administration. Serum drug concentrations were determined using an internally-validated method by a HPLC system equipped with a fluorescence detector, while ECGs were analyzed by a team of Japanese experts. The mean body weight (kg, mean±SD) of Filipinos was 54.3±8.2, whereas it was 59.4±9.1 for Japanese and 77.8±14.9 for Caucasians. The C_{max} (micro-g/mL) and T_{max} (h) of Filipinos (mean±SD) were 3.6±0.8 and 2.6±0.8, while being 4.3±1.3 and 1.0±2.2 in Japanese, and 2.9±1.0 and 2.1±0.8 in Caucasians, respectively. Furthermore, delta-QTcF (ms, mean±SD) at the C_{max} was 10.1±8.4 in Filipinos, and 13.1±6.4 in Japanese and 13.9±13.2 in Caucasians. These results indicate that there are no obvious ethnic differences in the C_{max} or QTcF interval prolongation induced by moxifloxacin among healthy adult Filipinos, Japanese and Caucasians, even though certain differences in the mean body weight and the T_{max} values were observed in these 3 ethnic groups.

231

Overview of Blood Pressure Assessment – Clinical Trials

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The assessment of blood pressure has been a primary cardiac safety biomarker throughout the clinical research development process. As a study endpoint, blood pressure has been used as both a primary efficacy endpoint in the development of anti-hypertension compounds, as well as safety endpoints in all therapeutic areas including CNS, infectious disease, and GI indications. There has been an ongoing evolution of the technology and methodology implemented in the collection of blood pressure within the clinical research environment. In some cases, change has been initiated by advancement in technology, changes in the regional regulatory & safety requirements, as well as study phase and design. The

progression and goal has been to obtain more accurate blood pressure readings for an individual patient and elimination of the "White Coat" effect that has been associated with blood pressure readings taken in an investigator clinic by a physician or nurse. Blood pressure evaluation has traditionally been completed in an office setting using a mercury or aneroid manometer, and assessed through auscultation using a stethoscope and Riva-Rocci methodology. This method has withstood the test of time, but technology and the drive to obtain a more accurate representation of a patient's blood pressure has lead to the implementation of a) ambulatory blood pressure monitoring (ABPM), b)automated – digital blood pressure devices in the office and/or home and c) central pressure evaluation – arterial compliance monitoring.

232

Effect of Bupropion on the Firing Rate of Dorsomedial Thalamic Nucleus of the Rats Forced to Swim

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A lack of coordinated activity between emotional memory structures and the prefrontal cortex is responsible for some of the symptoms of major depressive disorder. The dorsomedial nucleus of the thalamus (DM) is a critical connection between the amygdale and the prefrontal cortex hence; it is well positioned for involvement in major depressive disorder pathophysiology. The aim of this study was to explore DM firing rate in rats submitted to the forced swim rats (FST) and define the effect of bupropion, a selective dopamine reuptake inhibitor. A low-dose 15-day treatment with bupropion (5.0 mg/Kg) increased the latency to the first immobility period ($t=-3.031$, 20gl, $p<0.05$) and reduced the immobility time ($t=2.922$, 20gl, $p<0.05$) in FST without changes in crossing. The effect of 15-day bupropion treatment (5.0 mg/Kg) on the spontaneous neuronal firing rate of DM (single unit extracellular recording) was tested in an intact group of rats (n=10), a group subjected to FST (n= 10), a control group without FST (n= 12) and a vehicle group also forced to swim (n=10). Bupropion as the FST reduced the mean spontaneous firing rate of DM ($F_{3,119}= 9.719$, $p<0.001$). The treatment with bupropion did not promote additional changes in the rate of firing in this nucleus in FST rats. In conclusion, DM is not involved in the behavioral effects of bupropion, but it might participate in process of coping and decisions-making.

233

Progressive MPTP-non Human Primate Models that Recapitulate Non-motor Symptoms of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative condition that affects 1% of the population over 65. PD consists of a syndrome including tremor, rigidity, postural abnormalities and bradykinesia and a myriad of so-called non-motor symptoms such as neuropsychiatric symptoms, sleep disorders, sensory symptoms, as well as autonomic and gastro-intestinal dysfunction. No treatments exist for these non-motor symptoms. With this in mind, we have developed chronic MPTP non-human primate models that mimic some of the non-motor symptoms of the disease, namely cognitive dysfunction and sleep disorders. Chronic low-dose MPTP-treated monkeys develop impairments in performing spatial delayed response, delayed matching-to-sample, delayed alternation, object retrieval and discrimination reversal tasks, as well as a variety of specific attention and executive function impairments. These deficits are strikingly similar in nature to those described in Parkinson's disease patients. We will show the effect a number of treatments on these cognitive deficits using a fully automated touch screen testing system. Sleep disorders represent a prominent non motor symptom in neurodegenerative parkinsonian syndromes, affecting over 50% of patients with PD. These sleep alterations are primarily characterized by rapid eye movement (REM)

sleep behaviour disorder (RBD), excessive daytime sleepiness (EDS), sleep fragmentation and insomnia. We have used a radio-telemetric system allowing chronic and continuous recordings of sleep patterns to show that MPTP-treated monkeys also develop these sleep disorders. We will also show the effect of some dopaminergic treatments on sleep patterns in parkinsonian monkeys.

234

Role of the Liver Sinusoidal Endothelium in the Hepatic Disposition of Diazepam

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Liver sinusoidal endothelial cells (LSECs), which separate sinusoidal blood from hepatocytes, are very thin and fenestrated with pores 50-100 nm in diameter. We hypothesised that albumin bound medications undergo ultrafiltration by LSECs. This study evaluated the role of LSECs in the hepatic disposition of diazepam. The multiple indicator dilution technique was used in isolated perfused rat livers of young adult male Fischer 344 rats. Livers were perfused with Krebs-Henseleit bicarbonate buffer with 2% bovine serum albumin, equilibrated with 95% O_2/ 5% CO_2, at 1 ml/min/g wet liver. Injectate contained tracer amounts of ^{14}C-diazepam, ^{3}H-sucrose (non-extracted marker of extracellular space) and Evans Blue (marker of albumin), made up to 100 microliters with perfusate. Experiments were performed in intact animals (controls, n=5) and after treatment with Poloxamer-407 (0.05 g/kg i.p. 24 hours prior to liver perfusion, P-407, n=6). P-407 coats the LSECs causing dramatic loss of fenestrations. There was a significant increase in the ratio of recovery of diazepam to that of sucrose after P-407 treatment (0.13+/-0.09 controls, 0.34+/-0.20 P-407, $p<0.05$), indicating decreased diazepam extraction after a single pass through the liver. There was a trend towards reduced hepatic volume of distribution of Evans Blue as a ratio of that of sucrose after P-407 treatment (0.84+/-0.31 controls, 0.57+/-0.19 P-407, n.s.), which may be consistent with exclusion of albumin from the space of Disse. The results support ultrafiltration of protein bound drugs such as diazepam by LSECs, with important implications for ageing and cirrhosis, in which LSECs are thickened and have reduced porosity.

235

Pharmacokinetics of Whole Blood Ribavirin for Estimating Contraception Period after Stopping Interferon and Ribavirin Combination Therapy

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Interferon (IFN) and ribavirin (RBV) combination therapy for hepatitis C virus (HCV) eradication requires the patients to have contraception for 6 months after stopping the therapy because of possible deformity caused by RBV. This period based on the plasma RBV half-life (12 days) is enough to remove the RBV from plasma. However, intracellular RBV, which is accumulated as the phosphorylated metabolites in the tissue and cells including erythrocytes, may require much time to be removed. To confirm whether or not the contraception period (6 months) is enough for removing RBV phosphates, we investigated the pharmacokinetics of whole blood RBV (>90% RBV phosphates) after stopping IFN/RBV therapy. Methods: Fourteen HCV patients (M/F: 9/5, 52 ± 11 yr.) treated with IFN/RBV were enrolled. Six patients did not complete the combination therapy because of the adverse reactions. Plasma and whole blood concentration of RBV were monitored before and after stopping the combination therapy. Results and Discussion: RBV concentration for whole blood before stopping IFN/RBV was 30 times higher than that for plasma (307.9 ± 146.5 vs. 10.1 ± 4.6 microM). Elimination half-life for whole blood RBV was 1.8 times longer than that for plasma (23.9 ± 7.5 vs. 12.9 ± 4.4 days). The estimated days for removing RBV from whole blood and plasma after stopping IFN/RBV therapy were 176 (130 – 331) and 79 (51 – 121), respectively. These results suggested that 6 months was not enough to remove RBV phosphates from the blood after stopping combination therapy.

236

Association between Intestinal *CYP2C19* Genotypes and Tacrolimus Trough Levels with Proton Pump Inhibitors in Adult Living-donor Liver Transplant Patients

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Clinically relevant interactions between tacrolimus and proton pump inhibitors (PPIs) via CYP3A4 in subjects with CYP2C19 gene variants have been reported. Although the CYP2C19 is found to be expressed in the enterocytes of the human small intestine, the clinical importance of intestinal CYP2C19 on interaction between tacrolimus and PPIs remains unclear. In the present study, we examined the concentration/dose ratio of tacrolimus concomitantly administered with omeprazole or lansoprazole for 75 living-donor liver transplant patients considering CYP2C19 polymorphism both in the native intestine and the graft liver separately, because the genotypes of recipients themselves (intestine) and donors (liver) differed in some cases. The tacrolimus concentration/dose ratio ((ng/mL)/(mg/kg/day)) showed significantly higher in patients carrying functionally defect variants for intestinal CYP2C19 (median; 292.9, range; 93.3-994.5) than wild-type homo-zygotes (median; 153.2, range; 80.0-332.2) and hetero-zygotes (median; 98.1, range; 31.5-256.8) under co-administration of omeprazole in postoperative days 22-28 (P=0.043). In addition, among the patients carrying functionally defect variants for intestinal CYP2C19, the tacrolimus concentration/dose ratio concomitantly administered with omeprazole identified even higher values in those with intestinal CYP3A5*3/*3 compared to those with intestinal CYP3A5*1/*1 and CYP3A5*1/*3. In contrast, there were no association with tacrolimus trough levels and CYP2C19 polymorphism in the cases with lansoprazole-coadministration. These results suggest that the genetic variation in intestinal CYP2C19 may be important tacrolimus-omeprazole interaction, and the extent could be associated with polymorphisms of intestinal CYP3A5.

237

Polymorphisms of UDP-glucuronosyltransferase 2B7 and Multidrug Resistance 1 Genes in Patients of Hepatocellular Carcinoma Treated with Transcatheter Arterial Chemoemblization

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The glucuronidation of epirubicin is catalyzed by UDP-glucuronosyltransferase (UGT) 2B7, while the transportation of this drug is mediated by P-glycoprotein. We hypothesized that variants of the UGT2B7 and multidrug resistance 1 (MDR1) genes may affect therapeutic response in the patients of hepatocellular carcinoma (HCC) who are treated with epirubicin. The associations among the outcome of transcatheter arterial chemoemblization with epirubicin (TACE) for 100 HCC patients and variant status in their UGT2B7 and MDR1 genes were studied. The single nucleotide polymorphism (SNP) at nucleotide 802 in the UGT2B7 gene and SNPs at nucleotides 1236, 2677 and 3435 in the MDR1 gene were determined. The relationship of polymorphism in gene and survival time was calculated by Kaplan-Meier survival estimate. The results showed that the estimated median survival time (225 days) in the HCC patients bearing MDR1 3435TT polymorphism (N = 17) was significantly shorter than that (428 days) in those carrying the wild/3435CT polymorphism (N = 83) (hazard ratio = 2.77, P = 0.033). Such a phenomenon was not observed when the SNPs of UGT2B7 802, MDR1 1236 and MDR1 2677 were concerned. Our study indicates that homozygous C>T variation at nucleotide 3435 in the MDR1 gene is a determinant of poor prognosis for the HCC patients under TACE treatment.

238

Novel Heme Oxygenase Inhibitors and their Effects on Rat Cytochrome P450s 2E1 and 3A1/3A2

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Background: Heme oxygenase (HO) catalyzes the degradation of heme into biliverdin, carbon monoxide (CO) and free iron. The two major isoforms, HO-2 (constitutive) and HO-1 (inducible by various stressors such as heavy metals and reactive oxygen species) are involved in a variety of physiological functions, including cytoprotection, neuromodulation, and vascular regulation. Major tools used in exploring these actions have been metalloporphyrin analogs of heme that inhibit the Hos. However, these tools are limited by their lack of selectivity; they affect other heme-dependent enzymes, such as cytochrome P450s (CYPs), soluble guanylyl cyclase (sGC), and nitric oxide synthase (NOS). Our laboratory has been able to successfully synthesize a series of novel non-porphyrin HO inhibitors (QC-xx) that have little or no effect against sGC and NOS; and their effects on various CYP isoforms will be described. Methods: In order to determine these effects on enzyme activity microsomal preparations of two CYP isoforms (2E1 and 3A1/3A2) are incubated with varying concentrations of HO inhibitor and activity is determined by spectrophotometric analysis. Results: Some QC compounds demonstrate little to no inhibition at CYP 2E1 and/or CYP 3A1, while some others do inhibit these CYP isoforms. Discussion & Conclusion: Several structural modifications of the HO inhibitors correspond with decreases in CYP activity that are CYP isoform dependent. Further studies are underway in order is to identify which structural components are necessary for selective HO inhibition and lack of inhibitor action on CYPs, sGC, and NOS.

239

High Plasma Interleukin-1 Receptor Antagonist Levels in Schizophrenia Patients with Comorbid Substance Use Disorder

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Using meta-analysis, we have demonstrated previously establishment of an inflammatory syndrome in schizophrenia patients, illustrated by elevated circulating levels of interleukin-6 (IL-6) and interleukin-1 receptor antagonist (IL-1RA). Schizophrenia is frequently associated with comorbid substance use disorder (SUD). The goal of the present study was to investigate plasma levels of inflammatory cytokines in schizophrenia patients with SUD. The substances of abuse included mainly cannabis and alcohol. Patients were stabilized on various antipsychotic medications, and they were switched to quetiapine in order to minimize inter-subject variations in cytokine levels related to differences in antipsychotic treatments. Blood samples were withdrawn in patients at week 0 (n=29), week 6 (n=24), and week 12 (n=24) after quetiapine treatment, and in healthy volunteers (n=28). Plasma levels of IL-6, IL-1RA, and IL-17 were measured by sensitive ELISA. The results show elevated IL-6 and IL-1RA plasma levels in patients compared to healthy controls, whereas IL-17 levels were not affected. The lack of increases in IL-17 is consistent with the fact that this cytokine may play a pathogenic role in rheumatoid arthritis, which is negatively associated with schizophrenia. Interestingly, the increases in plasma IL-1RA observed in schizophrenia patients with SUD (6-fold) were more pronounced than those reported previously in schizophrenia without SUD (2-fold). In addition, IL-6 was positively correlated with depressive symptoms and with alcohol, while IL-1RA was positively correlated with cognitive deficits. These results suggest distinct pathophysiologic roles for IL-6 and IL-1RA in schizophrenia with comorbid SUD, and potential contribution of some substances of abuse in increased IL-1RA.

240

Peripheral Analgesic Synergy of a Ketorolac-Paracetamol Combination on an Inflammatory Model of Pain in Healthy Human Volunteers is due to a Pharmacodynamic Interaction

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Introduction: Non-steroidal anti-inflammatory drugs (such as ketorolac) and paracetamol are peripheral and central acting analgesics respectively. Their combination may provide additive pain relief. But data showing improved effectiveness are lacking. Methods: Pharmacokinetic/pharmacodynamic randomized, double-blind, placebo-controlled, crossover study evaluating the antinociceptive efficacy of an intravenous combination of ketorolac 20mg and paracetamol 1g compared to single agents in 11 healthy volunteers. Pharmacokinetics and sensory tests were performed 22 hours (maximal inflammation) after induction of a 7.5cm^2 sunburn inflammation on the forearm, using UVB (40 mJ/cm^2), and 1, 2, 4, 6 and 24h after drugs administration. Heat and mechanical pain thresholds in the erythema were used to detect the peripheral antihyperalgesic effect. RIII reflex and area of secondary hyperalgesia were used to assess the central effect. R/S-ketorolac and paracetamol plasma concentrations were measured with a stereoselective LC/MS method. Results: The combination significantly increased mechanical and thermal pain thresholds compared to placebo with a maximal median effect (±SEM) at 1-2 hours (+20.9±5.3 vs -8±5.7g,$p<0.037$) (+1.4±0.4 vs 0.0±0.3°C,$p<0.05$), whereas single agents had no effect. No central antinociceptive effect was detected. PK parameters (means) were not statistically different in combination and ketorolac arms (t1/2=4.9vs5.2h; 4.6vs5.1h and AUC=4.5vs4.7; 1.3vs1.3 for R and S-ketorolac respectively). Discussion: Our study showed the reliability of the UVB model to detect a peripheral synergistic analgesic effect of the ketorolac-paracetamol combination on primary hyperalgesia. This synergistic effect could not be explained by a pharmacokinetic interaction. Conclusion: The peripheral synergistic effect observed by combining ketorolac and paracetamol is probably due to a pharmacodynamic interaction.

241

Mechanism of the Atropine–resistant Contraction of Urinary Bladders in Aged Rats

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Urinary bladder contraction is mediated neurogenically by both cholinergic and nonadrenergic-noncholinergic pathways. Because muscarinic receptor stimulation plays a main role in voiding by contracting the detrusor muscle, muscarinic receptor antagonists are used in overactive bladder (OAB). However, atropine is not effective in aged people and in some diseases such as OAB, suggesting the increase in the atropine-resistant contraction (ARC). Recently it is postulated that ATP receptors are responsible for OAB. We studied whether ARC was enhanced by aging and whether purinergic component was associated with the increased ARC in aging. Urinary bladders were isolated from young (3 months) or aged (> 26 months) male Fischer 344 rats, and the electrical field stimulation (EFS)-induced contractions were recorded in the presence of atropine or propiverine. Atropine inhibited EFS-induced contractions by 55% and 25% in young and aged, respectively. Propiverine, another type of muscarinic antagonist, inhibited EFS-induced contractions by 56% in both young and aged bladders. After depleting ATP by repeated stimulation with alpha, beta- methylene ATP, ARCs were 45% and 75% in young and aged bladder, respectively. Both atropine and ATP-resistant contractions were 10% and 40% in young and aged bladders, respectively. These results indicate that the ARC was increased by aging. The purinergic component may explain only a part of ARC in aged bladder. Because propiverine can block Ca2+ channel in addition to antagonizing muscarinic receptors, it may be useful for treatment of diseases with ARC.

242

Pioglitazone Improves Dyslipidemia and Visceral Fat Deposition in Metabolic Syndrome Model Rats

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Background: Hypertension, dyslipidemia and hyperglycemia may well lead to cardiovascular events. As sucrose (Suc) loading on our metabolic syndrome rat, spontaneously hypertensive hyperlipidemic rats (SHHR) which is produced by ourselves, induce hyperglycemia, we used this metabolic syndrome models for the study on these risk factor. (Purpose) We studied the effect of pioglitazone in the dyslipidemia of SHHR treated with 15% Suc + high fat diet (HFD) and SD rats. Method: Suc, HFD and pioglitazone (3 mg/kg/day, s.c.) were administered for 2 months in male SHHR and SD rats. Plasma glucose and insulin levels of SHHR and SD rats treated with Suc+HFD were significantly increased compared with the control (Cont) SHHR and SD. Pioglitazone significantly decreased the plasma glucose and insulin in SHHR and SD rats treated with Suc + HFD. Plasma total cholesterol and visceral fatty tissue weight of SHHR treated with Suc + HFD were significantly increased than that of Cont SHHR. Pioglitazone also significantly decreased plasma cholesterol and visceral fat gain of SHHR, but not of SD rats. Further, pioglitazone improved lipid deposition in aorta of SHHR treated with Suc + HFD. And then, plasma high density lipoprotein (HDL) in the SHHR treated with Suc + HFD decreased than that of Cont SHHR. These decreased-plasma HDL level was significantly improved by pioglitazone in SHHR treated with Suc + HFD. Conclusion: These results may have suggested that pioglitazone improved dyslipidemia through the improvement of hyperglycemia of metabolic syndrome.

243

Effect of Diclofenac Sodium on Gastric Mucosa in Mice

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The detailed information on the adverse drug reactions of Diclofenac sodium is almost lacking in most laboratory animals including mice. The present study was conducted to investigate the effect of a selective non steroidal anti-inflammatory drug (NSAID), Diclofenac sodium on some blood parameters, (TEC, Hb, PCV and ESR) and gross and histological texture of stomach under different conditions. A total of 45 Swiss Albino mice were randomly assigned into five equal groups (n=9) and they all were fed with standard broiler pellet (25 gm/mice/day) throughout the experimental period of 40 days. Keeping 1 group as control, 4 groups were treated with Diclofenac sodium @ 3 mg/kg b.wt orally in empty or full stomach with or without Vitamin B_{12} @ 10 µg/Kg b.wt through intramuscular route. It has been observed that the oral administration of this drug significantly ($p<0.01$) decreased TEC, Hb and PCV with elevated ESR in the animals treated with Diclofenac sodium in empty stomach and in the animals treated with Diclofenac sodium plus Vitamin B_{12} in empty stomach. All the treated mice showed slight to moderate congestion of the stomach. A massive hemorrhage with ulceration in the stomach has been found in the animals having decreased TEC, Hb and PCV with elevated ESR. The anemic tendency in these animals might be due to serious depletion of intrinsic factor which is secreted from gastric mucosa and is responsible for Vitamin B_{12} absorption through gastrointestinal tract. It may be concluded from the study that Diclofenac sodium when administered in empty stomach, produces an adverse effect on gastric mucosa that may lead to anemia.

244

Electrocardiographic Effects of Scorpion *Leiurus quinquestriatus quinquestriatus* (H&E) Venom

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The scorpion Leiurus quinquestriatus quinquestriatus (Lqq) is responsible for the majority of dangerous and potentially lethal accidents particularly in children in Egypt. The cardiovascular effects are probably the most important aspect of envenoming and included myocardial damage, heart failure, arrhythmias, hypertension, hypotension and pulmonary oedema. Although both experimental and clinical studies were carried out regarding the effects of Lqq envenoming, little studies were published on the electrocardiographic (ECG) effect of the venom. In the present work, ECG studies were carried out in rabbits anaesthetized with urethane.

Additional experiments were carried out in rats. Lqq venom (1 mg/kg i.v. in rabbits) and (0.2 mg/kg i.p. in rats) was injected and serial ECG tracings were recorded using subcutaneous needle electrodes. The effects of the venom and treatment with propranolol, atropine, L-NAME and antivenom on the induced changes were determined. The venom by itself caused various electrocardiographic changes starting with bradycardia and myocardial ischemia. It progressed into inferior and anterior wall infarction, 1st degree heart block, some dropped and pre-excitation beats and left bundle branch block. The venom also caused ectopic foci low in the atrium or in the AV junction. The venom also caused some of its ECG wave abnormalities through electrolyte disturbance, mainly hyperkalemia and hypocalcemia. Pretreatment of the animals with atropine protected the animals from some of these changes, while pretreatment with propranolol or L-NAME markedly potentiated venom toxicity. When the prepared venom specific IgG or F(ab`)2 fractions were used, all effects of the venom were neutralized.

245

Hemodynamic Drug Interactions between Mirodenafil and Tamsulosin in Healthy Normotensive Male Subjects

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Introduction: Mirodenafil is a phosphodiesterase type 5 (PDE5) used for erectile dysfunction treatment. Tamsulosin, an alpha1-adrenergic receptor antagonist is drug for patients suffering with benign prostatic hyperplasia. The aim of the study was to investigate hemodynamic interactions of mirodenafil and tamsulosin. Method: A randomized, placebo-controlled, double-blind, two-period crossover study was conducted. Total 16 healthy normotensive male subjects were enrolled. Tamsulosin 0.2 mg was given daily for 7 days. After an interval of 3 hours mirodenafil 100 mg or placebo was administered. A two-week washout was applied between the two periods. Baseline blood pressure (BP) and heart rate (HR) in supine position were measured just before administration. BP and HR in supine and standing positions were recorded for 24 hours after dosing. Results: After mirodenafil administration, mean maximal changes from baseline versus placebo in supine systolic BP, diastolic BP, and HR were -1.2mmHg (95% confidence interval (CI) -5.3 to 2.9, P=.56), -1.1mmHg (95% CI -3.9 to 1.6, P=.39), and 3.3 beats per minute (95% CI 1.1 to 5.5, P<.01), respectively. Those in standing position were -4.3mmHg (95% CI -10.0 to 1.5, P=.13), -1.9mmHg (95% CI -5.5 to 1.7, P=.27), and 5.2 beats per minute (95% CI -0.1 to 10.5, P=.054), each. There were no significant differences in the number of subjects experiencing adverse events between 2 groups. Conclusion: Mirodenafil and tamsulosin coadministration did not induce clinically significant BP decreases in healthy subjects. Safety profiles after coadministration of mirodenafil and tamsulosin did not remarkably differ from those after tamsulosin dosing alone.

246

Influence of the Beta Adrenergic Receptor (AR) Genetic Polymorphisms on the Pharmacodynamics of Sevoflurane

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Objective: To investigate whether functionally important polymorphisms of beta1-AR and beta2-AR gene would influence pharmacodynamics in patients after general anesthesia with sevoflurane

Methods: Ninety ASA physical status I and II patients underwent slow inhalation induction of anesthesia with a face mask, using sevoflurane in 100% oxygen. After the induction, anesthesia was maintained with sevoflurane and ventilation was assisted by the face mask at the end-tidal concentration of 2.5% sevoflurane. Heart rate, QT intervals (QT_b, QT_h, and QT_f), and blood pressures (SBP, DBP, and MBP) were obtained as pharmacodynamic parameters of sevoflurane. Ser49Gly, Gly389Arg genotypes of beta1-AR, and Arg16Gly, Gln27Glu, Thr164Ile genotypes of beta2-AR were determined by polymerase chain reaction using restriction fragment length polymorphism analysis. Results: On the average, the homozygous patients for the Ser49Gly

genotype showed a greater decrease of heart rate in comparison to the patients with the heterozygous genotype (13.1 vs. 5.6 beats/min, p=0.0271). The heterozygous patients for the Arg16Gly genotype showed a greater decrease of DBP in comparison to the patients with AA or GG genotype (15.2 vs. 19.9 mmHg, p=0.0118). Three genotypes (AA, GA, GG) for Arg16Gly showed a statistically significant difference in a decrease of heart rate (10.8, 13.7 and 7.1 beats/min, respectively, p=0.0146). No differences were found among the Gly389Arg genotypes of beta1-AR, and the Gln27Glu of beta2-AR. All patients had only the wild type for the Thr164Ile genotype of beta2-AR. Conclusions: This study shows that the beta adrenergic receptor genetic polymorphisms may be associated with the pharmacodynamics of sevoflurane.

247

Effects of Gemfibrozil and Atorvastatin on the Pharmacokinetics of Repaglinide in Relation to *SLCO1B1* Polymorphism

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Hepatic uptake by organic anion transporting polypeptide 1B1 (OATP1B1) is an important step preceding the metabolism of repaglinide. A single nucleotide polymorphism of the *SLCO1B1* gene encoding OATP1B1, c.521T>C (p.Val174Ala), is associated with increased plasma repaglinide concentrations. To investigate the role of *SLCO1B1* polymorphism in drug interactions, a randomized cross-over study was carried out in 24 *SLCO1B1*-genotyped healthy volunteers. They ingested daily 1200 mg gemfibrozil, 40 mg atorvastatin, or placebo, followed by 0.25 mg repaglinide on day 3. The mean increase in the area under the plasma repaglinide concentration-time curve (AUC) by gemfibrozil was 1.56 (*P*=0.004) or 1.54 (*P*=0.002) times larger in individuals with the *SLCO1B1* c.521CC genotype (n=6) than in those with the c.521TC (n=6) or c.521TT genotype (n=12), respectively. Gemfibrozil increased repaglinide elimination half-life 1.43 times more in the c.521CC group than in the c.521TT group (*P*=0.047), but no differences were seen in the effect on peak plasma concentration (C_{max}). During the gemfibrozil phase, minimum blood glucose concentration after repaglinide intake was 19% (*P*=0.009) lower in the c.521CC participants than in the c.521TT participants. Thus, among patients using gemfibrozil, those with the c.521CC genotype are likely to be most suceptible to the prolonged blood glucose-lowering effect of repaglinide. In the c.521TT group, atorvastatin increased repaglinide AUC by 41% (*P*=0.001) and 18% (*P*=0.033), respectively. Atorvastatin did not significantly alter the effect of repaglinide on blood glucose concentrations. In conclusion, the extent of gemfibrozil-repaglinide interaction depends on *SLCO1B1* genotype. Atorvastatin raises plasma repaglinide concentrations probably by inhibiting OATP1B1.

248

Hypoxia Induced Apelin mRNA Expression in Astrocytes

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Objective: Apelin was identified as an endogenous ligand for orphan G-protein coupled receptor APJ. Recently, it has been suggested that apelin protects hippocampal neurons against excitotoxic injury. There are many kind of stress induced neuronal cell death including hypoxia, glutamate-excitotoxicity. It has been reported that apelin mRNA expression was induced by hypoxia in endothelial cells. Therefore, we hypothesis that apelin induced by hypoxia can protect neuronal cell death. In this study, we examined whether the expression of apelin mRNA is induced by hypoxia in central nervous system (CNS) cells, using neuroblastoma Neuro2a cells, oligodendrocytoma KG-1-C cells, microglia cell line BV-2 cells, and astrocytoma U-251MG cells. Methods and results: We investigated the induction of apelin mRNA expression by chemical hypoxia and hypoxia using RT-PCR and western blotting. We detected apelin mRNA expression in Neuro2a, U-251MG, and KG-1-C cells, not in BV-2 cells. Cobalt chloride (II), hypoxia inducible factors-1 (HIF-1) alpha inducer, induced apelin mRNA expression in U-251MG, not in Neuro2a and KG-1-C cells.

Moreover, in U-251 MG cells, cobalt chloride (II) induced the expression of apelin mRNA to same level as that of VEGF following the accumulation of HIF-1 alpha protein. In addition, hypoxia (oxygen concentration as low as 1%) also induced apelin mRNA expression following the accumulation of HIF-1 alpha protein. Conclusion: These results suggested that there is different sensitivity to hypoxia according to the type of CNS cells, and that apelin mRNA expression was induced by hypoxia in astrocytes, but not in other CNS cells.

249

The Evaluation of Low Dose Sulpiride as a Prokinetics on from the Viewpoint of Plasma Gut-regulatory Peptide Levels in Healthy Human

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Sulpiride, a dopamine receptor antagonist, is prescribed for gastroduodenal ulcer at a low dose (<150 mg), and for depression or schizophrenia at a high dose (>150 mg) in Japan. The pharmacological mechanism of its anti-ulcer effect is caused by improvement of mucosal microcirculation. Although the other dopamine receptor antagonists, such as metoclopramide and domperidone, are used as prokinetics, sulpiride has not been evaluated as a prokinetics. We studied the effect of sulpiride on the levels of plasma bioactive substance related to cytoprotection and gut-motility in healthy human. Sulpilide (150 mg) or placebo was orally administered in four healthy male volunteers. The blood samples were taken repeatedly from 0 to 240 minutes after administrations, followed by the extracting procedure, and submitted to the highly sensitive enzyme immunoassays as previously developed. All values were expressed as mean and statistically evaluated. Single administration of sulpiride caused significant ($p<0.05$) increases of plasma calcitonin gene-related peptide and vasoactive intestinal peptide, compared with placebo, which might be involved in the cytoprotective effect. Although sulpiride caused a drug concentration-dependent decrease of plasma gastrin levels, somatostatin levels were significantly increased compared with placebo. Furthermore, the increments of motilin release were positively correlated with the area under the drug concentration-time curve, which might indicate that sulpiride had the potential dose-dependent prokinetic effect. In this study, we revealed that the cytoprotective effect of sulpiride might depend on alteration of plasma bioactive peptide, and from the viewpoint of gut-regulatory peptide alteration, we indicated the potential of sulpiride as a prokinetics.

250

Pharmacokinetic and Pharmacodynamic Evaluation of Conivaptan Hydrochloride Injection Coadministered with Warfarin in Healthy Adults

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Introduction: Conivaptan (CNV), a vasopressin-receptor antagonist approved to treat euvolemic and hypervolemic hyponatremia in hospitalized patients, is a substrate and inhibitor of isoenzyme CYP3A4. Warfarin, the oral anticoagulant most used in hospitalized patients, is biotransformed by CYP2C9 and CYP3A4. This 2-period crossover study assessed pharmacokinetic (PK)/pharmacodynamic (PD) interactions between CNV and warfarin. Methods: In period 1, 22 healthy subjects were administered one 25-mg warfarin dose and underwent blood sampling and safety assessments through day 7, followed by a 3-day washout. In period 2, subjects received a CNV 20-mg loading dose followed immediately by continuous infusion of CNV 40 mg/d through day 4. One 25-mg warfarin dose was administered on day 3. Blood was sampled to assess R- and S-warfarin PK parameters. PD parameters included international normalized ratio and prothrombin time responses (AUC_{INR} and AUC_{PT}) and maximum responses (MAX_{INR} and MAX_{PT}). Full safety assessments were made. Results: All 22 subjects were evaluated for safety; 7 who completed treatment were evaluated for PK and PD. S-warfarin mean $AUC_{0\text{-}inf}$ and C_{max} were 14.4% and 16.9% greater, respectively, with CNV than without. Similarly, R-warfarin PK were unaffected by CNV. AUC_{INR} and AUC_{PT} were 1.5% and 1.4% greater, respectively, with CNV; MAX_{INR} and MAX_{PT} were affected minimally (<1%) by CNV. Excepting infusion-site reactions,

there were few other Aes, and there were no clinically meaningful changes in other clinical or laboratory evaluations, vital signs, or ECGs. Conclusion: Combined administration of CNV and single-dose warfarin produced minimal PK and no PD interactions in healthy adults.

251

Ligand- and Target-based Virtual Screening

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Virtual screening methods have emerged as an adaptive response to massive throughput synthesis and screening technologies. The main purposes of our research consist to screen virtually a large molecular database, in order to obtain some molecules closely related to the subtype-1 Cholecystokinin receptor which belongs to the G Protein Coupled Receptors family. For that, we have used the package which offers the possibility to associate both ligand- and receptor-based strategies. This platform called Virtual Screening Manager for Grids (VSM-G) is constituted of a preparation engine for both the targets and the molecules to be screened and of a docking funnel consisting of sequential different filters ranging from the faster, but less accurate rigid docking procedure, to the most accurate ones. At each step of the funnel, a fair percentage of non appropriate molecules may therefore be eliminated, so that the system is able to handle several million compounds along with several hundred targets, eventually yielding a small set of hit compounds. An application was made in the search for antagonists molecules of the cholecystokinin receptor. The molecules have been selected from Zinc database with a pharmacophoric substructure research. We have obtained four "candidate molecules" which will be proposed to the experimental biological trials.

252

Proteomic and Transcriptomic Analysis for Molecular Mechanism in Capsaicin-induced Apoptosis between Human Hepatocarcinoma and Human Neuroblastoma Cells

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It was found that endogenous ROS levels were increased during hepatocarcinoma (HepG2) apoptosis, whereas they decreased during neuroblastoma (SK-N-SH) apoptosis in response to capsaicin treatments. To clarify this different phenomenon, we used 2-DE-based proteomics to analyze the altered protein levels in both cells, with special attention on oxidative stress proteins before and after capsaicin treatments. The 2-DE analysis demonstrated that 23 proteins were increased and 26 proteins were decreased significantly in capsaicin-treated apoptotic HepG2 and SK-N-SH cells, respectively. The distinct effect of capsaicin-induced apoptosis on the expression pattern of HepG2 proteins includes the down-regulation of some antioxidant enzymes including aldose reductase, catalase, enolase 1, peroxiredoxin 1, but up-regulation of peroxiredoxin 6, cytochrome c oxidase, and SOD2. In contrast, most antioxidant enzymes were increased in SK-N-SH cells in response to capsaicin, where SOD2 might play a pivotal role in maintenance of low ROS levels in the course of apoptosis. Next, the global gene expression for oxidative stress and antioxidant defense genes was analyzed by the Human Oxidative Stress and Antioxidant Defense RT^2 *Profiler*™ PCR Array. Surprisingly, the profiles of 84 gene expressions related to oxidative stress and antioxidant defense were not significantly different in HepG2 cells between control and capsaicin-treated cells. In contrast, a number of oxidative genes were down-regulated in SK-N-SH cells, supporting the evidence of low ROS environment in apoptotic SK-N-SH cells after capsaicin treatment. It was concluded that the different relationship between endogenous ROS levels and apoptosis of two cancer cells presumably resulted from complicated expression patterns of many oxidative stress and antioxidant genes, rather than the individual role of some classical antioxidant enzymes such as SOD and catalase.

253

Stereoselective Determination of Venlafaxine, O-desmethylvenlafaxine, N-desmethylvenlafaxine and N-,O-didesmethylvenlafaxine by Liquid Chromatography with Electrospray Tandem Mass Spectrometric Detection

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A sensitive stereoselective method for simultaneous analysis of the S-(+)- and R-(-)-enantiomers of venlafaxine and its metabolites O-desmethylvenlafaxine, N-desmethylvenlafaxine and N-,O-didesmethylvenlafaxine with liquid chromatography and tandem mass spectrometric detection has been developed and validated. The compounds were isolated from the biological matrix by solid-phase extraction with a recovery in plasma, whole blood and rat brain ranging between 75-110, 63-107 and 82-112%, respectively. Chiral separation of the compounds was performed by reversed-phase high-performance liquid chromatography on a 250 x 2.1 mm Chirobiotic-V column. The mobile phase used consisted of tetrahydrofuran:10 mM ammonium acetate pH:6 (10:90; v/v) at a flow rate of 0.2 ml/min. To increase the sensitivity, 0.05% formic acid in acetonitrile was added postcolumn at a flow rate of 0.2 ml/min. The compounds were ionized in the electrospray ionization ion source of the mass spectrometer and detected using tandem mass detection. Calibration curves prepared from drug-free plasma were linear in the range of 1-1000 nmol/l for venlafaxine and O-desmethylvenlafaxine, and 0.5-500 nmol/l for N-desmethylvenlafaxine and N-,O-didesmethylvenlafaxine with a correlation coefficient <0.999. The lower limit of quantification was 0.5 nmol/l for venlafaxine and O-desmethylvenlafaxine, and 0.25 nmol/l for N-desmethylvenlafaxine and N-,O-didesmethylvenlafaxine. The intra- and inter-day variation coefficients were less than 5%. This LC-MS-MS method is highly specific, sensitive and accurate for the stereospecific analysis of the enantiomers of venlafaxine and its metabolites. The method was successfully applied for the determination of venlafaxine in patient, post-mortem, rat and mice samples.

254

Adrenal Response to Blood Collections from Restrained vs. Freely-moving Pigs

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Purpose: Plasma catecholamines and cortisol are stress biomarkers. Previous studies in rodents confirmed that catecholamines and corticosterone decrease when blood sampling is automated in freely-moving subjects. This study determined whether the same result would occur in pigs. Methods: Blood samples were collected from a jugular vein catheter and then analyzed to determine the concentration of epinephrine, norepinephrine or cortisol. Initially, each subject was either restrained during manual blood withdrawal from the catheter or unrestrained within a movement-responsive cage while programmed blood collections were performed automatically. After a rest ranging from one to several days, each pig was then switched to the other sampling method so that a comparison of plasma adrenal hormones could be compared within-subject. Blood volumes at each time point ranged from 0.5 to 1.0 mL. Samples were refrigerated until processed into plasma that was frozen at -80°C until analyzed. Analysis of the catecholamines was accomplished by liquid chromatography with electrochemical detection, while cortisol analysis was performed by LCMSMS. Results: The adrenal response of pigs to restraints was immediate, with plasma catecholamine concentrations more than an order of magnitude higher than the baseline observed in the same animals when they were sampled automatically. Plasma norepinephrine also increased in pigs being automatically sampled in the same room as other pigs that were vocalizing while being restrained during manual sampling. Baseline concentrations were similar to literature reports on average plasma catecholamine concentrations in humans. Conclusions: Plasma adrenal hormones are reduced when pigs are unrestrained and blood sampling is automated.

255

Involvement of Multidrug Resistance-associated Protein 4 (MRP4) in the Skeletal Muscle Distribution of Statins

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The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, are important drugs used in the treatment and prevention of cardiovascular disease. While statins are well-tolerated, up to 15% of patients prescribed this class of drug develop muscle aches and pain. These mild muscle side effects are considered a prelude to rhabdomyolysis, a severe, life-threatening form of muscle injury. Despite the prevalence of this side effect, little is known regarding the molecular determinants of statin distribution into skeletal muscle and its relevance to muscle toxicity. Here, we examined the role the membrane efflux transporter, MRP4, in regulating statin distribution in human skeletal muscle. The expression of MRP4 in human skeletal muscle was examined by quantitative real-time polymerase chain reaction, Western blot and immunofluorescence microscopy. In vitro models including transient heterologous expression of drug transporters in HeLa cells was employed to demonstrate statin efflux transport by MRP4. Chemical modulation of MRP4 function was examined in a model of differentiated, primary human skeletal myoblasts in culture. MRP4 is expressed in the sarcolemma of human skeletal muscle as well as in cultured myoblasts. We demonstrate that the non-metabolized statin, rosuvastatin, is a transport substrate of MRP4. In skeletal myoblasts, modulation of MRP4 function with transport inhibitors (MK-571 and non-steroidal anti-inflammatory drugs) resulted in increased intracellular levels of rosuvastatin. MRP4 appears to modulate skeletal muscle exposure to statins by preventing cellular drug accumulation. The involvement of this transporter in statin-induced myopathy remains to be determined.

256

Genetic Polymorphisms of the hOAT2 [SLC22A7] Drug Transporter and CYP3A Variants in Japanese Population

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Human organic anion transporter (hOAT) plays a pivotal role in the elimination and excretion of a wide range of organic solutes. To date, several OAT isoforms have been isolated and well characterized. Among them, hOAT2 is predominantly expressed and localized at the sinusoidal membranes of the human liver. In turn, CYP3A subfamily is a major drug metabolizing enzyme that highly expressed in the liver. Four CYP3A isoforms have been reported such as 3A4, 3A5, 3A7 and 3A43 to date. However, there is still limited data concerning the genetic polymorphism(s) of hOAT2 and CYP3A in Japanese population. To elucidate this, we analyzed genetic polymorphism of 3A enzymes (3A4*3, 3A4*18, 3A5*2, 3A5*3, 3A5*4, 3A5*6, 3A5*7, 3A7*1C, and 3A43*3) and hOAT2 (C329T, G571A, G1520A, rs36040909, rs2270860, rs2242416, rs2841647 and rs1574430) by PCR-RFLP and direct sequencing methods. Liver samples were collected by Showa University Hospital and genomic DNA was extracted. We also examined total CYP content, mono-oxygenase activities and Western blot analysis. This study was approved by the Ethic Committee of Showa University, and written informed consent was obtained from individual patients with hepatocellular carcinoma (HCV negative). The frequency of the CYP3A5*3 variant allele in Japanese was 0.9%. No genetic variants for the other CYP3A enzymes were observed. Of hOAT2 variants, no C329T, G571A, G1520A and rs36040909 variants were existed, whereas the other hOAT2 variants were detected (50%). However, there is no variant with amino acid changes in hOAT2. Our results may contribute to a better understanding of ethic differences in drug response.

257

Effects of Sophorae Radix-plus, Novel Formulas of Traditional Chinese Herbal Medicine, on Severe Atopic Dermatitis (Eczema)

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The efficacy and safety of novel formulas of traditional Chinese herbal medicine, Sophorae Radix-Plus, was investigated. Sixty-four severe atopic dermatitis (AD: eczema) patients received oral administration of the formula, Sophorae Radix-Plus 1, containing 10 herbs, combined with a lotion, Sophorae Radix-Plus 2, containing 7 herbs, and an ointment, Sophorae Radix-Plus 3, containing 8 herbs for 12 months as an open trial. The severity on the disease (Japanese clinical score; 0-4) and the severity of pruritus (pruritus score; 0-4) were judged by standardized scores. The clinical score of the all recruited patients before the treatment was 4. Both scores were significantly improved after 3 months of the treatment (p less than 0.01;non-parametric test) and then, both scores reduced slowly. Both the serum eosinophil ratio and serum IgE levels were high in AD patients and they were significantly improved at the end of the treatment (p less than 0.001). Many patients were cured after 12 months of treatment. Among 64 AD patients with traditional Chinese herbal therapy, 45 were markedly improved, 17 were improved, 2 were ineffective. There was no remarkable evidence of renal or hepatic toxicity or the other severe adverse effects. Thus, the present study indicated that this herbal treatment is clinically efficacious on AD.

258

Effects of Simvastatin, Atorvastatin and Pitavastatin on the Pharmacokinetics of Oral Midazolam in Healthy Volunteers

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Introduction: 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) are often used with other cardiovascular drugs to prevent coronary artery disease. To clarify the clinical impact of statins on CYP3A4 related drug interactions, we investigated the effects of various statins on the pharmacokinetics of oral midazolam (MDZ). Method: This was a randomized, open-label, crossover study with tree phases. Eleven healthy volunteers were enrolled and received either simvastatin (10 mg/day), atrovastatin (10 mg/day) or pitavastatin (2 mg/day) orally for 14 days in each phase. The pharmacokinetics of oral MDZ (15 μg/kg) were assessed on the day 14 in each trial phase. MDZ and its metabolite, 1'-hydroxy MDZ, in serum and urine samples were analyzed by LC/MS/MS spectrometry. Result: Statins used in this study did not affect the maximum concentration of MDZ [control: 7.0±3.1 ng/mL, simvastatin: 8.8±4.0 ng/mL, atorvastatin: 7.2±2.6 ng/mL, and pitavastatin: 6.7±1.7 ng/mL (mean±SD)], the area under the plasma concentration-time curve from 0 to 10 h (AUC[0-10]) of MDZ (control: 15.2±7.5 hr*ng/mL, simvastatin: 18.8±12.7 hr*ng/mL, atorvastatin: 17.5±6.3 hr*ng/mL, pitavastatin: 15.8±5.3 hr*ng/mL), and the AUC (0-10) ratio of MDZ/1'-hydroxy MDZ (control: 3.4, simvastatin: 3.6, atorvastatin: 3.5, and pitavastatin: 3.8. Conclusion: Our findings strongly suggest that the clinical dosage of these statins dose not affect both hepatic and intestinal CYP3A4 activities and that the drug interaction between these statins and CYP3A4 substrates is unlikely in clinical settings.

259

Effect of the Genetic Polymorphism of OATP2B1 on the Disposition of Fexofenadine in Healthy Volunteers

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Organic anion transporting polypeptide (OATP) 2B1 mediates the uptake of various drugs including fexofenadine. The objective of the present study was to investigate the contribution of the genetic polymorphism of OATP2B1 to the pharmacokinetics of fexofenadine. The pharmacokinetics of a single oral dose of 60 mg fexofenadine was assessed in 11 healthy male subjects. Subjects were divided into 2 groups based on OATP2B1 genotype for *3 allele (1457C>T). The pharmacokinetic parameters of fexofenadine were compared between subjects with OATP2B1*1/*1 (n = 5, group CC) and with *3 allele (n = 6, group CT+TT, CT: *1/*3, TT: *3/*3). AUC of fexofenadine was 1.5-fold greater in CC than in CT+TT ($0.05 < p < 0.1$). Also, CC showed 1.5 times higher Cmax compared with CT+TT, although the difference was not statistically significant. The terminal half-life was approximately 3 hours in both of the groups. The results of the present study suggest a potential association between the OATP2B1 genetic polymorphism and altered fexofenadine pharmacokinetics. The SNP of 1457C>T may decrease the transport capacity of OATP2B1 in the intestine, and thereby reduce the bioavailability of fexofenadine.

260

Microdose Pharmacokinetics of Nicardipine Using a High Performance Liquid Chromatography in Healthy Volunteers

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Introduction: A single microdose clinical trial is a useful tool in new drug development, however using radioactive drug is difficult in Japan. We compared pharmacokinetics of microdose and clinical dose of nicardipine, a CYP3A4 substrate using cold compound. Methods: 12 healthy male volunteer were administered 100 mcg and 20mg of non-radio labeled nicardipine orally in a randomized cross-over design by a 2 weeks' interval. Plasma concentration was measured by a LC/MS/MS method. Results: Nicardipine was absorbed rapidly after both dosages. Area under the curve of nicardipine corrected by dose was slightly higher in the case of the microdose. Small fluctuations of plasma concentration in terminal phases after the microdose were observed in subjects to whom clinical dose was administered first. Discussion: A cold microdose method was considered to be useful in evaluating pharmacokinetics of nicardipine, which is metabolized extensively by CYP3A4. The observed fluctuations in the terminal phase are considered to reflect release of nicardipine, which bound to the tissue after the first clinical dose, since such fluctuation was not found in the cases that were administered by microdose first. Conclusion: A cold microdose method is a possible approach to perform microdose clinical study in Japan.

261

Relationship between CYP2C19 Phenotype and Gene Mutation in Patient with Hepatic Cancer

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This study was conducted to assess whether the genotypic frequency of S-mephenytoin 4'-hydroxylase CYP2C19 was affected by hepatic carcinoma. Liver samples were donated from Japanese hepatic cancer patients who received partial hepatectomy; fifteen were hepatocellular carcinoma (HCC) and 22 were metastatic hepatic cancer (MC). Individual CYP2C19 genotype was determined by PCR-based amplification method followed by restriction fragment length analysis. Hepatic microsomal CYP2C19 activity was determined by S-mephenytoin 4'-hydroxylation (S-MP 4'-OH). Higher frequency of CYP2C19 gene mutation both in exon 4 and 5 were observed in MC patients compared to HCC patients. S-MP 4'-OH activity was relatively well correlated to the genotype in the samples from MC patients; most of the samples with CYP2C19 *1/*1 showed the comparable activity to that of healthy subject reported although the samples with mutation showed lower activity. In contrast, declined activity of the enzyme was observed in HCC samples without existence of any mutation. These

results suggested that low activity of CYP2C19 in MC patients were due to the enzyme deficiency caused by the mutation of CYP2C19 gene. It was also indicated that CYP2C19 deficiency or gene mutation might reflect the potentials of the initiation and/or metastasis of the carcinoma to the liver.

262

Pharmacodynamic Characterization of HM30181A, a Novel Inhibitor of MDR1 (P-glycoprotein)

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HM30181A was recently developed as a novel inhibitor of P-glycoprotein (multidrug resistance 1, MDR1, ABCB1) for reversing multidrug resistance and for facilitating oral uptake of co-administered cancer chemotherapeutic agents. This study was aimed to identify the pharmacodynamic characteristics of HM30181A at molecular levels. The inhibitory effects of HM30181A on MDR1 and several multidrug resistance-related proteins (MRPs) were determined by the ATPase assay of membrane vesicles and the cell-based flow cytometry. HM30181A showed the most potent inhibitory effect on the MDR1-mediated paclitaxel efflux in the ATPase assay (Ki = 0.58 nM) when compared with other already known inhibitors, such as cyclosporin A, XR-9576, and GF-120918. HM30181A also showed a strong inhibitory effect on the MDR1-mediated rhodamine123 efflux in 293FRT-MDR1 cells (IC50=12.63 nM). However, HM30181A showed a weak inhibitory effect on the BCRP1 and did not inhibit MRP1-, MRP2-, and MRP3-mediated xenobiotic transports up to a 100 microM concentration, indicating that HM30181A is a highly selective inhibitor of MDR1. The ATPase assay with a fixed concentration (1.4 nM) of HM30181A showed a typical non-competitive inhibitory pattern. The Emax of MDR1-mediated paclitaxel transport was decreased by HM30181A without affecting EC50, representing that HM30181A either irreversibly binds to the paclitaxel-binding site of MDR1 or inhibits MDR1 at allosteric binding sites. In conclusion, the present study shows that HM30181A is a highly potent and selective inhibitor of MDR1 and can be used for increasing the oral bioavailability of cancer chemotherapeutic agents, such as paclitaxel.

263

Novel Animal Model of Type 2 Diabetes

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Development of an appropriate animal model exhibiting human metabolic characteristics of Type 2 diabetes mellitus (T2DM) is crucial for study of the disease. Numerous models of T2DM in rodents have been created using genetic manipulation, dietary induction, chemical induction or a combination of the latter two. This study describes the development of a novel fructose-fed, streptozotocin (STZ)-induced rat model of T2DM. Wistar rats (5 weeks old) were fed a normal or high-fructose diet (60% fructose) starting day 0 until the end of the study. On day 14, half of the animals in the fructose-treated group were given STZ (60 mg/kg, I.V.). Blood samples were taken on day 35 for measurements of plasma glucose, insulin and triglyceride. Insulin sensitivity was determined by 2-h hyperinsulinemic euglycemic clamp on day 42. Rats treated with fructose-STZ (F-STZ) developed hyperglycemia, polyphagia and polydypsia, and they weighed less than control (CTL) and fructose (F) rats on day 42. These rats did not require insulin supplementation for survival but exhibited similar and lower plasma insulin than those in the CTL and F groups. In addition, the F-STZ group had significantly ($P<0.05$) higher plasma glucose and triglyceride and were significantly more insulin resistant than the CTL and F groups. This novel F-STZ rat model is stable for at least 4 weeks following injection of STZ, and it exhibits classic human symptoms of advanced T2DM, namely, insulin resistance, and elevated plasma glucose and triglycerides.

264

Pharmacokinetics and Local Action of Intravaginal DHEA in Postmenopausal Women

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The primary objective of that study was the evaluation of the systemic bioavailability of DHEA and its metabolites following daily intravaginal application of DHEA. In addition, the maturation index and the vaginal pH were measured to evaluate the local effect of DHEA. Forty postmenopausal women were randomized to receive a daily dose of one ovule of the following DHEA concentrations: 0.0%, 0.5%, 1.0% or 1.8%. After one week of treatment, the maturation value increased over pretreatment of 107% ($p < 0.01$), 75% ($p < 0.05$) and 150% ($p < 0.01$) in the 0.5%, 1.0% and 1.8% DHEA groups, respectively, while vaginal pH was significantly decreased at all doses. These local effects of DHEA were observed while the serum concentrations of estradiol and testosterone remained within the values found in normal postmenopausal women at all DHEA doses. Similar findings were observed for serum androstenedione, estrone, estrone-sulfate and dehydroepiandrosterone-sulfate (DHEA-S). Even at the highest 1.8% DHEA dose, serum DHEA was increased at the levels found in normal premenopausal women while the sum of the serum levels of the metabolites of androgens (ADT-G, 3alpha-diol-3G and 3alpha-diol-17G) were only increased to 66% of normal premenopausal values. The present data show that the intravaginal administration of DHEA permits to rapidly achieve the beneficial effects against vaginal atrophy without significant changes of serum estrogens, thus avoiding the increased risk of breast cancer associated with the current intravaginal or systemic estrogenic formulations and adding the local benefits on all the layers of the vagina of the recently recognized androgenic component of DHEA action in this tissue.

265

Glucocorticoid Receptor Gene Polymorphisms and ALL Outcome

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Corticosteroids (CS) drugs have been used extensively in the treatment of acute lymphoblastic leukemia (ALL), the most frequent pediatric cancer. Significant number of patients does not respond to CS and/or demonstrate serious side effects. CS exert their action through binding to the glucocorticoid receptor (GR), which acts as a transcriptional regulator of responsive genes, rendering GR polymorphisms potential modulators of a therapeutic response to CS. We have previously shown that the G variant of Bcl I polymorphism (C to G transition in intron 2) was associated with particularly dismal prognosis in children with ALL. Here we report the association of two other polymorphic sites in GR. 284 ALL patients of European descent treated in Ste-Justine Hospital were genotyped for two SNPs: -627 A/G promoter SNP (rs10482605) and 3'UTR GR-beta mRNA SNP (rs6198) in the AUUUA motif known to regulate mRNA stability. An association of AG+AA -627 genotypes and AA+AC 3'UTR genotypes with event free survival was observed with the hazard ratio HR = 2.04 (95% CI 1.03-4.06) and HR = 2.15 (95% CI 1.09-4.26), respectively, as estimated by the Cox regression analysis. Risk of event increased further in individuals with three event predisposing genotypes. Due to the linkage disequilibrium between studied loci, an association of the GR haplotypes with the disease outcome was found. In conclusion: we report an association of GR gene polymorphisms with poorer ALL outcome. The finding can be of importance for the prediction of the treatment response tailored to individual genotypes.

266

Influences and Interactions of Candesartan, Atenolol and Hydrochlorothiazide on Norepinephrine Levels in Hypertensive Patients

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Introduction: This study aimed at comparing the influences of a beta blocker and an angiotensin receptor blocker on plasma norepinephrine (NE) and spectral analysis of heart rate variability in hypertensive subjects. It also aimed at evaluating the influence of added thiazide diuretic therapy on the same parameters. Methods: This was a prospective randomized parallel-designed study with a 4 week placebo run in period. Subjects were treated with either atenolol 100 mg od (A100), candesartan 16 mg od (C16) or hydrochlorothiazide 25 mg od (HCT25) for 4 weeks. Treatments were then increased to A100+hydrochlorothiazide 12.5 mg od (HCT12.5), C16+HCT12.5 or HCT25+C16 for 4 more weeks. Blood pressure (BP), NE (supine and standing) and spectral analysis parameters were measured at trough and 4 hours post dose at the end of placebo run in, monotherapy and combination treatment periods. Results: Thirty nine subjects completed the study. Average age and BP were 54.7± 9.5 and 145.5± 13.0/ 94.5±5.6 respectively. BP significantly decreased with all 3 monotherapies and significantly more with combination treatments. Only HCT25 monotherapy and HCT12.5 added to C16 or A100 significantly increased standing NE parameters. All combination treatments significantly increased NE 4 hours post dose compared to placebo. No differences were found between treatment groups. Spectral analysis of heart rate did not provide additional information. Conclusion: Candesartan and atenolol do not increase NE in hypertensive subjects despite significant fall in blood pressure. Hydrochlorothiazide increases NE when given as monotherapy or even when combined to candesartan or atenolol.

267

Pharmacokinetics of Carvedilol Enantiomers in Patients with Hypertension Undergoing Continuous Ambulatory Peritoneal Dialysis

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Carvedilol, a racemic mixture e of the (+)-I- and the (-)-(S)-enantiomer, with nonselective β- and α1-blocking activity, is used for the treatment of hypertension and congestive heart failure. The β-receptor blocking activity of the (-)-(S)-carvedilol is about 200 times higher than (+)-I-carvedilol, whereas both enantiomers are equipotent α-blockers. The AUC of (+)-I-carvedilol is significantly higher than (-)-(S)- carvedilol (AUCR/S ratio 2.7) in healthy volunteers. The study describes the pharmacokinetics of carvedilol enantiomers in six patients (4 male, 2 female, age 36-53 years) with hypertension on continuous ambulatory peritoneal dialysis (CAPD). All patients received a single 25 mg oral dose of racemic carvedilol. Serial plasma samples were obtained from zero to 32 h. Carvedilol enantiomers concentrations in plasma were determined by LC-MS/MS using the chiral column Chirobiotic® V. The assay was linear over the range of 0.2-200 ng/ml with intra- and interassay precision (%CV) less than 15%. The pharmacokinetic parameters were calculated using the WinNonlin software. The Wilcoxon test ($P<0.05$) was used to evaluate the enantiomeric ratios differing from one. Data are expressed as median. Cmax (120.07 vs 97.73 ng/ml), AUC (497.94 vs 485.71 ng.h/ml), Cl/F (25.17 vs 27.89 l/h), Vd/F (116.57 vs 218.46 l) and t1/2 β (12.68 vs 13.79 h) values were not significantly different between (+)-I and (-)-(S)-carvedilol. The AUCR/S ratio was 0.97. In conclusion, hypertensive patients on CAPD have loss of the enantioselectivity in the kinetic disposition of carvedilol showing the importance of the application of enantioselective methods in pharmacokinetics studies of chiral drugs.

268

A Comparative Study of CYP3A4 Polymorphisms in Mexican Amerindian and Mestizo Populations

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Cytochrome P-450 3A4 (CYP3A4) contributes to the metabolism of approximately half the drugs in clinical use today. Adverse drug reactions,

therapeutic failure, and undesirable drug interactions in patients are a result of a complex of genetic and environmental factors. Drug-metabolizing enzymes (DMEs) play an important role in determining treatments. The aim of the present study was to determine the frequency of the *CYP3A4*1B, *2, *4, *5,* and **18* alleles amongst both Tepehuan Amerindians, a native group that has inhabited northern Mexico for thousands of years, and Mestizo Mexicans, and to compare the data with those of other populations. Genotyping experiments revealed that 8.8 and 8.0% of the Mestizo and Tepehuano subjects, respectively, carried the *CYP3A4*1B* allele which has been associated with a reduced capacity to metabolize drugs, carcinogens and endobiotics that are CYP3A4 substrates. Only one Mestizo subject was heterozygous for the *CYP3A4*2* variant, while *CYP3A4*4, *5* and **18* allelic variants were not detected in either group. On the other hand, the frequencies of the *CYP3A4*1B* variant in Mestizos and Tepehuanos were similar to those reported for Caucasians, but different from those observed for African and Asian populations. Such findings will aid in the assessment of appropriate strategies of drug therapy in Amerindian peoples. We recently reported findings of important differences in the frequencies of several CYP2D6 polymorphisms between Mestizos and Tepehuanos indigenous.

269

Vitamin D Receptor Gene Polymorphisms and Clinical Characteristics of Hemodialysis Patients

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Chronic kidney disease (CKD) patients often have secondary hyperparathyroidism (SHPT) and anemia, requiring treatment with vitamin D and erythropoiesis-stimulating agents (ESA). Vitamin D receptor (*VDR*) gene polymorphisms, thought to affect gene expression and response to vitamin D or ESA, have been associated with different treatment outcomes in SHPT and anemia. These effects have not been examined in the US Black and Hispanic populations. We conducted a pilot study to determine if VDR gene polymorphisms are associated with clinical outcomes and treatment in our hemodialysis (HD) patients. Seventy-one HD patients (76% Blacks; 21% Hispanics) were genotyped for two polymorphisms in the *VDR* gene [rs154410 (*Bsm*I) and rs10735810 (*Fok*I)] previously associated with SHPT and anemia. Genotype distributions of *Bsm*I and *Fok*I polymorphisms were determined, and patient demographics, laboratory parameters, and medication history were collected and compared across genotype groups. The genotype frequencies did not deviate from Hardy-Weinberg equilibrium (p=NS). No differences in hemoglobin, serum phosphorus, calcium and parathyroid hormone concentrations ($p>0.05$) were observed across genotype groups. Lower serum albumin concentrations ($p<0.05$) and IV vitamin D dose requirements ($p<0.05$) were observed in patients with the *Fok*I ff genotype compared to F allele carriers. Our analysis suggests that VDR gene polymorphisms may be associated with nutritional status and vitamin D dose in HD patients. Lower frequencies of the B and f alleles were observed in our study sample compared to Caucasian and Asian populations previously reported. Therefore, differences in VDR genotype distribution and association with clinical characteristics across racial and ethnic groups may exist.

270

Abeta Activation of STAT1/STAT2 and Increase in Mcl-1 Expression Protects against Abeta-induced Toxicity in PC12 Cells

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The presence of senile plaques (SP) is a major pathological hallmark of Alzheimer's disease and beta-amyloid peptide ($Abeta_{1-42}$) is a major component of SP. Abeta is known to cause lipid peroxidation, DNA fragmentation and caspase 3 activation that finally leads to apoptosis. But at the same time that Abeta produces its toxicity, cells may also develop defense mechanisms to protect against Abeta toxicity. We addressed this issue in the present study by using various cellular and molecular approaches. Results showed that Abeta produced a dose-dependent decrease of cell viability (0.01 to 1.0 μM) and a dose-dependent

increase in the expression of the anti-apoptotic gene Mcl-1 in PC12 cells. At higher doses of Abeta that cause more cell death, increase in Mcl-1 expression was not observed. Meanwhile, Abeta (0.1 μM) increased the phosphorylation of transcription factor STAT1 at Tyr701 and Ser727 and increased the phosphorylation of STAT2 at Tyr690. Abeta also significantly increased the luciferase activity of ISRE (the STAT1/STAT2-DNA binding site), but not that of STAT1 (the STAT1/STAT1-DNA binding site) and GAS (the STAT1/STAT3, STAT3/STAT3 and STAT1/STAT3-DNA binding site). Overexpression of the wildtype STAT1/STAT2 increased the cell viability and the expression of Mcl-1. But transfection of the STAT1/STAT2 mutant DNA (STAT1Y701FS727A/STAT2Y690F) antagonized Abeta-induced Mcl-1 expression. These results revealed a novel protective mechanism against Abeta toxicity. These results also provide a novel therapeutic implication that drugs that selectively activate STAT1/STAT2 signaling may protect against the toxicity of Abeta in neuronal cells.

271

Genetic Variations Associated with the Dermatologic Adverse Drug Reactions of Antiepileptic Agents

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Antiepileptic drug-related adverse drug reactions (ADRs) are a relatively common problem that complicates management of epilepsy patients. Studies have reported 10% or more of patients on antiepileptic agents have experienced skin rash. To find new genetic targets that are related to dermatologic ADRs of antiepileptic agents, a whole genome-based SNP analysis was performed using Affymetrix 5.0 SNPchip containing 500,568 SNPs and samples from 132 patients treated with antiepileptic agents showing skin rash and those from another 132 patients showing no signs of dermatologic side effects. The SNP selection criteria were 1) minor allele frequency > 0.05, 2) genotyping success rate > 0.98, and 3) P > 0.001 in the Hardy-Weinberg Equilibrium test from QQ plot. The P values of association between these SNPs and antiepileptic agent-related skin rash were calculated in dominant, recessive, and co-dominant mode. A total of 21 SNPs showed with P values under 0.000001. Among these, 11 SNPs were in intragenic regions while the remaining 10 SNPs were in intergenic regions. The present study suggests that some genetic polymorphisms are highly related to the dermatologic side effects of antiepileptic agents. Our findings also encourage further studies, particularly confirmatory studies with larger samples, to validate and analyze the association between these SNPs and antiepileptic agent-related ADRs.

272

Induction of Chemoprotective Phase 2 Enzymes by Ginseng and its Components

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Introduction: Phase 2 detoxification enzymes serve an important role in protecting against carcinogenesis and oxidative stress. Ginseng (*Panax* spp.) has been shown in animals to induce antioxidant phase 2 enzymes. Methods: Ginseng extracts and components were assayed for inducer activity of quinone reductase, a representative phase 2 enzyme, in Hepa1c1c7 cells. Ginseng extracts were analyzed for content of ginsenosides using a validated HPLC method. Results: Korean red *Panax ginseng* extracts demonstrated the most potent phase 2 enzyme induction activity (76,900 U/g and 27,800 U/g for two commercial preparations). The ginsenoside-enriched HT-1001 American ginseng (*Panax quinquefolius*) extract was the next most potent inducer, with activity of 15,900 U/g, followed by raw American ginseng root with activity of 8,700 U/g. Neither a polysaccharide-enriched extract of American

ginseng nor a commercial white *Panax ginseng* preparation showed any inducer activity. None of the 8 pure ginsenosides tested showed any inducer activity. There was no correlation between ginsenoside content and phase 2 enzyme induction. Protopanaxadiol and protopanaxatriol, deglycosylated ginsenoside metabolic derivatives, showed potent induction activity (approximately 500,000 U/g each). Synthetic panaxytriol was over 10-fold more potent, with an induction potency of 5,760,000 U/g. Conclusions: Various extracts of ginseng induce phase 2 enzymes. Minor components are likely to be the most potent inducers. Discussion: Clinical studies may be warranted to ascertain whether ginseng extracts or components induce phase 2 enzymes in humans, since this may contribute to the cancer chemoprotective activity of ginseng or lead to drug interactions with medications metabolized by phase 2 enzymes.

273

Proteomic Analysis for 3T3-L1 Adipocyte Differentiation in Response to Chitosan Oligosaccharides

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Firstly, we performed a proteomic analysis using two-dimensional gel electrophoresis (2-DE) between 3T3-L1 pre-adipocyte and mature adipocytes to discover biomarkers during adipocyte differentiation. Four proteins (VDAC, PRPS, GPDH, and Vinentin) were found, for the first time, significantly regulated during the adipogenesis. Next, we also conducted a differential proteomic analysis to clarify the molecular mechanism for the suppressive effect of chitosan oligosaccharides (CO) during differentiation of adipocyte 3T3-L1. Cell differentiation was significantly inhibited by CO at the concentration of 4 mg/mL. Protein mapping of adipocyte homogenates by 2-DE revealed that numerous protein spots were differentially altered in response to CO treatment. Out of 50 identified proteins showing significant alterations, 6 were up-regulated and 44 were down-regulated by CO treatment in comparison to control mature adipocytes. The levels of FDS, DOCK9, and CLIC1 were significantly reduced (>2-fold) with CO treatment. These results have not previously been examined in the context of adipogenesis, and thus can be used as novel biomarkers. It was concluded that the inhibitory effect of CO on adipocyte differentiation was mediated by C/EBP-alpha and PPAR-gamma pathway through significant down-regulations of important adipogenic molecules such as FABP and GLUT4.

274

MRP4 Polymorphisms Associated with Lamivudine Responses in Chronic Hepatitis B

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Lamivudine (2', 3'-dideoxy-3'-thiacytidine, 3TC) is one of the commonly prescribed antiviral agents for hepatitis B viral infection and the patients treated with lamivudine show a diverse inter-individual variance in the drug responses. This inter-individual variance has been ascribed to the viral factors such as YMDD mutation. Here, we report that a host factor, genetic polymorphisms in the MRP4 (multidrug resistance associated protein 4, *ABCC4*) gene, is associated with the inter-individual variance of lamivudine response. MRP4 is a member of ABC (ATP binding cassette) transporter superfamily and is known to transport nucleoside analogues including lamivudine. Ninety-eight patients who had been administered lamivudine were included in this study and divided into two groups according to the loss of HBV-DNA at 24 weeks. Among the analyzed 13 SNPs in the MRP4 gene, two SNPs at the promoter region and the 3' UTR showed significant associations with lamivudine response. Moreover, the haplotype harboring these two SNPs showed a strong association with the poor drug response (p=0.002). The results from logistic regression analysis with major clinical factors revealed that the MRP4 genotype is an important factor determining the lamivudine response (OR 4.9, 95% CI 1.5~15.7, p=0.008). Therefore, we suggest that the genetic polymorphisms in the MRP4 gene need to be considered in prescribing lamivudine and individual dosage determination.

275

ET_B Receptor Agonist, IRL-1620, Increases Cerebral Blood Perfusion in Rats

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IRL-1620, a highly selective ET_B receptor agonist, is a synthetic analogue of ET-1 and can produce both vasodilatation as well as vasoconstriction. Interaction of IRL-1620 with ET_{B1} receptors on endothelial cells leads to vasodilatation, while its interaction with ET_{B2} receptors on smooth muscle cells leads to vasoconstriction. However, effect of IRL-1620 on cerebral blood perfusion has not been studied. The present study was conducted in urethane anesthetized rats to determine the effect of IRL-1620 on cerebral blood perfusion using a laser Doppler blood perfusion meter. Cerebral blood perfusion increased on an average of 11.38% and 26.08% following intravenous administration of 1.6 and 5.0 µg/kg dose of IRL-1620, respectively. The effect lasted for more than 45 min. An increase in cerebral blood perfusion by IRL-1620 could be completely blocked by pretreatment with, ET_B receptor antagonist, BQ-788 (1 µg/kg, i.v.). On the other hand, cerebral blood perfusion increased on an average of 24.49% when IRL-1620 (5.0 µg/kg, i.v.) was administered to rats pretreated with ET_A receptor antagonist, BMS182874 (15.0 mg/kg, i.v.). It is possible that the presence of tight junctions in cerebral blood vessels did not allow intravenously administered IRL-1620 to reach the cerebrovascular smooth muscle cells and that led to selective stimulation of ET_{B1} receptors producing an increase in cerebral blood perfusion.

276

Complementary use of In-life Quantitative Microdialysis, Brain and Plasma Exposure to Help Validate the Concept of a Peripherally Acting Cannabinoid Agonist in Rat

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In animal models, cannabinoid receptor (CB) agonists reduce the hyperalgesia and allodynia that develop after nerve injury. In man, their use as analgesics is limited by their psychoactive properties, which are primarily mediated by CB1 receptors in the CNS. The aim of this study was to characterize the distribution kinetics in rat brain and plasma of a peripherally-restricted CB agonist (AZ1) in an effort to provide insight into its site of action as an analgesic. Quantitative microdialysis was performed in rats at doses producing robust efficacy without CNS side effects as measured in a rat neuropathic pain model. Microdialysate was collected over 30 min intervals for 3.5 h. Total brain and plasma exposures were assessed in satellite animals under similar conditions. Three hours following a 60 micromol/kg oral dose of AZ1, free brain levels (as measured by microdialysis) were 25 nM, over 10-fold lower than the estimated free plasma levels (200-400 nM). The total brain to plasma ratio was likewise estimated to be low (0.05 – 0.03). In agreement with a peripherally restricted compound, microdialysate concentrations increased slowly over time, potentially as a result of efflux as the compound was found to be a Pgp substrate in MDR1-MDCK cells. In conclusion, AZ1 produces full analgesic efficacy with free plasma levels 2-5 fold above the rat CB1 EC50 (81nM) and free brain levels of 25 nM, providing evidence that a separation between peripherally-mediated analgesia and centrally-mediated side effects is possible with peripherally restricted CB agonists.

277

Diet-induced Hyperglycemia and Hypertriglyceridemia in Streptozotocin-treated Rats

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Diabetes mellitus is a metabolic disorder in which plasma glucose is elevated above the normal range. Streptozotocin (STZ) is a chemical commonly used to induce the development of type 1 diabetes in rodents. The feeding of rodents with a diet high in carbohydrate content has been

shown to produce metabolic abnormalities such as hyperlipidemia, glucose intolerance and insulin resistance. This study investigated if feeding of STZ-pretreated rats with a high fructose diet induced metabolic abnormalities as well as hyperglycemia thereby making the animals a suitable type 2 model of diabetes. Wistar rats (5 weeks old) were fed a normal or high-fructose (60% of caloric intake) diet starting day 0 until the end of the study. On day 14, all of the fructose-fed rats and half the rats fed a normal diet were given STZ (60 mg/kg, I.V.). Blood samples were taken on day 35 for measurements of plasma glucose, insulin and triglycerides. Insulin sensitivity was determined by hyperinsulinemic euglycemic clamp on day 42. Rats treated with fructose as well as STZ had significantly higher plasma glucose and triglycerides ($P<0.05$) than rats given only STZ. Both the STZ and fructose-STZ groups did not require insulin supplementation for survival, had lower plasma insulin and developed insulin resistance relative to the control group. These results show that fructose feeding in STZ-pretreated rats causes severe hyperglycemia, insulin resistance and hypertriglyceridemia, characteristics of type 2 diabetes.

278

Effects of Polymorphism of *MDR1, SLCO1B1* and *CYP3A4* on the Pharmacokinetics of Simvastatin in Korean Subjects

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Objectives: To investigate the effects of the polymorphism of *CYP3A4*4*, *MDR1* and *SLCO1B1* on the pharmacokinetics of simvastatin in Korean Subjects. Methods: 13 subjects received 40mg of daily oral dose of simvastatin from day 1 to 8, and the pharmacokinetics of simvastatin and simvastatin acid were evaluated on day 8. Blood samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 13, 17, 24, 36 and 48 hours after the last dose. Plasma concentrations were analyzed by the HPLC–MS/MS method. AUC, C_{max}, t_{max}, and $t_{1/2}$ were determined by a non-compartment method using WinNolin. We investigated the genotypes of *CYP3A4*4*, 3 SNPs of *MDR1*, c.1236C>T, c.2677G>A/T, and c.3435C>T, and 3 SNPs of *SLCO1B1*, c.388A>G, c.521T>C, and g.-11187G>A. Haplotypes of *MDR1* and *SLCO1B1* genotypes were assembled using the Haploview 3.2 program. Differences in pharmacokinetic parameters between the genotype or haplotype groups were tested using the Kolmogorov-Smirnov test or the Kruskal-Wallis test. The P-value < 0.05 was considered statistically significant. Results: In subjects with the *MDR1* c.1236TT genotype, the mean AUC_{48h}, AUC_{inf} and C_{max} of simvastatin acid were higher than those with the *MDR1* c.1236CC or c.1236CT genotype by 67.2% (p=0.0152), 66.2% (p=0.0152) and 61.8% (p=0.0676), respectively. Other genotypes had no significant effect on simvastatin and simvastatin acid's pharmacokinetics. Conclusions: This study shows that the polymorphism of *MDR1* markedly affects the pharmacokinetics of simvastatin acid, reflecting that genetic variability in P-glycoprotein could have significant influence on the balance of risks and benefits of the simvastatin treatment.

279

Pharmacokinetics, Tissue Distribution and Excretion of CQP-Propionic Acid (A Novel Discovered Anti-malarial Chemical) in Rats

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Introduction: Malaria is one of the severe epidemics in the world, about 40 % of the world's population faced to the threat of malaria. Despite tremendous efforts, the morbidity hasn't obviously been declined over the last 50 years. Many antimalarial drugs are, mainly, ineffective due to the malaria resistance. Recently, a new chemical, CQP-propionic acid possessing the antimalarial effect was isolated from human urine after oral dose of piperaquine phosphate. Methods: The in vitro antimalarial activity assay of CQP-propionic acid was evaluated by the Rieckmann's method. The pharmacokinetic properties, tissue distribution and excretion of

CQP-propionic acid were investigated after oral dose in rats. And, its plasma protein binding was measured by equilibrium dialysis method. Results and Discussions: The development of ring stage parasite into the schizont stage was inhibited when the concentration of CQP-propionic acid was more than 10.0 μmol/L. The CQP-propionic acid was rapidly absorbed, extensively distributed and quickly eliminated in rats. The highest concentrations were found in stomach, followed by kidney, plasma and intestine and the lowest in the liver. Approximately 25 % of the total dosages were measured in urine and feces till 72 h post-dose, whereas almost no any CQP-propionic acid was excreted by bile until 24 h post-dose, respectively. The binding ratio with rat and human plasma protein were 44.07 – 47.52 % and 39.61 – 48.25 %, respectively. Conclusion: The preclinical pharmacokinetic properties of CQP-propionic acid in rats would be very useful for human. And, this chemical may be as a new candidate reagent for antimalarial.

280

CYP2D6 Genetic Polymorphism and Debrisoquine Hydroxilation in Four Hispanic Populations

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Introduction: Interethnic differences in cytochrome P450 polymorphism might be partially responsible for the variations in drug disposition between ethnic groups. The *CYP2D6* gene is highly polymorphic with alleles causing absent (Poor metabolizers: PMs), decreased, normal (Extensive Metabolizers: Ems), and increased activity. Aims: To determine differences on CYP2D6 activity (evaluated by debrisoquine hydroxylation polymorphism) and genotype in three Hispanic populations of healthy volunteers. Methods: Debrisoquine metabolic ratio (MR) were determined in urine collected 8 h following a 10 mg oral to 125 healthy Nicaraguan Amerindians (Nas), 129 Cuban-Mestizos (CMs) and 131 Cuban-Caucasians (CCs). A group of 925 Spanish Caucasians was used as control (SCs). The *CYP2D6* genotype was analysed by PCR and PCR-RFLP techniques for the *CYP2D6 *3, *4, *5, *6, *10, *17* and duplicated alleles to 98 healthy Nicaraguan Amerindians (Nas), 121 Cuban-Mestizos (CMs), 123 Cuban-Caucasians (CCs) and 142 Spanish Caucasians (SCs). Results and Discussion: As PMs were classified 5.6% of Nas, 5.3% of CCs and 4.9% of SCs respectively. This proportion was slightly higher than in CMs (3.9%). However, the median metabolic ratio among Ems was higher ($p<0.001$) among the Nas than the CCs, CMs and SCs. The MR correlated with the number of *CYP2D6* active genes in the American studied populations ($p<0.001$). The percentages of Non-functional (**3-*6*) and increased activity (multiplications) *CYP2D6* alleles were lower ($p<0.05$) in the Nas compared with the other three populations. Moreover, the percentage of *CYP2D6* with diminished activity (**10* and **17*) was higher ($p<0.05$) in CMs compared with others.

281

San-Huang-Xie-Xin-Tang Protects against Microglia-mediated Neuroinflammation: An *In Vitro* Model for Neuroprotective Herbal Medicine

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Microglia-mediated inflammation plays an important role in the pathway leading to neuronal cell death in a number of neurodegenerative diseases. Many herbal medicines and dietary supplements sold as aids to improve memory or treat neurodegenerative diseases or have other favorable effects on the CNS. San-Huang-Xie-Xin-Tang (SHXT) is a traditional Chinese herb medicine which is confirmed and possessed potent anti-inflammatory effects. In this study, we investigated the neuroprotective effects of SHXT on activated microglia-like cells mediated-neurotoxicity in human dopaminergic neuroblastoma SH-SY5Y cells. In co-culture conditions, we employed lipopolysaccharide (LPS) stimulated BV-2 cells as a model of activated microglia. SHXT diminished LPS-induced overexpression of iNOS, COX-2 and

gp91^{phox}, and production of nitric oxide, intracellular ROS and TNF-alphạin BV-2 cells. SHXT also reduced LPS activated microglia-mediated neuronal death and microglia-induced overexpression of nNOS and gp91^{phox} on SH-SY5Y cells. In conclusion, the neuroprotective effects of SHXT are mediated, at least in part, by decreasing the inflammation induced by microglia. These results also suggested that herb medicines possess the benefits on the inhibition of microglia-mediated inflammation might be potentially used to the treat neurodegenerative disease.

282

Combined Glutathione-S-Transferase M1 and T1 Genetic Polymorphism as Increased Risk Factors for Drug-induced Idiosyncratic Liver Injury (DILI).

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Background & Aims: Individual vulnerability to drug-induced liver injury (DILI) might result from deficiencies in the detoxification process, which determines the level of exposure to the reactive metabolite. To evaluate whether a genetically-determined reduction in the ability to detoxify electrophilic compounds, such as that expected among individuals with glutathione S-transferase (GST) null genotypes, might play a role in determining the risk for DILI and its clinical expression. Methods: Genomic DNA from 154 patients (mean age 53 year, 80 women) with a diagnosis of DILI as assessed with the CIOMS scale and 250 gender-and age-matched healthy controls were analyzed. A multiplex polymerase chain reaction (PCR)-based method was used to detect *GSTM1* and *GSTT1* gene deletions. Results: Carriers of double *GSTT1- M1* null genotypes had a 2.70-fold increased risk of developing DILI as compared to non-carriers (OR = 2.70; 95% CI = 1.45-5.03; p=0.003). The OR for DILI patients receiving antibacterials, NSAIDs and cardiovascular drugs were 3.52 (p=0.002), 5.61 (p=0.001) and 3.74 (p=0.024), respectively. Patients with amoxicillin-clavulanate hepatotoxicity (n=32) had a 2.81-fold increased risk (p = 0.037). Patients classified by the combined *GSTT1* and *GSTM1* null genotypes did not differ with regard to the type of injury, clinical presentation and outcome, except for the predominance of women in the combined null genotype (p<0.001). Conclusions: The double null genotype for *GSTT1* and *GSTM1* might play a role in determining the susceptibility to develop DILI, as a general mechanism that occurs regardless of the type of drug involved, and predominantly in women.Partially funded by Spanish Medicine Agency, FIS 07/0064/0016 and EC07/90910

283

Intrathecal Morphine Induced Scratching is not Relieved by H1-Antagonists

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It is known that i.v. injections of morphine can increase plasma levels of histamine and this has been thought to cause skin flushing and itching in facial, neck and anterior thoracic areas in patients. Interestingly, intrathecal (i.t.) injections of morphine in non-anesthetized mice cause a dramatic behavioral syndrome showing mainly as intense scratching and biting of fore limbs and hind limbs, which may be due to a severe pruritus. We have investigated whether this response was diminished or abolished with antihistamines. To this end, 96 BALB/c mice were separated randomly into 8 groups of 12 animals each. Two control groups were pretreated. 30 minutes before morphine i.t., with isotonic saline by s.c. injection. Three other groups received diphenhydramine (s.c.) at dose levels of 6.3, 12.5, and 25 mg/kg respectively. The remaining three groups received pyrilamine (s.c.) at 12.5, 25 and 50 mg/kg. After 30 minutes each mouse was injected with 2 mg/kg morphine (i.t.). All animals pretreated with saline, diphenhydramine or pyrilamine at the identified

dose levels which then received (i.t.) morphine displayed the behavioral scratching, biting syndrome which was unaffected by the antihistamine. These experimental results suggest that histamine release may not follow applications of morphine (i.t.), or that other histamine receptors may be involved in the response, or alternatively other substances, such as serotonin, may be liberated. It is noteworthy that initial studies involving applications of histamine (i.t.) to mice did not produce any scratching behavior, which may imply that histamine itself may not be involved in this phenomenon.

284

Effect of Amiloride on the Protective Action of Newer Antiepileptic Drugs in the Mouse Maximal Electroshock-induced Seizure Model

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The objective of this study was to assess the effect of amiloride (a potassium retaining diuretic) on the protective action of some newer antiepileptic drugs (AEDs: lamotrigine [LTG], oxcarbazepine [OXC], and topiramate [TPM]) in the mouse maximal electroshock seizure (MES) model. Electroconvulsions were produced by constant current (25mA, 0.2s) delivered via auricular electrodes by Hugo Sachs generator. The anticonvulsant effects of newer AEDs administered alone and in combination with amiloride were determined by calculating their median effective doses (ED_{50} values), protecting 50% of the animals against MES-induced seizures. Acute adverse effects of AEDs in combination with amiloride were determined in the chimney, passive avoidance, and grip-strength tests. Total brain concentrations of newer AEDs were estimated with HPLC technique to ascertain pharmacokinetic or pharmacodynamic characteristics of interactions between drugs. Amiloride (at 75 and 100 mg/kg, i.p.) significantly potentiated the antielectroshock action of LTG, OXC and TPM in mice. The drug at lower dose of 50 mg/kg had no impact on the anticonvulsant action of the tested AEDs. Amiloride (75 mg/kg, i.p.) in combination with the studied AEDs did not produce acute adverse effects in experimental animals, as determined in the chimney test, passive avoidance task and grip-strength test. Moreover, amiloride (75 mg/kg, i.p.) significantly increased total brain concentrations of LTG, OXC and TPM, indicating pharmacokinetic nature of observed interactions. In conclusion, amiloride pharmacokinetically enhanced the anticonvulsant effects of LTG, OXC and TPM against MES-induced seizures- therefore a special caution is advised for epileptic patients treated with newer AEDs and amiloride.

285

The Single Nucleotide Polymorphism in the Nicotinic Acetylcholine Receptor Alpha4 Subunit Gene Influences Nicotine Dependence in Japanese Smokers

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Achievement of smoking cessation is difficult due to nicotine dependence (ND). We previously presented that the smokers with CYP2A6 high-activity (CYP2A6 *1/*1, *1/*9, *1/*4 and *9/*9) show severer ND, compared with those with low-activity (*4/*9 and *4/*4) because the polymorphisms of CYP2A6, the main nicotine metabolizing enzyme, alter the pharmacokinetics of nicotine. In this study, we addressed gene polymorphisms related with ND from the pharmacodynamic aspects. The study subjects were 293 Japanese male smokers with CYP2A6 high-activity from the two populations. The 1st population consisted of the 135 workers in the same office and the others for the 2nd were from several institutions. The ND was assessed based on the Heaviness of Smoking Index. Genotyping was performed with PCR-based methods. Chi-square test was used for the association analyses. First, we analyzed 37 polymorphisms from 18 ND-candidate genes in the 1st population. Nicotine acetylcholine receptor alpha 4 subunit (CHRNA4) gene polymorphism rs2273504 (G/A) could be the most significantly associated with ND because the frequency of the subjects with

G/A or G/G (74.8%, n=107) was higher than those with A/A (42.9%, n=28) (P<0.005, OR=3.95, 95%CI=1.66-9.40). Confirming the possibility, we replicated the same tendencies in the 2nd (G/A or G/G, 73.0%, n=126; A/A, 50.0%, n=32; P<0.05, OR=2.70, 95%CI=1.22-6.00) and the pooled populations (G/A or G/G, 73.8%, n=233; A/A, 46.7%, n=60; P<0.001, OR=3.22, 95%CI=1.79-5.79). Conclusively, the CHRNA4 gene polymorphism rs2273504 influences ND in the smokers with CYP2A6 high activity. CHRNA4 genotyping, in combination with CYP2A6, could contribute to individualized smoking cessation pharmacotherapy.

286

Caspase-9 is a Substrate of Caspase-4

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Apoptosis is a process of fundamental importance to cellular homeostasis. Caspase-4 is a member of the caspase-1 family, which is involved in cytokine maturation, and participates in apoptosis induced by Fas ligands and endoplasmic reticulum (ER) stress. Recently, we find that ER stress inducer tunicamycin activates caspase-3, -4, and -9 in human neuroblastoma SH-SY5Y cells, and that caspase-4 cleaves procaspase-9. In this study, we examined whether caspase-4 can directly activate caspase-3 or caspase-9. When the lysates of SH-SY5Y cells were incubated with human recombinant active caspase-4, both of caspase-9 and -3 were activated. A recombinant procaspase-9 mutant (D315A) was resistant to digestion by recombinant active caspase-4. In contrast, a caspase-9 mutant (D330A) was digested by the caspase-4. This finding means that caspase-4 activated caspase-9 via cleavage of caspase-9 auto-processing site (Asp315). These results suggest that caspase-4 may have two functions for induction of apoptosis. One is that a caspase-4-cleaved fragment of caspase-9 accelerates apoptosis, and the other is that caspase-4 facilitates apoptosis by activation of caspase-3 directly.

287

Influence of Efavirenz on the Pharmacokinetics of Ketoconazole in HIV-infected Patients

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Introduction: Efavirenz is both an inducer and inhibitor of CYP3A4. Ketoconazole, is metabolized by the CYP450. Co-administration of both drugs is necessary for treatment of the opportunistic fungal infection in HIV-infected patients. Objective: To investigate the effect of efavirenz on the pharmacokinetics of ketoconazole in HIV-infected patients. Methods: Twelve HIV-infected patients were assigned into a one-sequence, two period pharmacokinetic interaction study. In phase one, the patients received 400 mg of ketoconazole as a single oral dose on days 1; in phase two, they received 600 mg of efavirenz once daily in combination with 150 mg of lamivudine and 30 or 40 mg of stavudine twice daily on day 2 to 16. On day 16, 400 mg of ketoconazole was added to the regimen as a single oral dose. Ketoconazole pharmacokinetics were studied on days 1 and 16. Results: Pretreatment with efavirenz significantly increased the clearance of ketoconazole by 202% (10.76±11.17 vs 32.46±36.73 L/h). C_{max} and AUC_{0-24} were significantly decreased by 44% (10.62±4.23 vs 5.95±3.20 μg/ml) and 72% (68.53±52.89 vs 19.18±9.76 μg.h/ml), respectively, The $t_{½}$ was significantly shorter by 58% (5.00±3.99 vs 2.08±1.27 h). Discussion and conclusion: Efavirenz has a strong inducing effect on the metabolism of ketoconazole. The dosage of ketoconazole should be monitored during co-administration with efavirenz in order to optimize the clinical outcome.

288

Pharmacogenetic Determinants of the Lipid Lowering Responses to Simvastatin in Chinese Patients with Hyperlipidaemia

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Introduction: There is large variation in lipid lowering responses to statin therapy due to a combination of phenotypic and genotypic factors. We examined the effects of some common single-nucleotide polymorphisms (SNPs) on lipid responses to simvastatin. Method: Chinese patients suitable for treatment with high dose simvastatin had plasma lipids measured before and after treatment with simvastatin 40 mg daily for at least 6 weeks. They were genotyped for 18 SNPs for common variants in 10 candidate genes potentially related to simvastatin pharmacokinetics or pharmacodynamics. Results: Data were obtained from 95 patients reporting good compliance (mean ± SD age 57.5 ± 10.6 years, 42 male, 50 with familial hypercholesterolaemia, 26 with diabetes). The adiponectin 45T>G genotype distribution matched previous reports (TT:TG:GG 41:42:12) and was significantly related to absolute reductions in LDL cholesterol and total cholesterol and was almost significant for percentage reduction in total cholesterol (TT:-38.4%, GT:-35.6%, GG:-32.6%; p=0.053) but not for LDL cholesterol. Two LDL receptor (LDLR) polymorphisms 2052T>C and 1866C>T were also related to absolute reductions in total cholesterol and LDL cholesterol but not to percentage reductions. The ABCG2 421C>A polymorphism tended to be related to lipid changes and this was significant for absolute and percentage changes in HDL cholesterol ($p<0.05$). Conclusion: The adiponectin 45T>G and LDLR polymorphisms and possibly the ABCG2 421C>A polymorphism appear to have a modest influence on the lipid responses to simvastatin but most effects were of borderline significance. These findings need to be confirmed in larger samples with our ongoing studies.

289

Placebo-controlled, Randomized, Double-blind Clinical Trial to Evaluate the Efficacy and Safety of Acitretin plus Pioglitazone in Patients with Severe Plaque Psoriasis

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Introduction: We have earlier demonstrated in a randomized, double-blind trial that pioglitazone is efficacious in plaque psoriasis (Shafiq et al 2005). We hypothesized that addition of pioglitazone to acitretin may enhance the efficacy of acitretin. Methods: This was an investigator-initiated double-blind, randomized, placebo-controlled, clinical trial to evaluate the efficacy and safety of combination of acitretin (25mg) and pioglitazone (15mg) in patients with severe plaque psoriasis. Patients aged 18-65 years having more than 20% body surface area involvement were included. Institutional Ethics Committee approval was obtained and after obtaining written informed consent, 22 patients were randomly assigned to acitretin+placebo group while 19 patients were assigned to acitretin+pioglitazone group with follow-up at 2, 4, 8 & 12 weeks. Efficacy was evaluated by observing change in psoriasis area and severity index (PASI) score. Statistical analysis was done using SPSS and Mann-Whitney U-test or Fisher's-Exact was used. P value <0.05 was considered significant. Results: The treatment groups were comparable in baseline variables. After 12 weeks of therapy, the reduction in PASI score was significantly greater in pioglitazone group [median (range) 4.8 (1.1-24.3)] in comparison to placebo group [8.0, (0.9-39.1); P = 0.045]. At 12 weeks, reduction in PASI score was 64.2% (95% CI, 49.2-79.3) in pioglitazone group as compared to 51.7% (95%CI, 38.7-64.7) in placebo group. Majority of adverse events were not serious. Discussion and conclusion: Pioglitazone may provide a convenient, efficacious, and relatively safe drug for combining with acitretin as an alternative to other currently available more toxic anti-psoriatic agents.

290

Validation of a Sensitive LC-MS/MS Method for Quantitation of Sufentanil in Patients Submitted to Cardiac Surgery

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Introduction: Opioids are a large class of drugs that have long been used for the control of labour pain. They provide analgesia during labour after intravenous, epidural or subarachnoid administration. Sufentanil is the most potent synthetic opioid currently prescribed. Objectives: The objective of the study was to develop a LC-MS/MS method for quantitation of sufentanil in patients submitted to cardiac surgery. Methodology: The analyses were carried out using a C_{18} column at 40 ºC with a mobile phase of acetonitrile: 0.5% ammonium acetate 1M + 0.25% formic acid (70:30) at a flow rate of 1.0 mL/min (split 1:5). The mass spectrometer equipped with an electrospray source in positive mode, was set up in multiple reaction monitoring, monitoring the transitions 387.0>238.0 (sufentanil) and 337.0>188.0 (fentanil, IS). Results: The chromatographic separation was obtained within 2.0 min and it was linear in the concentration range of 0.05-500 ng/mL. Good precision and accuracy were obtained and the stability studies of sufentanil in plasma showed results within the acceptable range. An excellent recovery of sufentanil at low, medium and high quality control samples by liquid-liquid extraction was obtained. Conclusions: A simple and fast LC-MS/MS method for the determination of sufentanil in human plasma was developed and validated. This method involves a single step liquid-liquid extraction procedure, using fentanil, a commercially available substance, as IS. The data obtained for the optimized LC-MS/MS method showed specificity, linearity, precision and accuracy. Moreover, the proposed method was successfully applied for the quantitation of sufentanil in patients submitted to cardiac surgery.

291

The Effect of Chronic delta^9-THC Treatment in Rats: Examination of Pharmacokinetic Parameters and Expression Levels of Key Metabolic Factors

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Background and Purpose: The rat Δ^9-tetrahydrocannabinol (Δ^9-THC) drug discrimination model is frequently used pre-clinically to investigate the abuse potential of new chemical entities. It is therefore important to understand the potential pharmacokinetic (PK) modulations associated with chronic Δ^9-THC treatment in this model. We examined the effect of chronic Δ^9-THC on the expression of CYP2A1, CYP2C9, CYP2C11, CYP3A2, OATP2, PXR and UGT2B1. Potential PK modulations were evaluated using morphine as a probe drug. Experimental approach: Rats were treated daily with Δ^9-THC injected intraperitoneally (5-8 µmol/kg) for 7 days. Following oral (50µmol/kg) or intravenous (3µmol/kg) administration of morphine, blood and urine samples were collected for bioanalysis using LC-MS/MS. Liver and brain were collected for mRNA expression studies using real-time RT-PCR. A group of vehicle-treated rats was used as control. Key results: Following chronic Δ^9-THC treatment: (1) In the liver, more than 2 fold decreases in mRNA expression were observed for CYP2A1, 2B2 and 2C11. CYP3A2, UGT2B1, PXR and OATP2 remain unchanged. (2) The overall PK profile of morphine was not significantly altered. Conclusion: These results suggest that chronic Δ^9-THC treatment leads to changes in key cytochromes P450 in the rat. However, these changes did not alter the PK of morphine as UGT2B1 levels were unaffected and because CYP2C11 mediates one of its minor metabolic pathways. Further studies using other probe drugs metabolized by CYP2A1, 2B2 or 2C11 are warranted to evaluate the in vivo impact of the modifications induced by chronic Δ^9-THC on metabolizing enzymes.

292

Distribution of Human Flavin-containing Monooxygenase 3 Polymorphisms in Different Ethnic Populations

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The human flavin-containing monooxygenase isoform 3 (FMO3) catalyzes the metabolism of many drugs (e.g. clozapine, ranitidine and sulindac) and endogenous compounds. The *FMO3* gene is polymorphic and a number of allelic variants causing decreased catalytic efficiency have been identified. Genetically determined interindividual variability in the enzyme activity may affect the therapeutic outcome of its substrates. To evaluate the distribution of *FMO3* polymorphisms in major ethnicities and their subgroups, randomly selected healthy volunteers from Europe (410 Swedes, 279 Italians, 300 Turks), Asia (300 Japanese) and Africa (1203 individuals from 5 sub-Saharan countries) were genotyped for three common single nucleotide polymorphisms in the coding region of the gene (E158K, V257M and E308G) using TaqMan allelic discrimination assay. The prevalence of K158 was high in all three major ethnicities (Asian 22.7%, Caucasian 38.6%, Africans 45.3%). G308 was second most common for Caucasians (14.2%) and Asians (21.0%) but was rare among Africans (0.7%). M257 was most frequently detected in Asians (14.5%) compared to Caucasians (6.6%) and Africans (3.4%). G308 was linked to K158 in all populations. The linkage disequilibrium between the two loci was strong in Asians but absent in Africans. The heterozygosity and homozygosity for K158/G308 were considerably more prevalent ($P<0.001$) among Swedes (23.4%, 6.6%) and Japanese (32.0%, 4.7%) than among Italians (15.1%, 1.1%) and Turks (8.0%, 0.0%). Hence, FMO3-mediated metabolic capacity may exhibit large variability not only across major ethnicities but also between the subgroups within the same ethnic population.

293

Modelling the Interaction between Alpha-adducin Polymorphisms and the Effect of Hydrochlorothiazide on Hypertensives from a Virtual French Population

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The prediction of the treatment effect in relation with some population features can have important consequences on decision making. In Caucasian populations, polymorphisms of alpha-adducin have been linked to a salt-sensitive form of essential hypertension, suggesting that carriers of at least one mutant allele (Trp) could benefit more than wild-type homozygotes from diuretic treatment [1]. We aimed to predict the effect of hydrochlorothiazide in accordance to different genotypes. We constructed a therapeutic model describing the effect of hydrochlorothiazide (HCTZ) on systolic blood pressure (SBP). The model couples sub-models of pharmacokinetics, pharmacodynamics, a feedback reaction and an undesired effect. The therapeutic model was applied to hypertensive men (n=89835) and women (n=45107) in the age range 35 to 64 years) from a virtual French population [2]. Each subject had been assigned an alpha-adducin polymorphism in agreement with hypertensive status. Results in wild-type homozygotes (G/G) and carriers of the Trp allele (G/W-W/W) were compared. Allelic frequencies were in accordance to the Hardy-Weinberg equilibrium for Caucasian populations[1, 3]. HCTZ 25 mg/day lowered SBP by 12.4 ± 1.5 vs 12.1 ± 1.5 mmHg in G/W-W/W and G/G women, respectively (t-test, $p<0.001$); and by 12.2 ± 1.4 vs 12.0 ± 1.4 mmHg in G/W-W/W and G/G men, respectively (t-test, $p<0.001$) These results illustrate the success of our model in reproducing the effect of an interaction between the treatment and the genotype G/W-W/W. Next steps will be to explore the effect of this interaction on cardiovascular mortality. Our approach has large perspectives of exploitation in cardiovascular prevention. [1]Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, Glorioso N, Lanzani C, Manunta P, Righetti M, Rivera R, Stella P, Troffa C, Zagato L, Bianchi G. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997 May 10;349(9062):1353-7.[2]Marchant I. Boissel J-P., Kassaï B., Bejan T., Massol J., Vidal Ch., Amsallem E., Naudin F., Galan P., Chernichow S., Nony P., Gueyffier F. SCORE predicts better than Framingham the cardiovascular death in French population. Submitted to the Eur Heart J. [3]Manunta P, Cusi D, Barlassina C, Righetti M, Lanzani C, D'Amico M, Buzzi L, Citterio L, Stella P, Rivera R, Bianchi

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294

Association between *CYP3A5* Genotypes and Biotransformation of Tacrolimus in Living-donor Liver Transplant Patients

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A calcineurin inhibitor tacrolimus is metabolized by CYP3A subfamilies in the liver and small intestine. The allele frequency of *CYP3A5**3, which causes the loss of mature enzyme is 60% in the Japanese, and relates with a large interindividual variation of tacrolimus pharmacokinetics. Some metabolites are detected by the monoclonal antibodies against unchanged form tacrolimus, which is used in the clinical situations. Therefore, some data derived by immunological methodologies may overestimate the tacrolimus concentration, and associate with its pharmacological interindividual variation. In the present study, we have examined the relationship between the *CYP3A5* genotypes and blood concentration of tacrolimus, M-I, M-II and M-III focusing on the variation in their profile. The patients were enrolled in this study with written informed consent. The unchanged tacrolimus and its metabolites were measured using LC/MS/MS method. Genotyping of *CYP3A5**3 was carried out with PCR-RFLP. Among 72 cases, M-I, M-II and M-III were found in 56, 48 and 55 cases. The blood concentration of M-III was markedly lower than the others. The blood concentration of M-I and M-II tended to be higher in *CYP3A5**1 expressors and defects, respectively. Because tacrolimus and M-II have a similar pharmacological effect and cross-reactivity by the monoclonal antibody, M-II might be a component of blood tacrolimus concentration by the immunological methods. Therefore, blood concentration of M-II might be a potential marker relating the interindividual variation of pharmacological responsiveness against tacrolimus after liver transplantation.

295

MDR1 Genotype-dependent Pharmacokinetics of Nabumetone

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Nabumetone, a widely used NSAID, is a prodrug of the main active metabolite, 6-methoxy-2-naphtylacetic acid (6-MNA), which acts as a potent COX2 preferential inhibitor. Almost complete first-pass metabolism after absorption leads to very low plasmatic concentrations of the parent compound after a single dose in most people. Metabolism of nabumeton consists of three metabolic pathways O-demethylation, reduction into a ketone structure and oxidation of the side chain producing acetic acid derivates, but hepatic enzymes involved remain unknown. Mean absolute bioavailability of 6-MNA is approximately 36% after oral administration of nabumetone. The aim of our study was to determinate, whether functional MDR1 polymorphism 3435C/T influences bioavailability of the drug. Fifteen young, healthy volunteers were administred a single dose of 500mg of nabumetone followed by 12 blood sampling over 96 hours post dose for pharmacokinetic profiles. The subjects were genotyped for MDR1 3435C/T polymorphism by PCR-RFLP. The mean Cmax and AUC parameters of 6-MNA were significantly lower in wild-type homozygous subjects in comparison with 3435TT homyzogotes. Mean values ±SD for Cmax were 85.7±35.4 mg/l, and 116.5±13.7 mg/l, and for $AUC_{0\text{-}96}$ 3750±1275 mg.h/l and 5028±571 mg.h/l in 3435CC and 3435TT homyzogotes, respectively. The pharmacokinetics of nabumeton is affected by MDR 1 polymorphism 3435C/T after a single dose. The clinical importance of this observation needs to be confirmed after a repeated dosing.

296

The GATC Project: Identification of Novel Genetic Markers of Cisplatin Induced Hearing-loss in Children

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Adverse drug reactions (ADRs) are potentially life-threatening responses to medications. Children are at greater risk for ADRs, because >75% of approved drugs used in children are untested in pediatric populations. Adverse drug reactions in pediatric oncology regimens are especially severe and account for 22% of all pediatric oncology hospital admissions. Cisplatin is one of the most effective anti-cancer agents for the treatment of solid tumors. However, a major dose-limiting toxicity of cisplatin is severe, permanent hearing loss. In previous studies the risk of developing cisplatin ototoxicity was increased with younger age and higher cumulative dose, but the contribution of genetic risk factors remains undetermined. We aimed to evaluate genetic markers predictive of hearing loss in children treated with cisplatin. DNA samples and detailed clinical information from cisplatin induced hearing-loss cases (n = 60) and drug-matched controls (n = 44) were collected through the GATC nation-wide ADR surveillance network. DNA samples were genotyped for a panel of 3072 single nucleotide polymorphisms (SNPs) in 220 genes. A significant genetic association with increased hearing loss risk was found for patients carrying variants in a key drug metabolizing enzyme (P-value=0.0001; OR=infinity). In addition, variants in known drug transporters were also associated with cislatin ototoxicity (P<0.002; OR=3). Our results confirm findings that cisplatin-hearing loss is associated with a genetic susceptibility. The identification of genetic variants contributing to cisplatin ototoxicity is essential in the development of diagnostic markers to reduce the incidence of hearing loss, and make cisplatin treatment safer for children.

297

Lactobacillus Helveticus Fermented Milk Containing Bioactive Tripeptides Reduce Arterial Stiffness in Hypertensive Subjects

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Background: The milk casein-derived biologically-active tripeptides, Ile-Pro-Pro and Val-Pro-Pro, have been shown in many experimental and clinical trials to have antihypertensive effects. Part of the antihypertensive effect seems to be related to reduced angiotensin formation by e.g. ACE inhibition. The effects of these tripeptides on arterial stiffness have not so far been studied. Objective: The present study was aimed to find out whether the antihypertensive effect of the peptides is related to the improved vascular function. Design and method: In this double-blinded intervention, 89 hypertensive subjects received daily either *Lactobacillus helveticus* fermented milk with a low concentration of tripeptides (200 ml/d) for 12 weeks and with a high concentration (400 ml/d) for the following 12 weeks, or identical placebo product without the peptides. Arterial stiffness testing was performed by use of pulse wave analysis at the beginning and at the end of each intervention period and blood pressure was measured by using office blood pressure measurement. Results and conclusions: At the end of the second intervention period, the aortic augmentation index in treated subjects had decreased significantly compared to the controls (p=0.013). Systolic and diastolic blood pressure decreased more in the *Lactobacillus helveticus* group compared to the control group, but the difference between the groups was significant only with the males (p=0.005 and p=0.002). The results demonstrate that long-term treatment with *Lactobacillus helveticus* fermented milk containing bioactive peptides has a beneficial effect on arterial stiffness in hypertensive subjects.

298

Multiple-dose Applications of Rotigotine Transdermal Patch to Evaluate the Pharmacokinetics, Safety, and Tolerability in Healthy Subjects

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Introduction: Rotigotine is a transdermal D3/D2/D1 dopaminergic receptor agonist patch for the treatment of Parkinson's disease and Restless Legs Syndrome. The objective was to determine the pharmacokinetic characteristics, safety and tolerability of repeated-dose applications of the rotigotine transdermal patch in healthy young male and female Korean subjects. Methods: This was a randomized, double-blind, placebo-controlled, multiple-dose study. Forty-eight healthy subjects (24 male and 24 female) were randomized to either rotigotine or placebo treatment (20 rotigotine and 4 placebo per gender). Rotigotine patches were applied daily with a dose of 2 mg/24 h from Days 1 to 3 followed by 4 mg/24 h from Days 4 to 6. Serial blood and urine samples were collected to determine rotigotine and its metabolites during their hospitalized period (from Days 1 to 9). Safety and tolerability assessment was performed. Results: Approximately 50% of the total drug content was delivered to the skin within 24 h. For rotigotine, the geometric mean $AUC_{0\text{-}24h,ss}$ were 5.88 and 13.74 ng*h/mL and $C_{max,ss}$ were 0.35 and 0.84 ng/mL during treatment with rotigotine 2 mg/24 h and 4 mg/24h, respectively. The mean terminal elimination half-life was 5h and total body clearance was about 12 L/h/kg. Common AEs reported in rotigotine-treated subjects included nausea, headache and dizziness, which are typical dopaminergic side effects. No clinically significant changes were observed in safety assessments. Conclusion: Rotigotine PK parameters $AUC_{0\text{-}24h,ss}$ and $C_{max,ss}$ values increased with dose. Repeated daily application of rotigotine transdermal patch was safe and well tolerated in healthy subjects.

TUESDAY, JULY 29, 2008
STREAM 3:
MEDICINES AND SOCIETY

299

Concomitant use of Cardiovascular Related Medications in Diabetic Patients Receiving Rosiglitazone

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Background: During the past year attention has been drawn to the potential for an increase in cardiovascular risk associated with the use of the thiazolidinedione rosiglitazone, which resulted in an advisory warning to health professionals from Health Canada in June 2007. Objectives: To describe the prescribing pattern amongst type 2 diabetic patients, with a particular focus on the concomitant use of cardiovascular (CV) medications in patients receiving thiazolidinediones. Methods: Using the IMS Canada database of prescription medications filled at community pharmacies, a cohort of diabetic patients was identified, based on their use of diabetes medications. All drug transactions for the cohort between Dec. 1, 2003 and Nov. 30, 2006 were analyzed. Results: Preliminary results are available for patients in the Toronto region. Rosiglitazone was used by 14% of the sample population. These patients also received nitrates 8%, platelet aggregation inhibitors 5%, ACEi or ARB 37%, beta blockers 14%, calcium channel blockers 25%, thiazides 10% and HMG CoA reductase inhibitors 59%. There were no differences in concomitant CV related drug use between rosiglitazone users and non-users except for cholesterol-lowering drugs, thiazide diuretics, and ARB, which were higher in the rosiglitazone patients. Conclusion: A substantial proportion of rosiglitazone users may have additional CV risks beyond the presence of diabetes. As this data represents rosiglitazone use prior to the Health Canada warning, a similar analysis should be done one year later to determine if patients particularly at increased risk for CV events are less likely to be prescribed rosiglitazone than in the past.

300

Using Criteria to Select Pharmaceutical Topics for Health Technology Assessment (HTA) in Canada

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Selecting relevant HTA topics is a critical step to inform policy development effectively. In 2005, CADTH undertook an HTA prioritization project aiming at developing a criteria-based process for selecting HTA topics. Methods: A systematic review of international HTA agencies' prioritization frameworks was undertaken to compare methods for HTA priority setting and identify prioritization criteria. Members of CADTH Advisory Committee on Pharmaceuticals (ACP), composed of jurisdictional Pharmaceutical Directors (or delegates), were then surveyed on their criteria for funding pharmaceutical products. Results: Eleven prioritization criteria common to all HTA agencies were identified. From these, six core criteria were agreed to by ACP members (disease burden, clinical impact, availability of alternative drugs, budget impact, economic impact, and evidence). In 2006, CADTH implemented an enhanced topic prioritization process. This process involves screening topic proposals to ensure they are linked to a policy decision. Briefing papers are prepared for each topic proposal considered for HTA work, based on the six most important criteria identified by ACP members. These briefing papers are also used to generate a preliminary ranking of all topic proposals. Briefing papers are then submitted to ACP members for the purpose of selecting HTA topics for the following two quarters. In 2008, this process was further enhanced to include jurisdictional context information related to the proposed topics. Conclusion: The enhanced topic prioritization process helps Canadian publicly funded drug plans selecting HTA topics most relevant to them. In addition this approach ensures that limited resources available for pharmaceutical HTA work are used efficiently.

301

Initial Pharmacist Experience with the Ontario-based MedsCheck Service

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Background: In April 1st 2007, community pharmacists in Ontario began providing a new medication review service called MedsCheck. MedsCheck is intended to help patients better understand their medication therapy, ensure that medications are being taken as prescribed, and establish a medication history. This study explored the initial experiences with MedsCheck and identified barriers and facilitators to the implementation of MedsCheck. Methods: This was a sequential explanatory mixed methods study. Community pharmacists practicing in Hamilton, ON completed a semi-structured mailed survey. A subsample of participants also participated in a semi-structured telephone interview. Results: Eighty-eight pharmacists returned a survey and 13 participated in an interview. Respondents reported that it took 30.0 minutes (11.2 SD; Min 10, Max 60) on average to complete a MedsCheck review. Barriers to providing the service included lack of time, physical space and lack of patient awareness of and interest in the service. Facilitators included pharmacist overlap coverage, scheduling reviews during slower times, pharmacists personally inviting patients, reducing paperwork, and using electronic or paper tools. Discussion: The MedsCheck Program was well received. However, there were numerous barriers identified; most notably, lack of time and a workflow not conducive to an appointment based 20-30 minute service. Changes within the pharmacy can improve the implementation of this service. Conclusion: This study provided information on how to facilitate implementation of MedsCheck. The results of this study can help pharmacists improve the delivery of MedsCheck or similar services in community pharmacies.

302

Application of Clinical Pharmacology in Pharmacoeconomics: The Effect of Molecular Polarity and Receptor Affinity of HMG-CoA Reductase Inhibitors (Statins) on their Economic Outcomes

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Background: Evidence from clinical studies on statins has demonstrated that significant differences exist among various statins regarding clinical efficacy and safety. The hydrophilic or lipophilic nature of statin molecules plays a significant role in the pharmacodynamic and pharmacokinetic properties of statins.Objective: To estimate cost savings (direct and indirect costs) of simvastatin as a lipophilic statin versus rosuvastatin as a hydrophilic statin from a Canadian perspective. Method: The benefit of reducing LDL-C was incorporated into an economic analysis through a reduction in cardiovascular (cardiac and cerebrovascular) events. Data for LDL-C reduction from a head-to-head RCT [Am Heart J 2002;144:1036-43]; rosuvastatin (starting 5mg) versus simvastatin (starting 20mg) with up-titration doses; and distribution of cardiovascular risk for users [N = 100,000, duration 5 years] in Canadian population [Clin Invest Med 2007;30:E63-E69].Results: Rosuvastatin and simvastatin can prevent 3161 (95%CI) (2926, 3396) and 2857 (2656, 3058) deaths, respectively. Furthermore, cardiovascular events can be reduced using rosuvastatin and simvastatin by as many as 8197 (7539, 8855) and 7407 (6855, 7959), respectively. Reduction in cardiovascular morbidity can be translated to $160,667,246 CDN (148,385,842, 172,948,650) and $145,171,581 (134,188,480, 156,154,682) direct cost savings for the Canadian healthcare system. Total indirect cost savings from cardiovascular mortality and morbidity reduction due to rosuvastatin and simvastatin therapy were estimated to be as much as $216,900,782 (136,751,926, 457,716,881) and $193,078,202 (123,395,844, 391,963,268), respectively. Conclusion: This study demonstrated that molecular properties of rosuvastatin and simvastatin can affect the economic outcomes of hypercholesterolemia treatment with these drugs through diverse clinical efficacy and safety.

303

Interaction between *Lycium barbarum* (GoJi) and Major Phase I Metabolism Enzymes

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Purpose: GoJi berry (*Lycium barbarum*) has been used as Traditional Chinese Medicine and functional food in China for over 3000 years. Early studies show that GoJi tea may interact with drugs such as warfarin by inhibiting cytochrome P450 (CYP) 2C9. This study was undertaken to characterize the effect of GoJi products on cytochrome P450- and flavin-containing monooxygenase (FMO) 3-mediated metabolism. Methods: Samples were either obtained from locally cultivated berries (GoJi juice, LGJ) or from commercial sources (dry GoJi, DGB and Goji juice, CGJ). Cold/hot water extracts and 80% ethanol extracts (50 mg/ml) of DGB were prepared fresh. Aliquots were examined for their effect on CYP2C9/19, 2D6, 3A4/5/7, CYP19 and FMO3-mediated metabolism. LGJ and CGJ were also studied for mechanism based inhibition (MBI) on CYP3A4 by using established *in vitro* bioassay. Results: LGB and CGJ caused strong (75%) inhibition of the CYP450- and moderate (30-60%) inhibition on FMO3-mediated metabolism. Compared to juice, the GoJi water extracts and ethanolic extracts had a lower inhibitory (30%) potential with these enzymes. Goji did not have an MBI effect under these conditions. Conclusions: GoJi juice has the potential to affect the safety and efficacy of most therapeutic products; however the traditional way of consuming GoJi as a tea or tincture would not be expected to affect clinical safety and efficacy of drugs or other NHPs. Further studies are warranted to determine if these effects are clinically significant.

304

Drug Restrictions Policy: Experience in an Australian Hospital Setting

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Introduction: The Drug and Therapeutics Committee (DTC) of Austin Health controls the hospital formulary. The DTC considers factors such as safety, efficacy and quality of evidence but is also challenged by budgetary constraints when deciding on addition of a drug to the formulary. The Austin Health Drug Restrictions Policy is a DTC initiative and stipulates that non-formulary drugs or 'restricted' formulary drugs can only be prescribed outside of the restrictions when approved by a Clinical Pharmacologist, and/or via a High Cost Drug Meeting. The objective of this study was to assess the economic impact of the Austin Health Drug Restrictions Policy. Methods: Over a 6 week period, Pharmacists and Clinical Pharmacologists were asked to note details of all requests, including outcome, for drugs that were not on the Austin Health formulary or formulary drugs that were prescribed outside of the Policy i.e., for an unapproved indication. Results: During the audit period, there were 87 requests for a non-formulary drug or non-Austin Health approved indication for a drug. Fifty-six percent of the requests were changed to a hospital approved alternative drug or ceased as a result of pharmacists' recommendations, with the remaining requests being accepted. Discussion: Based on an average overnight inpatient stay of 7 days, standard once-off dose or course, the cost saving associated with the changeover to hospital-approved alternatives was estimated to be over $40,000 per annum. Conclusion: The Austin Health Drug Restrictions Policy resulted in a cost saving to the hospital.

305

Antibiotic Drugs Usage in Croatia during 6-year Period

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The aim of our study was to identify and analyze changes in the use of antibiotic drugs in Croatia during the six-year period (2001-2006). The data on antibiotic drugs utilization during the studied period were obtained from the Croatian National Health Insurance (CNHI). According to the WHO Collaborating Centre for Drugs and Statistics Methodology, the data were calculated and presented in defined daily doses (DDD)/inhabitant/year. Antibiotic drugs utilization increased 25.67% during the investigated time period, e.g. from 6.31 to 7.93 DDD/inhabitant/year (2006 vs. 2001). The most prominent increase according to the previous year was 16.48% (2002) and 13.88% (2003). Antibiotics utilization was on the same level or slightly decreased in 2005 (-2.8%) and 2006 (-2.64%). Amoxicillin in combination with clavulanic acid represents the most prescribed antibiotic in Croatia with continuous increase in usage (2001= 0.19; 2006= 2.06). The next most prescribed antibiotics are: amoxicillin and cefalexin. Penicillins represent the most widely used antibiotic class followed by cephalosporins and macrolides. Croatia is among the countries with the high rate of antibiotics utilization in Europe. The characteristic utilization pattern is a decreasing trend of narrow-spectrum penicillin usage. The analysis showed considerable antibiotics utilization increase in 2002 and 2003 and the explanation is legal change (the new Insurance Act). The introduction of different measures (administrative and educational) resulted in utilization stagnation afterwards. Adequate managing of antibiotic utilization, which includes an analysis of prescribing regimens, educational and other interventions must be further provided.

306

Improving Medication Safety in Hospitalized Children: An Effective Model for Sustained Change

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Introduction: Evidence about effective strategies to improve paediatric medication safety is limited. We evaluated the effectiveness of an innovative improvement model and assessed long term impact on medication incidents (MI) and adverse drug events (ADE) in hospitalized children. Methods: Prospective time series study in a paediatric referral hospital over 4 years (2003-2007). The improvement model included: 1) Guidelines for safe prescribing, implemented using a multi-faceted, evidence-based model with demonstrated effectiveness; 2) Improved multi-disciplinary communication; and 3) Ward based clinical pharmacists. Impact on MI (error) and ADE (harm) was measured using standard definitions and a multi-method approach. Prospective data were triangulated from: 1) reports by nursing and medical staff; 2) reports by hospital pharmacists; and 3) intensive chart review. All data were reviewed by a multi-disciplinary panel, including causality assessment for ADEs. Results: 1867 patients and 8438 medication orders were reviewed. ADEs decreased by >50% in the first year (19.22/100 patients at baseline vs 10.43/100 patients in 2004) and this was maintained at 4 years (8.59/100 patients in 2007). The greatest reductions were seen in potential ADEs which decreased from 12.26/100 patients at baseline to 4.60/100 patients at 4 years. Total MI decreased from 4.51/100 orders at baseline to 2.78/100 orders at 4 years, with specific reductions in prescribing related MI (4.07/100 orders at baseline vs. 2.05/100 orders at 4 years). Conclusions: Our model was effective in reducing medication errors and harm in hospitalized children, with sustained improvement over 4 years. Our methods may be applicable to other settings and strategies.

307

Sedative Antihistamines, not to be Prescribed to Infants?

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Introduction: Recently, over the counter cough and cold syrups marketed for infants were taken off the market in North America. These syrups often contain sedating antihistamines, which were repeatedly associated with sudden infant death in case reports. As these agents are frequently used in hospitals, we aimed to systematically review whether existing evidence on safety and efficacy also warrants restriction of prescribing in this setting. Methods: We searched the literature using PubMed/Medline, Embase and Google. Initial search terms were "antihistamines", "safety", "efficacy", "adverse event", "infant". Additional papers were identified in Pubmed through 'related articles' and from paper references. Results: This initial search identified 19 papers useful for our review. Of these papers 8 were case reports (14 cases), 3 review articles, 1 comparative study, 2 letters and 3 editorials. The case reports only reported cases of death in infants after antihistamine use as part of cold and cough medication at home after an (un)intentional overdose and/or during upper respiratory tract infection. An association of these drugs with non-fatal cardiovascular events and neuroleptic malignancy syndrome has been suggested. The sedative and anitpruritic efficacy has not been proven. Discussion: Antihistamines prescribed to hospitalized children less than 2 years of age are not proven as either unsafe or efficacious. Conclusion: Evidence is lacking to support the claim that sedating antihistamines in general should not be used in hospitalized young infants. Further research is needed to settle the controversy on efficacy and safety of these drugs.

308

Large Scale Anonymized Longitudinal Patient Data and its Role in Better Understanding Canadian Health and Healthcare

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The power of large scale anonymized patient level information can be harnessed to examine the disease treatment of Canadians, by age, gender, and geography. Longitudinal prescription data (LRx) were examined to obtain a better understanding of therapeutic treatment choices. To demonstrate the value of LRx information, over 17 million lives were analysed cross-sectionally to develop relevant treated-prevalence estimates, and further analysed longitudinally for concomitant treatment across three disease states: Cholesterol, Diabetes, and Cardiovascular

diseases. Population projections from Statistics Canada were used, to assess the impact of the demographic wave on treated demand for Cholesterol medication over the next 15 years. The analysis shows treated prevalence for hyperlipidemia varies regionally from just under 10% in Alberta, to over 17% in Newfoundland. By age, it exceeds 5% by the time Canadians reach age 43, and peaks at over 37% at age 75. At age 43 over 40% of those being treated for hyperlipidemia are being treated for hyperlipidemia alone, however by age 75, 55% of those being treated for hyperlipidemia are also being treated for cardiovascular disease. Over the next 15 years the number of Canadians being treated for hyperlipidemia is expected to increase by 50%, and more than double in those over age 65. Although the focus of this work has been on one particular disease state, the power of such databases can be leveraged against almost any disease state affecting Canadians. LRx Information is a powerful tool for governments, pharmaceutical manufacturers, medical researchers, and other healthcare professionals.

309

Potential Drug Interactions with Bacopa monniera: A Lab Study

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Introduction: Recently, the interactions of herbal medicine with synthetic drugs came in to focus of particular interest. Herbal medicine may cause significant toxicity with additive, synergistic or antagonistic effect when taken in combination with allopathic drugs. Objective: To investigate the potential interaction between *bacopa monniera* extract (BME) and fluoxetine (FXT) on 2-week, chronic, oral, once daily, administration in rats. Materials and methods: Healthy, male albino, wistar rats were divided in to eight groups of six each. Control group-I was administered distilled water. Group II received drug FXT 20mg/kg/day. Group III, IV and V received standardized water soluble BME containing 20% of total bacosides in doses of 20, 40 and 80 mg/kg/day respectively, while Groups VI and VII and VIII received combinations of FXT along with BME in three different doses for 2 weeks. Spontaneous motor activity (SMA), motor coordination, forced swimming test (FST) and chronic fatigue tests were evaluated. Biochemical parameters were checked in blood and homogenized brain tissue samples. Results: Compared to control groups, test groups VI, VII and VIII showed dose dependent reduction in SMA, a significant reduction in immobility period in FST plus chronic fatigue test and reduced lipid peroxidation and increased glutathione level. No major changes were observed in motor coordination in any groups. Discussion & Conclusion: There is a possibility of interaction between BME and FXT. More tests are needed to confirm the fact. Precautions are advised while using this combination. Potential interactions of *bacopa monniera* with newer antidepressants are matter of further study.

310

Using Horizon Estimation to Know Whether your Literature Search is Complete

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Cochrane Reviews and meta analyses have detailed literature searches; few state the search completeness or horizon. Ordered database searches were: CochraneI, PLUS(P), Medline(M) and Cochrane RenalI. Distinct journals found in these searches were put in cells of a 2^4 contingency table with one cell missing. Data were entered into SAS 9.1, modeled with Poisson regression from search P to R, yielding a missing cell estimate and its 95% Confidence Interval (CI). The known cells plus the missing cell estimate give a horizon estimate and its 95% CI. SearchI detected 102 journals. Search (P) detected 149 new journals; 251 total, with 17 journals missed [95%CI: 8, 32] and the horizon estimate was 268 journals [259, 283]. Search (M) gave 197 new journals; 448 total. The model estimated 1 journal missing [0, 1] and the horizon was 449 [448, 449]. SearchI gave 18 new journals; 466 total. The missing cell estimate was 21 [12, 36], so the horizon estimate was now 487 [478, 502]. The four searches obtained (466/487 or) 96% of the complete list of journals. An optimal search

order would have been MPR and found all 466 journals with horizon estimate 489 [479, 505] since 0 new journals would have been found with the C search done last. Horizon estimation using Poisson regression can estimate how many journals are missed with this 4 search strategy. Here 21 were missed for a completeness of 96%. This information should be added to the search report of Cochrane Reviews and meta analyses.

311

Acetaminophen Oral vs. Rectal. Is Rectal Administration as Effective as Oral? A Meta-analysis

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The use of rectal suppositories of acetaminophen, a commonly administered antipyretic and analgesic agent in children is controversial since several studies demonstrated unpredictable pharmacokinetics. Objective: To determine, based on published studies, the efficacy of rectal verses oral acetaminophen as treatment of fever and of pain. Data Sources: A search was conducted in Medline, Pubmed and the Cochrane database as well as major pharmacological textbooks and the references of all the included studies for studies comparing oral and rectal administration of acetaminophen. Study Selection: Randomized and quasi-randomized controlled studies comparing rectal and oral administration of acetaminophen were included. Reviews, letters, or studies that compared combined treatments or additional drugs were excluded. Results: Temperature reduction: Four studies met the inclusion criteria. The decline in temperature one hour after administrating acetaminophen was no different in rectal verses oral administration (Weighted mean difference [WMD] –0.14; 95% CI –0.36-0.08, p for heterogeneity = 0.49). There was no difference in the decline of temperature 3 hours after administration (WMD –0.1; 95% CI –0.41-0.21, p= 0.84), or in the maximum decline of temperature (WMD –0.1; 95% CI –0.24-0.04, p=1.0) or in the average time to temperature reduction of 1 degree (WMD -0.06; 95% CI –1.34-1.23, $p<0.01$). We did not perform a metanalysis comparing rectal and oral acetaminophen for pain reduction as only one study fulfilled the inclusion criteria. Conclusions: Rectal and oral acetaminophen are comparable in respect to temperature reduction. The American Acadamy of Pediatrics recommendation to refrain from rectal acetaminophen in children should possibly be revised.

312

Ability of Clinical Pharmacologist Service to Improve Physician and Patient Adherence to Clinical Guidelines in Refractory Hypertension

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To evaluate a clinical pharmacologist consultation service for refractory hypertension (RH) management in Belarus (an Eastern European country) we retrospectively analyzed 200 randomly selected cases of ambulatory patients with RH consulted at the Vitebsk Center for RH (RH-Center) during 2004-2006 years. Adherence to and effectiveness of the recommended treatment regimen according to 2003 European Society of Hypertension / European Society of Cardiology guidelines for the management of arterial hypertension were evaluated during 12 months of follow-up. In the patient sample studied, the recommended treatment regimen was preserved in 126 cases (63%), and in 82 of them (65%) the treatment effectiveness was judged as sufficient (systolic BP change by 10% or more). In the last 74, the recommended regimen was substantially changed by general practitioner (30 patients) or by patient himself (44 cases), the lack of grounded reasons for that being registered in 22 (73%) and 19 (43%) cases, correspondently. The most common feature of physicians' and patients' non-compliance was cancellation or intermittent administration of thiazide diuretics. Former use of clonidine was an important marker of patient non-compliance. The rates of patients' applying for emergency service and hospitalization both in adherent and non-adherent portion of the sample were significantly lower compared with 12-months period preceding the consultation at the RH-Center. Thus, the first in Belarus implementing

of clinical pharmacologist consultation service can improve management quality of the most problematic group of hypertensive patients. However, general practitioners' irrational behavior has been highlighted as an important barrier which should be addressed in a systematic fashion.

313

The Safety of Intrauterine Exposure to Proton Pump Inhibitors during the First Trimester of Pregnancy

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Introduction: Little data exist on the safety of proton pump inhibitors (PPI's) use during pregnancy. Aim: Assess the safety of PPI's use in the first trimester, by linking computerized databases. Methods: A computerized database for medications dispensed between 1998 and 2007 to women registered in "Clalit" Health Maintenance Organization, southern district of Israel, was linked with computerized databases containing maternal and infant hospitalization records from the district hospital. The following known confounders were controlled for in the statistical analysis: maternal age, ethnic group (Jews, Bedouins), maternal diabetes and smoking, maternal fever before and around delivery and parity. Results: A total of 117,960 infants were born during the study period; 658 of them were exposed to one or more Ppi's during the first trimester of pregnancy (614 to omeprazole, 31 to lansoprazole and 17 to pantoprazole). Exposure to PPI's as a group and to omeprazole individually, was associated with a slight increased risk of premature delivery (OR= 1.28, 95% CI: 1.06 – 1.557; OR=1.30, 95% CI: 1.07 – 1.59, respectively). Exposure to PPI's was not associated with an increased risk for congenital malformation (OR=1.18, 95% CI: 0.86 – 1.62), low birth weight (<2500grams) (OR=0.97, 95% CI: 0.75 – 1.26), or perinatal mortality (OR=0.85, 95% CI: 0.32 – 2.29). Discussion: This study reports on more infants exposed to PPI's during the first trimester than all other studies combined. Conclusion: Intrauterine PPI's exposure is not associated with an increased risk of congenital malformations, low birth weight or perinatal mortality. More research is needed to corroborate the apparent risk for prematurity.

314

Drug Information Portal on the Web

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The National Library of Medicine (NLM), part of the USA National Institutes of Health (NIH/HHS) provides a wide range of Web resources, including PubMed, Medlineplus, ClinicalTrials, and the TOXNET system of toxicology-related databases. A new addition to the NLM collection is the Drug Information Portal (http://druginfo.nlm.nih.gov) to informative, user-friendly information on over 15,000 drugs (194,000 unique drug names and their synonyms). On the Drug Portal, links to sources include NLM, NIH, and other USA government agencies. Current information regarding consumer health, clinical trials, biomedical literature and physical properties and structure is easily retrieved by searching on a drug name. A varied selection of focused topics in medicine and drug-related information is also available. Pharmacological actions using NLM's MeSH categories are included and are searchable, allowing grouping of drugs related to a category or classification. A spellchecker is incorporated into the Drug Portal searching to make suggestions for misspelled drug names. The ChemIDplus search and retrieval architecture is used for the Portal, and links to the full ChemIDplus record allow further exploration of the drug in other databases. The Drug Information Portal gathers together a wide range of resources in one site, making it easier for users to quickly find the information they need about a drug.

315

New Drugs: Why the Slower Uptake?

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Background: The impact of new medicines as a cost driver has diminished substantially in recent years. The objective of this analysis was to see if the market share of recent entrants could provide insights for this uptake slowdown. Methods: We analyzed the number of new chemical entities (NCEs) introduced each year between 1999 and 2004, and measured their combined market share. Then, we looked at three different aspects: the number of NCEs introduced the targeted patient populations, and the reimbursement environment. The analysis was conducted using the Brogan national private drug plan database. Results**:** Between 1999 and 2004, there was a decline in the uptake as measured by the combined market shares of new products. The drugs launched in 1999 reached 5% of the total market within 4 years while those introduced in subsequent years accounted for less than 2%. The number of innovative compounds between 2000 and 2006 was fairly stable whereas the proportion of drugs for small populations appears to be on a slow rise. Lastly, there was a sharp decline in the proportion of new drugs listed by public plans over the past few years. This may have influenced medical practices and affected private drug plans. Conclusion: It is not evident that innovation has declined substantially. More new drugs, however, seem to be targeted towards smaller populations. The reduced listing of NCEs by public formularies seems to be one cause of this slowing down, and may have discouraged some from introducing new treatments in Canada.

316

Increased Adverse Events and Treatment Discontinuation in Patients Switched from Branded Alendronate to Generic Alendronate

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Introduction: Generic alendronate was introduced in Canada in July 2005 and within 2 months the conversion from brand to generic alendronate was almost complete. The generic has a different dissolution profile compared to brand alendronate which may impact gastrointestinal (GI) adverse events (AE). Methods: A chart review study examined the GI AE profile of postmenopausal women 50 years of age and older who were on brand alendronate prior to July 2005 and who were subsequently switched to generic alendronate. Patients on brand alendronate were on stable doses that were well tolerated. Discontinuation of alendronate and initiation of other therapies was also recorded. Results: A total of 173 women from a single clinic that specialized in treating patients with osteoporosis were analyzed. The average age of the patients was 68.9 years (9.43) at the approximate time of switching. Results indicated that following the switch, 16.5% (29/173) of patients on generic alendronate had a total of 35 GI Aes. Of the 35 GI Aes, most patients complained about stomach pain (20%; 7/35), nausea (11.4%; 4/35), reflux (11.4%; 4/35) and GI upset (11.4%; 4/35). Alendronate was discontinued in a majority of these patients. Preliminary data on bone mineral density also indicate an increase in the frequency of therapy changes due to declining bone mass. Discussion: Generic alendronate may not be as well tolerated as brand alendronate and should not be considered equivalent in all individuals. This may have implications for treatment adherence and effectiveness, as well as the preferential reimbursement of generic alendronate.

317

Prescribing Behaviours of Psychiatrists in Turkey – A Survey Study

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Introduction: Information sources for drugs are various but their influence on the physician's

attitudes while prescribing is not well known. The aim of the study was to determine influence of pharmaceutical information sources on the prescribing attitudes of psychiatrists working in different academic positions and clinics in Turkey. Methods: 111 non-randomly selected psychiatrists rated the influence of main factors of rational prescribing on their prescribing habits. They have also rated the information sources according to their importance for getting theoretical information and prescribing the drugs. Their trainings on various fields and knowledge on the principles of evidence based medicine were also documented. Results: Psychiatrists see the drug guide as the most common source for the prescription of old drugs. The articles were the most common source for the theoretical information of new drugs while internet, textbooks and personal experience were effective on their decision about prescribing the new drugs. Psychiatrists think their colleagues see pharmaceutical representatives as the most common source of information. Meta analysis results are accepted as the most convincing evidence. GP's generally refer to the information sources at least once weekly. Discussion: The sources of information are different for the new and old drugs for psychiatrists. They usually prefer internet and textbooks but their personal experience also counts. Conclusion: It is interesting to realize that although personally they describe the importance of various sources for the information for prescribing but they think that their colleagues are usually informed by the pharmaceutical respresentatives.

318

Prescription Cost Reduction in Tertiary Health Care in the Hill State of India

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Introduction: In developing countries due to poor socio-economic status & limited health budget, majority of the patients can't afford to purchase costly prescribed drugs. With low cost prescriptions, patient compliance can be improved & favorable therapeutic outcome can be achieved. Therefore the present study was proposed to explore the possibilities of prescription cost reduction in tertiary health care. Methods: Prescriptions were collected randomly from outdoor patients of internal medicine & pediatrics, of a tertiary care teaching hospital of Himachal Pradesh, during April & May, 2007 & were analyzed for cost of prescribed drugs with there most economical alternative brands. Results: Total no. of encounters was 223 of internal medicine & 240 of pediatrics. Total no. of drugs prescribed 561 from internal medicine & 384 from pediatrics. The average prescription cost was Indian Rupees 213 for internal medicine & 124 for pediatrics. Total drugs prescribed by generic were 65(6.88%), from essential drugs list 373(39.47%), as injections 16(1.69%), antibiotics 114(12.06%), fixed dose combinations (FDCs) 359(37.99%) & vitamins & minerals 84(8.89%). Overall cost of drugs prescribed by generic was 1.33%, from essential drugs list 26.15%, as injections 0.6%, antibiotics 16.06%, FDCs 42.34% & Vitamins & minerals 26.07%. Discussion: Prescriptions of drugs as generic & from essential drugs list was less which should be encouraged to make the treatment more economical. Vitamins & minerals (8.89%) costing 26.07% & FDCs (37.99%) costing 42.34% may have limited therapeutic value & should be discouraged. Use of most economical substitutes of the same salt resulted in significant prescription cost reduction from 5.7% to 81.84% (average36.92%) for internal medicine & 5.97% to 89.98%(average36.52%) for pediatrics. Conclusion: Drugs were mainly prescribed by brand names. Preference to most economical alternative brand of the same salt can reduce prescription cost significantly. Awareness among prescribers about the rational use of drugs may have supraadditive effect in making the treatment cost effective.

319

Incidence Pattern of Poisoning with Aluminium Phosphide: A Common Pesticide in India

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Introduction: Aluminium phosphide (AlP), a highly effective and economical fumigant pesticide, is commonly used by large grain store-houses and farmers in India. Due to easy

availability, it is commonly abused for self-poisoning. Though several precautionary measures in packaging and distribution have been taken, there is lack of reliable and adequate data on change in AlP poisoning pattern in India. Methods: Retrospective case-series analysis was done of AlP exposure in north India as recorded by direct telephonic recording at National Poisons Information Centre, All India Institute of Medical Sciences. Results: Total 9460 queries were received (Jan1999–Dec2007). Of these, 300 (3.2%) were relating to AlP poisonings; 74% from National Capital Region (Delhi) and 26% from rest of north India; 95% were from attending physicians and 5% from patient attendants; 4% sought general information on suspected AlP exposure however 96% were confirmed cases of AlP poisoning. Male: female ratio was 55:45 and 15–35 years age group was maximally affected (73%). Majority were of suicidal intent (82.6%) followed by accidental exposure (10%) and others (7.4%). Outcome was serious in 46%cases, non-serious in 34% and unknown in the remaining. Seriousness of the outcome was not significantly related to reason of poisoning (p=0.2). No significant change was found in the incidence of poisoning in any year. Conclusion: Despite several measures, there is no significant change in the incidence of AlP poisoning and its outcome in last nine years. This analysis highlights the need for targeted poisoning prevention efforts and specific antidote development.

320

Comparative Study of Type II Collagen and Glucosamine Plus Chondroitin for Therapeutic Efficacy in Arthritic Horses

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Arthritis is a debilitating disease that commonly affects millions of horses. The present investigation evaluated arthritic pain in horses receiving daily placebo, undenatured type II collagen (UC-II, 80, 120, or 160 mg/day), and glucosamine and chondroitin (5.4 g/day and 1.8 g/day, respectively, bid for the first month, and thereafter once daily) for 150 days. Horses receiving placebo showed no change in arthritic condition, while those receiving 80, 120, or 160 mg UC-II exhibited significant reduction in arthritic pain ($P<0.05$). UC-II at 120 mg dose provided optimal effects. With this dose, reduction in overall pain was from 5.7±0.0.42 (100%) to 0.7±0.42 (12%); and in pain upon limb manipulation from 2.35±0.37 (100%) to 0.52±0.18 (22%). With glucosamine + chondroitin, although reduction in pain was significant compared to pretreated values, but the efficacy was significantly less comared to that observed with UC-II. UC-II was found to be twice as effective as glucosamine and chondroitin in arthritic horses. Clinically, physical condition, and liver (ALP, GGT, and bilirubin), kidney (BUN and creatinine), and heart (CK) functions remained unchanged, suggesting that these supplements were well tolerated. These findings demonstrated that UC-II was significantly more efficacious than glucosamine and chondroitin in arthritic horses.

321

Development and Provision of Patient Specific E-Pharmacological Services 24 Hours a Day in Stockholm Metropolitan Health Region

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Background: Prescribers need e-pharmacological services. Stockholm Health Care Region (2 million) has established e-pharmacological services. Methods: An infrastructure for e-prescribing has been built during 10 years. The e-prescribing service links the desk of the physician to any pharmacy computer desk in Sweden (9 million) through one single server. A server linking maximum four different EMRs presents the complete list of prescribed drugs. A web based window integrated into EMRs (Janus window) provides to producer independent drug information (www.janusinfo.se) and has patient

specific pop-ups for a. drug-drug interactions, b. pregnancy and c. nursing warnings for drugs and for adjustments of dosage of d. renally eliminated drugs. Database information is shown either in pop-ups (i.e. drug-drug interactions) or through janusinfo. Results: Electronic prescriptions have increased from 1.5 % in 2003 to 85% in 2007 in Stockholm. A paper prescription costs 4 USD as compared to 1.4 USD for an electronic prescription. One user reported: "E-prescribing is the best innovation in our professional lives as doctors since the introduction of EMR". Janus Window is integrated into 8 of 14 EMRs in Stockholm with 8000 daily visitors today (12 in 2002). The complete list of drugs contains data from 225000 patients today (one third have prescriptions from two or more health care units). Janusinfo was rated as objective and containing clinical valuable information by 90% of users. Conclusion: E-pharmacological services change the practice of medicine. The implementation and maintenance require commitment, leadership and a medical quality system functioning 24 hour a day.

322

Antibiotic Utilization Prevalence: Prospective Analysis and Comparison of Two Pediatric Departments in a Tertiary Care University Hospital

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Introduction: The aim of this study was to analyze prospectively antibiotic utilization pattern, comparing two in-patient pediatric units in a tertiary care university hospital. Methods: Data on individual antibiotic utilization pattern were collected from 70 and 83 hospitalized children in pediatric A and B respectively. Their electronic charts were viewed once weekly for each admission unit over a 12-week period during 2007 according the prescription-point prevalence method. Results: The antibiotic volume/patient (number of prescriptions) was 108 and 136 for Pediatric A and B, respectively, resulting in 1.54 and 1.63 antibiotic courses/patient for each of the two units, respectively ($p>0.1$). The total defined daily dose (DDD), and drug utilization 90% (DU90%) index were 68.52, 71.07 (p=ns) and 61.82 and 64 ($p<0.02$) for Pediatric A and B, respectively. The drug cost 90% index (DC90%) placed Gancyclovir and Voriconazole in the first place in Pediatric A and B (p=ns), respectively; while Cephtriaxone and Ampicillin were in first place when the DU90% index was applied in both admission units, respectively. Pediatric A and B were similar considering their patients admission diagnosis (p=ns). Discussion and Conclusions: No significant difference between pediatric A and B was found in the anti-microbial cost analyses, DDD and DC90% indexes; while a slight significant difference was depicted when the DU90% index was analyzed using the prescription-point prevalence methodology. The active intervention of a pediatric infectious disease specialist in both admission units had a positive impact in cost containment in a tertiary university hospital.

323

Increasing Duration of Inadequate Empiric Antibiotic Therapy is a Risk Factor for In-hospital Mortality among Solid-organ Transplant Patients with Infections

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Background: The incidence of inadequate empiric antibiotic therapy (IET) and its clinical importance as a risk factor for hospital mortality in solid-organ transplant patients remains unknown. Methods: This retrospective cohort study evaluated all patients admitted to a transplant unit over a 2-year period. Therapy was considered adequate when the organism cultured was found to be susceptible to an antibiotic administered within 24 hours of the index sample collection time. Univariate and multivariate regression analyses were conducted to determine associations between potential determinants, IET, and mortality. Results: IET was administered among 169/312 (54%) transplant patients with infections. The in-hospital mortality rate for patients receiving at least one episode of IET was significantly greater than those receiving adequate therapy (24.9% vs. 7.0%; RR, 3.55; $p < 0.001$). Regression analysis demonstrated that an

increasing duration of IET (adjusted OR at 24h, 1.33; $p < 0.001$), ICU-associated infections (adjusted OR, 6.27; $p < 0.001$), prior antibiotic use (adjusted OR, 3.56; $p = 0.004$), and increasing APACHE-II scores (adjusted OR, 1.26; $p < 0.001$), were independent determinants of hospital mortality. Discussion: The increased mortality rates associated with IET suggest that starting empiric therapy at the earliest signs of infection may be beneficial. This will require a higher degree of clinical suspicion, both in terms of timely therapy and antibiotic coverage for organisms that are not typically suspected. Conclusions: IET is common and appears to be an important determinant of hospital mortality in the solid-organ transplant population.

324

Pharmacogenetics: From Research Results to Practical Guidelines

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Introduction: The translation of pharmacogenetics (PGx) to the clinic has proven to be difficult. Therefore the Royal Dutch Association for the Advancement of Pharmacy (KNMP) established the Pharmacogenetics Working Group, consisting of experts with day-to-day clinical experience to develop PGx based therapeutic recommendations. These recommendations are integrated into the Dutch drug information database, the G-Standaard. This database is integrated into computerised systems for drug prescribing and dispensing. Methods: The collection of the data includes a search in Pubmed with fixed search terms. The assessment includes the assignment of a code for the evidence and for the severity of the effect. Results were reported in a comprehensive data sheet. Based on this data sheet, a therapeutic recommendation is made per genotype or phenotype. The recommendations appear on the screen of the healthcare professional during prescription or dispensing if the specific gene-drug-interaction occurs, and in this way it supports decision making. Results: To date, the G-Standaard includes therapeutic recommendations for 40 drugs, for polymorphisms of CYP2D6, CYP2C9, CYP2C19, VKORC1, UGT1A1, TPMT and Factor V Leiden. The recommendations include dosage adjustments, advice for an alternative drug, for performing TDM or an alert for increased risk of adverse drug events or diminished effectiveness. Conclusion: The clear cut therapeutic recommendations and the accessibility during electronic drug prescribing and medication control may present an important step forward in the clinical application of PGx information. The data sheets containing the literature on which the recommendations are based, ensure the transparency of the recommendations.

325

Development of Off-label use in Children after a New Therapeutically Significant Class of Medicines Becomes Available for Adults

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Widespread off-label use of medicines in children is well documented. Aim of the study was to investigate how off-label use of a therapeutically significant new medicine develops over time. Methods: Triptans were chosen, migraine being a common disorder in children, and the only indication for triptans. Data on all persons prescribed triptans 1994-2006 was extracted from the nationwide Social Insurance Institution's Prescription Register. Number of persons in outpatient care prescribed a triptan in different age groups was compared over time. Results: Sumatriptan was the most commonly prescribed triptan for children (8991/15276). From 1994 (n=195) to 1997 (n=592) the number of children over 12 year prescribed sumatriptan off-label increased more rapidly than the respective total number. The growth of sumatriptan off-label use leveled after launch of other triptans. When nasal sumatriptan was labeled for children over 12 years in 2003, prescribing to younger children (6-11 years) increased two-fold 2003-2004. In 2006, 241 children <12 yrs were prescribed sumatriptan and 930 children other triptans off-label, while

633 children over 12 year were prescribed sumatriptan labeled for them. Discussion: Sumatriptan off-label use started slower than in adults, demonstrated catch-up after more experience was available and leveled before becoming labeled in the age group. Labeling extended off-label use to younger children. New triptans further increased the overall off-label use. Conclusion: When a new therapeutically significant medicine becomes available in adults, off-label use in children seems to start slowly, show catch-up growth and, if labeling is delayed, reach steady state before labeling.

326

Did a Simple Education Program Change the use of Psychotropic Medications in Nursing-homes for the Elderly?

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An audit of all nursing-home residents in Hawke's Bay, New Zealand in October 2005 (n = 1053) showed increased antipsychotic and antidepressant medication use but decreased benzodiazepine use compared to a similar audit eleven years previously. Following this a series of educational seminars and papers were provided to nursing-home staff, general practitioners, pharmacists and hospital doctors, particularly highlighting the risks of antipsychotic and hypnotic medications in the elderly. In March 2007 an identical audit was performed to establish whether there had been any changes to prescribing practice. Accessing community pharmacy data-bases, the number of residents on regular psychotropic medication and the classes of psychotropic medications that they were on was audited. A total of 1076 residents were audited. The use of agents in different levels of nursing care and the specific agents being used were also analysed. The results showed that between October 2005 and March 2007 there had been a reduction in prescription of hypnotic medications (from 16.6% of residents to 13.9%) and of antipsychotic medications (from 24.1% to 21.1%). These results suggest that the education program may have had a beneficial effect on prescribing of psychotropic medications. However, it is possible that other factors (such as medical media coverage of the risks of these agents) are responsible for the changes. Further educational seminars are scheduled to restate educational messages and present the results of the 2007 audit.

327

Drug Claims by Seniors: An Analysis Focusing on Utilization of Potentially Inappropriate Medications, 2000–2006

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It is important that seniors, who are more at risk for adverse effects due to complex drug therapies and age-related changes to the way drugs are processed by the body, have access to safe, appropriate and effective drug therapies. This analysis was intended to identify trends in potentially inappropriate medication use among seniors. Claims level data from the National Prescription Drug Utilization Information System (NPDUIS) database, were analyzed to identify claim patterns for seniors on public drug programs in Alberta, Saskatchewan, Manitoba and New Brunswick, with a focus on claims for drugs on "the Beers list." This list, developed by Dr. Mark H. Beers, is an internationally recognized list of drugs identified as "potentially inappropriate" to prescribe to seniors due to an elevated risk of adverse effects. Specifically, we calculated the proportion of seniors on public drug programs using drugs on "the Beers list," and also examined chronic use and chemical specific usage rates.The rate of Beers drug use among seniors on public drug programs decreased from 34% in 2000/01 to 27% in 2005/06. In all four provinces, among seniors with drug claims, the rate of chronic Beers use is highest among women and seniors over the age of 85. Although this study does not infer the appropriateness of individual claims, these are drugs that, according to Beers, should be avoided in seniors, when possible. As growth in Canada's senior population continues, so will the need to monitor the use of potentially inappropriate drugs.

328

Potentiality of a Newer Oral Antihyperglycemic Combination Therapy over Conventional One

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The Aim of this study is to perceive the "Potentiality of a newer oral antihyperglycemic combination therapy over conventional ones" & "aid and assist" the people with type 2 diabetes mellitus. Main objective is to compare efficacy of newer oral antihyperglycemic combination therapy over conventional ones in the therapy of type 2 diabetes mellitus by finding out the effect of these two varied combinations on glycemic control by monitoring fasting plasma glucose (FPG), glycosylated haemoglobin (HbA_1c), the Lipid Profile and the body mass index (BMI). Major Findings – In the glibenclamide plus metformin combination therapy (n = 23) group FPG & HbA_1c decreased during the therapy significantly up to − 49.06 mg/dL & − 0.90 % respectively during the 4 months of study period. In the glimepiride plus metformin combination therapy group (n = 15) FPG & HbA_1c decreased during the therapy significantly up to − 55.44 mg/dL & − 1.28 % respectively during the 4 months of study period. It was found that combination therapy with metformin plus glimepiride is more effective than glibenclamide plus metformin in improving glycemic control in type 2 diabetes mellitus and is a beneficial adjunct to diet/exercise in management of type 2 diabetes mellitus. At the end of the study period in glimepiride combination therapy more patients were observed to have ≤ 8% of HbA_1c (60.00 %) as compared to glibenclamide combination therapy (39.13 %). Reduction in BMI and in total and LDL cholesterol level were not too significant.

329

Effects of Korean Traditional Dance, Talchoom, on Physical and Mental Function in Elderly People

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During aging, there is a gradual decrease in the ability to maintain physical and mental activity. Therefore, an increasing number of people are becoming health conscious trying to maintain a quality life. A need for a suitable fitness program that is interesting, artistic and culturally oriented toward Korean values and requirements was felt. Talchoom, Korean traditional dance, must strive to achieve full range of motion for all the major muscle groups and the ability of muscles to work hard for increasingly longer periods of time without fatigue. Keeping this need in perspective, talchoom was introduced to Korean elderly for the benefits of physical and mental function. Thirty subjects (21 men and 9 women) between the age of 50 to 70 years old were participated this study. The subjects carried out the talchoom program for 60 minutes each time, 3 times / week for 10 weeks. Blood chemistry, hormonal assay, uroflowmetry, equilibrium sense test and WHOQOL-BREF questionnaire (brief version of the WHO quality of life measure) conducted in baseline and after talchoom program. No clinically relevant changes were observed in blood chemistry and hormonal assay. In case of uroflowmetry, residual urine volume was decreased in normal range after dance program. And equilibrium sense test and WHOQOL-BREF questionnaire were significantly better than baseline. This study show dance program of talchoom improves equilibrium sense and quality of life. Our results indicate that talchoom helps develop physical and mental function in elderly people.

330

Novel Approach for Intensive Pharmacovigilance Studies: Application to Hepatitis C Treatment in Mexican Patients

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As clinical trials do not provide all the necessary information, post-marketing surveillance, including active pharmacovigilance, is required to

yield an adequate risk/benefit perspective for a given drug. Hence, our objective was to design a novel approach for active pharmacovigilance trials allowing it to identify adverse events in routine clinical practice for a specific medication in a defined disease. Peginterferon alpha-2a was the selected medication, chronic hepatitis C being the disease. The first approach was to identify the prescribing physicians in Mexico. Then, physicians were invited to participate. Participation implied authorization to clinical monitors to: (a) create a census of patients currently on treatment, (b) prospective review of the clinical charts for adverse events, (c) if required, answering a questionnaire. Data were consolidated on a monthly basis and submitted to statistical analysis. Adverse events of special interest will be studied in detail by a specific questionnaire. Up to date, 942 patients (54% male) from 28 institutions have been enrolled. Genotype 1 was observed in 62.95%, whereas 25.05% of the patients exhibited genotype 2-3. Genotype was unknown for 110 patients. High viral load was identified in 62.46% of all patients. Most frequent adverse events were: asthenia 21.76%, adynamia 19.50%, headache 9.26% and arthralgia 8.32. Until now, no serious adverse event has been identified. This trial includes the participation of an academic institution (CINVESTAV-IPN), the Mexican Regulatory Agency (COFEPRIS) and a pharmaceutical industry (Roche-Syntex) and thus represents a novel approach for pharmacovigilance.

331

A Predictive Model for Antidepressant use in Santiago, Chile

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Even though Santiago de Chile is a capital city with high prevalence of both antidepressant use and self-medication, factors associated to antidepressant consumption are unknown. The purpose of this study was to investigate medical and socio-demographic factors that may be used to identify patients at risk of antidepressant use in Santiago, Chile. A household cross-sectional study was carried out interviewing a representative and probabilistic sample of 1000 subjects, aged 15 years or older, residing in Santiago, Chile. Data were analyzed identifying significant factors of antidepressant use ($p<0.05$), confounding variables and interactions. A predictive model was developed using logistic regression, and then validated using data from 30 sets of a retrospective random cohort of 600 subjects each. This predictive model includes those factors that were strongly associated with antidepressant use: female gender, high income, age between 40 to 59 years, with private health insurance, more than 12 years of higher educational studies, single, family history of antidepressant use, and diagnosed diseases. Sensibility was 71.1%, specificity was 66%; positive and negative predictive values were 33.5% and 90.5%, respectively. Factors found and included in the present model are not in accordance with those reported about patients who could be susceptible to antidepressant use. This study represents the first step toward understanding the role of socio-demographic and clinical variables in accessibility to antidepressant use among individuals of Santiago, Chile. Also, this model could help to identify inequity as well as to propose a health strategy for support given to a population considered at risk.

332

CYP2D6 Function and Tamoxifen Therapy in Breast Cancer: An Evaluative Survey of Canadian Cancer Care Agencies and their Translation of Pharmacogenetic Research into Clinical Practice

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Introduction: Recent studies in pharmacogenetics of CYP2D6 and tamoxifen use have shown nearly a doubling of risk in breast cancer reoccurrence in subjects with reduced CYP2D6 metabolism. The evaluation of CYP2D6 metabolism in patients may allow clinicians the ability to personalize treatment with tamoxifen and aromatase inhibitors as well as accurately asses the significance of drug interactions. This study surveys the Canadian cancer care landscape to determine the extent of the translation of this new, clinically relevant scientific data into practice. Methods: A survey of

Cancer cancer care agency published practice guidelines and public databases were reviewed. Subsequently Canadian medical oncologists were surveyed via mail. Results: Preliminary results show that published guidelines are silent on the use of pharmacogenetic testing prior to initiating tamoxifen therapy, and cautions about the concomitant use of moderate CYP2D6 inhibitors are absent. Discussion: Ontario, British Columbia and Nova Scotia are silent on the role of pharmacogenetic testing of patients who may be starting tamoxifen therapy. B.C. Cancer Agency states that CYP2D6 metabolism causes variation in endoxifen levels but does not report on its clinical significance. British Columbia and Ontario suggest the use of venlafaxine, a weak inhibitor of CYP2D6, in the treatment of tamoxifen side effects. Modified guidelines could benefit 47% of patients by identifying their poor metabolizer status, or avoiding medications that further reduce an intermediate metabolizer's CYP2D6 activity (Kirchheiner, 2004)

333

Rapid Point of Care Tests (POCT) for Methanol and Acetaminophen- a Pilot Study

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Acetaminophen (APAP) and methanol (MeOH) overdose, if not treated promptly can lead to serious complications and death. For these poisonings, treatment is available. Presenting symptoms may or may not be present making rapid identification by laboratory method critical. Methanol measured by gas chromatography (GC) is available only in a few of the teaching hospitals. To our knowledge neither of these tests are available as POCT. Objective: Evaluate POCT for the detection of APAP and MeOH. Method: 12 sera from two different MeOH overdoses and 17 sera that were positive for APAP by Abbott or Cobas Integra were analyzed by POCT. In addition to the commercially available quality control samples, five and ten negative patient sera for MeOH and APAP respectively, were also analyzed. Cross reactivity to ethanol, was checked with 40mmol spiked ethanol standard. Testing technician was blind to the original results. Results: MeOH: Nine of the eleven sera that were positive by POCT were positive by GC. Two of the remaining positives by POCT were just below the detection limit of the GC suggesting that POCT may be more sensitive than GC. There was concordance with the remaining patient and control samples. Spiked ethanol did not give a false positive result. Readings were taken at two minutes. APAP: All patient and QC samples were in concordance with the original test results. Readings were taken at seven minutes with detection limit at 200umol. Conclusion: Methanol and acetaminophen POCT can be used in the emergency department to triage the poisoned patient.

334

Comparison of Ca Blocker with and without ACE Inhibitor on the Survival of Hemodialysis Patients with Hypertension

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Aims: To determine whether ACE inhibitor further improves the survival rate of hypertensive hemodialysis patients with Ca blocker. Methods: One hundred two hypertensive patients enrolled in the maintenance hemodialysis program were studied for ten years period. Sixty eight patients received Ca blocker alone, and thirty four patients had both Ca blocker and ACE inhibitor for the whole period. Difference in survival was analyzed by using Cox proportional hazard models. Results: There were no significant differences in the predialysis blood pressure between two groups during the ten years period. Thirty three patients (49 %) died in the Ca blocker group while fourteen patients (42 %) died in the group with the combination therapy. In the multivariable models adjusting for the background data and biomarkers, the following items strongly predicted the risk of all-cause death: with ACE blocker (risk ratio: 0.397), Fe (0.968) and RBC (0.982). In the cardiovascular events case, HDL-cholesterol was founded in addition to above variables.

Conclusion: The present study suggests that ACE inhibitor for the prognosis of hypertensive hemodialysis patients with Ca blocker could decrease the risk of death including cardiovascular events.

335

How do Evidence and Value Impact Decision Making? EVIDEM – A Practical Framework Applying Multicriteria Decision Analysis (MCDA) to Structure and Facilitate Healthcare Decisionmaking

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Introduction: Healthcare decisionmaking is a complex process that requires the simultaneous consideration and integration of disparate types of information and value judgments. Objectives of this study were to develop a framework to guide the organization of components of decisionmaking and to translate this framework into practical tools structuring and quantifying assessment of healthcare interventions to facilitate decisionmaking. Methods: A conceptual framework was developed that segregated components of decisionmaking into three: 1) quality of evidence available; 2) intrinsic value of the healthcare intervention; and 3) extrinsic or system-related value. Using this framework, practical tools to assess healthcare interventions were designed drawing on current decisionmaking processes for drug reimbursement globally and an extensive review of the literature. Results: A Quality Matrix was designed to quantify the quality of evidence available for an intervention; five elements defining quality were clustered by three criteria and 12 components covering the range of evidence required for decisionmaking. Scoring was based on international standards in each discipline. To quantify the intrinsic value of an intervention, a multi-criteria decision analysis (MCDA) matrix (Value Matrix) was designed encompassing 15 key value components. Scoring allows inclusion of the perspectives and weighted values of representative healthcare stakeholders. An integrated process to apply matrices was established. Preliminary findings of pilot testing with Canadian stakeholders in the context of drug reimbursement will be presented. Conclusions: The EVIDEM methodology is a practical collaborative framework that can be applied to assess quality of evidence, define explicit data needs, and support healthcare decisionmaking.

336

Medication Errors Made by Health Care Professionals. Analysis of the Finnish Poison Information Centre Data between 2000 and 2007

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Introduction: Aim of the study was to analyse extent, type and time trends of medication errors by health care professionals leading to a call to the Finnish Poison Information Centre (FPIC). Methods: The FPIC database of all calls (277,300) received between 1 June 2000 and 31 May 2007 was analysed in terms of medication errors. Results: Of 189,956 acute human poisoning calls, 1270 (0.7%) concerned medication errors (n = 1275), of which 779 (60.9%) involved administration of a wrong drug, 429 (33.6%) of a wrong dose and 70 (5.5%) an erroneous route of administration. 34.6% of the calls originated from nursing homes for the elderly, mentally challenged and/or dementia patients. Most commonly involved were central nervous system medicines (290; 53.3%), cardiovascular medicines (83; 15.3%), and systemic anti-infectives (44; 8.1%). In children, the most common error was wrong dose, while in adults, it was wrong drug. The number of calls concerning medication errors, corrected for the total number of calls increased steadily, and the absolute number doubled during the study period. The number of medication errors was greatest during the summer months and in December. Discussion: The study is based on spontaneous reports and is likely to underestimate true frequency of medication errors. The overrepresentation of inquiries from nursing homes may partly be explained by not having a doctor available for consultation. Conclusion: Medication errors seem to occur frequently in nursing home like facilities especially during the

holiday seasons, and are different in children and the elderly.

337

Health Care Resources Utilisation by Patients with Psoriasis

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Background/Objective: Psoriasis is a chronic inflammatory skin disease with substantial impact on quality of life, however, little is known about the economic burden of psoriasis in Canada. The objective of this study was to characterize patients with psoriasis and to estimate their relative health care resources consumption. Methods: This study was performed with data from the Quebec provincial medical and drug plans (RAMQ administrative databases). Psoriasis patients (ICD-9 codes: 6961, 6968 or 6969) were identified (psoriatic arthritis excluded). Comorbidities, assessed by an updated VonKorff Chronic Disease Score (CDS), and health care resource utilisation during the one-year period from July 1st 2006 to June 30, 2007 were compared between patients with psoriasis and a control group of patients without psoriasis matched for age and sex in a 1:1 ratio. Subgroup analyses were also performed with patients having used a psoriasis treatment (systemic medication or phototherapy). Results: Compared with the control group, a higher level of comorbidities was found in the 8,881 psoriasis patients (CDS: 3.2 vs. 2.7; p<0,001) and outpatient resources were used with more intensity (p<0.001). Among these patients, 1,596 had received psoriasis treatment (mean age: 55.6). They had a higher level of comorbidities (CDS: 4.2 vs. 2.7; p<0,001) and total cost of health care resources were 64% higher than for the non-psoriasis control group (CDN$2,329 vs. CDN$1,421; p<0.001). Conclusions: Patients treated for psoriasis have a higher level of comorbidities, use more medical resources and incur higher health care cost than patients without psoriasis.

338

Indirect Comparison Meta-analyses and the Importance of Adjusting for a Common Comparator: An Empirical Perspective from Analyses of Pharmacological Agents in Neuropathic Pain

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Introduction: In absence of head-to-head trials, researchers can perform indirect comparisons of controlled studies using a meta-analysis framework. Several studies have established that a naïve approach, where clinical efficacy is assessed independently of comparator-controlled effect, is methodologically flawed and can potentially bias the appraisal. The present study compared results from adjusted and unadjusted indirect comparisons of drugs used in the management of neuropathic pain, focusing on anticonvulsants (Acs: gabapentin, pregabalin) and serotonin-norepinephrine reuptake inhibitors (SNRIs: venlafaxine, duloxetine). Methodology: Randomized, controlled trials were identified through a systematic literature search conducted in MEDLINE, EMBASE, and Cochrane Library. Efficacy was compared using partial response (≥30% pain score reduction) or full response rates (≥50% pain reduction). Meta-analyses were performed using CMA software, v.2.0. Results: Using Relative Response Ratio (RR), the unadjusted meta-analysis suggested a greater response with SNRIs over Acs ($_{unadj}RR_{AC\text{-}SNRI}$ = 0.77). However, adjustment for placebo response revealed opposite results ($_{adj}RR_{AC\text{-}SNRI}$ = 1.55 [1.27,1.90], indicating that pain reduction with Acs is significantly superior to that reported with SNRIs. A similar disparity was reported for partial response, with $_{unadj}RR_{AC\text{-}SNRI}$ = 0.83 compared to $_{adj}RR_{AC\text{-}SNRI}$ = 1.36 (1.13,1.64). Conclusions: This case study underscores the importance of adjusting for placebo effect in meta-analyses, providing a statistically robust approach to conduct indirect studies.

339

Utilization Patterns of Extended Release Niacin and other Lipid Modifying Drugs in Regular Clinical Practice

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Background: The full benefits of lipid modifying therapy can only be realized if patients attain appropriate dose and remain persistent. The objective of this study was to evaluate utilization patterns with extended release niacin (Niaspan®) vs other lipid modifying drugs (LMD). Methods**:** A random sample of patients with ≥1 dispensation of LMD (niacin, statins, bile acid sequest, ezetimibe, fibrates) between Jan-04 and Feb-07 was obtained from RAMQ. Index date (Idate) was date of the first LMD dispensation. Patients who received index drug in the year preceding the Idate were excluded. Persistence was analyzed using the Kaplan-Meier method and Cox proportional hazards model estimated risk factors for early discontinuation. Results: Among 26,862 patients included, 867 (3%) received niacin (mean age 62 years, 75% male, 54% seen by general practitioner, 9% LMD free 1 year prior to Idate). Within 1 year post Idate, compliance was 62% among niacin patients v.s. 75% among statin patients, and persistence was 36% for niacin patients, v.s. 47% for statin patients. One year after Idate, daily niacin dose was 954 mg among persistent patients (n=312); 7.7% of patients reached 2g. Among patients initiated on niacin with a daily dose of 0.5g -1g, the risk of discontinuation was lower for men (rate ratio (RR):0.74; 95%CI: 0.61-0.90) and higher for patients initiating 1g daily dose (RR:1.24 ; 95%CI: 0.96-1.59). Conclusions: In this cohort of initiated patients on LMD, compliance and persistence with niacin remain below that of statins while discontinuation rates remain high and dose attainment suboptimal.

340

How are Type 2 Diabetes Patients who use Insulin Different from their Non-insulin Using Peers?

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Introduction: Patients with type 2 diabetes require pharmacological treatment for optimal glycemic control and to reduce diabetic complications. The analysis compared experiences and beliefs of insulin users with non-insulin users. Methods: In this cohort study, patients with type 2 diabetes were recruited through community pharmacies from 4 provinces (NS, QC, ON, BC) as they were filling a prescription for any oral hypoglycemic medication. Consenting patients were interviewed by telephone. Chi-square analyses were used to compare categorical variables, ANOVA for continuous variables. Results: 801 patients were interviewed; mean age 63 years; 54% male; and a 9 year mean duration of diabetes. Patients were using a median of 2 diabetes medications and a median of 7 prescription medications. 16% were using insulin and insulin users had a longer duration of both diabetes (15yrs) and duration of drug therapy (14yrs) compared to non-users (8yrs and 7yrs, respectively) ($p<0.0001$). A larger percentage of insulin users checked their own blood glucose (99% vs 84%, $p<0.0001$), had their feet checked annually (59% vs 44%, $p=0.002$), and were using an ACEi (84% vs 71%, $p=0.002$). More insulin users reported having experienced a hypoglycemic reaction (72% vs 43%, $p<0.0001$) and a smaller proportion of insulin users felt their diabetes was adequately controlled compared with non-users (70% vs 84%, $p<0.0001$).

341

Proton Pump Inhibitors and the Risk of Osteoporosis Related Fractures

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Introduction: The use of proton pump inhibitors has been associated with an increased risk of hip fracture. We sought to further explore the relationship between duration of PPI use and osteoporosis-related fractures, defined as fractures of the hip, vertebra, and wrist. Methods: We used administrative claims data from Manitoba, Canada to identify all cases of fractures of the hip, vertebra, or wrist occurring between 1996-2004, who were each matched with 3 controls on age, sex, and medical comorbidity. Odds ratios for the risk of hip fracture and all osteoporosis-related fracture were calculated for durations of PPI exposure ranging from more than one to more than seven years. Results: 15792 cases of osteoporosis related fracture were matched with 47289 controls. We did not detect a significant association between osteoporosis related fracture and durations of PPI use for PPI exposures for durations of 4 years or less. However, duration of PPI exposure exceeding 5 years and greater were associated with increased odds of osteoporosis-related fractures (OR >5 years: 1.27, >6 years: 1.47, >7 years; 2.42, all p-values < 0.05). Discussion: Duration of PPI exposure exceeding five years is associated with a significantly increased risk of osteoporosis-related fracture. Further study is required to determine the clinical significance of this finding at to determine the value of osteoprotective medications in long-term PPI users.

342

Utilization Review of Antibiotics Use in Pediatric Upper Respiratory Tract Infections (URTI)

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The emergence of bacterial strains that are increasingly resistant to antimicrobial agents is a growing national and worldwide concern. Antibiotic use rates are higher in young children and respiratory infections account for the largest part of antimicrobial drug prescription. The objective of this study was to evaluate the appropriate use of antibiotics for URTI in young children across seven utilization criteria in the province of Quebec. Data were obtained from medical records of children between the ages of 3 months and 6 years, who have received medical care in an emergency setting. Overall, 55% of children received an antibiotic treatment after consulting for URTI. Approximately 50% of the children received first-line recommendation as initial antibiotic treatment and 19% received the recommended second-line treatment. We observed under utilization of amoxicillin high dosage which is recommended in otitis and, for children over two years old, duration of therapy of ten days even though a treatment of 5 to 7 days is now considered satisfactory. Although most cases of rhinosinusitis are viral, 88% of children have received an antibiotic in non-complicated rhinosinusitis. When an antibiotic was prescribed in pharyngitis-tonsillitis a diagnosis test was prescribed for only 39% of children. On the positive side, most of the children who consulted for a viral infection did not receive antibiotics, except in bronchitis. In conclusion, the results of this study illustrate that inappropriate use of antibiotics still persists for some URTI but has improved in non-complicated viral infections.

343

Decision Model for the Evaluation of New Therapeutic Target in Patients with Schizophrenia

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Aim: Pharmacological strategies for schizophrenia have received increasing attention due to the development of new and costly drug therapies. A decision model, useful for evaluating the benefits and cost-effectiveness ratios, is built and applied in the Canadian context. Objectives: To develop a decision model for patients newly diagnosed for schizophrenia**.** Methods: To develop a computer Markov model based on data from the literature and Canadian Health Statistics which is used to compare the effectiveness of new therapeutic target and/or to estimate the pricing of new drug development. Seven discrete disease states were defined within the model and patients' movements

between these disease states enables10 disease courses to be identified. The model simulated the disease course over a 5-year period. The validation was done by comparing Canadian predictions obtained with those of published model. Results: Predicted probabilities in Canadian context for each state defined as well, stable, low dependency, high dependency and death were 29.7%, 24.1%, 31.5%, 12.3% and 2.4%, respectively. The corresponding figures were similar to those obtained from published model e.g. 29.6%, 20.8%, 38.8%, 9.8% and 1.0%. The impact of intervention that reported on schizophrenia from literature data resulted in less intense use of resources based on significant improvement as lower transition probabilities into high dependency states and higher transition probabilities into better health states (well and stable) were at 41.2%, 25.8%, 24.3%, 6.4% and 2.3%, respectively. Conclusion: We conclude that this model is useful to predict benefits and cost-effectiveness ratios of new therapeutic target and/or to estimate the pricing of new drug development for schizophrenia.

344

Trends in Utilization of Medications for Diabetes in Manitoba, Canada

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Introduction: Few studies have evaluated the utilization of drugs for diabetes in the population over time. We sought to describe the utilization of medications for diabetes in Manitoba, Canada (population 1.1 million). Methods: Data were derived from anonymized health care administrative databases in the Population Health Research Data Repository, housed at the Manitoba Centre for Health Policy. Incident and prevalent utilization per quarter year of drugs for diabetes was determined for the population of Manitoba (>18 years old) by identifying all prescriptions for drugs for diabetes (metformin, sulfonylureas, insulin, acarbose, glitazones, meglitinides) dispensed in Manitoba from 1995-2005. When acarbose, glitazones and meglitinides were added to the Manitoba formulary, these agents required special authority for coverage. Results: Prevalent metformin use rose from 5 to 36 users per 1000 population to become the most commonly prescribed drug in this group. Increases prevalent users were also observed from 1994-2005 for insulin (7 to 11 per 1000) and the glitazones (0.4 to 8 per 1000). Utilization of acarbose, meglitinides and glitazones also increased, but remained low compared to other agents. A greater number of persons used combination therapies from 1994-2005 (4 to 22 per 1000) as compared to oral monotherapy (14 to 23 per 1000) or insulin monotherapy (7 per 1000 throughout). Utilization of all drugs was greatest in those aged 65-84 and lowest in those aged 19-44. Conclusion: Utilization of metformin is increasing, as is the utilization of newer agents and combination therapies. Special authority listing of newer agents kept utilization low.

345

Cost-effectiveness of Etanercept in Canada for the Treatment of Severe Psoriasis

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Objectives: To evaluate the cost-effectiveness of etanercept versus supportive care (i.e. non-systemic therapy) for treating patients with severe psoriasis in Canada. Method: We adopted a published German-based Markov model to the Canadian setting. The patient population considered in this evaluation were severe patients who have plaque psoriasis with an initial Psoriasis Area and Severity Index (PASI>10) and a Dermatology Life Quality Index (DLQI) >10. Efficacy and changes in quality of life data were derived from randomized clinical trials. Resource utilization data were gathered from a survey of 12 dermatologists in Ontario and Quebec. The evaluation was undertaken from an Ontario Ministry of Health perspective. The main outcome of interest was the 10-year incremental cost per Quality-Adjusted-Life-Year (QALY) gained of etanercept compared to supportive care. All post one year costs and outcomes were discounted at 5%. A probabilistic sensitivity analysis (PSA) was performed in addition to univariate sensitivity

analyses (Sas). Results: The incremental cost effectiveness ratio was $83,880/QALY over a 10-year time horizon when etanercept was compared to standard supportive treatment. The PSA indicated that the probability of etanercept being cost-effective was 0.98 when compared to supportive care if society or decision makers were willing to pay $100,000 per QALY gained. Sas indicated that the cost-effectiveness improved with severity and that drug costs and utility values were the key drivers of the model. Conclusion: Consistent with findings from Germany and in the UK, etanercept is a cost-effective alternative in the treatment of severe psoriasis in Canada.

TUESDAY, JULY 29, 2008
STREAM 4:
CLINICAL PHARMACOLOGY IN SPECIAL POPULATIONS

346

The Effectiveness of Discontinuing Iron-containing Prenatal Multivitamins on Reducing the Severity of Nausea and Vomiting of Pregnancy

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Background: Nausea and vomiting of pregnancy (NVP) is experienced by the majority of pregnant women, and can negatively affect a women's quality of life. It has been suggested in observational studies that iron-containing prenatal multivitamins may increase the severity of NVP. Objective: The objective of this study was to determine whether decreasing iron exposure can mitigate NVP symptoms. Study Design: Data were collected from a prospective cohort at the Motherisk Program in Toronto. Women (n=97) seeking advice on managing severe NVP were advised to discontinue prenatal multivitamin administration and switch to folic acid, an adult multivitamin or a children's chewable multivitamin. Results: Two-thirds (63 out of 97) ($p<0.001$) of those women qualitatively reported an improvement in NVP symptoms after discontinuation of iron-containing prenatal multivitamins. These findings were verified quantitatively using both the PUQE ($p<0.001$) and Well-being ($p<0.001$) scoring systems. Conclusions: This is the first interventional study showing that discontinuation of iron results in improvement of NVP symptoms. Our data suggest that avoiding iron-containing prenatal multivitamins in the first trimester is effective in improving NVP symptoms in the majority of pregnant women suffering from morning sickness.

347

Association between Drug Burden Index and Physical Function in Older Australian Men

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This study aimed to evaluate the association between physical performance and the Drug Burden Index (DBI), a measure of a person's total exposure to anticholinergic and sedative medications that includes the principles of dose-response and maximal effect, in older Australian men. DBI has been associated with physical and cognitive performance in older people in the Health ABC study in the USA. A cross sectional survey was performed on a random sample of community dwelling older men enrolled in The Concord Health and Ageing in Men Project (CHAMP), Sydney, Australia. The functional outcomes included gait speed over 6m, 20cm narrow walk speed, balance and the Instrumental Activities of Daily Living (IADLs). Analyses were performed with SAS statistical software (Version 9.1). At baseline, the study population consisted of 1705 men. A total of 1527 (90%) subjects reported taking medications. Of these, 19% had been exposed to anticholinergic and 12% to sedative drugs. The average DBI was 0.16±0.35. After adjusting for confounders (sociodemographics, comorbidities, cognitive impairment, depression), DBI was associated with slower walking speed ($p=0.009$) and narrow walk speed ($p=0.0009$), balance difficulty ($p=0.007$) and poorer performance on IADLs ($p=0.0003$). Association with physical function was stronger for the sedative than for the anticholinergic component of DBI, after adjusting for confounders. Average DBI in CHAMP

participants is very similar to that of community dwelling older people in the Health ABC study in the USA. Higher DBI is associated with poorer physical function in community dwelling older Australian men.

348

The Association between Canadian Folic Acid Flour Fortification and the Incidence Rate of Early Childhood Cancers

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Introduction: In 2005, approximately 1,000 Canadian children under the age of 15 were diagnosed with cancer. In case-control studies, folic acid (FA) supplementation was associated with prevention of brain cancers (BC), Acute Lymphoblastic Leukemia (ALL) and embryonal cancers (EC). However, no study has examined effects of FA intervention. We examined the impact of FA fortification of flour on incidence rates of ALL, BT, EC and Wilms' tumor (WT) in Ontario. Methods: This was a population-based, cross-sectional study of Ontario children using the Pediatric Oncology Group of Ontario's cancer registry. The incidence rate of cancers in children aged 0-4 and 5-9 was compared before and after FA fortification. Data was analyzed using comparative statistics and time series analysis. Results: In both age groups, there were no differences in the incidence of ALL (0-4: 6.41 vs. 6.81, p=0.33 ;5-9: 3.12 vs. 3.34, p=0.44), BT (0-4: 2.03 vs. 1.93 p=0.64 ;5-9: 2.13 vs. 1.94, p=0.40) and EC (0-4: 5.03 vs. 4.90, p=0.73 ;5-9: 1.16 vs. 1.21, p=0.73) after FA fortification. The incidence of WT was reduced by 30% post FA fortification in the 0-4 age group (1.94 vs. 1.43, p=0.02). Discussion: The results do not corroborate previous findings; however, those studies could not isolate the effect of FA. Ontario is the only region with FA fortification and a network of pediatric oncology centres submitting to a central database, allowing us to study this population. Conclusion: FA fortification was associated with a 30% reduction in WT. Further studies are required to corroborate these results.

349

Candesartan Improves Left Ventricular Diastolic Function and Left Ventricular Hypertrophy in Patients with Essential Hypertension

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Objectives: To study the effects of the AT1 receptor blocker Candesartan on left ventricular hypertrophy (LVH) and LV diastolic function in hypertensive patients. Patients and Methods: Forty hypertensive patients were randomized to receive candesartan cilexetil (n=18) or telmisartan (n=22) for 6 months. They had LVH (interventricular septal thickness (IVSTd) $\geq$ 12 mm) and diastolic dysfunction (E/A ratio less than 1 and deceleration time (DT) greater than 250 ms) were evaluated by echo Doppler studies. Results: After 6 months of candesrtan treatment IVSTd decreased from 16 ± 104 to 14 ± 103 ($p<0.01$) , LVMI decreased from 162 ± 21 g/m^2 to 140 ±20g /m^2 ($p<0.01$),E / A ratio increased from 0.90 ± 0.8 to 1.2 ± 0.7 ($p<0.05$),DT was reducd from 270 ± 20 to 235 ± 18 ($p<0.01$). Under telmisartan IVSTd decreased from 15 ± 1.2 to 914 ± 1.4 ($p<0.01$), LVMI decreased from 160 ± 20 g/m^2 to 138 ± 21 g/m^2 ($p<0.01$),E/A ratio increased from 0.95 ± 1.3 to 1.3 ± 0.8 ($p<0.05$), DT was reduced from 260 ± 15 to 240 ± 12 ($p<0.01$) . Conclusions: Candesartan and telmisartan treatment caused significant regression of LVH and a significant improvement of LV diastolic function and both are equally effective.

350

Erythrocyte Antioxidant and Antiplatelet Effect of Statins in Hyperlipidemic Indian Patients

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Introduction: A prospective randomized study to assess the effects of simvastatin and atorvastatin

on erythrocytic oxidative stress and platelet aggregation in hyperlipidemic subjects Methods: Twenty statin naïve patients were administered either atorvastatin or simvastatin 10 mg daily each for 8 weeks and evaluated for erythrocytic oxidative stress (TBARS, antioxidant enzymes) and platelet aggregation. Six control subjects were assessed for baseline parameters. Results: Baseline erythrocytic oxidative stress was significantly higher in the hyperlipidemics compared to healthy controls (TBARS 294.1 ± 126.8 vs. 99.4 ± 22.50 units; p= 0.002) and SOD levels 359.5 ± 176.3 units vs. 647.07 ± 384.5 units, p= 0.01). There was no significant difference in catalase or glutathione peroxidase levels. Platelet aggregation with ADP and epinephrine was not significantly different between the hyperlipidemics and the control. Eight weeks after treatment there was a significant decrease in lipid peroxidation in both the statins compared to baseline. TBARS levels decreased 38.3% in atorvastatin group and 28.2 % in simvastatin group (p= 0.005). SOD, catalase and glutathione peroxidase levels increased by 32.1%, 90.9%, 19.8% (p= 0.01, 0.005, 0.005) respectively with atorvastatin group and with simvastatin it was 32.9%, 12.6% and 36.9% (p= 0.01, 0.01. 0.05) respectively. Platelet aggregation decreased significantly to both aggregants. The decrease to ADP and epinephrine induced aggregation was 38.7%, 58.0% respectively with atorvastatin and 14.8% and 24.5% with simvastatin respectively (p< 0.05). Both statins were well tolerated. Conclusion: The results of this preliminary study suggest comparable and significant antihyperlipidemic, antioxidant and antiplatelet effects in hyperlipidemic Indian subjects.

351

Comparative Assessment of alpha-Lipoic Acid and Vitamin E on Nerve Conduction in Type-2 Diabetes with Peripheral Neuropathy

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Introduction: Diabetic neuropathy (DN) is increasingly viewed as neuronal damage due to oxidative stress. Antioxidant, alpha lipoic acid (ALA) and Vitamin E (VE) diminishes increased oxidative stress and shows improvement in nerve dysfunction. Hence, the present study was carried out to assess and compare potential benefits of ALA and VE on nerve conduction in Indian DN patients. Methods: A total of 40 type-2 diabetic patients with peripheral neuropathy between the age group of 35-65 yrs were enrolled for randomized, double-blind, placebo-controlled study. Patient received orally 600 mg (n = 10) of ALA, 800 mg (n = 20) of VE or placebo (n = 10) daily for 3 months. Measurement of HbA1c, FPG and 2hr-PG along with nerve conduction parameters in their basal state and after 3 months of treatment were studied. Results: Motor Nerve Conduction improved significantly in 6/5 of 15 studied electrophysiological parameters after 3 months in patients on ALA/VE supplementation. Latency of peroneal, median, and ulnar motor nerves (P less than 0.047) after ALA and VE supplementation, NCV of peroneal, median, and ulnar motor nerves (P less than 0.043) on treatment with ALA and NCV of median, and ulnar motor nerves (P less than 0.045) on treatment with VE improved significantly, whereas glycemic indexes did not show any significant changes during the study. Discussion: The motor nerve conduction showed significant improvement in both treatment groups. No significant improvement was observed in sensory nerve conduction. Conclusion: ALA is more effective in improving motor nerve conduction in comparison to Vitamin-E.

352

Study of the Anti-proliferative Effects of Phthalides from *Angelica sinensis* on Human Colon Cancer Cells

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Angelica sinensis (Oliv.) Diels is a Chinese medicinal herb with several pharmacological actions relating to its phthalide content. This study aims to investigate the anti-proliferative effect of *A. sinensis* to assess its potential use in the chemotherapy of colorectal cancer. The phthalides studied were n-butylidenephthalide, senkyunolide A and z-ligustilide. Human colon cancer HT-29 and normal CCD-18Co cell lines were used. Cell

viability and proliferation were assessed by MTT and [^{3}H]-thymidine incorporation assays, respectively. Median effect analysis and combination indices were used to determine whether there were synergistic, additive or antagonistic effects among components in the extracts and mixtures. All three phthalides dose-dependently decreased cell viability in both cell lines, with a higher potency observed in HT-29 cells. Senkyunolide A, z-ligustilide and n-butylidenephthalide dose-dependently decreased cell proliferation with IC_{50} values of 54.2±5.1, 60.6±6.8 and 236.9±18.2 μM, respectively in HT-29. Extracts of *A. sinensis* and *Ligusticum chuanxiong*, which also contained these three phthalides but in different proportions, and two mixtures of the three phthalides in the same ratios as found in the herbal extracts, were examined. *A. sinensis* extract showed synergistic effects for the inhibition of HT-29 cell proliferation, while phthalide mixtures and *L. chuanxiong* extract showed antagonistic effects. All three phthalides showed potential as anti-cancer drug candidates. The unique combination of compounds in *A. sinensis* is worthy for further investigation since it contains other bioactive components that may act synergistically with phthalides to produce anti-proliferative effects in colon cancer cells.

353

Estimation and Study of High Sensitive C-Reactive Protein (hs-CRP) Levels in Indian Hypertensive Dyslipidemic Patients

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Introduction: Hypertension and dyslipidemia are major risk factors for development of atherosclerosis. They are modifiable cardiovascular risk factors that coexist frequently. Several markers have been evaluated as predictors of coronary vascular disease. High sensitivity C-reactive protein (hs-CRP) appears to be an excellent marker of persistent atherosclerosis & coronary heart disease. So, the objective of present study was to evaluate C-reactive protein as a persistent marker of cardiovascular disease.Method – Sixty patients with hypertension & dyslipidemia were selected from OPD/Wards of Medicine, Guru Nanak Dev Hospital, Amritsar, India and were randomly divided into two groups A and B of 30 patients each. Group A were administered Ramipiril (5mg) plus Atorvastatin (10mg) and Group B were given Amlodipine (5mg) plus Atorvastatin (10mg). Treatment was continued for 12 weeks and investigations were conducted at beginning & 12th weeks of study. Results – There was a non-significant decrease in levels of hs-CRP $p = 0.0723(t = 1.865)$ and $p = .2110(t = 1.279)$; a significant decrease in levels of total cholesterol $p<0.0001(t = 9.892)$ and $p<0.0001(t = 22.667)$; a significant decrease in levels of systolic blood pressure $p<0.0001(t = 10.706)$ and $p<0.0001(t = 13.589)$ and levels of diastolic blood pressure $p<0.0001(t = 8.548)$ and $p<0.0001(t = 13.924)$ at the end of 12week in group A and B respectively. Conclusion – C-reactive protein is a relatively moderate predictor of coronary heart disease in Indian population. Recommendations regarding its use in predicting the likelihood of coronary heart disease may need to be reviewed in large population.

354

Risks of Statins use during Pregnancy: A Systematic Review

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Introduction: Drugs of the class known as statins are used successfully in the treatment of hypercholesterolemia. Statins are amongst the most extensively investigated and prescribed pharmaceutical agents in current clinical use. The available body of data regarding exposure to statins during pregnancy is relatively small and controversial. On the basis of limited animal data, the FDA assigned statins to pregnancy category X. Methods: We reviewed medical publication databases (MedLine, EMBASE) for all studies addressing the effect of statins on pregnancy from 1983 to 2007. Key search words included: pregnancy, statins, teratogens. Results: Statins have been identified as potential teratogens on the basis of theoretical considerations and small case series. The risk for an exposed pregnancy seems to be small, if present at all, and does not by itself warrant termination of pregnancy. Although the paucity of human data does not support the

unrestricted use of statins during pregnancy, there is not evidence to suggest that accidental exposures are associated with increased risk of malformations. Conclusion: Even though the information data available to date is not conclusive, overall it suggests that statins are unlikely to be major teratogens. Nevertheless, until more data are available, statins should be avoided during pregnancy, and pregnant women exposed to cholesterol-lowering drugs, should be monitored closely. Statins should be used during pregnancy only if the benefits clearly outweigh the risks. If a woman becomes pregnant while taking this drug, discontinuation of therapy should be discussed and the woman should be informed about potential hazards to the fetus.

355

Comparative Study between the Eradication Rates of Helicobacter Pylori using Two Different Ten Days Triple Drug Regimens

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Objective: Research was designed to find eradication rate of H. pylori in Nepal. Methods: Two ten-days triple therapy Omeprazole 20 mg, Amoxicillin 1000 m and Clarithromycin 500 mg versus, Regimen II Omeprazole 20 mg, Tinidazole 500 mg and Clarithromycin 500 mg, all oral BD therapy to cure H. pylori. All medications were stopped after ten days regimen and patients were followed after four weeks of stopping medication. Repeated endoscopy was performed to confirm the eradication of organism. Results: 17%of patients using Regimen I suffered from adverse effects, diarrhea 60%, rashes 20% and vomiting 20%. Due to the severity of the adverse effects there was a dropout of 5% patients, suffering from rashes and vomiting. Regimen II, minor adverse-effects were reported, 42% having metallic taste, 29% nausea and 29% anorexia. However, in the Regimen II 80 patients, out of which fifty (62%) male and thirty (38%) female, with mean age of thirty-five years. The chief complaint was found to be epigastric pain/burning in 91.5% and the common endoscopic diagnosis was found to be gastritic erosion 79%. Out of 80 patients, 62 patients completed the study. The eradication rate of the Regimen I was 78.1 % and Regimen II was 73.3%. (p=0.660) was found between the eradication rates of the two regimens. Conclusion: The eradication rate 80%. Hence both the regimens of ten days duration are low efficacy regimen.

356

Comparative Evaluation of Mixed Amphetamine Salts Extended Release and Short/Intermediate Acting Stimulates for Treatment of ADHD in Children

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Introduction: The objective of the study was to compare the efficacy of mixed amphetamine salts (MAS-ER) extended release versus short/intermediate (S/I) acting stimulants as a treatment of ADHD in children. Methods: In an open-label, randomized, prospective study design, 142 children who were seen in a pediatric outpatient clinic were assigned to receive once-daily MAS-ER (group 1) or S/I acting stimulants methylphenidate or amphetamine (group 2). Diagnosis of ADHD was made by a structured interview with the parents and by psychometric tests including the Continuous Performance Test (CPT), the long version of the Connor's Rating Parental Scale (CRPS-L), SNAP-IV Teacher and Parent Rating Scale of 18 items (SNAP-18) and the Text Revision of the Diagnostic & Statistical Manual for Mental Disorders (DSM IV-TR) criteria. Duration of treatment was 6 weeks, and children were follow-up weekly until completion of the study. Results: 122 patients completed the study. Following 6 weeks of treatment with MAS-ER or S/I acting stimulants, children in both groups showed clear improvement while on medication as documented by the different psychometric evaluations. However, a differential response on the subscales of the CPT, CPRS-L, and SNAP-IV, was noted between groups. For example, the average clinical confidence index, omission errors, percentile of omission and error of commissions decreased more significantly ($P<0.05$) in the MAS-ER group than in the S/I acting stimulants group. Discussion/Conclusions: The mixed amphetamine salts extended release formulation was significantly superior to S/I

acting stimulants in reducing core symptoms of ADHD in children in a 6-week treatment period.

357

Effect of Nevirapine (NVP) and Efavirenz (EFV) Based Antiretroviral Therapy on Liver Enzymes in Ugandan HIV/AIDS Patients

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Background: Varying grades of liver enzyme elevations (LEE) have been associated with the use of Nevirapine (NVP) and Efavirenz (EFV). This study compared the grades and factors associated with Alanine amino transferase (ALT) and Aspartate amino transferase (AST) LEE in NVP and EFV HIV/AIDS treated patients. Methods: A retrospective analysis of eighty four (84) patients attending Rakai Health Science Program was done in two arms; fifty-five (55) and twenty nine (29) patients on NVP and EFV regimens respectively. Clinical and laboratory data at three month intervals for a 12 month observation period was extracted using a standardized data collection tool. The data was analysed using SPSS. Results: More female patients were on NVP 51(85%) than EFV regimens ($p < 0.001$). Creatinine levels were higher in the NVP than EFV treatment group ($p = 0.017$). No significant differences in LEE were observed between the NVP and EFV arms. No case of severe LEE detected. Mild LEE occurred in the first three months in the NVP more than the EFV group. Female, gender and Stavudine containing regimens were associated with LEE. Discussion: Mild self-limiting LEE were observed in the first 3 months of NVP or EFV treatment probably due to immune reconstitution that has been reported to occur at initiation with NNRTIs and retonavir.Conclusion: The liver safety of EFV and NVP regimens are comparable. Monitoring of AST and ALT in the first three months of initiating NVP or EFV based regimens is necessary. Key words: Liver enzymes, Nevirapine, Efavirenz, HIV/AIDS

358

SC Administration of Vitamin A in ELBW Neonates

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Background: The majority of very low birth weight (VLBW) neonates, birthweight (BW) < 1,500 g, are born with low serum vitamin A levels (<30 mcg/100 mL, normal 30-60 mcg/100 mL). The protracted duration of vitamin A deficiency in this population may have a significant impact on the development of eyes, lungs, GI tract and the cardiovascular system. Supplementation trials have utilized the IM route for repeated dosing of vitamin A. This route is not ideal and may account for its poor acceptance. An alternative route that would not require repeated, painful injections is desirable.
Objective: To determine the safety and efficacy of repeated subcutaneous (SC) administration of vitamin A through a small gauge, indwelling (Insuflon ®) catheter. Methods**:** Newborns < 1,500g received 4,000 units/kg of SC vitamin A every 48 hours X 3 through an indwelling catheter. Monitoring was performed daily to check for possible side effects of vitamin A (eg, bulging fontanelles, skin abnormalities, increased liver span). Results: Eleven VLBW neonates (BW 975 $\pm$ 252g, Gestational Age 27.6 $\pm$ 1.7 weeks) (mean $\pm$ SD) received SC vitamin A beginning on day 20 $\pm$ 3.2 day of life. Serum vitamin A increased 5.2 $\pm$ 4.8 mcg/100 mL after 3 SC doses. No systemic or local side effects were observed. Conclusion: This pilot study suggests that repeated administration of SC Vitamin A through an indwelling catheter may offer an alternative to the painful IM route. Additional studies are required to pursue this hypothesis.

359

Therapeutic Approach to Early and Late-onset Neonatal Sepsis at Fudan University Children's Hospital

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Introduction: Neonatal sepsis is one of the leading causes of infant death in developing countries. The development of a rational approach to screening and treatment is important to the

reduction of infant mortality. Methods: A retrospective analysis was undertaken to evaluate antibiotic choices, blood culture protocol, and pathogen prevalence in neonatal sepsis at Fudan University Children's Hospital, Shanghai, China. Neonatal cases designated as sepsis admitted January 2003 – March 2007 were identified and divided into early (EOS) and late-onset sepsis (LOS) (0-7 days and >7 days post-birth). Results: 211 cases were identified. Blood culture status, pathogens identified, and antibiotic data were collected. In total, 27 antibiotics were used monotherapeutically. Blood was cultured for 86.8% of EOS and 90.8% of LOS; 26.6% of EOS and 46.8% of LOS resulted in positive cultures. *Coagulase-negative staphylococcus* was the most common pathogen cultured for both EOS (38.1%) and LOS (58.8%). *Escherichia coli* and *Enterococcus sp. w*ere the second most common pathogens cultured for EOS (both 14.3%), and *E coli* was for LOS (9.80%). Cefotaxime was the most frequently used first-line antibiotic in EOS (54.9%) and LOS (43.3%), followed by piperacillin in EOS (16.5%) and cefazolin in LOS (16.7%). Conclusion: In this sample most neonatal sepsis cases were late-onset. Cefotaxime was the first-line antibiotic choice for both EOS and LOS. The common North American first-line treatment choices gentamicin and ampicillin for EOS and cloxacillin for LOS were not used. Further study of common pathogens and their sensitivity profiles is needed to support optimal antibiotic choice.

360

Population Pharmacokinetics of Flurbiprofen in Children after Intravenous and Oral Dosing

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Aims: Flurbiprofen is a commonly used NSAID in pediatric pain therapy. However, pediatric pharmacokinetics of flurbiprofen is poorly described. We have studied the pharmacokinetics of flurbiprofen after intravenous and oral dosing in 64 generally healthy children aged 3 months - 12 years undergoing day-surgery with spinal anesthesia. Methods: After ethics committee approval and informed, written, parental consent the children were given either a single intravenous dose of flurbiprofen axetil, 1 mg/kg (corresponding to 0.74 mg/kg of flurbiprofen) (n = 27), or a single oral dose of flurbiprofen, 1 mg/kg (n = 37). A total of 304 blood samples (1-7 per child) were obtained up to 20 hours after the dosing. Flurbiprofen concentrations were measured by gas chromatography with mass spectrometric detection. Population pharmacokinetic modeling was performed with NONMEM software. Results: A good fit for plasma concentrations was obtained by using a 2-compartment model. Weight was an important covariate for clearance, and for central and peripheral volume of distribution. The mean bioavailability of per oral syrup was 82%. Discussion and conclusions: Population pharmacokinetic modeling enabled us to calculate the pharmacokinetic parameters for flurbiprofen in this pediatric study with sparse plasma sampling.

361

Clinical Pharmacology in Paediatrics

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Pharmacokinetic and Pharmacodynamic behavior differ greatly in paediatric age group compared with "normal" adult populations. The objective of this study is to demonstrate the difference in pharmacology of drugs in paediatric age group compared with normal adult population, using amino penicillin (amoxicillin) as an example. Methods: Standard pharmacokinetic study was used. Multiple doses of amoxicillin in therapeutic range were administered orally to one hundred paediatric patients with upper respiratory tract infection between the ages of two and six years and the response gotten compared to that of one hundred adult patients with same condition. Blood samples were collected over specified intervals and serum levels of the drug measured. Results: Ninety six percent of the adult population had peak serum concentrations after two hours as against thirty five percent of the paediatric population. Discussion: It appears that the absorption, distribution and metabolism of the drug (amoxicillin) was delayed in the paediatric

age group .While majority of the adult population had peak serum concentrations after two hours, only thirty five percent of the paediatric age group had achieved such concentration after two hours.
Conclusion: The labeling for a product should reflect the data pertaining to the effect of age and/or development on the pharmacokinetics and pharmacodynamics (if known) obtained from the studies conducted. The clinical pharmacology section should state differences in absorption, distribution, metabolism and excretion, if any, between the adult and paediatric populations. The dosage and administration section should describe dosing adjustments for paediatric patients according to age and/or body weight.

362

Pharmacokinetics of Intravenous and Oral Midazolam in Plasma and Saliva in Humans: Usefulness of Saliva as a Matrix for CYP3A Phenotyping

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Aims: Midazolam plasma clearance is established for CYP3A phenotyping. Saliva would be an alternative matrix, but its usefulness for phenotyping with midazolam has not been demonstrated. Our aims were to compare the kinetics of midazolam in plasma and saliva and to find out the suitability of saliva for phenotyping.
Methods: We conducted an open-label, randomized, 2-way cross-over study in 8 subjects treated with 2 mg midazolam i.v. or 7.5 mg p.o. under basal conditions and after CYP3A induction with rifampicin. Results: Under basal conditions and i.v. administration, midazolam, 1'-hydroxymidazolam, 4-hydroxymidazolam and 1'-hydroxymidazolam-glucuronide were detectable in plasma, and midazolam and 1'-hydroxymidazolam in saliva. After rifampicin, the AUC of midazolam (plasma, saliva) and 1'-hydroxymidazolam (plasma) had decreased significantly. The concentrations of midazolam were two orders of magnitude lower in saliva than plasma, reflecting high plasma protein binding. There was a significant correlation between the midazolam concentrations in plasma and saliva both under basal conditions (R=0.864) and after treatment with rifampicin (R=0.842). After oral administration and under basal conditions, midazolam, 1'-hydroxymidazolam and 4-hydroxymidazolam were detectable in plasma and saliva. After treatment with rifampicin, the AUC of midazolam (plasma) and 1'-hydroxymidazolam (plasma, saliva) had decreased significantly. The parameters separating best between basal conditions and post-rifampicin were: 1'-hydroxymidazolam-glucuronide/1' hydroxymidazolam at 20-30 min (plasma) and the AUC of midazolam (saliva) after i.v., and the AUC of midazolam (plasma) and of 1'-hydroxymidazolam (plasma and saliva) after oral administration. Conclusions: Saliva appears to be a suitable matrix for non-invasive CYP3A phenotyping using midazolam as a probe drug.

363

Population Pharmacokinetics of Vancomycin in Korean Pediatric Patients

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Introduction: The pharmacokinetics of vancomycin (VCM) is known to differ between pediatrics and adults. This study aimed to describe the population pharmacokinetics (PPK) of VCM in Korean pediatric patients and to evaluate the predictive performance of the PPK model. Methods: Data were collected retrospectively from therapeutic drug monitoring reports and randomly allocated to either index data set (n=92) or validation data set (n=27). Index data set consisted of 159 concentration measurements. The PPK parameters of VCM were estimated by nonlinear mixed-effect modeling with first-order conditional estimation and interaction method. The influence of covariates were assessed. The predictive performance of the model was evaluated using validation data set. Bias and precision were assessed by mean prediction error (ME) and root mean squared prediction error (RMSE). Results: A two-compartment model was adopted and the final PPK parameters were as follows; Clearance(CL) (L/h) = $0.0722 \times Wt^{1.3}$ (kg) × (1 - (s-Cr) (mg/dL) × 0.506), Central volume of distribution(V_d) (L) = $0.0709 \times Wt^{1.61}$ (kg), k_{12} (1/h) = 0.288, k_{21} (1/h) = 0.14.The coefficients of variation of interindividual

variability in CL and central V_d were 25.5% and 18.6%, each. The residual variability was 23.5%. The ME and RMSE were -0.016 and 2.171 mg/L, respectively. Conclusion: A PPK model of VCM in Korean pediatric patients was developed. Wt and s-Cr were included as significant factors affecting VCM disposition. The predictive performance of this model was reasonably acceptable. The PPK parameter values obtained in this model may be used to optimize VCM therapy.

364

Tolerability and Pharmacokinetics of s New Anticancer Agent CKD-732 in Korean Patients with a Progressive or Recurrent Solid Tumor

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Objective: To investigate tolerability and pharmacokinetic characteristics of CKD-732, a new synthetic analog of fumagillin, in Korean patients with advanced or recurrent solid tumor who failed in the standard therapy. Methods: Each cycle of the treatment consisted of twice-a-week, one-hour intravenous infusions of CKD-732 over two weeks, followed by a one-week washout. Blood samples were collected at the first (day 1) and fourth infusion (day 11) of the first cycle, and the first infusion (day 43) of the third cycle, each collected at 0 (pre-dose), 0.5, 1, 2, 3, 5, 7, 9, 13, 49hr after the start of infusion. Plasma drug concentrations were measured by the high-performance liquid chromatography/mass spectrometry. The area under the concentration-time profile from time 0 to the last measurable concentration (AUClast), the maximum concentration (Cmax), the time of Cmax (Tmax) and half-life (T1/2) were analyzed by noncompartmental methods. Results: A total of 19 patients were enrolled. Tolerability was tested with the following dose escalation scheme: 1mg/m^2 (n=3), 2mg/m^2 (n=3), 5mg/m^2 (n=4), 10mg/m^2 (n=7), and 15mg/m^2 (n=2). For CKD-732 sampled on day 1, AUClast (ng·hr/mL) and Cmax (ng/mL) per unit dose (1mg) were 3.80±2.04 and 2.97±1.69 2 (mean±SD), and Tmax (hr) and T1/2 (hr) were 0.97±0.13 and 1.70±0.92, respectively. The metabolite (M11) and the samples of day 11 and 43 are in process of analysis. Conclusion: CKD-732 was well tolerable in Koreans up to 15mg/m^2 and showed pharmacokinetic characteristics comparable with those of previous fumagillin studies. Further studies are needed for better understanding of characteristics of CKD-732.

365

Factors Associated with Compliance to the Treatment of Osteoporosis

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Introduction: Previous research suggested that adherence to osteoporosis therapies is low. The objective of this study was to explore the relationship between compliance and the convenience of the schedule, quantity of medication, use of a reminder system, difficulty in paying for the medication, side effects, patients' outcome expectations, and quality of life. Methods: This was a cross-sectional study that used self-reported data from both patient groups participating in a randomized controlled trial, the Falls, Fracture and Osteoporosis Risk Control and Evaluation Study. Compliance to treatment and patients' outcome expectations were measured by the Medication Compliance. To determine which variables were associated with compliance, logistic regression analyses were conducted Results: A total of 201 patients were included in the analysis. The odds of being compliant for those who find their medication schedule as "very convenient" was 3.1 (95% CI:1.3-7.4) that of those who found it "somewhat" or "not at all" convenient. The odds of being compliant for those who strongly agreed or agreed with the outcome expectation that "osteoporosis medications will decrease one's risk of the disease" was 13.2 times (95% CI: 1.3-139.4) that of those who strongly disagreed or disagreed with the statement. Conclusion: We identified two factors that were associated with compliance: i) convenience of the patient's medication schedule and, ii) the outcome expectation that "osteoporosis medication will decrease one's risk of getting osteoporosis or having it progress". Improving the convenience of taking osteoporosis medication and providing further patient education regarding the benefits of osteoporosis medication may increase compliance.

366

Pathogenic Sensitivity Profile of Neonatal Sepsis Cultures at Fudan University Children's Hospital: A 6-year Retrospective Analysis

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Introduction: Overuse of antibiotic contributes to increasing antimicrobial resistance. Early identification of the antibiotic sensitivity profile of pathogens is important in order to initiate effective control over antibiotic choice in suspected neonatal sepsis. Methods: A retrospective analytical study evaluated the sensitivity profile of pathogens cultured in neonatal sepsis cases at Fudan University Children's Hospital in Shanghai, China. All admitted patients designated as neonatal sepsis (December 2001 - March 2007) were included and divided into early (EOS) and late-onset sepsis (LOS) (0-7 days and >7 days post birth, respectively). A total of 118 cases with positive blood cultures were identified and antibiotic sensitivities were established. Results: *Coagulase-negative staphylococcus* was the most common pathogen cultured for both EOS (62%) and LOS (64%), and *Escherichia coli* was the second most common for both EOS and LOS (8%). The sensitivity rates of *CoNS* were: vancomycin (99%), gentamicin (72%), cefazolin (71%), amoxicillin/clavulanate (70%), and cefaclor (66%). The sensitivity rates of *E. coli* were: amoxicillin/clavulanate (100%), imipenem/cilastatin (100%), cefaclor (76%), cefotaxime (67%), and piperacillin (54%). Conclusions: In this sample positive blood cultures were found in 39.6% of patients with clinical sepsis. Both *CoNS* and *E.* coli pathogens demonstrated sensitivity to a number of antimicrobials. Further study of common pathogens and their sensitivity profiles will support implementation of more effective empirical antibiotic choice in neonatal sepsis at Fudan University's Children's Hospital.

367

Pharmionics in Uganda: Methodologic Innovation for Study of HIV Positive Adolescents

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Introduction: Excellent adherence to antiretroviral therapy is essential in preventing adherence-related viral mutations, especially among antiretroviral naïve individuals. The adolescent population is deemed high risk for non-adherence, but few data are available for HIV positive individuals. As antiretroviral coverage increases throughout Africa, adolescents become a high risk for adherence-related mutations. Furthermore, traditional methods for assessing adherence are relatively weak and difficult to interpret. A novel method to assess adherence in the developing world is necessary to study adherence-related outcomes and implement adherence improving interventions. Methods: A longitudinal cohort of HIV positive patients will be randomly selected from a single clinic in Kampala, Uganda. Adherence will be monitored for 12 months using eCAPs, a novel method of adherence measurement using microchip technology embedded in medication vials, recording date and time of opening. Primary outcomes will be proportion of patients in predefined adherence strata. Secondary outcomes will include progression to alternative ARV therapy. Social and demographic risk factors associated with poor adherence will be assessed. Results: Funding for this study has been finalized and ethical approval of the protocol has been granted both in Canada and Uganda. Enrollment will begin in March 2008 and continue until March 2009. Discussion: This will be the first study to our knowledge using eCAPs technology to assess adherence in an HIV positive adolescent cohort in a developing country. Results of this study will be used to design further interventions aimed at improving adherence for those at highest risk of non-adherence. Conclusions: Technological advances are enabling researchers to better answer critical questions related to medication adherence.

368

Maternal use of Codeine during Breastfeeding and Neonatal Opioid Toxicity

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Background: A large number of women receive codeine for obstetric pain while breastfeeding. Following fatal opioid poisoning in a neonate whose codeine prescribed mother was a cytochrome P450 2D6 (CYP2D6) ultrarapid metabolizer (UM), we examined the prevalence of opioid toxicity and characteristics contributing to central nervous system (CNS) depression in breastfed infants and mothers exposed to codeine. Methods: All cases counseled by the Motherisk Program (Toronto, Canada) regarding codeine use during breastfeeding between 2004 and 2007 were followed up. Clinical characteristics and maternal genotyping for CYP2D6 and UDP-glucuronosyltransferase 2B7 (UGT2B7) were collected. Results: Mothers whose infants exhibited CNS depression (n= 17) consumed a mean 59% higher doses of codeine than those without CNS depression (n = 55). [1.62 $\pm$ 0.79 mg/kg/d vs. 1.02 $\pm$ 0.54 mg/kg/d (p= 0.004.)] The lowest maternal codeine dose associated with neonatal toxicity was 0.63 mg/kg/d. There was 71% concordance between maternal and neonatal CNS depression. Two mothers whose infants exhibited severe neonatal toxicity were CYP2D6 Ums in combination with UGT2B7*2/*2 genotype. Conclusions: Codeine is not safe during breastfeeding for all suckling infants; one fifth of babies exhibited CNS depression temporally related to codeine exposure during breastfeeding. Breastfeeding mothers who are CYP2D6 Ums should avoid codeine. Maternal dose of codeine should not exceed 0.63 mg/kg/d for more than a few days. In any case of neonatal CNS depression, codeine exposure should be discontinued and the infant examined by a physician.

369

Identification of Risk Factors Predicting Problem Drinking in Pregnancy: The Motherisk Experience

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Introduction: Approximately 20% of women drink some alcohol during pregnancy, making effective screening of all pregnant women an essential part of prenatal care to improve identification and reduce risks. "Risk" or problem drinking is commonly used to describe non-dependent drinking that can still result in adverse consequences for the drinker. This is the most prevalent pattern of alcohol use in pregnancy, but also the most difficult to identify as most women typically lead high functioning lives. The study objective was to determine risk factors most predictive of prenatal alcohol use, and thereby allow for easier identification of at-risk women. Methods: Women calling the *Alcohol Helpline* for information regarding their alcohol use in pregnancy were included. Concern surrounding reporting bias in this Motherisk cohort is unlikely as study participants called and provided exposure details of their own volition to the service. Problem drinking was defined by TWEAK score of more than 2. Three groups in the study consisted of non-alcohol drinkers (100), non-problematic drinkers (106) and problem drinkers (105). Univariate and multivariate analysis were done to determine independent variables predictive of problem drinking. This was a prospective, observational, comparative study. Results: Women at risk are often non-compliant with psychiatric therapy ($p<0.001$), typically binge drink ($p=0.002$), tend to recognize pregnancy late ($p=0.034$), are not highly educated ($p=0.011$) and may continue drinking upon recognition ($p<0.001$). Conclusion: It appears that problem drinkers differ from non-problem drinkers in several risk factors mentioned, however, only some are effective in potentially identifying at-risk women.

WEDNESDAY JULY 30, 2008
STREAM 1:
NEW THERAPEUTIC APPORACHES

370

Glycyl-L-Glutamine Inhibits the Rise in Extracellular Dopamine and DOPAC Concentrations Evoked by Acute Morphine Administration in the Nucleus Accumbens

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Glycyl-L-glutamine (Gly-Gln) is an endogenous dipeptide that is synthesized from beta-endorphin post-translationally. Previously, we showed that Gly-Gln prevents acquisition of morphine conditioned place preference, a behavioral test of morphine reward. Gly-Gln also inhibited morphine tolerance, dependence and withdrawal but it did not interfere with morphine analgesia. In this study, we tested the hypothesis that Gly-Gln attenuates the rewarding effects of morphine by inhibiting morphine-induced dopamine release in the nucleus accumbens. Extracellular dopamine and DOPAC concentrations were sampled by microdialysis and analyzed by HPLC with electrochemical detection. Guide cannulas were implanted in the nucleus accumbens (NAc) and left lateral ventricle of male Sprague-Dawley rats stereotaxically. Approximately 24 h later a microdialysis probe was inserted into the NAc and perfused at 2 ul/min. Gly-Gln (1, 3, 30 or 100 nmol/5µl) or saline was administered i.c.v., morphine (2.5 mg/kg i.p.) was injected 2 min later and extracellular dopamine and DOPAC concentrations were sampled at 20 min intervals. Morphine administration increased extracellular dopamine concentrations by approximately 600% within 40 min. Gly-Gln pretreatment inhibited the rise in extracellular dopamine in a dose-related manner. The lowest significantly inhibitory dose was 1 nmol Gly-Gln; the response was blocked completely by 100 nmol. The morphine-induced rise in extracellular DOPAC concentrations was similarly inhibited. Gly-Gln did not affect dopamine or DOPAC concentrations in control animals. These data support the hypothesis that Gly-Gln abolishes the rewarding effect of morphine by inhibiting the ability of morphine to stimulate dopamine release in the NAc.

371

YM-244769, A Novel NCX3-Selective Inhibitor, Efficiently Protects against Hypoxia/Reoxygenation-induced Neuronal Cell Damage

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We investigated the pharmacological properties and interaction domains of *N*-(3-aminobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy} nicotinamide (YM-244769), a novel potent Na^+/Ca^{2+} exchange (NCX) inhibitor, using various NCX-transfectants and neuronal and renal cell lines. YM-244769 preferentially inhibited intracellular Na^+-dependent $^{45}Ca^{2+}$ uptake via NCX3 (IC_{50} = 18 nM); the inhibition was 3.8- to 5.3-fold greater than for the uptake via NCX1 or NCX2, but it did not significantly affect extracellular Na^+-dependent $^{45}Ca^{2+}$ efflux via NCX isoforms. We searched for interaction domains with YM-244769 by NCX1/NCX3-chimeric analysis and determined that the α-2 region in NCX1 is mostly responsible for the differential drug response between NCX1 and NCX3. Further cysteine scanning mutagenesis in the α-2 region identified that the mutation at Gly833 markedly reduced sensitivity to YM-244769. Mutant exchangers that display either undetectable or accelerated Na^+-dependent inactivation, had markedly reduced sensitivity or hypersensitivity to YM-244769, respectively. YM-244769, like KB-R7943, protected against hypoxia/reoxygenation-induced cell damage in neuronal SH-SY5Y cells, which express NCX1 and NCX3, more efficiently than that in renal LLC-PK_1 cells, which exclusively express NCX1, whereas SN-6 suppressed renal cell damage to a greater degree than neuronal cell damage. These protective potencies consistently correlated well with their inhibitory efficacies for the Ca^{2+} uptake via NCX isoforms existing in the corresponding cell lines. Antisense knockdown of NCX1 and NCX3 in SH-SY5Y cells confirmed that NCX3 contributes to the neuronal cell damage more than NCX1. Thus, YM-244769 is not only experimentally useful as a NCX inhibitor

that preferentially inhibits NCX3, but also has therapeutic potential as a new neuroprotective drug.

372

Validation of an Analytical Methodology for the Quantification by High Performance Liquid Chromatography of Quercetin in a Vegetal Matrix

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Quercetin, a widely distributed flavonoid, has been reported to have positive effects in combating or helping to prevent cancer. However, there is little information available as to how quercetin may affect human cancer cells. Development of the analytical techniques that allow determinate the amount of quercetin present in different plants foods is an essential first step in the process of establish its contribution in the cancer treatment. Quercetin exposure in human populations results from the dietary intake of various plant foods. High concentrations of quercetin have been found in tea, onions, apples, and red wine. We report the development of a high-performance liquid chromatography (HPLC)-based assay for quantification of quercetin in white and red onions (*Allium cepa*).This was a satisfactory process and let us to establish that 1906 mg of quercetin were enclosed per Kg of white dry onion and 2701 mg of quercetin in red onion per Kg.

373

Screening of Some Synthetic Ninhydrin Complexes for Antiallergic Activity

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Allergic disorders are greatest challenge for the new drug discovery. It was evident that allergic disorders are mediated through free radicals and involvement of COX/LOX pathways. In this effort some new synthetic ninhydrin adducts with proven analgesic, anti-inflammatory and antioxidant action were screened for antiallergic activity by in-vivo and ex-vivo mast cell degranulation models in Swiss albino mice and Wistar rats. Prior approval was obtained from the institutional animal ethics committee. Study was carried out by sheep serum induced active and passive anaphylaxis, compound 48/80 induced mast cell degranulation and paw edema models. One of the compound naphthol ninhydrin complex (NNC) showed dose dependent reduction in mesenteric mast-cell degranulation in active, passive and ex-vivo models in rats. Oral doses of ketotifen were significantly more potent than NNC but efficacy was comparable. However, in ex-vivo mast cell degranulation model, NNC was more potent than ketotifen, perhaps on account of the greater direct contact with the cells. NNC also showed dose dependent inhibition of compound 48/80 induced mast cell degranulation and paw edema. In one of the experiment using intact human erythrocyte it was also showed that NNC readily crosses the cell membrane. Several general pharmacology screening tests revealed no untoward actions except marginal CNS depression at doses above what was needed to prevent mast cell degranulation. Ease of synthesis, low systemic toxicity, absence of peripheral adverse effects, high cellular penetration etc makes the compound, NNC a promising lead candidate for further study.

374

A Putative Intervention on Amadori-induced Apoptosis in Human Peritoneal Mesothelial Cells

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Introduction: Diabetic patients undergoing continuous ambulatory peritoneal dialysis (CAPD), have more complications than non-diabetic ones. In the last years, hyperglycaemia has emerged as a culprit for the poor outcome of CAPD, as long-term hyperglycaemia has been related to impaired functionality of peritoneal

membrane (PM). Loss of peritoneal mesothelial cells (HPMCs) seems to be a hallmark in complications associated to this therapy. Indeed, apoptosis has been previously reported as consequence of hyperglycaemia in HPMCs. We recently showed that Amadori adducts induce apoptosis in HPMCs, through changes in several pro-apoptotic molecules that, in turn, regulate mitochondrial cell-death signaling pathway. Aims: This study evaluates whether bio-flavonoides and polyphenolic compounds, common constituents in most foods and beverages of plant origin, as well as different antidiabetic agents, such as PPARgamma agonists, clinically used to treat patients with type-2 diabetes, may to prevent Amadori-induced apoptosis in HPMCs.
Methods: HPMC were isolated from omental tissue from 20 different donors free of any cardiovascular or peritoneal disease and non-taking anti-inflammatory drugs or antioxidants, and different cellular and molecular biology experimental approaches were used. Results: Our data showed that, different diet flavonoids and polyphenols significantly prevented; HHb-induced iNOS promoter activity, HHb-induced p53 protein levels or caspase-9 and caspase3/7 activities in HPMCs. On the other hand, different PPARgamma agonists block HHb-induced caspase-3/7 and caspase-9 activities depending upon the agonist structure. Conclussion: These results raise the hypothesis that, these compounds may be turn as a relevant tool to prevent Amadori-induced effects on mesothelial cell-death.

375

Efficacy of Fenugreek Galactomannan Formulations in the Obesity Management

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Objective: This study investigated the effect of Fenugreek galactomannan formulations on weight loss and the composition of biochemical parameters related to weight loss Methods: We conducted a seven week, double blind placebo trial where 25 obese subjects treated conventionally by a low fat diet were randomly assigned to take either fenugreek formulations in capsules or placebo. To achieve moderate caloric restriction (1800-2000Kcal/Day), an individual dietary counselling was also done. The drug and the placebo capsules were indistinguishable both for the patient and the physician. There were five types of capsules named Fenugreek (F), Fenugreek +phaseolamine (F+P), Fenugreek + Commiphora mukul + ayurverdic fruit mix (F+G+A), Commiphora mukul+ ayurverdic fruit mix (G+A) and placebo. Results: For active drugs groups, weight loss was maintained through out the end of the experimental period and the mean decrease in body weight comprised between 4.83±1.07% and 5.87±0.71% was significantly different from that of placebo group 2.2% ±1.05% ((P<0.05). An improvement in blood pressure was observed with fenugreek formulations in capsules. The primary blood pressure end point (systolic blood pressure) decreased significantly with G+A (12.42%; P<0.02), Fenugreek (13% (P<0.05) and the diastolic blood pressure with F (8.53 ±4.2mmHg; P<0.05), F+P (13.15±6.16mmHg; P<0.05), F+G+A (5.1±2.13mmHg; P<0.05). There were significant changes in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides when the active formulations in capsules were compared with the placebo. Conclusion: Fenugreek formulations are safe and effective in weight reduction. They may help .retard, prevent or treat obesity.

376

Atypical Patterns of Opiates in Urine Samples from Heroin Addicts

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Heroin is hydrolyzed to 6-monoacetylmorphine (6-AM) and 6-AM is further to deacetylated by carboxylesterase to morphine. The morphine is mainly conjugated to morphine-3-glucuronide and morphine-6-glucuronide. Acetylcodeine (AC) is an impurity of illicit heroin synthesis and is metabolized to codeine, codeine-6-glucuronid and norcodeine. Urine samples taken after heroin intake are expected to contain 6-AM, morphine, codeine and conjugates thereof. The aim of this investigation was to compare results from our newly developed opiate LC-MS/MS method with data from GS-MS. The LC-MS/MS method using direct injection after dilution of urine with internal standards, reversed-phase chromatography and

selected reaction monitoring and direct measurement of 6-AM.The GC-MS method involved acid hydrolysis, solid-phase extraction, and analysis by selected ion monitoring. Seventy (26%) samples of 273 urine samples were positive for opiates by LC-MS/MS and of these samples 17 (24%) samples had no content of codeine and 5 (7%) samples also had no detectably content of morphine. These 17 samples were analyzed with GC-MS and 16 samples showed morphine, 5 samples codeine and one sample was confirmed negative. The reason for this atypical content with no morphine in samples positive for 6-AM (LC-MS/MS) and unexpected discrepancy between no content of codeine (LC-MS/MS) and a relatively high concentration of codeine found by using GC-MS could suggest a yet not discovered interaction and/or metabolic defect. Further investigation of these atypical patterns is important for a deeper understanding of factors involved in the metabolism of opiates. Our data also demonstrate the importance of measure 6-AM when doing opiate confirmation.

377

Cardioprotection by Stimulation of Glycogen Synthesis at Reperfusion

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The relative rates of myocardial glycolysis and glucose oxidation determine proton (H+) production from glucose metabolism and thereby alter intracellular acidosis and the potential for Na+ and Ca2+ overload. This study tested the hypothesis that stimulation of glycogen synthesis by inhibition of glycogen synthase kinase-3 (GSK) with the selective inhibitor, SB-216763 [SB], will partially repartition glucose away from glycolysis, reduce H+ production and improve post-ischemic recovery. Isolated working rat hearts were perfused with Krebs solution containing 100 microU/ml insulin, 1.2 mM palmitate and 11 mM [3H/14C] glucose. SB (3 micromol/L), given either before global ischemia or at reperfusion, significantly improved the recovery of left-ventricular (LV) function compared with vehicle-treated hearts (63.6±7.4%, n=6, P<0.01 and 66.9±7.3%, n=6, P<0.01 vs. 29.8±7.3%, n=10, respectively). During reperfusion, glycogen synthesis increased (by 118%, P<0.01). Glycolysis and H+ production decreased by 62% (P<0.05) and 70% (P<0.05), respectively. In aerobically-perfused glycogen-replete hearts (n=7), SB (n=7) had no significant effects on either LV work or glucose metabolism. When aerobic hearts (n=7) were depleted of glycogen by substrate-free perfusion to a level similar to that measured at the onset of reperfusion, SB (n=7) accelerated the rate of glycogen synthesis (by 40%, P<0.05) and reduced glycolysis (by 31%, P<0.01) and H+ production (by 38%, P<0.01), independent of changes in LV function. Thus, SB-induced stimulation of glycogen synthesis and inhibition of glycolysis during reperfusion are not a consequence of improved LV function. Rather, our study indicates that stimulation of glycogen synthesis during reperfusion by GSK inhibitors contributes to their cardioprotective mechanism.

378

Therapeutic Effects of Ellagic Acid against Toxcicity of AFB_1 in Chicken Hepatocytes

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Aflatoxin B_1 (AFB_1) is the most powerful liver toxin. The aim of this study was to investigate the possible protective role of ellagic acid (AE), a natural phenolic compound, on AFB_1-induced toxicity using biochemical approaches in chicken liver cell suspensions (CLCS). Ross & Ross broiler male 7-d old chickens were randomly divided into two groups of 10 each. The control group received a basal diet (BD); group EA received BD plus EA (4.0 g/kg feed) during seven days. The effects of EA on AFB_1 toxicity were evaluated using CLSC (10^6/mL) exposed to different concentrations of AFB_1 (0, 5, 10, 20 μg/mL) for 30 min. Celular integrity, enzymatic activity of glutathione S–transferases (GST) and alanine aminotransferase (ALT), as well as reduced glutathione (GSH) and total proteins concentrations were evaluated. Addition of AE to diet significantly increased (P<0.01) the detoxification route in treated CLCS. Based on the control, the GST activity was three times larger;

GSH availability increased four times with 10 and 20 μg AFB_1 /mL; ALT activity decreased, suggesting a reduction of damage by AFB_1 to exposed CLCS. Our results suggest that consuming AE has a protective effect in CLCS against the negative effects and biochemical alterations induced by AFB_1. Furthermore, the addition of EA had no effect on body weight, feed intake, convertion index, and hepatocyte chemistry and integrity.

379

Biochemical Evaluation as Indicator of Dermal Reconstitution in Wound Healing of Skin Treatment with Hyaluronan

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Introduction: To assess the dermal reconstitution in open wounds treated with Hyaluronan jelly (HA) were determined biochemical indicators such as alpha amino and total amino acid content in dermis samples. Methods: Treatment: jelly with HA at 2,4,8%, placebo and control during 5th and 7th days respectively to treat the wound, after were collected similar area of dermis of each treatment and acid hydrolysis were made with the objective of quantified the alpha amino acid groups from nitrogen content and total amino acid with an Analyzer. Results: The values of alpha amino nitrogen were most high for the jelly to 4% to 7th day with 4, 32 mg.area^{1}, followed by concentration to 8 %. Glycine was the most abundant at 7th day with 310 and 402 μg.area^{-1} in the jelly to 4 and 8 % with a hydroxyproline of 10, 3 and 13, 3 μg.area^{-1} this is a direct relationship between the behaviour of the two treatments and two assays it coincides with histology matrix results in collagen fibres of the jelly to 4 % obtained a 100 % response were obtained. Discussion: The alpha amino nitrogen and glycine quantitative values indicate collagen synthesis and dermal reconstitution, the quantitative hydroxyproline value shows adequate response, because this amino acid is a marker biochemical for this. Conclusion: Correlation between the biochemical and histological results in the dermis of jelly 4 % of HA showed higher results of dermal reconstitution than other treatments.

380

An Anti-oxidant Capacity of Chinese Herbal Medicine "Wu Ling San" in Experimental Granulomatous Nephritis Model

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Oxidative stress mediates a wide range of renal impairments, from acute renal failure, obstructive nephropathy, diabetic nephropathy and glomerular damage to chronic failure and hemodialysis. Therefore, interventions favoring the scavenging of reactive oxygen species (ROS) should attenuate or prevent the oxidative stress, thereby militating against the subsequent renal damage. Traditional herbal medicine, Wu Ling San (WLS) is widely used in Asia to coordinate water balance in patients suffering from nephritis and nephritic syndrome. However, the mode of WLS in the regulation of renal oxidative stress still remains unknown. We used adenine-fed rat models of human chronic renal failure, in which the deposition of crystals of the abnormal adenine metabolite 2,8-dihydroxyadenine in the kidney induced granulomatous nephritis. In the present study, we found that taking WLS in powdered diet (350mg/kg/day, oral intake;14 days) significantly reduced ROS level in the serum of the nephritis rats. WLS attenuates the increase of monocyte chemoattractant protein-1 (MCP-1), which mediate initiation and persistent of granulomatous inflammation, in both serum and urine. Furthermore, a real-time PCR demonstrated that WLS ameliorated MCP-1 messenger upregulation in these renal tissues. Our findings suggest that WLS might protect renal impairment via ROS by its antioxidant activity, and buffer inflammatory reaction by reducing nephritis inflammatory cytokines, MCP-1. This conservative treatment with traditional herbal medicine that used for renal disease experientially; WLS may be a potential alternative novel treatment for kidney

inflammation as well as familial nephritis especially for the Alport syndrome.

381

A Prospective Double Blind Placebo Controlled Study to Determine whether Absorbatox is Associated with Lower Incidences of Gastric Events in Volunteers using NSAIDS

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Introduction: Clinical upper gastro-intestinal events are present in 2 to 4 percent of non-steroidal anti-inflammatory (NSAID) users. Naproxen is known to cause gastric mucosal breaks in 80 percent of patients. The aim of this study was to investigate the possible benefits of Absorbatox, a physically enhanced alumino-silicate, in the prevention of NSAID induced gastric events. Method: In this 14 day double blind placebo controlled pilot study, 24 healthy volunteers were enrolled. Volunteers were randomized to receive orally either 1500 mg Absorbatox or placebo three times daily, plus 500 mg naproxen twice daily. All volunteers underwent gastroscopic evaluation of their stomach linings prior to and on day 14 of the study to evaluate gastric events and the status of the gastric mucosa. Volunteers also filled in a daily symptom diary. Results: Absorbatox resulted in a significant reduction in clinical symptoms (i.e. 36 percent discomfort reduction ($p<0.001$); and 41 percent heartburn reduction ($p<0.001$)) as well as significant reduction in gastric events (50 percent reduction in gastritis ($p<0.03$); and 10 percent mucosal erosion reduction). Discussion and conclusion: Absorbatox is a non-absorbable substance with potential benefit in protecting against NSAID induced gastric events. The exact mechanism of action is not clear but may be due to its binding to hydrogen ions and biologically active amines and nitrates.

382

The Effects of Flunixin Meglumine and L-NAME on the Carrageenan-induced Hyperalgesia in Rats

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Introduction: Flunixin meglumine (FM) is a potent non-steroidal anti-inflammatory drug used in veterinary clinical practice. This study aimed to determine the effect of FM, L-NAME and their combination on carrageenan-induced hyperalgesia in rats. Methods: Hyperalgesia was induced by intraplantar administration of carrageenan (0.5%) into the rat hind paw. Electronic von Frey apparatus was used to determine paw withdrawal threshold (g). FM and N^G-nitro-L-arginine methyl ester (L-NAME) were administered by subcutaneous route (s.c.) 2.5 h after i.pl. injection of carrageenan. L-arginine (s.c.) was administered 15 min later. Results: FM (0.095-0.12 mg/kg) produced a significant dose-dependent anti-nociceptive effect on the carrageenan-induced hyperalgesia. Also, L-NAME (2.5 and 5 mg/kg) produced anti-nociceptive effect, while higher dose of L-NAME (7.5 mg/kg) did not produce any significant effect. However, when FM (0.095 mg/kg) and subeffective dose of L-NAME (7.5 mg/kg) were co-administered after induction of inflammation, the anti-nociceptive effect was significantly increased in comparison with the effect of FM alone. L-arginine (10 mg/kg) significantly inhibited the anti-nociceptive effects of both FM and FM and L-NAME combination. L-arginine (10 mg/kg) itself did not produce significant effect on carrageenan-induced hyperalgesia. Conclusions: These results indicate that in carrageenan-induced hyperalgesia in rats subeffective dose of L-NAME significantly increased the anti-hyperalgesic effect of FM. Subeffective dose of L-arginine inhibited the anti-hyperalgesic effects of both FM and FM and L-NAME combination. The inhibition of production of nitric oxyde might be involved in the mechanism of the anti-hyperalgesic effect of FM in a model of carrageenan-induced hyperalgesia in rats.

383

Advanced Therapeutic Approach of the *Plantago Sp.* Extracts – *Microbial Activity*

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The aim of the paper is to present the results of the experimental research concerning the effect on some pathogenic bacteria of three main solid extracts E1, E2, E3, isolated from *Plantago sp*. from alcoholic and aqueous media. In the first step, the dish diffusive Kirby Bauer method was applied for "in vitro'' testing of the biological effects of the three extracts by using the following pathogenic microorganisms: *Staphiloccocus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacilus sp*. Each biological test was made by more repetitions and the Student method was applied for results validation. The biological tests results showed that the E1 had no biological effect, E2 had a moderate bactericidal effect and the E3 presented a significant bactericidal effect. In the second step the solid extracts E1, E2, E3 were characterised by physico-chemical analyses. For this purpose, qualitative and quantitative analyses were performed by the energy dispersive X-ray fluorescence and by atomic emission with inductively coupled plasma respectively. The obtained results indicated the presence of C, H, P, and some microelements in all the solid extracts. The infrared spectrum analysis performed for all the extracts indicated the presence of polysaccharides chains in E1, the existence of flavonoides in E2 and the presence of irridoid compounds in E3. In conclusion, the biological and physico-chemical tests indicate a significant bactericidal effect of the extract E3 with irridoid compounds content.

384

Single-agent Therapy for Low Risk Gestational Trophoblastic Neoplasia (LRGTN): A Preliminary Report on a Randomized Clinical Trial to Compare Pulse-methotrexate versus Pulse-dactinomycin

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The efficacy of single-agent chemotherapy for patients with low risk gestational trophoblastic neoplasia (LRGTN) with methotrexate or dactinomycin is well established, but efforts continue to reduce the toxicity, the patients time and cost of treatment. In a randomized clinical trial, we evaluated and compared the efficacy, toxicity and cost effectiveness of pulse-methotrexate versus pulse-dactinomycin as single-agent therapy in LRGTN. Forty low-risk GTN patients were randomly assigned to receive pulse-methotrexate (30 mg/m2 weekly intramuscularly) or pulse-dactinomycin (1.25 mg/m2 every two weeks intramuscularly). Treatment continued if no major toxicity was encountered and beta human chorionic gonadotropin (beta-hCG) values were lower than 5mIU/m2 in three consecutive weeks. Seventy percent of methotrexate group and 90% of dactinomycin group responded to treatment (100% remission was achieved with no recurrence in one-year follow-up). The mean time to response was 43 days for methotrexate and 66 days for dactinomycin group. Patients achieved remission after receiving an average of 8 courses of therapy in methotrexate versus 6 courses in dactinomycin group. The average cost of treatment per course was about 7 US$ for methotrexate and 62 US$ for dactinomycin group. There were no cases of major toxicity in methotrexate or dactinomycin groups. Based on our results, LRGTN treatment with dactinomycin is somewhat more effective than that with methotrexate, but methotrexate is more cost-effective for both patients and the health system. As the effectiveness of both pulse-methotrexate and pulse-dactinomycin does not differ significantly, pulse-dactinomycin is recommended as first-line treatment.

385

Comparative Hypoglycemic Efficacy of Different forms of Stevia Rebaudiana Bartoni Leaves in Rats with Streptozotocin Induced Diabetes Mellitus: Therapeutic Study

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The leaf of Stevia is used as a folk medicine for the treatment of diabetes. In the present study, the comparative efficacy of powder, juice and aqueous extract of leaves of Stevia has been evaluated for its hypoglycemic action in

streptozotocin (55 mg/kg, as single i. p. injection) induced diabetic rats using aqueous solution of glimepiride (15 µg/kg/day p.o.) as a standard hypoglycemic agent. The rats were divided into six groups, two was control (normal and diabetic) and others were treated. The powder, juice and aqueous extract of leaves of Stevia was administered orally in induced diabetic rats at doses of 150 mg, 750 mg and 2.0 ml/ kg/day, respectively, for 21 days. Blood samples were collected from the tail vein before (0^{th} day) and also at 7^{th}, 14^{th} and 21^{st} day after drug administration and blood glucose was analyzed by using Accu-Check Advantage blood glucose system (strip method). The data was compared statistically by using Student's *t*-test. The forms of leaves of Stevia at 21^{st} day produced 2.85, 17.40 and 20.31 % reduction in blood glucose of diabetic rats, respectively and that of was 36.72 % with the glimepiride. The active compound(s) of Stevia is probably mediated this action through enhance secretion of insulin from the beta-cells or through extrapancreatic mechanism and less absorption of powder form in intestine may be responsible for its less effectiveness. The present study indicated that the aqueous extract and juice of leaves of Stevia possess potent hypoglycemic activity although less potential than glimepiride.

386

A Novel Series of Triple Re-uptake Inhibitor Potential Antidepressants

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Triple re-uptake inhibitors (TRIs), drugs that block transporters for serotonin (SERT), norepinephrine (NET), and dopamine (DAT) are in various stages of development as potential antidepressants. We report on a new series of 25 TRIs. Methods: Standard organic syntheses were used to make 25 compounds, all but 2 of which were pure enantiomers. *In vitro* tests included radioligand binding to human transporters for all compounds; and for a subset of compounds, inhibition of hERG and CYP2D6. A subset of compounds was tested in the forced swim test (FST) with rats and in the tail suspension test (TST) with mice. Results: A broad range of transporter affinities was obtained with the most potent compounds exhibiting sub-nanomolar K_d's at SERT or at NET, and ~10 nM at DAT. Rank-order of potencies for different compounds of the series included: NET>SERT>DAT; DAT>SERT>NET; SERT>DAT>NET; and SERT>NET>DAT. Compounds tested at hERG were negative, while some tested compounds were relatively potent at inhibiting CYP2D6. Compounds that were tested showed antidepressant-like activity in FST, TST, or both. Discussion: Relatively small changes in structure and stereochemistry resulted in marked changes in potency in the various *in vitro* tests for these compounds. Conclusions: At least five of these new compounds are ready for preclinical toxicology testing for the first studies in humans.

387

Nitroxyl (HNO) is a Novel Antihypertrophic Mediator in Neonatal Cardiomyocytes: Role of cGMP-Dependent Antioxidant Actions

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Nitric oxide (NO)/cGMP signaling is an important antihypertrophic mechanism in the heart. HNO, an alternate redox form of NO insensitive to reactive oxygen species (ROS), may offer new promise for the treatment of cardiovascular disorders. The impact of HNO on either cardiac hypertrophy or its triggers (including ROS) has however not been sought. We tested the hypothesis that HNO prevents hypertrophy in cultured neonatal rat cardiomyocytes. The HNO donor Angeli's salt (sodium trioxodinitrate) prevents the hypertrophic response to angiotensin II (Ang II) and its induction of NADPH oxidase. Angeli's salt restored cell area, protein synthesis, expression of beta-myosin heavy, glycogen synthase kinase-3beta (a negative regulator of hypertrophy) and Nox2, as well as superoxide generation, from 186±10% in the presence of Ang II alone to 105±6%, from 146±7% to 106±6%, from 2.3±0.4-fold to 1.4±0.5-fold, from 63±13% to 132±72%, from 3.8±0.6-fold to 1.1±0.4-fold and from 3.1±0.7-fold to 1.1±0.2-fold, versus paired

controls, respectively (all $p<0.05$ on one-way RM ANOVA). These effects are mimicked by cGMP-elevating agents, B-type natriuretic peptide (BNP) and 8-Br-cGMP. Co-incubation with either KT5823 or ODQ attenuates both the antihypertrophic and antioxidant effects of Angeli's salt and BNP. Further, the HNO scavenger L-cysteine (but not carboxy-PTIO) block the antioxidant actions of Angeli's salt. Moreover, Angeli's salt does not generate NO. In conclusion, our results suggest HNO prevents cardiac hypertrophy, and cGMP-dependent suppression of cardiomyocyte NADPH oxidase contributes to these antihypertrophic actions. Hence, novel redox forms of NO that are ROS-insensitive yet exploit NO/cGMP-dependent signaling may represent innovative pharmacotherapy for cardiac hypertrophy.

388

Green Tea Polyphenols as Potential Treatment of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a frequent inherited muscular disorder. DMD patients show muscular weakness, that progresses towards paralysis and leads to death at age 20-30. DMD is caused by mutations in the gene encoding dystrophin, a cytoskeletal protein that normally contributes to the stabilization of the muscle fibre membrane during muscle activity.We investigated the ability of Green Tea Extract (GTE) and its major component, (-)-epigallocatechin gallate (EGCG), in improving mdx5Cv mouse muscle function and histology. Three-week old mdx5Cv mice were fed for either 1 or 5 weeks or 15 months on control chow or on chow containing GTE or EGCG. Normal C57Bl/6 mice were used as control. Muscle histology showed that GTE and EGCG reduced muscle necrosis after 1 week; fibrosis occurring late in the disease was strongly attenuated in 15-month old mice. Electrically-evoked properties of the triceps surae muscles were recorded. Phasic and andomi forces of treated mdx5Cv mice were increased up to 94%, close to values of normal mice. In addition, muscles from treated mdx5Cv mice exhibited a 30 to 50% increase in resistance in a fatigue assay. These results suggest that administration of GTE or EGCG to mdx5Cv mice caused a delay in muscle necrosis, and stimulated muscle adaptation towards a slower, more resistant phenotype. In vitro studies showed potent anti TNF-a and TGF-b activities. Investigations regarding the mechanisms of action are ongoing. In view of these encouraging results, we are looking for partners willing to perform clinical studies in DMD patients.

389

Novel 5'-(phenyl)-phosphonate Derivatives of Zidovudine (AZT): Synthesis, Cytostatic Activity and Preliminary Studies on Metabolism

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AZT, originally designed as an antitumor agent, is well known as highly potent antiviral drug, especially against HIV retroviruses. Recently, however, AZT has emerged as the anticancer drug e.g. in the treatment of advanced colon cancer and in breast cancer therapy. Phosphoramidate pronucleotides seems to be effective for the intracellular delivery of nucleoside 5'-monophosphates (MP) as regards to their *in vitro* cytostatic activity showed in human tumor cell models. A series of ten newly synthesized 5'-(phenyl)-phosphonates of 3'-azido-3'-deoxythymidine (AZT) (1FF-10FF), desired as the potentially pronucleotides, were studied against KB, MCF-7 and HeLa tumor cells, based on the DR and D, NCI, NIH Bethesda programs with modifications. Two most active compounds; 5'-(phenyl)-allyl-phosphonate of 3'azido-3'-deoxythymidine (3FF) with IC_{50} = 1,3 microg/cm^3 (0,00277 mol/L x 10^{-3}) against KB cells, and 5'-(phenyl)-dimethyl-phosphonate of 3'-azido-3'-deoxythymidine (7FF) with IC_{50} = 0,35 microg/cm^3 (0.00072 mol/L x 10^{-3}) against MCF-7 cells, were qualified for *in vitro* investigation using a mixture of insect cell-expressed human drug metabolizing cytochrome P450 isoforms (CYPs:1A2, 2C8, 2C9, 2C19, 2D6 and 3A4)

model, as to related to their activities in human liver microsomes (HLM). The results performed with cDNA-expressed cytochrome P450 isoforms showed considerable increase of cytostatic activity against KB and MCF7 tumor cells for compound 3FF with IC_{50} = 0.16 microg/cm^3 (0.0003 mol/L x 10^{-3}), in comparison to non-activated control samples. It is proposed that the influence of drug metabolizing CYP450 isoforms plays important role in chemical reduction of phenylphosphonates of AZT to reactive intermediate – 3'-amino-3'-deoxythymidine phosphonates which enhance in vitro cytostatic activity.

390

Camellia Sinensis Extract: A Potent Agent against Gingival Epithelial Cells Damage-induced by Polycyclic Aromatic HydroCarbons

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Background: Nicotine can induce reactive oxygen species (ROS), the most important substances implicated in gingival tissues inflammation. ROS is found in gingival tissues as well as plasma and saliva from periodontitis patients. Furthermore, gingival crevicular fluid (GCF) antioxidant capacity is significantly decreased in patients with periodontal disease. Green tea catechin mediates T cellular NF-kappa B inhibition and exerts oxidative-stress protection in periodontitis. Eventhough, previous researches considering both ROS and antioxidant capacity in periodontal disease are sparse, but the effect of green tea on the polycyclic aromatic hydrocarbons (PAHs), environmental toxicants found in cigarette smoke and the outcome of ROS are still unknown. Methods: The unique extract of *Camellia sinensis (CS)* was prepared by our laboratory protocol and total catechin content of the extracts were determined using high performance liquid chromatography. The effect of *CS* extract on gingival epithelial cells (GECs) viability was determined by the MTT assay. Intracellular H2O2 production was analyzed by flow cytometry. Results: We demonstrated approximately 80 percent of catechin content in our *CS* extract. We found that *CS extract* dose- and time- dependently attenuated PAHs-induced H2O2 production in viable GECs. Conclusion: Our findings suggest that PAHs–induced the production H2O2 in GECs could be inhibited by *CS* extracts. Discussion: *CS extract* may act as one of the free radical scavengers when GECs were chemically damaged. The reverse effects of *CS* extract on PAHs-induced superoxide production may imply for an effective and economical method of ROS reduction and contribute to promote periodontal health development.

391

Reversing Prediabetic Insulin Resistance by a Synergy of Drugs Mimicking a Feeding Signal

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Following a meal, the glucose-disposal response to a bolus of insulin is dramatically potentiated in all species tested. This Meal-induced Insulin Sensitization (MIS) occurs as a result of insulin causing the release of the putative hormone, Hepatic Insulin Sensitizing Substance (HISS), which acts selectively on skeletal muscle to stimulate glucose uptake. Insulin-stimulated HISS release occurs only in the fed state and requires two permissive feeding signals: hepatic parasympathetic nerve activation and elevation of hepatic glutathione (GSH). The absence of MIS leads to postprandial hyperglycaemia, hyperinsulinaemia, and shifting from nutrient storage as glycogen in skeletal muscle towards lipids. In Sprague-Dawley rats, a 9-week sucrose-supplemented diet resulted in the absence of HISS release and, consequently, absence of MIS. Mimicking the nerve signal with a muscarinic agonist, bethanechol, caused a small restoration (23%) of MIS whereas the GSH replenisher, N-acetyl-L-cysteine, was without effect. Co-administration of the two compounds resulted in a synergistic and complete restoration of MIS. When the drugs were given prior to administration of a liquid test meal (gastric injection, anaesthetized rats) the dynamic insulin response, determined using the Rapid Insulin Sensitivity Test, was increased by 53% in rats that had previously been made insulin resistant by supplementing their diet with 35%-sucrose-water. This therapeutic approach formed the basis for a

successful phase 2 human clinical trial, and is the first approach to focus on MIS and to demonstrate that reversal of the MIS-defect can be achieved by the pharmacological provision of both feeding signals in a prediabetic animal model.

392

A Novel Option for Treatment of Chronic Heart Failure: First Results of a Multicenter Randomized Clinical Study

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Patients with symptomatic chronic heart failure and reduced left ventricular ejection fraction (LVEF) have a high possibility of deterioration despite therapies with angiotensin-converting enzyme inhibitors, beta-blockers, and aldosterone antagonists. A randomized, open-label, 3-arm, multicenter, crossover, clinical trial has been designed to test the ability of a new antibody-based medication to improve the efficacy of "standard" therapy. By now 74 out of 90 patients (NYHA II-IV, LVEF ≤45%) were randomized to oral ultra-low doses of antibodies to angiotensin (AT1) receptor (cardos, n=25, 1 tablets x 3 times a day), valsartan (n=26, 80 mg a day) or their combination (n=23) as an add-on to "standard" therapy during the first 6-month treatment stage. The outcome measures were improvement in LVEF, exercise tolerance, Kansas City Cardiomyopathy Questionnaire (KCCQ). By month 3, LVEF increased by 11.7%, 6.5% in patients receiving cardos or valsartan; exercise tolerance assessed in 6-min walking test improved by 19.3%, 12.0%; KCCQ total score – by 9.7% and 7.3% respectively. By month 6, the preliminary results were inconsistent due to a small number of patients completed the stage (36 out of 90). The combination of cardos and valsartan did not significantly change the efficacy of the drugs taken separately. The adverse event rate was lower in cardos group. Thus, cardos was at least as efficacious as valsartan in the first 6-month stage. The combination of cardos and valsartan does not seem to be advantageous over monotherapy, however, the final results along with the second treatment stage data (after crossover) are to come.

393

Altered Heart Specific microRNA Expression in Rat Model of Heart Failure

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MicroRNAs (miRNAs) are endogenous small noncoding RNAs that negatively regulate the expression of genes in a variety of eukaryotic organisms. miRNAs have been reported to play important roles in diverse biological and pathological processes. But it's not known the roles of miRNAs in the pathological process of heart failure (HF). To investigate whether heart specific miRNAs were involved in HF, we assessed mir-1,mir-133,mir-206 and other related genes expression in the Sprague-Dawley rat model of heart failure generated by ligating the left anterior descending coronary artery. 4 weeks after myocardial infarction, heart failure was elicited in SD rat model characterized by left ventricular remodeling and impaired systolic and diastolic function. RT-PCR showed that the transcripts of rat mir-1, mir-133 and mir-206 were up-regulated in time-dependent manner at 1,2,4,6 weeks after myocardial infarction, which were consistent with the expression of mature mir-1, mir-133 and mir-206 detected by real-time PCR assay. However, the transcripts of DGCR8, exportin-5 and connexin43 were not changed significantly during the process of rat HF. Western-blotting assay showed that connexin43, the target of mir-1/mir-206, was decreased in time-dependent manner during rat HF. Together, the above results indicated that mir-1, mir-133 and mir-206 were complicated in the process of HF without the regulation by DGCR8 and exportin-5 which were related with the miRNAs maturation, and further study of mir-1, mir-133 and mir-206 in the development of HF may be of potential therapeutic implications.

394

KR-31378, A Novel mitoK$_{ATP}$ Channel Opener, Reduces Infarct Size and Arrhythmias in an Anesthetized Rat Model of Ischemia and Reperfusion-induced Heart Injury

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Mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channel is known to be an end-effector of ischemic preconditioning, the most powerful endogenous protective mechanism against lethal ischemia of heart. We determined the infarct-reducing and antiarrythmic effects of KR-31378, a highly cardioselective mitoK_{ATP} channel opener, in an anesthetized rat model of 30 min-ischemia/2 hr-reperfusion-induced heart injury together with its cellular mechanisms. KR-31378 (1 mg/kg, i.p.), administered for 1, 3 or 5 consecutive days, once a day, significantly reduced infarct size in left ventricle from animal model prepared 24 hr after the final dosing (IZ/AAR: 58, 37, 42 and 22% for control and groups treated for 1, 3 and 5 days, respectively). These effects were completely antagonized by a concomitant treatment with 5-hydroxydecanoate (5HD), L-NAME or wortmannin, a mitoK_{ATP} channel antagonist, a nitric oxide synthase inhibitor and a PI3-Kinase inhibitor, respectively. KR-31378 also significantly decreased the occurrence of various types of arrythmias including ventricular tachycardia (VT), ventricular fibrillation (VF) and ventricular premature beats (VPBs), the effects being reduced by these antagonists in a similar pattern. Both Akt and phosphorylated-Akt (p-Akt) were decreased in the area at risk of infarction (AAR) of left ventricle in line with the decrease in infarct size. The decrease in Akt and p-Akt in AAR was abolished by a concomitant treatment with 5HD and L-NAME, whereas it remained unchanged after wortmannin. These results indicate that KR-31378 exert significant cardioprotective effects, via mitoK_{ATP} channel, PI3-Kinase-Akt pathway and nitric oxide, either alone or in a signaling network, with potential for the efficient pharmacological preconditioning.

395

Results of a First-in-Man Proof of Concept Study of Anti-CD4mAb-Fragment (EP 1645) for Imaging Chronic Inflammation in Patients with Active Rheumatoid Arthritis

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Rheumatoid arthritis (RA) as a disease with extensive economic impact on the healthcare system requires early and effective drug treatment to preserve patients` quality of life. Therefore, an effective and reliable diagnostic tool is necessary to identify patients which can be treated with an advantageous risk-benefit ratio. As a diagnostic tool for this task a CD4mAb-Fragment (EP 1645) is under development. To demonstrate efficacy, safety and tolerability of i.v. injections of EP 1645, an open single-dose, adaptive design, phase I study is performed in otherwise healthy volunteers (50-80 years, 5 to 9 volunteers), who suffered from joint disease due to rheumatoid arthritis (min. 5 criteria according to ACR scheme). The results of this trial indicate that EP 1645 well confirms scintigraphically clinical disease. EP 1645 was safe and well tolerated. Aside the spots of obvious clinical disease, activity is located time-dependently in kidney, liver, spleen and bladder (elimination path). In one case, activity was displayed aside the sites of obvious clinical disease (hands) also in two joints of the feet. Further investigations have to prove if EP 1645 displays either sub-clinical sites of inflammation or inflammation prior to obvious clinical disease. Safety and tolerability of EP 1645 has to be confirmed in trials with larger numbers of patients during clinical development.

396

Urotensin-II Plays a Major Vasoconstrictor Role in Pulmonary Hypertension of the Newborn Secondary to Meconium Aspiration Syndrome

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Introduction: Meconium aspiration syndrome (MAS) disrupts normal perinatal decreases in pulmonary vascular resistance (PVR) and is the most common cause of pulmonary hypertension of the newborn (PHN). The pathophysiology of MAS-related PHN is incompletely understood, and in particular, the potential role of urotensin-II, the most potent vasoconstrictor yet identified, is unknown. The aim of this study was to determine the role of urotensin-II in a new perinatal model of PHN secondary to MAS, by combining measurement of circulating urotensin-II levels with urotensin-II receptor blockade studies. Methods: 19 fetal lambs were instrumented under general anaesthesia with aortic, pulmonary trunk and left atrial catheters and pulmonary trunk/left pulmonary artery transit-time flow probes, then randomly allocated to one of 4 treatment groups: *1)* control(n=5), *2)* control plus specific urotensin-II receptor blockade with palosuran (10 mg/kg/hr infusion; n=5), *3)* tracheal instillation of 20% human meconium (3 mL/kg; n=5), *4)* meconium instillation plus palosuran(n=4). Haemodynamics and plasma urotensin-II levels were measured for 6 hours after delivery during mechanical ventilation. Results: PVR decreased progressively after birth in control lambs (p=0.01) in association with an increase in urotensin-II levels (p=0.03), but was unchanged after urotensin-II receptor blockade. By contrast, PVR increased after birth in MAS lambs (p=0.02), in conjunction with an exaggerated rise in urotensin-II levels (p=0.007 vs control). However, the PVR increase was attenuated in MAS lambs after urotensin-II receptor blockade ($p<0.001$). Conclusions: These findings suggest that urotensin-II normally acts as a pulmonary vasodilator after birth, but assumes a major vasoconstrictor role in MAS-related PHN. Furthermore, urotensin-II receptor blockade improves pulmonary haemodynamics in this model of MAS.

397

Is TNF-alpha Still Important Target in Treatment of Heart Failure?

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Introduction: Inflammatory cytokines may play a pathogenic role in the development of congestive heart failure (CHF). Plasma levels of Tumour Necrosis Factor alpha (TNF-alpha) can be used as biochemical surrogates for titrating treatment of CHF patients and for determining appropriate therapeutic target levels for anti-TNF-alpha therapy. Methods: Circulating levels of TNF-alpha were measured using enzyme-linked immunosorbent assay kits (Quantikine, R&D Systems) in 25 patients with various degrees of heart failure (due to dilated cardiomyopathy or coronary artery disease) and 15 healthy controls. Results & Discussion: Patients with CHF had increased plasma concentrations of TNF-alpha, which correlated with functional class. CHF patients due to dilatated cardiomyopathy had higher values of TNF-alpha. As expected, patients with acute failure had higher values of this cytokine than chronic failure patients, but the question is – what is happening with permanently increased levels of TNF-alpha in CHF patients? Among patients with chronic heart failure, CHF patients due to dilated cardiomyopathy showed higher values of TNF which could make them a possible candidates for anti-cytokine therapy. Conclusion: The persistent immune activation in CHF patients appears to be unmodified by traditional cardiovascular treatment and anti-inflammatory strategies need to be focused on patients with chronic heart failure.

398

Effects of the Beta 3-Adrenergic Receptor Agonist CL-316243 on Bladder Micturition Reflex in Spontaneously Hypertensive Rats

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Beta-3 adrenoceptor (AR) activation was reported to increase bladder capacity in animal models of pathological bladder instability and hyperreflexia. The present study investigated whether beta3 AR activation produces urinary bladder relaxation in hypertensive SHR rats in comparison with its normotensive control (WKY) by examination of the bladder rhythmic contraction and whether beta3 AR activation acts on the bladder afferent pathway by examination of visceromotor reflex (VMR) and pressor responses to urinary bladder

distension (UBD). A cannula (PE 50) was placed into the bladder via the urethra in anesthetized female rats. Rhythmic bladder contraction was studied by monitoring the bladder pressure following saline infusion. VMR and pressor responses were studied by measuring abdominal, myoelectrical activity and carotid arterial blood pressure subsequent to repeat phasic UBD (60 mmHg, 20 seconds). The selective beta3 AR agonist CL-316243, dose-dependently inhibited the rhythmic contraction (ED50 0.1 mg/kg, i.v.) in SHR rats. However, using the same model in WKY rats, CL-316243 was ineffective at 1mg/kg. Interestingly, CL-316243 (3mg/kg, i.v.) failed to attenuate VMR and pressor responses to UBD in both SHR and WKY rats, indicating that activation of beta3 AR did not change bladder afferent path. In support, Taqman analysis showed the mRNAs of beta3 AR subtypes were highly expressed in the detrusor tissue, but undetectable in primary sensory neurons. Our results suggest that beta3 AR agonist CL-316243 acts on the detrusor muscle to improve bladder compliance and increase urine storage in SHR rats.

399

Effects of Bradykinin and Bradykinin Receptor Blocker on Ca^{2+} Responses via Protease-activated Receptors in Endothelial Cells

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Background and aim: Protease-activated receptors (PARs) are activated by serineprotease (SP)-induced cleavage of their extracellular N-terminus. PARs are expressed in platelets, endothelial cells, neurons and myocytes, and involved in inflammation and platelet aggregation. It has been reported that bradykinin (BK) inhibits thrombin-induced platelet aggregation; however, its mechanism remains unclear. Aim of this study was to declare how BK inhibits SP-induced PAR activation by evaluating endothelial Ca^{2+} responses via PARs. Methods: We used primary culture porcine endothelial cells and measured intracellular Ca^{2+} levels using fura-2/AM. Thrombin, plasmin, kallikrein and trypsin, were used as SPs. Results: SPs increased intracellular Ca^{2+} levels, however, secondary application of SPs didn't cause Ca^{2+} increases even 60min after washout of SPs, which suggests activation of PARs by SP is irreversible. BK inhibited SP-induced Ca^{2+} responses; however, secondary application of SP didn't cause Ca^{2+} increases after washout of BK and SP, which suggests BK inhibits PAR activation, even when N-terminus is cleaved by SPs. Interestingly, BK receptor blocker, HOE140, also completely inhibited SP-induced Ca^{2+} increases, demonstrating inhibitory effect of BK on SP-induced Ca^{2+} responses could be independent of BK-stimulated signaling pathway. Substance P, which shares common intracellular signaling pathway with BK, didn't affect SP-induced Ca^{2+} responses, which suggests inhibitory effects of BK on PARs could be independent of the common signaling pathway with substance P. Conclusion: These findings suggest that BK directly inhibits PAR activation without affecting SP-induced N-terminus cleavage and BK-related intracellular signaling pathway, and that BK receptor blocker also exerts inhibitory effect on PARs.

400

An Investigation of the Possible Anti-inflammatory Properties of Acomplia (Rimonabant, a Cannabinoid CB1 Receptor Antagonist) Compared to Anandamide

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Various studies indicated that CB1 antagonists possess anti-inflammatory properties. The aim of this study was to investigate the anti-inflammatory action of Acomplia *in vitro.* The effects of Acomplia and anandamide were determined on the (i) growth of HUVEC and lymphocyte cultures (ii) expression of CR3 by stimulated phagocytes (iii) excretion of IL-1, IL-6, IL-8, IL-10, IL-12p70 and TNF-alpha by stimulated HUVEC and human macrophages after treatment with the experimental compounds. Both Acomplia and anandamide inhibited the growth of HUVEC and lymphocyte cultures in a dose related fashion with Acomplia being more toxic than anandamide. However the significant effects were seen at concentrations above 25µM. No effects were observed on the expression of CR3 by human neutrophils. Acomplia inhibited the

production of IL1beta by human macrophages at 0.5uM. An inhibition was also observed at 0.5µM of TNF-alpha production by HUVEC cultures. However this inhibition did not reach significance. Synergistic effects between Acomplia (0.5 and 1.0µM) and anandamide (5 and 10µM) were observed on the production of IL-8 by HUVEC cultures. No other effects were observed on cytokine production at concentrations less than 5uM. Most of the significant effects documented in this study were obtained at non physiological concentrations. The only significant finding was the inhibitory effect Acomplia had on the production of IL1beta by human macrophages. Because IL-1beta plays an important role in the up regulation of adhesion molecules on endothelial cells, which is associated with inflammation, the results from this study indicate a possible mechanism by which Acomplia inhibits inflammatory reactions.

WEDNESDAY, JULY 30, 2008
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401

Efficacy of Ezetimibe and Statin Coadministration in the Management and Prevention of Hypercholesterolemia: A Meta-analysis of Randomized Clinical Trials

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Background: The efficacy of statin and ezetimibe coadministration in hypercholesterolemic patients has been established; however, the magnitude of this efficacy varies in the literature. This study synthesizes evidence from randomized controlled clinical trials (RCTs) in order to assess the efficacy of statin/ezetimibe therapy in patients with hypercholesterolemia. Methods: Included studies were RCTs that assessed the efficacy of ezetimibe coadministered with a statin in hypercholesterolemic patients who were previously on statin monotherapy, or who were either treatment-naïve for lipid-lowering medications (LLMs) or were washed out of any LLMs prior to study entry. The primary efficacy measure was the percent change from baseline in LDL-C. Secondary efficacy measures were changes in total cholesterol (TC), triglycerides (TG), HDL-C, and TC/HDL-C ratio. Results: The addition of ezetimibe to ongoing statin therapy produced a mean percent reduction in LDL-C of -27.2% (95%CI -27.9% to -26.4%) whereas a mean percent reduction in LDL-C of -4.5% (95%CI -5.3% to -3.8%) was observed in patients remaining on statin monotherapy. For treatment-naïve patients or patients washed out of LLMs, statin/ezetimibe coadministration produced a mean percent LDL-C change of -51.5% (95%CI -51.9% to -51.1%) compared to a mean percent LDL-C reduction of -40.4% (95%CI -40.9% to -40.0%) in patients treated with statin monotherapy. At equivalent statin doses, statin/ezetimibe coadministration was more efficient than statin monotherapy at reducing LDL-C. Conclusion: Evidence from this clinical review shows that the coadministration of ezetimibe with statins is efficient in producing clinically important reductions in serum LDL-C.

402

HIV-1 Viral Envelope Glycoprotein Gp120 Produces Oxidative Stress and Regulates the Expression of Multidrug Resistance Proteins (Mrps) in Glial Cells

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Brain HIV-1 infection is associated with pathological events (i.e., oxidative stress) that may lead to a chronic neurodegenerative condition known as HIV-1 encephalitis (HIVE). *In vitro*, an oxidative stress response can be induced in cultured cells by exposure to HIV-1 viral envelope protein gp120. However, it is unknown if expression and/or activity of Mrp isoforms are altered in response to gp120 treatment. Mrps play an important role in the extrusion of endogenous substrates [i.e., glutathione (GSH), glutathione disulfide (GSSG)] involved in defense against

reactive oxygen species (ROS) and oxidative stress. Altered GSH/GSSG export may disrupt cellular redox equilibrium and contribute to oxidative damage during HIVE. Our goal was to investigate functional expression of Mrp isoforms in gp120-treated astrocyte cultures. Fluorescence of 2',7'-dichlorofluorescein (DCFH), an indicator of ROS, was increased in primary cultures of rat astrocytes triggered with gp120. Immunoblot analysis demonstrated increased expression of heat shock protein 70 and iNOS, further suggesting an oxidative stress response in gp120-treated astrocytes. RT-PCR and immunoblot analysis demonstrated increased Mrp1 mRNA (2.3-fold) and protein (2.2-fold) respectively. In contrast, Mrp4 mRNA transcript or protein expression was not changed. Cellular retention of BCECF, an established Mrp substrate, was reduced (2.0-fold) in gp120-treated astrocytes, suggesting increased Mrp-mediated transport. As expected, GSH and GSSG export were enhanced in gp120-triggered cell. These data suggest that gp120 can modulate the functional expression of Mrp1, but not Mrp4, in cultured astrocytes. Studies are currently ongoing to examine the relationship between transporter expression and oxidative stress in glial cells.

403

Quantifying the Impact of a Drug on Gastric Emptying: Measuring the Pharmacodynamic Effect in Clinical Trials

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Introduction: Many drugs impact on the rate of gastric emptying (GE), the effect of which may be beneficial or detrimental. Accurate quantification of the rate of GE is critical to supporting proof of concept, label claims and predicting the impact on co-administered drugs. Methods: Three methods for measuring GE were compared: scintigraphy, paracetamol (acetaminophen) absorption and ^{13}C-octanoic acid breath test. A review of the methods was performed to identify the merits of each technique. Results: Scintigraphic quantification of GE was introduced in 1966. A gamma emitting radionuclide is incorporated into the solid and/or liquid phase of a meal and a gamma camera is used to acquire regular images. It is a direct and non-invasive method resulting in defined parameters, including time to 50% emptied. The link between the rate of GE and rate of paracetamol absorption was reported in 1973. Standard pharmacokinetic parameters following paracetamol administration are determined to provide an indirect measure of liquid phase GE. ^{13}C-octanoic acid breath test is a novel method, proposed in 1993 and still being refined. ^{13}C is incorporated into the solid phase of a meal and breath samples taken at regular intervals. The rate of exhalation of $^{13}CO_2$ is an indirect measure of solid phase GE, but only after corrections and modelling are applied. Discussion and Conclusions: Scintigraphy provides accurate, detailed and clinically relevant data. It is the gold standard for the assessment of GE and the only direct and non-invasive measure that permits the assessment of both solid and liquid phase emptying.

404

Frequency Distribution of CYP3A4 Gene Polymorphisms in a Mexican Prostate Cancer Population

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Cytochrome P450 3A4 (CYP3A4) is the predominant isoform expressed in human adult liver and intestine. It has a significant role in the metabolism of approximately half of drugs in use today. In addition, CYP3A4 catalyzes the 6 beta-hydroxylation of a number of steroids including testosterone. Up to 40 inter-individual variations in the expression levels of CYP3A4 have been observed in human liver, as well as 10-fold variation in metabolism of CYP3A4 substrates *in vivo*. This variability is due to several factors, including environmental and genetic determinants. *CYP3A4* gene has several allelic variants, but only few have been correlated with functional changes or diseases. Testosterone exposure has been implicated in prostate carcinogenesis, and genes that alter its metabolism, such as CYP3A4, have been associated with prostate cancer susceptibility. Therefore, *CYP3A4* gene polymorphisms may be

overrepresented in a prostate cancer population. In the present study were determined the allelic frequencies of six *CYP3A4* variants. DNA samples obtained from the peripheral blood cells of 100 individuals diagnosed with prostate cancer and 200 healthy male donors were used in this case-control study. The *CYP3A4* polymorphisms were analyzed by RFLP and Real Time PCR methodologies. *CYP3A4*4, *5, *6,* and **18* were not detected, whereas *CYP3A4*2* and **1B* variants were observed in 4% and 16% of the individuals, respectively, from the case group. Although gene frequencies from the control group have not been yet determined, comparison with previous studies suggests that these polymorphisms are not associated with prostate cancer risk within the Mexican population.

405

Effects of Pioglitazone on Erectile Dysfunction in Sildenafil Poor-responders

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Introduction**:** The effects of pioglitazone on sildenafil responsiveness in men with erectile dysfunction (ED) and a history of poor response to sildenafil were assessed. Methods**:** In a double-blinded study, 38 men aged 47 ± 1.5 years with moderate-to-severe ED and poor response to sildenafil were randomly assigned to take pioglitazone 30 mg once daily or placebo for 9 weeks. Erectile function (EF) scores assessed by EF domain of International Index of Erectile Function (IIEF) along with responses to Global Assessment Questions (GAQs) were major outcome measures. Serum levels of total testosterone (T), dehydroepiandrosterone sulfate (DHEAS), glucose, lipid profile and liver function tests were minor outcome measures. Results: Pioglitazone significantly improved major outcome measures compared with placebo. More decrease from baseline of total cholesterol was observed with pioglitazone than placebo. In 84% of the sildenafil poor-responders, at least one of the associated risk factors of ED was found. There was undiagnosed hypercholesterolemia in 34% of the subjects. Serum levels of T, DHEAS, glucose and other parameters remained unchanged in both groups. Pioglitazone was well tolerated either alone or in co-administration with sildenafil. Conclusions: Pioglitazone increased sildenafil response to improve ED of men with prior sildenafil failure and seems to be safe according to the present preliminary small study. This improvement is regardless of fasting glucose and sex hormones levels.

406

rSNP Analyses of Organic Ion Transporters in the Kidney

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Organic cation transporters (OCTs), organic anion transporters (OATs) and H^+/organic cation antiporters (MATEs) play crucial roles in the renal secretion of various ionic drugs. The mRNA expression of transporters can be a key factor regulating interindividual differences in the pharmacokinetics of drugs. However, the source of variations in mRNA levels of transporters is unclear. In the present study, we focused on single nucleotide polymorphisms in the promoter region (regulatory SNPs (rSNPs)) as candidates for the factor regulating mRNA levels of these transporters. We sequenced the promoter regions (about 1 kb) of OCT2 and OAT1-4 in 63 patients, and investigated the effects of the identified rSNPs on transcriptional activities using luciferase reporter constructs and mRNA expression of renal tissues from nephrectomized patients. In the OCT2 promoter region, one deletion polymorphism (-578_-576delAAG) was identified (allele frequency: 8.7 %). -578_-576delAAG significantly reduced OCT2 promoter activity ($p < 0.05$) and carriers of -578_-576delAAG showed lower OCT2 mRNA levels. There was no rSNP in the OAT1 and OAT2 genes. The five rSNPs of OAT3 and one rSNP of OAT4 were unlikely to influence mRNA expression and promoter activity. When the rSNP of *MATE1* gene was searched in the proximal promoter region (about 100 bp), we found a novel rSNP (G-32A), which belongs to a Sp1-binding site. This rSNP exerted a decrease of Sp1-binding and promoter activity about 50%. This is the first study to investigate

the influences of rSNPs on the mRNA expression and promoter activities of organic ion transporters in the kidney.

407

Use of Radiolabeled Antibodies against Cardiac Myosin Heavy Chain for the Detection of Acute Myocardial Infarction

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Myocardial infarction is a leading cause of morbidity and mortality worldwide. Diagnosis, localization, and measuring the size of the infarct with precision are crucial variables when initiating proper treatment. Cardiovision™ is a monoclonal antibody to cardiac myosin heavy chain that is radiolabeled with Technetium 99m. When cardiac cells are damaged, as during an acute heart attack, cardiac myosin heavy chains become accessible to these antibodies for binding. The infarct is then directly visualized using nuclear medicine. Due to its specific binding affinity to injured cardiac myosites, radiolabeled antibodies is an innovative tool for the diagnosis of acute myocardial infarction. We have successfully completed phase I initial safety studies and phase II clinical trials for drug efficacy. Presently, 82 patients have been enrolled in the phase III trials and completion is expected in the near future.

408

The Mechanisms Underlying Sulfamethoxazole Metabolites Regulated Cytokine Production

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For many decades, sulphonamides together with trimethoprim have been used as antimicrobial agents against bacterial and protozoan infections in immunocompromised patients. During bacterial infection, proinflammatory cytokines including tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6), as well as anti-inflammatory cytokines such as IL-10 are produced by macrophages. Here, we investigated the mechanisms and effects of sulfamethoxazole metabolites, which are related to hypersensitivity reactions, on cytokine production in primary human differentiated blood macrophages (PBMac). PBMac were incubated with the drug metabolites for 30 minutes and followed by the addition of bacterial endotoxin (LPS) for another 15 minutes to 3 hours. The results from the Real time Quantitative RT-PCR showed that the mRNA expressions of IL-6 and IL-10 in metabolites-treated cells were downregulated, compared to the LPS treatment alone. We also demonstrated the protein levels of IL-6, IL-10 and TNF-alpha were suppressed. To delineate the molecular mechanisms involved in the metabolites-induced cytokine production, we measured the activity levels of mitogen-activated protein kinases (MAPK), which are known to be key signaling modulators in cytokine induction. The results illustrated that sulfamethoxazole metabolites abrogated the LPS-induced MAPK phosphorylation, concomitant with their effects on cytokine downregulation. Furthermore, sulfamethoxazole metabolites could inhibit the LPS-activated nuclear factor-kappa B (NF-kappa B) functions. In conclusion, our data demonstrated that in addition to their antimicrobial effects, sulfamethoxazole metabolites may play a role in limiting the propagation of uncontrolled inflammation, via the suppression of MAPK and NF-kappa B activities, in microbial infections.

409

Effectiveness of Ezetimibe 10mg/day Co-Administered with Statins versus Statin Dose Doubling in Patients with Coronary Artery Disease (CAD) who are not at Target LDL-C on Statin Monotherapy: The EZE (STAT)^2 Trial

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Introduction: Patients with Coronary Artery Disease (CAD) who are not at recommended LDL-C target levels require effective and aggressive lipid lowering treatment. For these patients, coadministration of ezetimibe with a statin may be more effective in the management of hyperlipidemia when compared to doubling of the statin dose. Methods: Patients with or at high risk for CAD with above target LDL-C while on statin monotherapy were randomized to ezetimibe 10 mg/day co-administered with a statin (EZE+*Statin)* or statin dose doubling (STAT^2). Results: Of the 936 patients enrolled, 398 (EZE+*Statin*: 262. STAT^2: 129) had confirmed CAD. Mean (SD) age was 65.0 (11.0) years and 302 (69.6%) were male. The mean percent reduction in LDL-C was -29.3% (95% CI -31.2% to -27.3%) in the EZE+*Statin* group and -14.7% (95% CI -17.8% to -11.7%) in the STAT^2 group. The mean percent reduction in the EZE+*Statin* group was significantly higher compared to STAT^2 (P<0.001). In the EZE+*Statin* group, 76.4% and 37.4% of patients achieved target LDL-C <2.5 mmol/L and <2.0 mmol/L compared to 45.5% and 17.1% in STAT^2 patients respectively (P<0.001). The odds ratio of achieving target LDL-C was 3.9 (95% CI 2.5 to 6.2) for LDL-C <2.5 mmol/L and 2.9 (95% CI 1.7 to 5.0) for LDL-C <2.0 mmol/L in favor of the EZE+*Statin* group. Conclusion: For patients with CAD who are not at target LDL-C with statin monotherapy, ezetimibe coadministered with a statin is significantly more effective in reducing LDL-C and achieving target LDL-C levels when compared to doubling the statin dose.

410

GlycoPEGylated G-CSF XM22 Demonstrates Sixty Percent Higher Bioavailability and Thirty Percent Greater Neutrophil Response in Comparison to Pegfilgrastim in Healthy Volunteers after Single Body Weight Dependent and Fixed Dose

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Introduction: XM22 is a glycoPEGylated recombinant human G-CSF intended to be dosed once per cycle for the reduction of duration of severe neutropenia and incidence of febrile neutropenia in cancer patients undergoing chemotherapy. Methods: Two single center, randomized, single-blind studies of XM22, administered by the s.c. route were conducted to assess safety, pharmacokinetic profile and pharmacodynamic properties based on absolute neutrophil count (ANC) and CD34+ cell count. Single escalating body-weight (bw) dependent doses (study 1) and a fixed dose (study 2) were administered to male or female healthy volunteers. Results: 53 or 36 healthy volunteers, respectively, were enrolled in the studies and completed 21 day follow-up. Injections were generally well-tolerated with no discontinuations due to adverse events or serious adverse events. XM22 at a dose of 100 mcg/kg bw or 6 mg fixed dose resulted in a 60% increased bioavailability compared to the same dose of pegfilgrastim. ANC area over baseline effect curve (AOBEC) was approximately 30% greater after XM22 in both studies compared to the same dose of pegfilgrastim. CD34+ and antibody data will be discussed. DISCUSSION: Single body-weight dependent doses up to 100 mcg/kg and a fixed dose of 6 mg of XM22 were generally well-tolerated with regard to the expected side effect profile. Conclusions: XM22 demonstrated a 60% higher bioavailability and a 30% greater neutrophil response in comparison to pegfilgrastim in healthy volunteers after single body weight dependent and fixed dose treatment. Therefore, XM22 may become a promising therapeutic alternative to pegfilgrastim.

411

Combined Bayesian Analysis and Genotyping Approach in Testosterone Doping Tests

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Background: To discriminate exogenous from endogenous testosterone, the urinary testosterone glucuronide / epitestosterone glucuronide ratio (T/E) is measured. We have demonstrated a strong association between a UGT2B17 gene deletion

polymorphism (*del/del*) and urinary testosterone glucuronide levels. Future anti-doping tests will use Bayesian individual-based, rather than population-based, T/E cut-off ratios. Hypothesis: Sensitivity and specificity of the T/E test will increase by combining Bayesian analysis and UGT2B17 genotype information. Material and method: Testosterone enanthate (500 mg) was injected into 52 healthy Caucasian male subjects. Two baseline, and 12 additional T/E ratios were determined by gas chromatography-mass spectrometry in urine samples. Genotyping was performed by Real-time PCR analysis. Results: Bayesian individual-based cut-off ratio limits, using one or two baseline T/E ratios, was ~0.6 for *del/del* and ~6.0 for *ins/ins* + *ins/del* individuals. Using these, sensitivity increased substantially for the *del/del* group. Adding genotype information did not significantly change individual cut-off ratios for *ins/ins* + *ins/del* individuals after one or two baseline T/E ratios. However, individual cut-off ratios for all *del/del* individuals were markedly reduced with only one baseline T/E ratio. Using two baseline T/E ratios even lower individual cut-off ratios were observed in 35% of the del/del subjects. Discussion: UGT2B17 genotype information significantly improves sensitivity of the Bayesian analysis of T/E ratios. Discrimination between a baseline T/E value and a positive one will be difficult for *del/del* individuals without UGT2B17 genotype information. Conclusion: Sensitivity and specificity of the T/E ratio test is markedly increased by using Bayesian analysis and genotype information.

412

Pharmacokinetics and Pharmacodynamics of Brivanib Alaninate (BMS-582664), An Oral Dual Inhibitor of VEGFR and FGFR Signaling Pathways, in Patients with Advanced/Metastatic Solid Tumors

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Background: Brivanib is an oral prodrug of BMS-540215, a dual tyrosine kinase inhibitor of VEGFR and FGFR signaling. Part A of this Phase I study was to define the MTD of brivanib dosed orally qd in patients with advanced or metastatic solid tumors. Part B investigated expanded doses/schedules for safety, PK, and PD. Methods: In Part B, sequential cohorts were treated with 320 mg qd (320C), 800 mg 5 days on/2 off (800I), 800 mg qd continuously (800C), or 400 mg bid (400B). PK was measured on Days 1, 8, and 26. DCE-MRI to assess inhibition of tumor permeability/vascularity (AUC_{60}) was performed at baseline (X2), around peak on day 2 and at trough on days 8 and 26 (D8,D26). Exposure-response analysis was performed using logistic regression. PD was assessed on two serum biomarkers (sVEGFR2, and Collagen IV) at baseline, D8 and D26. Results: Eighteen patients were treated in part A, and 50 in part B. The MTD was determined as 800 mg PO qd. PK was linear between 180-800 mg. Brivanib 400B and 800C produced the greatest change on DCE-MRI; some recovery was observed on Day 8 with intermittent dosing (800I). Probability of AUC_{60} response increased with (AUC_{tau}) of BMS-540215. The greatest change in serum biomarkers was seen on D26 in the 3 cohorts receiving 800 mg/day. Conclusions: Brivanib has dose-proportional PK with doses of up to 800 mg/day. PD changes were greatest at 800 mg/day. Brivanib 800 mg qd (800C) is recommended for future phase II/III studies.

413

Phenpocoumon Associated Gastrointestinal Bleedings - Influence of Genetic and Non-genetic Factors

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The anticoagulant treatment with phenprocoumon is characterised by a high variability in the dose regimen and associated with high risk for bleeding complications. The dose variability is at least in part attributed to polymorphisms of the metabolizing enzyme CYP2C9 and of the target

enzyme vitamin K epoxide reductase complex 1 (VKORC1). However, there is only limited information whether this genetic variability and other non-genetic factors are also a risk factor for bleeding complications after phenprocoumon intake. Methods: The CYP2C9*1,*2,*3 haplotypes, the VKORC1 polymorphisms 1173C>T, 4811G>A, 10179A>G and non-genetic factors (age, gender, comedication) were assessed in 90 patients with phenprocoumon associated gastrointestinal bleedings in comparison to 73 patients without bleedings after phenprocoumon (German National Pharmacovigilance study) and an age and gender matched regional control group (n=153, SHIP study). Results: The CYP2C9 haplotypes did not influence the phenprocoumon doses. The frequency of CYP2C9*2 and *3 among patients with phenproucomon associated gastrointestinal bleedings was significantly higher than in the regional control group. The frequency of the VKORC1 polymorphisms was identical in all groups. Carriers of at least one 1173T allele required significantly higher weekly doses of phenprocoumon (17.9 ± 8.7 mg versus 15.3 ± 8.9; p=0.023). The age, gender, comedication of CYP2C9 substrates and NSAIDs were not an additional risk factor for gastrointestinal bleedings. Conclusions: The CYP2C9 but not the VKORC1 genotyp seems to be a risk factor for phenprocoumon associated gastrointestinal bleedings.

414

CYP3A4*1B Gene Polymorphism and the Verapamil/Cyclosprine Interaction in Pediatric Renal Allograft Recipients

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Introduction: Verapamil (VP) is known to alter cyclosporine (CsA) bioavailability. However, not all patients respond to this interaction. Hence, we conducted a prospective open study to examine the impact of CYP3A4*1B genotype on the VP-CsA interaction. Methods: Children with stable renal allograft function for a minimum of 6 months receiving CsA with or without VP were included. In the first study visit, a two-point (2h and 12h) CsA pharmacokinetic profile was obtained, along with DNA sample for CYP3A4*1B genotyping by real time PCR. Serum creatinine and IL-2, TGF-beta1, and TGF-beta2 protein levels (ELISA) were also determined. After the initial visit, patients were either withdrawn of VP (if the patient was ALREADY receiving VP) or started on VP 2mg/kg/day (if the subject was NOT receiving VP). Two weeks after the discontinuation or the introduction of VP, evaluations were carried out as for the first visit. Results: Twenty-one patients were included, mean post-transplant time was 4.8 years, mean CsA dose being 3.8mg/Kg/day. Five patients had the CYP3A4*1A allele, and showed no changes in CsA bioavailability, creatinine or IL-2 and TGF-beta serum levels during the study, whereas 16 patients with CYP3A4*1B allele showed statistically significant increase in CsA bioavailability, and reduction in serum creatinine while receiving VP. No significant difference was observed in IL-2 and TGF-beta protein levels. Conclusion: Patients with CYP3A4*1B, but not CYP3A4*1A allele, benefit from the VP-CsA interaction. Our data suggest that genotype may be a useful predictor of the VP-CsA interaction outcome.

415

Targeted Deletion of Murine Oatp1b2 Transporter: Loss of Hepatic Thyroid Hormone Uptake Results in Aberrant Glucose Homeostasis

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Organic Anion Transporting Polypeptide Transporters (OATPs) mediate the cellular uptake of numerous endogenous and exogenous compounds. Oatp1b2 is the murine orthologue of human OATP1B1 and OATP1B3, hepatic transporters with broad substrate specificity which include drugs, bile acids, and hormones. In order

to more fully understand the *in vivo* relevance of these hepatic OATP transporters, we utilized a mouse model in which *oatp1b2* had been disrupted. Absence of Oatp1b2 expression was confirmed by real-time PCR and immunohistochemistry. AffyMetrix-chip based global mRNA expression analysis revealed differential regulation of genes associated with glucose homeostasis in the oatp1b2$^{-/-}$ mice. With oral glucose tolerance testing, oatp1b2 $^{-/-}$ mice showed a significant delay in the time to normalization of plasma glucose levels. Furthermore oatp1b2$^{-/-}$ mice had normal insulin levels and elevated hepatic glycogen storage. Interestingly, knockout animals exhibited higher plasma T_4 and T_3 levels while hepatic expression of thyroid hormone target genes was paradoxically lower compared to wildtype animals, thus suggesting an important role of Oatp1b2 in the hepatic uptake of T_4 and T_3. In fact, using a reporter assay, we noted activation of the thyroid hormone receptor (TR) was markedly greater in cells over-expressing Oatp1b2. In summary, we identified an unexpected phenotype in which failure of adequate hepatic thyroid hormone uptake by Oatp1b2 resulted in elevated plasma glucose level due to reduced liver TR activation of genes involved in glucose metabolism. Our findings add important new insights to the role of transporters in hormone receptor activation and the etiology of chronic diseases such as diabetes.

416

Effect of Uremic and Hemodialyzed Human Serum on Hepatic Cytochrome P450 Expression and Activity

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Cytochrome P450 (P450) activity and expression is decreased in patients with end-stage renal disease (ESRD). It has been shown that dialyzable uremic toxins could be implicated in these decreases. Recent studies indicated that hemodialysis (HD) can prevent the inhibition of P450 although the mechanism remains to be defined. The aim of this study was to evaluate the effect of hemodialysis on P450 expression and activity using a rat hepatocytes culture model. 12 ESRD patients treated with chronic HD (high-flux, 4-hour sessions) and 12 control subjects were enrolled in this study. Serum samples were collected immediately pre- and post-HD. Normal rat hepatocytes were isolated and incubated for 24 hours in Williams' E medium containing 10% serum from ESRD patients or control subjects. P450 protein and mRNA expressions were determined via Western blotting and Real-time PCR respectively. CYP3A2 activity was determined by DFB metabolism. Incubation of normal hepatocytes with pre-HD serum resulted in downregulations of 44, 27 and 35% ($p<0.01$) of CYP1A1, 2C11 and 3A2 protein expressions compared to control. CYP2C11 and 3A2 mRNA expressions and CYP3A2 activity were similarly downregulated by pre-HD serum. Conversely, post-HD serum had no effect on CYP1A1, 2C11 and 3A2 expressions (>95% of control value, p=NS). These results indicate that hemodialysis, via its detoxificating benefits, can normalise P450 expression. Moreover, these results confirm our recents studies suggesting that dialysable uremic toxins are responsible for the decrease in drug metabolism observed in ESRD patients.

417

Enantioselective Pharmacokinetics of Cyclophosphamide in Lupus Nephritis Patients with CYP3A Activity Evaluated *in vivo*

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Intravenous pulse therapy with cyclophosphamide (CYC) is an established treatment for systemic lupus erythematosus and other autoimmune diseases. It is a chiral drug, administered as a racemic mixture. (S)-(-)-CYC is more effective in

killing ADJ/PC6 plasma cell tumor in mice. Eleven patients with lupus nephritis (creatinine clearance 51-155 mL/min/1.73m^2) received pulse therapy with CYC in monthly infusions with 0.75 to 1.3g CYC administered as a two-hour infusion. Subjects also received midazolam (MDZ) 1mg i.v. bolus. Blood samples were drawn until 24 hours after the beginning of CYC infusion. CYC enantiomers and MDZ were analyzed in plasma by LC-MS/MS. Pharmacokinetic (Pk) parameters of CYC enantiomers and MDZ were obtained by monocompartimental model. Enantioselectivity in CYC Pk was evaluated using the Wilcoxon test. Relationship between CYC Clearance (Cl) and MDZ Cl (CYP3A activity) was evaluated by orthogonal regression and correlation analysis. Pk parameters (median values) observed for (R)-(+) and (S)-(-)-CYC were, respectively: Distribution Volume = 29.32 and 29.09L; Area under plasma concentration versus time curve = 136.44 and 155.33microg.h/mL, Elimination half life = 5.38 and 6.34h, Cl = 3.66 and 3.22L/h. Except for Vd, there were significant differences ($p<0.05$) between CYC enantiomers in Pk parameters. Correlation coefficient between MDZ and (S)-(-)-CYC Cl was 0.6334 ($p=0.05$) and between MDZ and (R)-(+)-CYC Cl was 0.0393 ($p>0.05$). Our results suggest that the Pk of CYC is enantioselective with plasma accumulation of (S)-(-)-CYC. Correlation analysis also suggests that (S)-(-)-CYC is preferably metabolized by CYP3A in lupus nephritis patients.

418

On the Pharmacokinetics of Parent Drugs and their Active Metabolites. Pharmacokinetic and Biostatistics Considerations on the Best Model

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Introduction: Pharmacokinetics of drugs and their active metabolites are important to model and predict since the activity is a resultant of two or more chemical entities existing in the same time in living body. Choice of the best model is in the same time a mathematical, statistical, biopharmaceutical and clinical problem. Methods: Pharmacokinetic data were obtained in the frame of bioequivalence studies for nicergoline and pentoxiphyline. Parent drugs were administered to lots of 24 healthy volunteers in standard two sequences, two periods, two sequences cross-over studies. Plasma levels of the nicergoline metabolites MELUOL and LUOL, of pentoxifylline and its metabolites, I (1-[5-hydroxyhexyl]-3,7-dimethylxanthine) and V (1-[3-carboxypropyl]-3,7-dimethylxanthine), were performed by HPLC and respectively LC/MS methods. Pharmacokinetic and statistical analyses were undertaken using the softwares TopFit and Kinetica. Results: Plasma levels were fitted with monocompartmental, bicompartmental and tricompartmental models solutions. Statistical tests Schwarz, Imbimbo and Akaike were applied for decision concerning better model. All test indicated that monocompartmental and bicompartmental are better than tricompartmental models. Disscussion: Although the parent compounds distributes in the second, lipid compartmental ann are metabolized, their pharmacokinetic follow an apparently very simple model. This contradiction was explained as coming from a particular hierarchy of the transfer and metabolizing constants, issued from physicochemical properties of active substances. Conclusion: In case of drugs with significant metabolism, conclusion concerning the best model is in the same time a problem of biopharmacy, pharmacokinetics, physiology and biostatistics.

419

Drug-induced Hypersensitivity Syndrome: New Biological Markers

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Introduction: Hypersensitivity syndrome (HS) is characterised by fever, rash and at least one organ involvement, occurring 1 to 8 weeks after drug introduction. The aim of the study was, through a series of HS, to identify new markers. Methods: For 3 years in our hospital, 19 cases of HS were

analyzed. Plasma concentrations of ferritin, its glycosylated form, triglycerides, LDH and 25(OH) vitamin D3 were determined. Results: Imputed drugs included cotrimoxazole, sulfasalazine, rifampicin, carbamazepine, diaminopyridines. Of the 10 women and 9 men, 11 had phototype III or IV. Edema helped to evoke the diagnosis in half patients. Minor rash was only observed in immunosuppressed patients. Four patients had myocardial dysfunction, with increased NT-proBNP levels. LDH, ferritin and triglycerides were generally increased, suggestive of macrophage activation syndrome. Glycosylated ferritin determined in six patients was down, as observed for Still disease. Severe vitamin D deficiency (≤ 10 µg/L) was observed in 9/14 patients. The three patients with the highest values of ferritin, above 5000 µg/L, had the lowest values of vitamin D. Discussion: The similarities between hypersensitivity syndrome, still disease and reactive haemophagocytosis suggest common pathways. Interleukin-18 may be the link because this cytokine plays a pathogenic role in Still disease, is correlated with ferritin levels, and its synthesis can be inhibited by vitamin D. Our results are consistent with the inhibition of inflammatory diseases by vitamin D and the negative correlation between ferritin and vitamin D levels. Conclusion: In case of suspected HS, we propose systematic determination of: ferritin, triglycerides and LDH for macrophage activation syndrome, NTproBNP for myocardial dysfunction, and vitamin D since deficiency could contribute to disease severity.

420

Chronic Treatment by Angiotensin II (Ang II) Induces Differential Alterations in PDE1A and PDE4D Expressions in Rat Left Cardiac Ventricles (LV)

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The renin-angiotensin system actively participates to the development of heart failure. PDEs regulate cardiac function by specifically hydrolysing cyclic nucleotides. The objective is to evaluate the participation of PDEs in heart failure induced by chronic treatment of rats with Ang II. Rats of 12-week age were treated during 14 days by Ang II (0.4 mg/kg/d) using osmotic mini-pumps. The cAMP- and cGMP-PDE isozyme activities were measured on nitrogen powdered LV using specific inhibitors for PDE1 to PDE5 families. PDE-mRNA expression was determined by Real-Time PCR (qPCR) and PDE-protein expression was determined by western blot. Ang II induced hypertension and cardiac hypertrophy without tachycardia. In control rat LV, cAMP-PDE and cGMP-PDE activities were mainly and respectively due to PDE4 (64%) and PDE2 (62%). In Ang II treated rat LV, PDE4 activity was specifically increased (+20%). This increase was abrogated by addition of PKA inhibitor. qPCR and western blot revealed that PDE4D-mRNA was decreased by 54%, with a 20% decrease in PDE4D-protein. cGMP-PDE1 (+167%), PDE2 (+85%) and PDE5 (+100%) activities were also increased. PDE1A-mRNA was increased (+47%) and associated to a 30% increase in PDE1A-protein. Cardiac hypertrophy induced by Ang II is mainly associated with increased PDE1A expressions and activity and with a decrease in PDE4D mRNA and protein expression. The increase in PDE4 activity could result of a PKA-dependent phosphorylation, indicating differences in phosphorylation state for PDE4. The different contributions of PDE1 and PDE4 suggest that both cAMP and cGMP are implicated in the cardiac effects induced by Ang II.

421

Influence of *Mangifera Indica* l. Steambark Extract (Vimang) on Blood Coagulation Indicators: Preliminary Evidences

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It has been developed in Cuba a standardized whole-crude extract of steam bark of mango tree (*Mangifera indica* L., Anacardiaceae) with antioxidant, anti-inflamatory, and immunomodulatory properties. It contents polyphenols, mangiferin (major component), selenium, copper and zinc. The ethnomedical use of the mango stem bark aqueous extract in Cuba has been documented widely and it has been extensively used in cancer, diabetes, asthma,

infertility, lupus, prostatitis, prostatic hyperplasia, gastric disorders, arthralgies as the more frequent diseases. Antioxidant effect of the standard MSBE has been tested both in vitro and in vivo and no prooxidant effect was observed. A prospective pilot study was done to investigate the effect of short-term, moderate dosage (900 mg/day) supplementation of an extract of *Mangifera indica* L. (Vimang™) on coagulation profile. Objects of our exams were twelve patients (30 – 75 years old) who had no blood coagulation disorders and who had abstained from any medication in the preceding 10 days. We excluded all patients who had history of warfarin or other anticoagulant therapy use, liver disease, kidney disease or other known coagulopathy. Studies were performed on each patient the day before the start of treatment, a week and a month after the treatment. Some haematological variables were determined to them: blood hemoglobin concentration, hematocrit, glycemia, creatinine, total bilirrubin, glutamic piruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT) and coagulogram. Non parametric tests were applied to the results. Not significant difference in time was found, in none of the cases the p-value associated to the calculated statistic was statistically significant considering level $p<0.05$ which indicates Vimang™ didn't modify studied blood variables.

422

Drug-induced Phospholipidosis: A Novel Mechanistic Hypothesis

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Drug-induced phospholipidosis is caused by chronic dosing with lipophilic cationic drugs from various classes like amiodarone and fluoxetine and consists of the presence of large vacuoles that contain multiple concentric layers of membranes in cells ("onionoid" or multilamellar bodies). Concerns about alteration of tissue functions have prompted the FDA to establish a working group on phospholipidosis. We used human cultured cells (HEK 293, vascular smooth muscle cells) to probe the mechanism of phospholipidosis induced by the anti-arrhythmic drugs procainamide and amiodarone (microscopy, biochemistry). V-ATPase-mediated concentration of these drugs in vacuoles is a cellular response to both drugs in 4 h, but at different concentration ranges (mM and micromolar, respectively). Drug-induced vacuolization was prevented by the V-ATPase inhibitor bafilomycin A1. The vacuoles initially contain concentrated solution of drugs (as shown by the violet fluorescence of amiodarone) and are of macroautophagic nature (labeled with the late endosome/lysosome markers Rab7 and CD63 and partially with the autophagy effector LC3; parallel to LC3 II formation in cells). The contents of a vacuole subset slowly (> 24 h) became positive for Nile red staining (phospholipidosis-like response). We propose that amine drugs are subjected to a V-ATPase-mediated "pseudo-transport" into acidic cell vesicles that swell by an osmotic mechanism. In the absence of drug washout, multiple cycles of macroautophagy (with addition of 2 membrane layers per cycle) explain the formation of multilamellar bodies. This sequence of events can be demonstrated with many weak bases, but is a clinically relevant only for the most lipophilic drugs, like amiodarone.

423

PK/PD Modeling, Biomarkers and Clinical Endpoints: The use of Cluster Analyses, Logistic Regressions, and Receiver Operator Characteristics (ROC) for Efficient Drug Development

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Introduction: The escalating costs and low productivity of drug development have been well documented over the past several years. The FDA in its Critical Path initiative and other agencies are calling upon new methods such as PK/PD modeling, biomarkers and clinical endpoints to improve development processes. Advanced statistical methods such as cluster analyses (CA), logistic regressions (LR), and Receiver Operator Characteristics (ROC) may be combined with PK/PD modeling methods to provide additional insights for the analysis of biomarkers and clinical endpoints. Methods: PK/PD modeling, along with CA, LR, and ROC techniques were used to analyze biomarker data and binary clinical

endpoints. Case-studies in various therapeutic areas are presented (e.g., CNS, cancer, and rheumatoid arthritis). Results: In case-study 1, ROC analyses provided insights on the early qualification of biomarker(s) and cut-off levels that correlated with clinical response 4 months after study start. Implications of the early detection of responders according to key biomarkers lead to the development of an adaptive trial. In case-study 2, PK/PD modeling combined with LR and ROC methods determined an optimal drug exposure in specific populations that maximized the clinical response. In case-study 3, CA quickly detected correlations between drug exposure, biomarkers and clinical outcome, and resulted in very rapid development of a mixed-effect LR model and the development of a PK-biomarker-clinical response model. Discussion and Conclusion: Research groups implementing PK/PD modeling and statistical tools such as CA, LR, and ROC methods should have a distinct competitive advantage, including additional insights and guidance for effective drug development.

424

Variability of Efavirenz Population Pharmacokinetics with MDR-1, CYP2B6, and CYP3A5 Polymorphisms

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Background: Efavirenz, an antiretroviral drug metabolized by polymorphic enzymes, exhibits pharmacokinetic variability causing varied clinical responses. Low and high plasma concentrations among HIV patients result into virologic failure and CNS toxicities respectively. Factors for efavirenz pharmacokinetic variability which include sex and ethnicity are poorly understood. We examined the genetic polymorphic effect of CYP2B6, CYP3A5 and MDR1 on efavirenz population pharmacokinetics among Ugandans. Methods: Efavirenz concentration samples (402) from 121 participants were analyzed by HPLC. Study participants were characterized for 26 SNPs in CYP2B6 (n =7), CYP3A5 (n =5) and MDR1 (n =14), genes by PCR-RFLP and micro-assay. Non-linear mixed effect data modeling approach in NONMEM software was used to determine covariate effects on efavirenz pharmacokinetics. Results: Inclusion of CYP2B6 (516G>T, *11), and MDR1 rs2032582 (Ala893Ser) and rs36008564 (Ile261Val) in the model explained 11%, 4%, 6.2% and 1.3 % of the between-subject variability in oral efavirenz clearance. Sex as a covariate decreased unexplained between-subject variability (CV %) in peripheral oral volume of distribution from 28.3 to 47%, while MDR1 rs3600564 and rs3842 reduced relative efavirenz bioavailability variability by 83% and 10% respectively. The high peripheral oral volume of distribution among female participants could be explained by high body fat content. Presence of MDR1 at absorptive and secretory sites explains its polymorphic effect on efavirenz bioavailability. CYP2B6*11 exhibited about 40% of 516G>T effect on efavirenz pharmacokinetics. Conclusion: These results suggest that CYP2B6 (516G>T, *11), as well as MDR1 rs2032582, 36008564, rs3842 and 3435G>T polymorphisms and sex influence efavirenz pharmacokinetics.

425

Comparison of Bupivacaine with Fenthanyl and Ropivacaine for Labor Intermittent Epidural Analgesia with Top Expulsion Dose

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Introduction: The study compared the onset of analgesia, duration of labor stages and concentration of total bupivacaine and ropivacaine in maternal plasma. Methods: 20 patients received intermittent epidural analgesia with bupivacaine and 100 microg fenthanyl and 20 patients with ropivacaine. All parturients received one top dose of anesthetic in expulsion. Results: There is no significant difference for the duration of the first stage of labor between groups (bupivacaine 112.5±14.5 min, ropivacaine 117.5±19.22 min). In bupivacaine group a significant decrease in epidural doses induces a significant decrease in comfort interval. Expulsion dose of bupivacaine was 33.25±1.08 mg. Concentration of total bupivacaine remains constant (0.62±0.01

microg/ml, 0.65±0.01 microg/ml, 0.67±0.02 microg/ml), with a peek after top doses 0.99±0.05 microg/ml). Decrease in plasma concentration was significant after 2 hours from expulsion with 294.61 min concentration half life. In ropivacaine group: a significant decrease of ropivacaine doses from 17.6±0.49 mg to 13.6±0.7 mg makes no modification in comfort interval after each injection. Concentration of total ropivacaine was significantly higher after second and third epidural doses (0.87±0.05 microg/ml). The highest concentration was 1.1±0.06 microg/ml. Ropivacaine elimination half life was 229.42 min). Disscution and Conclusion: The study confirms that bupivacaine is similar with ropivacaine. A small decrease in doses during first stage of labor determines a significant decrease of successive comfort interval. The peek of maternal blood concentration of bupivacaine and ropivacaine after top doses is followed by a specific decrease rate.

426

Ginkgo Biloba Protects against Ischaemia-Reperfusion-induced Gastric Lesions in the Rat

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The typical standardized Ginkgo biloba (GBE) extract contains Ginkolides, bilobalide and flavone glycosides. Flavone glycosides include quercetin, 3-methylquercetin and kempferol. Quercetin, myrcetin and the flavone fraction have antioxidant and free radical scavenging effects. Male Wistar rats (240 ± 10g), maintained on raised mesh bottom cages to prevent cropohagia were deprived of food 24h before experiments. GBE (40-320mg/kg, P.O.) was given 30 min before procedure. Control groups received the same volume of distilled water, P.O. Ischemia and reperfusion-induced gastric lesions were produced as described by Andrews *et. al*, (1994). Vascular permeability was determined using Evans blue dye as described previously (Lange *et. al*, (1994). The total surface area of erosions in ischemia alone was 0.5 ± 0.13 mm^2 and this was significantly higher 13.4± 3.8 after reperfusion. Pretreatment with GBE produced dose-dependent and statistically significant reductions in ischemia-reperfusion-induced gastric lesions (control, 13.4 ± 3.8, GBE, 80mg/kg, 5.4± 0.6, 160mg/kg, 3.6± 0.5, 320 mg/kg, 1.9 ± 0.2). Additionally, pretreatment with ginkgo biloba (80-320 mg/kg, P.O. produced a dose-dependent and significant reductions in the extravasations of Evans blue (vascular permeability) in the stomach (Control, 7.7± 0.9, 80 mg/kg, 5.4 ± 0.5, 160 mg/kg, 3.9 ± 0.3, 120 mg/kg, 2.1± 0.2). Ginkgo biloba extract protects against ischemia-reperfusion-induced gastric lesions and increases in vascular permeability. Oxygen-derived free radicals may be involved in the pathogenesis of these ischemia-reperfusion-induced gastric lesions.

427

Analytical Method for the Rapid Analysis of Valsartan in Human EDTA K_2 Plasma by LC/MS/MS

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Purpose: Valsartan is the second of a class of drugs known as angiotensin receptor blockers (ARBs) that are used for treating high blood pressure. The purpose of this work was to develop and validate a fast, specific and robust method for the determination of valsartan in human EDTA K_2 plasma. Methods: Valsartan and its internal standard valsartan-d_9 were extracted from human EDTA K_2 plasma by protein precipitation using methanol. Analysis was performed on a MDS Sciex API 4000 tandem mass spectrometer with TurboIonSpray interface. Positive ions were measured with m/z $436.3 \rightarrow 291.5$ for valsartan and $445.3 \rightarrow 300.5$ for IS. The chromatographic run time was 2.0 minutes on a Zorbax SB-C18 50 X 4.6 mm column. The mobile phase was a mixture of Milli-Q type water / methanol (30/70) with 2 mM ammonium formate, pH 3.00. Results: This assay was validated over a nominal range of 20 to 10000 ng/mL. Linearity over the calibration range was $\geq$ 0.9978. The between-run accuracy ranged from 98.80 to 102.21% with precision ranging from 4.16 to 6.43%. The within-run accuracy ranged from 97.60 to 101.50% with precision ranging from 2.09 to 4.16%. The recovery of valsartan and its internal standard was 100%. No matrix effect on quantitation was observed. Valsartan was found to be stable in

human EDTA K_2 plasma after 24 hours at room temperature for short term stability, after 49 days at -20°C and -80°C for long term stability, after 95 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -20°C and -80°C. Dilution integrity and matrix selectivity were also demonstrated. Hemolysis effect was evaluated. Conclusions: This method is fast, reproducible and was successfully applied for the analysis of clinical samples. Over 5500 study samples were analysed with accuracy ranging from 97.04 to 106.60% and precision ranging from 1.49 to 6.14%.

428

Grapefruit Juice Polyphenols Interaction with Simvastatin and Pravastatin: *in vitro* Intestinal Permeability Characterization

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Introduction: Statins are well characterized HMG CoA reductase inhibitors. Their low oral bioavailability can be explained by an important intestinal or hepatic first pass effect. The aim of our work was to study this two statins and their *in vitro* modulation by 3 polyphenols of grapefruit juice on intestinal permeability. Such modulation could explain polyphenols influence on statins clinical effect. Methods: The transcellular transport: apical (A) to basolateral (B); of two statins was evaluated by the intestinal apparent permeability coefficient (Papp) in caco-2 cells in presence or absence of 3 polyphenols (naringenin, quercetin, bergamotin). To explain the involved mechanisms, the two statins and the flavonoids were incubated in presence of various membranes carriers' inhibitors such as MDR1, MRP2 and OATP inhibitors. Interaction of statins, polyphenols and membrane transporters was investigated in capillary cytometry by measuring intracellular fluorescent substrat release of treated cells. Results: The Papp A->B of two statins was increased by polyphenols: 4 times by Quercetin and Naringenin and 3 times by Bergamotin. Polyphenols are modulators of the membrane transporters MDR1, MRPs and OATP. Simvastatin is a MDR1 and MRP substrate but not for OATP like is pravastatin. Conclusion: The 3 tested polyphenols increase intestinal permeability of statins. They contribute to enhanced statins oral bioavailability. These properties should be taken into account when statins are co-administered with grapefruit juice so to adjust doses in order to avoid adverse effects. These results emphasize the accuracy our new model of intestinal drug absorption and mechanism of food drug interaction.

429

Comparison of Pharmacokinetics of Mycophenolic Acid and its Glucuronide between in Patients with Lupus Nephritis and with Kidney Transplantation

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Introduction: Mycophenolate mofetil (MMF) has been utilized to treat lupus nephritis (LN). In LN patients, pharmacokinetics of mycophenolic acid (MPA), an active metabolite of MMF, is not fully characterized. The aim of this study is to evaluate pharmacokinetics of MPA and its glucuronide (MPAG) in LN patients through comparing with them in kidney transplant (KT) patients. Methods: This study enrolled 6 LN patients (WHO, class IV and V) and 24 KT patients [8 patients treated with tacrolimus (Tac) and 16 patients with cyclosporine (CyA)]. Pharmacokinetic parameters of MPA and MPAG and reciprocal of serum creatinine (ReScr) were compared among LN, Tac-, and CyA-treated patients. Results: Interindividual variability was observed in pharmacokinetics of MPA and MPAG in LN as well as KT patients. MPA area under the curve $(AUC)_{0\text{-}12\ h}$ was significantly lower by 52% in LN than Tac-treated patients (P<0.01). In contrast, MPAG $AUC_{0\text{-}12\ h}$ was significantly lower by 57% in LN than CyA-treated patients (P<0.01). $AUC_{0\text{-}12\ h}$ ratio of MPAG to MPA was also significantly lower by 47% in LN than CyA-treated patients (P=0.02). MPA AUC ratio of 5-12 h to 0-12 h tended to increase by 24% in LN compared with CyA-treated patients. ReScr was significantly higher in LN than KT patients. Conclusion: Pharmacokinetics of MPA and MPAG were different between LN and KT

patients. The difference of renal function may be responsible for that of their pharmacokinetics. In addition, inhibition of enterohepatic recirculation by CyA may also be involved in this difference.

430

Implication of Seric Factors in the Pharmacokinetics of Fexofenadine in Patients with End-stage-Renal-Disease

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End-stage-renal-disease (ESRD) causes a decrease in the non-renal elimination of drugs. Recent studies in rats with surgically-induced ESRD have demonstrated a reduction in the expression and activity of intestinal efflux transporters p-glycoprotein (P-gp) and multidrug-resistance-related-protein 2 (MRP2) and hepatic influx transporter organic-anion-transporting-polypeptide 2 (Oatp2) which can increase drug bioavailability and decrease biliary excretion. These impairments are shown to be in response to factors accumulating in the serum of nephrectomized rats. A recent *in vivo* study in humans demonstrated that the area under the curve of fexofenadine was significantly increased in ESRD patients while its clearance (CL/F) appeared decreased. The goal of this study was to determine if these pharmacokinetic changes in humans are associated to factors accumulated in the serum of ESRD patients. Hepatocytes and enterocytes were incubated with 10 % plasma from 10 ESRD patients and 5 control subjects for 48h. Protein and mRNA expressions of P-gp, Oatp2 and Oatp3 were assessed. In enterocytes incubated with ERSD plasma, we found a significant 50 % decrease in P-gp protein expression, while Oatp3 remained unchanged. In hepatocytes, we found a significant 40 % increase in P-gp protein expression and a significant 30 % decrease in Oatp2. These results are similar to previous reports using serum from nephrectomized rats.This study indicates that the increase in bioavailability and decrease in CL/F of fexofenadine observed in patients with ESRD could be due to alterations in the expression and activity of influx and efflux drug transporters caused by seric factors accumulating in ESRD patients.

431

Single Dose Comparison of Folate Pharmacokinetics: 1.1 mg versus 5 mg Folic Acid

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The importance of folic acid before and early in pregnancy to reduce the risk of neural tube defects (NTDs) has been well documented. A 2001 study suggested that daily supplementation with 5 mg folic acid, among women of childbearing age, is needed for maximum protection against NTDs and potentially other malformations. The objective was to compare the single dose pharmacokinetics of 5 mg versus 1.1 mg folic acid, contained in 2 prenatal multivitamins, and to estimate its contribution to steady-state folate levels. The pharmacokinetics of 1.1 mg folic acid had been determined in a previously published study. The method was replicated among 6 healthy, non-pregnant women who were given 5 mg folic acid to ingest. Serum folate concentrations were measured at various times over 10 hours post-ingestion. Standard pharmacokinetic parameters were calculated and compared with Student's t-test. The mean AUC of serum folate after oral administration of 1.1 mg and 5 mg folic acid were 147.6 ± 52.8 (ng/mL)·hr and 997.5 ± 271.9 (ng/mL)·hr, respectively ($p<0.0002$). A 5-fold difference was detected in peak concentrations (C_{max}) between the 2 groups ($p<0.0005$), alongside a slight difference in mean times to peak (T_{max}) ($p=0.02$). The estimated steady-state serum folate contribution produced by 1.1 mg and 5 mg of folic acid were 6.2 ± 2.2

ng/mL and 41.6 ± 11.3 ng/mL, respectively ($p<0.0002$). Single dose administration between 1.1 mg and 5 mg folic acid demonstrated linear pharmacokinetics, with a 5-fold difference between the 2 doses in supplement contribution to steady-state levels, under ideal compliance.

432

A Phase I Study of a Novel Combination Drug - AMT in Healthy Male Subjects

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Introduction: Auron Misheil Therapy (AMT) is a mixture of approved pharmaceuticals in low therapeutic doses (human insulin and chlorpheniramine) and herbal components (aqueous camomile extract). In vitro and In vivo pharmacological studies reveal anti-cancer effects of AMT in several tumour types in addition to a very favourable safety profile. This Phase I study carried out in healthy volunteers, assessed the safety and tolerability, pharmacokinetic properties and pharmacodynamic properties of single rising doses of AMT, for the first time under controlled conditions. Methods: The study was conducted in a single rising dose, double-blind, placebo-controlled design. Three groups of 8 male volunteers received one of 3 doses of AMT (0.011, 0.033 or 0.066 ml AMT/kg body weight i.m.; n=6 per group) or placebo (n=2 per group). Blood samples were taken from the subjects for pharmacokinetic, pharmacodynamic and safety assessments. Additionally tolerability and safety was monitored throughout the study. Results: AMT was shown to be well tolerated, revealing no severe or serious adverse events. There were no unexpected pharmacokinetic or pharmacodynamic results for any of the three components of AMT. Conclusions: The study provided important pharmacokinetic, pharmacodynamic and safety data of AMT, and supports further controlled clinical investigation in cancer patients. Phase II studies have been initiated to evaluate the efficacy, pharmacodynamics, safety and tolerability of AMT in patients with different types of cancer.

433

Effect of Tenryocha (*Rubus suavissimus* S. LEE) Tea on Human CytoChrome P4503A4-mediated Metabolism *in vitro* and *in vivo*

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Tenryocha (Rubus Suavissimus S. LEE) tea is consumed as an herbal beverage, especially to prevent and to relieve pollen allergy. This study was conducted to evaluate the possible herb-drug interaction with Tenryocha tea via cytochrome P450 3A4 (CYP3A4) in vitro and in vivo. The inhibitory effect of Tenryocha tea on CYP3A4 activity was investigated using midazolam 1'-hydroxylation in pooled human liver microsomes. The oral pharmacokinetics of simvastatin was determined after administration of simvastatin 10 mg with 200 mL of Tenryocha tea or water in a randomized two-way crossover study in 8 Japanese healthy men with a week washout period between each phase. Either Tenryocha tea or water was further ingested three times on the day before and once after simvastatin administration. In vitro study showed the potent inhibitory effect of Tenryocha tea (2.5v/v%) on midazolam 1'-hydroxylation with 35% of the residual activity. In contrast there was no significant difference of simvastatin pharmacokinetic parameters between Tenryocha tea and water treatment group. The geometric mean ratio of Tenryocha tea to water treatment was 0.94 for simvastatin AUC0-24 and 1.29 for the Cmax. The difference of arithmetic mean between both treatment group for the t1/2 and CL/F were 0.83 h and 37.5 mL/h, respectively. Tenryocha tea was a potent inhibitor of CYP3A4 activity in human liver microsomes, however, it was unlikely to have any appreciable effect in humans. Further studies are required to assess whether Tenryocha tea affect CYP3A4-mediated metabolism under different conditions, with various concentration, frequency or timing of its ingestion.

434

Pharmacodynamic Effects Assessed with Pupillometry in Healthy Subjects after Single and Multiple Doses of Escitalopram

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Escitalopram is an allosteric selective serotonin reuptake inhibitor. The serotonergic effect of escitalopram on pupillary reflexes was assessed as a biomarker using pupillometry. The study was a randomized, double-blind, placebo-controlled, cross-over design using single and multiple doses of 10 mg escitalopram and placebo. Static and dynamic pupillometry was measured before and 1, 2, 2.5, 3, 3.5, 4, 5, 6, 7 and 8 hours after medication by a handheld infrared pupillometer (PLR100, Neuroptics®). The area under the effect curve (AUEC) was calculated using linear trapezoidal method. Thirteen subjects gave written consent and completed the study. Treatment was well tolerated; eight subjects experienced adverse effects: six during escitalopram treatment and two during placebo. Most of the adverse effects were of mild severity, none were serious or unexpected. Evaluation of pupillometry data before and after the first and the last (of eight daily doses) of escitalopram revealed that $AUEC_{0\text{-}8h}$ of amplitude of pupil light reflex relative to maximum pupil diameter was statistically significantly decreased by 8.8% (95% CI: 4.2-13.4; p=0.001) after multiple doses of escitalopram compared to placebo. This finding could be explained by activation of central $5HT_{1A}$ heteroceptors evoking a release of noradrenaline, affecting the pupil light reflex in two ways: inhibition of the parasympathetic activation by α_2 receptors on Edinger-Westphal nucleus and by a central increase in the sympathetic outflow via α_1 receptors.

435

Combined Genotype of Norepinephrine Transporter T-182C and Alpha2c Adrenergic Receptor Deletion Polymorphisms is Associated with the Response to Beta-blockers in Dilated Cardiomyopathy

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Introduction: Recent clinical trials have clearly demonstrated that the administration of beta-blockers substantially improves the prognosis of the patients with chronic heart failure (CHF). However, inter-individual variation in therapeutic efficacy has been observed in the clinical use of beta-blockers. Norepinephrine level in synaptic cleft is mainly regulated by presynaptic norepinephrine transporter (NET) and alpha2 adrenergic receptor (alpha2AR) in human hearts. We investigated the association between the response to beta-blockers and the polymorphisms of these two genes; NET T-182C and alpha2cAR 322-325 insertion (Ins)/deletion(Del). Methods: Patients with CHF due to idiopathic dilated cardiomyopathy (n=126) were genotyped by PCR-based methods. We measured left ventricular fractional shortening (LVFS) by echocardiography before and 6 months after the beta-blocker treatment. Results: Improvement of LVFS was inversely correlated with pre-treatment LVFS (r=-0.398, p<0.001), suggesting that beta-blocker does not improve LV function in the patients with high pre-treatment LVFS. Therefore, we exclude the patients with pre-treatment LVFS ≥20% (n=26) in polymorphism analysis. Patients with NET -182CC genotype showed lower LVFS improvement than those with NET -182T allele (CC:4.8±7.3, TT+TC:10.4±9.4%, p=0.025), while alpha2cAR 322-325 Ins/Del did not influence on LVFS improvement. Concerning combined genotype of these polymorphisms, LVFS improvement was lower in the patients with NET -182CC/alpha2cAR Ins/Ins genotype than those with other genotypes (3.1±6.2, 10.5±9.3%, respectively, p=0.006). Conclusion: These results suggest that combined genotype of NET T-182C and alpha2cAR 322-325 Ins/Del is the predictive marker of the response to beta-blockers. Genotyping of these polymorphisms may provide clinical insights into individual differences in the response to beta-blockers in CHF.

436

Deferiprone Penetration into Organs and Iron Removal from Target Tissues by Chelation

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Introduction: Characterization of chelator penetration into organs, tissues and cells and of iron distribution in animal models is essential for assessment of therapeutic potential. We studied: (A) deferiprone (DFP) tissue penetration, and plasma and organ kinetics in naïve rats; (B) iron distribution in iron-loaded rats, and (C) iron removal from target tissues by DFP monotherapy, desferrioxamine (DFX) monotherapy, and combination (DFP+DFX). Methods: Study (A): DFP content was measured in the plasma and organs of rats given 150 mg/kg DFP orally. Studies (B) and (C): Rats were given iron-dextran, 100 mg/kg intraperitoneally twice weekly for four weeks and treated (five times weekly; 127 days, 89 doses) with 60 mg/kg DFX subcutaneously (Group #1, N=4); 100 mg/kg DFP orally (Group 2, N=4); DFX + DFP (Group #3, N=4); Fe alone (Group #4, N=3); iron naïve (Group #5, N=4). Terminal studies: tissue histology, transmission electron microscopy (TEM), X-ray-micro-array analysis, and iron determination. Results: Iron dextran pretreatment produced cellular and mitochondrial iron accumulation in heart and liver. DFP freely penetrated tissues, including the heart and brain (plasma:tissue ratio typically 1:0.5-1). DFP (46%) and DFP+DFO (48%) resulted in non-significantly greater reductions in heart iron than did DFO (30%). DFP+DFO (45%) reduced liver iron significantly more than DFO (24%) or DFP (33%) (p=0.029). Discussion and Conclusions: Combination therapy in this animal model was superior to either chelator alone in reducing hepatic iron, whereas DFP appeared to be as effective as DFP+DFO in reducing heart iron; both showed a trend to a greater effect than DFO.

437

Induction of Lung Fibrosis by Methotrexate in C57BL/6J Mice

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Methotrexate (MTX) has been used as the first-line disease modifying antirheumatic drug (DMARD) in patients with early progressive rheumatoid arthritis (RA). In contrast, MTX has several severe side effects, such as hepatotoxicity, nephrotoxicity, myelosuppression, and pulmonary toxicity. However, the pathogenic mechanism of MTX-induced pneumonitis is still not known. The purpose of the present study is to examine whether the oral administration of MTX could be induced pulmonary fibrosis and apoptosis in mouse lung epithelial cells. C57BL/6J mice were treated with 200 μL sterile water containing MTX 3 mg/kg by oral administration. After 21 days, 28, and 35 days later, hydroxyproline content, pathologic, and immunohistochemical analyses were performed. Apoptosis was quantified by terminal deoxynucleotidyltransferase-mediated UTP end-labeling (TUNEL) assay. The MTX-treated animals exhibited a significant increase in lung hydroxyproline and collagen deposition when compared with control animals. Drug-treated mice had abnormal histology including areas of septal thickening with cellular infiltration of the interstitial and alveolar spaces, whereas the control mice had histologically normal. Furthermore, the apoptosis level was increased in the alveoli of MTX-treated animals. Our results thus indicate that the oral administration of 3 mg/kg MTX treatment during days 35 induces a fibrotic response with apoptosis, and suggests that this animal model may take advantage of the elucidation of the further detailed mechanism of MTX-induced pulmonary toxicity.

438

Clinical Impact of *NAT2*19*, which Works as a Low-activity Allele Expressing Slow Acetylator Phenotype

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Introduction: Genotype of N-acetyltransferase 2 (NAT2) is an important biomarker. The slow acetylator genotype, homozygous low-activity

alleles, predict the adverse effects of isoniazid and sulphasalazine. NAT2*19 exhibits reduced enzyme activity in vitro and, therefore, has been believed to be a risk factor of the side effects; however its clinical significance was still unproved so far. Here, we examined if NAT2*19 had any clinical impact on drug-induced hepatic dysfunction as well as plasma concentration of isoniazid during multi-drug treatment for tuberculosis. Methods: After written informed consent was obtained, four hundred and thirty one Japanese patients with pulmonary tuberculosis were genotyped for *NAT2*4*, **5, *6, *7,* and **19*. Clinical phenotype of *NAT2*19* carriers were assessed according to their clinical profile and plasma concentration of isoniazid. Drug-induced hepatic dysfunction was determined according to the international criteria. Results: Allele frequency of *NAT2*19* was 0.0035. Clinical profiles of three *NAT2*19* carriers were as follows. A patient with *NAT2*7/*19* exhibited isoniazid-induced hepatic dysfunction during multi-drug antituberculosis treatment with isoniazid, rifampicin, pyrazinamide and ethambutol according to international guideline. AST and ALT increased fourteen days after commencing the regimen and reintroducing isoniazid. In contrast, no adverse reactions were observed in two patients carrying *NAT2*4/*19*. Apparent clearance of the patient with *NAT2*4/*19* was equivalent to that of *NAT2*4/*5, *4/*6* and **4/*7* carriers. Conclusion: *NAT2*19* is classified as a slow acetylator allele with low NAT2 activity. This is the first clinical characterization of *NAT2*19* allele.

439

Pharmacokinetics and Toxicodynamics of Lomefloxacin in Mice

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Fluoroquinolones, widely used antimicrobial drugs, sometimes cause phototoxicity. The objective of this study was to investigate the pharmacokinetics (PK) and the toxicodynamics (TK) of lomefloxacin (LFX), a model fluoroquinolone compound, and to develop a PK/TD model to characterize the relationship between the PK parameters and phototoxicity. LFX (10, 30, 100 mg/kg) was administered to male ICR mice via iv bolus injection and exposed to the long-wave ultraviolet light (UVA) for 0.5-4 h. An increase in ear thickness was measured to assess LFX-induced phototoxicity, and plasma samples were collected and analyzed to assess LFX pharmacokinetics. LFX exhibited linear disposition within the dose ranges used, which was well-characterized through the use of a two-compartment model. Ear thickness was monotonously increased for 96 h after the end of UVA irradiation. The ear thickness at 96 h after irradiation was increased in a dose and irradiation time dependent manner, and this value was compared with PK parameters. There was a high correlation between the area under the concentration-versus time curve (AUC) of LFX in plasma during the irradiation and the ear thickness at 96 h (r=0.933). Similarly, a good correlation between the AUC in auricle and the ear thickness was also observed (r=0.968). In contrast, no relationship was observed between the Cmax of LFX and the ear thickness. The results of the present study indicate that the LFX-induced phototoxicity can be predicted from the AUC and the prediction is applicable to clinical use of fluoroquinolones.

440

The Effects of Ozone Exposure on Pro and Anti-apoptotic Gene Expression in Cultured Human Epithelial HeLa Cells

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While the biological effects of the gaseous oxidant ozone (O3) are associated with toxicity, it has also been used for therapeutic purposes in alternative medicine. In biological systems ozone elicits dose-dependent oxidative stress, which may induce adaptation. We recently demonstrated that repetitive exposure of cultured cells to ozone may induce adaptation, inhibited by the Akt inhibitor (-)-deguelin. In the current study we measured the modulating effects of single and repetitive exposures of human epithelial HeLa cells to ozone on the expression of genes encoding for anti-apoptotic (Akt, Bcl2, Creb, NF-kappa B and BDNF) and pro-apoptotic (Bax, Caspase 3 and 8) proteins. HeLa cells were exposed to control or

ozone-saturated glucose-free Krebs-Henseleit (gf-KH) solution for 4 x 5 minutes (spaced 4 hours), followed by 16 hours incubation in culture medium and then a 25-minute exposure to control or ozone-saturated gf-KH. Thereafter the cells were lysed immediately (0 hour) or after 8 hours and gene expression determined with real-time PCR. When exposed to ozone for 4 x 5 minutes alone, genes for Bcl-2, Creb, BDNF and caspase-3 were down-regulated at 0 hour, but these returned to pre-treatment values at 8h. However, when treated with ozone 4 x 5 minutes + 25 minutes, Akt, and Creb were upregulated at 8 hours. In conclusion and in line with previous results, the current data suggest that anti-apoptotic mechanisms may be involved in the adaptive effects following repetitive exposure to ozone. This adaptation may be preceded by the down-regulation of pro- and anti-apoptotic enzymes.

441

Implication of NMDA Receptors in the Antidyskinetic Activity of Cabergoline, CI-1041 and Ro 61-8048 in MPTP Monkeys with Levodopa-Induced Dyskinesias

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The present study assessed striatal NMDA glutamate receptors of MPTP monkeys as a model of Parkinson's disease in rel'tion with L-DOPA-induced dyskinesias (LID). In a first protocol, four MPTP-monkeys receiving L-DOPA/Benserazide alone for one month developed dyskinesias. Four other MPTP-monkeys received L-DOPA/Benserazide plus CI-1041 an NMDA antagonist selective for NR1A/NR2B assembly and four other treated with L-DOPA/Benserazide with a small dose (0.015 to 0.35 mg/kg) of cabergoline a dopamine D2 receptor agonist; only one in each group developed mild dyskinesias at the end of the treatment. In the second protocol, a kynurenine 3-hydroxylase inhibitor Ro 61-8048, increasing kynurenic acid and kynurenine that antagonize NMDA receptors, with L-DOPA/Benserazide developed less dyskinesias than MPTP monkeys treated with L-DOPA/Benserazide. Glutamate receptors were investigated by autoradiography using [^{3}H]CGP-39653 (NR1A/NR2A antagonist) and [^{3}H]Ro25-6981 (NR1A/NR2B antagonist). Striatal [^{3}H]CGP-39653 specific binding was unaltered in all experimental groups. By contrast, MPTP lesion decreased striatal [^{3}H]Ro25-6981 specific binding; dyskinetic monkeys had [^{3}H]Ro25-6981 specific binding higher than non-treated MPTP monkeys, and non-dyskinetic MPTP monkeys had lower specific binding than the dyskinetic ones. Maximal dyskinesias scores from both experiments were correlated with [^{3}H]Ro25-6981 specific binding in the rostral striatum (caudate nucleus: $R = 0.695$, $p = 0.0005$; putamen: $R= 0.677$, $p = 0.0007$) and in the caudal putamen ($R = 0.470$, $p=0.032$) but not in the caudal caudate nucleus ($R = 0.245$, $P = 0.284$). Hence, the present results have shown that LID and its prevention with drugs affecting glutamatergic activity were associated with modulation of NR2B/NMDA glutamate receptors.

442

One-year Follow-up of Cardiovascular Outcomes of Newly Detected Diabetic Patients Treated with Metformin or Pioglitazone (The ANSWER Trial)

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Introduction: We are conducting a randomized trial to compare the effects of Antihyperglycemic metformin with pioglitazone on cardiovascular Endpoint Reduction). The results of 48 patients who have completed a 1-year follow up are presented here. Methods: Newly detected diabetics (duration of diabetes 6 months), not on any medication were randomized to receive pioglitazone or metformin in addition to gliclazide given to all patients. The doses of medications are uptitrated at regular intervals. The primary endpoint is a combination of cardiovascular death, acute coronary event, congestive heart failure (CHF) and cerebrovascular accident (CVA). The secondary outcomes are each of the endpoints dealt individually in addition to new onset hypertension, changes in lipid profile and development of triopathy. Results: A total of 110

patients have been enrolled in the study. One-year follow-up of patients are available for 48 patients. The baseline demographic and biochemical characteristics were similar in the two groups. After 48 weeks of follow up, four patients (2 each in metformin and pioglitazone) groups developed silent ischemia. No patient has developed symptoms/signs suggestive of CHF or CVA. A statistically nonsignificant increase was noted in the number of patients who developed hypertension in metformin (5/23) group as compared to pioglitazone group (1/25)(p=0.091). At the end of one year, a non-significant improvement in the TC, LDL, HDL and TG was better in the pioglitazone group as compared to metformin group. Discussion and Conclusion: One year follow-up results of ANSWER trial show no significant difference in primary or secondary outcomes.

443

Protective Effect of Rosiglitazone on the Acid-induced Oxidative Stress of Cultured Feline Esophageal Epithelial Cells

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It was reported that drugs of the thiazolidinedione class, which are peroxisome proliferator-activated receptors-gamma (PPAR-gamma) agonists, have a potent antioxidant antiinflammatory effect. In the present study, to determine whether rosiglitazone (RSG), a selective and potent PPAR-gamma agonist, has protective effect against the oxidative stress in esophagus, we investigated the effect of RSG on the generation pathway of reactive oxygen species (ROS) by acidified medium exposure in cultured feline esophageal epithelial cells (CFEEC). Intracellular hydrogen peroxide generation was measured using fluoroprobe, 2'7'-dichlorofluorescein diacetate in CFEEC exposed to acidified medium (pH 5.5). NAD(P)H oxidase activity was determined by tetrazolium salt radical detector. Expression of NADPH oxidase (NOX), superoxide dismutase (SOD) and catalase, which are enzymes implicated in ROS generation and degradation, was determined by Western blot. Acidified medium significantly increased intracellular hydrogen peroxide generation in comparison with control, and this effect was significantly inhibited by RSG. Acidified medium increased NAD(P)H oxidase activity and NOX expression, but it decreased the expression of SOD and catalase. Pretreatment of RSG significantly increased the expression of SOD and catalase reduced by acidified medium, but did not affect on the NAD(P)H oxidase activity and NOX expression. These results suggest that RSG inhibits acidified medium-induced ROS generation through increase of expression of SOD and catalase in CFEEC. Therefore, RSG may protect epithelial cells against acid-induced oxidative stress in esophagus.

444

Polymorphism of the Hepatic Influx Transporter Organic Anion Transporting Polypeptide 1B1 is Associated with Increased Cholesterol Synthesis Rate

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Introduction: Organic anion transporting polypeptide 1B1 (OATP1B1) is an influx transporter, which mediates the hepatic uptake of several statins and endogenous compounds, such as bile acids. Our aim was to investigate the influence of polymorphism in the *SLCO1B1* gene, encoding OATP1B1, on the markers of cholesterol synthesis and absorption during baseline, and as affected by different statins. Methods: In a crossover study, thirty-two healthy volunteers with different *SLCO1B1* genotypes ingested a single dose of fluvastatin, pravastatin, simvastatin, rosuvastatin, and atorvastatin with a washout period of at least one week. Plasma total cholesterol, markers of cholesterol synthesis (desmosterol, lathosterol, and squalene) and absorption (cholestanol, campesterol, sitosterol, and avenasterol) were measured up to 12 h. Results: The mean fasting plasma desmosterol to cholesterol ratio was 40% higher in subjects with the *SLCO1B1* c.521CC variant genotype than in

those with the c.521TT reference genotype (P=0.022). The genotype had no effect on cholesterol absorption markers. All statins decreased the lathosterol to cholesterol ratio, but no significant differences existed between *SLCO1B1* genotypes in the response to different statins. Discussion and Conclusions: The low activity *SLCO1B1* c.521CC genotype is associated with an increased cholesterol synthesis rate, possibly due to reduced OATP1B1-mediated hepatic bile acid uptake. The short-term effects of statins on cholesterol synthesis were not associated with the *SLCO1B1* polymorphism.

445

Human Equilibrative Nucleoside Transporter 2 (hENT2) Mediates the p53-Dependent Genomic Response of Chronic Lymphocytic Leukemia Cells to Fludarabine

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Fludarabine is used in the treatment of chronic lymphocytic leukemia (CLL). We have analyzed the molecular mechanisms implicated in fludarabine action in a set of CLL patients bearing wild type p53 but showing different sensitivity to this nucleoside drug. The transcriptomic response triggered by fludarabine was analyzed, firstly by performing a high throughput analysis of gene expression in two sensitive and two resistant cases, and secondly by monitoring the response to fludarabine of a subset of previously identified genes (20) in cells from 10 CLL patients. Eleven of the twenty genes *(TYMS, BAX, TIGAR, FAS, TNFSF7, TNFSF9, CDKN1A, SESN1, PPM1D, OSBPL3,* and *WIG1*) were up-regulated in a manner that proved to be significantly correlated with the *ex vivo* sensitivity of CLL cells to fludarabine. Interestingly, this transcriptomic response was poorly sensitive to the hENT1 nucleoside transporter inhibitor NBTI. This is consistent with the other ENT-type isoform expressed in CLL cells (hENT2) playing a major role in fludarabine uptake into CLL cells. In summary, early events related to drug uptake processes may contribute to the variability in fludarabine responsiveness in wild type p53 CLL patients.

446

Amiodarone and its Pharmacogenetic Background

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Introduction: Amiodarone is an important antiarrhythmic agent, which possesses unique pharmacokinetic properties. Three major proteins (CYP2C8, 3A4 and P-glycoprotein) involved in transport and metabolism of amiodarone were found to be genetic polymorphic and have interindividual activity among population. Methods: Plasma levels of amiodarone and desethylamiodarone were determined by an HPLC-UV system. The genotypes of P-glycoprotein were determined by PCR–RFLP among 56 patients treated by amiodarone during long term therapy and who met the inclusion criteria. Results: Carriers of homozygous genotype of variant alleles 3435T, 2677T, 1236T, -76A MDR1 in exons 26, 21, 12 a 17 reached higher plasma levels of amiodarone (1.0159 mg/l; 0.8787 mg/l; 0.8787 mg/l; 0.8070 mg/l respectively) than patients with either heterozygous genotype (plasma levels of amiodarone 0.6449 mg/l; 0.6185 mg/l; 0,5999 mg/l; 0,6345 mg/l respectively) or homozygous wild type genotype (plasma levels of amiodarone 0.6697 mg/l; 0.7918 mg/l; 0.8207 mg/l; 0.8022 mg/l respectively). Discussion: Risk of potentially serious non–cardiac side effects after administration of amiodarone increases substantially if its plasma levels exceed therapeutic range. SNPs in *MDR1* gene of P-glycoprotein could have possible influence on amiodarone plasma levels and on its toxicity. Conclusion: Our results show that SNPs in *MDR1* gene could have the influence on the plasma levels of amiodarone, mainly in exon 26.

447

Pharmacogenetic Relevance of Cytochrome P450 2C9*3 Isophorm in a Tenoxicam Bioequivalence Study

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Genetic differences in metabolizing enzymes can directly induce a higher intersubject pharmacokinetic variability, conditioning study bioequivalence results. We analyze pharmacokinetic variability of tenoxicam related to cytochrome P450 (CYP) 2C9 in an open, randomized, crossover, phase I study with 18 healthy Spaniard volunteers. Significant increases were found when CYP2C9*2 or *3 (single nucleotide polymorphisms group, SNPg) results were compared with wildtype (wt) genotype (mean±SD) in: Mean residence time (MRT) (143±93 hours vs. 84±19 hours, respectively) ($p<0.05$) and half-life time (t½) (101± 68 vs. 60±14 hours) ($p<0.05$), requiring a larger % to calculate area under the curve from the time of dosing extrapolated to infinity ($AUC_{0\text{-infinity}}$) (17 % vs 6%) ($p< 0.05$). In fact, 2C9*2/3 profile has the major significant highest increases in MRT (446 hours), t½ (245 hours), longest % AUC extrapolated (58%) ($p< 0.001$) and thus, $AUC_{0\text{-infinity}}$ (407 microgram x h/mL) ($p<0.001$). Although our tenoxicam bioequivalence study safety was optimal, allele CYP2C9*3 associated to valuable tenoxicam concentrations following a wash period of 21 days. This is related to a reduced elimination velocity that could increase the risk of adverse events. Collectively, our findings suggest that CYP2C9*3 genotype significantly alter tenoxicam metabolism. If we randomized the subjects using genotype information, or if we exclude the CYP2C9*3 population, it would result in a lesser pharmacokinectic variation, giving us the possibility to calculate smaller sample sizes in bioequivalence studies using population data. We consider a useful approach to genotype for CYP2C9 prior to a bioequivalence study performed with drugs metabolized by this cytochrome.

448

Analysis of R-Selegiline, N-Desmethyl Selegiline, L-Amphetamine and L-Metamphetamine in Human EDTA K_2 Plasma using Tandem Mass Spectrometry Detection

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Purpose: Selegiline is a levorotatory acetylenic derivative of phenethylamine. It is an irreversible inhibitor of monoamine oxidase (MAO), an intracellular enzyme associated with the outer membrane of mitochondria. It is used in the treatment of Parkinson's disease. The purpose of this work was to develop and validate a specific and robust method for the determination of R-Selegeline (SEL) and its three major metabolites N-desmethyl selegiline (DES), L-amphetamine (AMP) and L-metamphetamine (MET) in human EDTA K_2 plasma. Methods: SEL, DES, AMP, MET and their respective deuterated internal standards (SEL-d_8, DES-d_{11}, AMP-d_6 and MET-d_5) were extracted from human EDTA K_2 plasma by liquid-liquid extraction using chlorobutane. Analysis was performed on a MDS Sciex API 4000 tandem mass spectrometer with TurboIonSpray interface. Positive ions were measured with m/z $188.1 \rightarrow 91.0$ for SEL, $174.1 \rightarrow 91.0$ for DES, $136.2 \rightarrow 91.0$ for AMP, $150.1 \rightarrow 91.1$ for MET, $196.2 \rightarrow 93.1$ for SEL-d_8, $185.2 \rightarrow 98.1$ for DES-d_{11}, $142.1 \rightarrow 93.0$ for AMP-d_6 and $155.1 \rightarrow 91.1$ for MET-d_5. The chromatographic run time was 4.2 minutes on a Zorbax SB-C18 50 X 4.6 mm column. A gradient of acetonitrile and Milli-Q type water with ammonium formate 3 mM was used to elute the compounds. Results: This assay was validated over a nominal range of 10 to 1000 pg/mL for SEL, 50 to 5000 pg/mL for DES and 20 to 8000 pg/mL for AMP and MET. Linearity over the calibration range was $\geq$ 0.9978 for all analytes. The between-run accuracy ranged from 98.15 to 102.42% with precision ranging from 2.78 to

6.06% for all analytes. The within-run accuracy ranged from 90.47 to 109.08% with precision ranging from 0.99 to 14.03% for all analytes. The recovery of all analytes and internal standards was greater than 72%. No matrix effect on quantitation was observed. SEL, DES, AMP and MET were found to be stable in human EDTA K_2 plasma after 25 hours at room temperature for short term stability, after 5 days at -20°C and -80°C for long term stability, after 104 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -20°C and -80°C. Dilution integrity, hemolysis effect and matrix selectivity were also demonstrated. Conclusions: This method is accurate, reproducible and was successfully applied for the analysis of 2215 clinical samples.

449

Pharmacokinetics of Ivermectin after Maternal or Fetal Intravenous Administration in Sheep

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In pregnant sheep at 120 – 130 days of gestational age, a study was undertaken in order to characterize the pharmacokinetics and transplacental exchange of Ivermectin after maternal or fetal intravenous administration. Eight pregnant Suffolk Down sheep of 73.2 ± 3.7kg body weight (bw) were surgically prepared in order to insert polyvinyl catheters in the fetal femoral artery and vein and amniotic sac. Following 48 h of recovery, the ewes were randomly assigned to two experimental groups. In group 1, (maternal injection) 5 ewes were treated with an intravenous bolus of 0.2 mg ivermectin/kg bw. In group 2, (fetal injection) 3 ewes were injected with an intravenous bolus of 1 mg of ivermectin to the fetus through a fetal femoral vein catheter. Maternal and fetal blood and amniotic fluid samples were taken before and after ivermectin administration for a period of 144 h post-treatment. Samples were analyzed by liquid chromatography (HPLC). A computerized non-compartmental pharmacokinetic analysis was performed and the results were compared by means of the Student t-test. The main pharmacokinetic changes observed in the maternal compartment were increases in the volume of distribution and in the half-life of elimination ($t_{½\beta}$). A limited maternal-fetal transfer of ivermectin was evidenced by a low fetal C_{max} (1.72 ± 0.6 ng/mL) and $AUC_{0\text{-infinity}}$ (89.1 ± 11.4 ng.h/mL). While the fetal administration of ivermectin resulted in higher values of clearance (554.1 ± 177.9 mL/kg) and lower values of $t_{½\beta}$ (8.0 ± 1.4 h) and mean residence time (8.0 ± 2.9 h) indicating that fetal-placental unit is highly efficient in eliminating the drug as well as limiting the transfer of ivermectin from the maternal to fetal compartment.

450

Population Pharmacokinetics of Valganciclovir in Solid Organ Transplant Recipients

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Introduction: This study aimed at describing the pharmacokinetics (PK) of ganciclovir (GCV) after oral valganciclovir (VGC) for prophylaxis or treatment of CMV infection in kidney (K), lung (Lu), heart (H) and liver (Li) recipients, at exploring the magnitude of PK variability and at identifying the influence of clinical covariates on drug disposition. Methods: A population pharmacokinetic analysis was performed with NONMEM® on 439 plasma samples from 65 transplant patients (41 K, 12 Lu, 10 H, 2 Li). 59 patients received oral VGC prophylaxis, 9 oral VGC treatment and 2 iv GCV treatment. VGC prophylactic dosage was 900 mg VGC qd in H and Lu and 450 mg qd in K recipients (adjusted to renal function). VGC therapeutic dosage was 900 mg bid (adjusted to renal function and GCV blood levels). GCV levels were measured monthly at trough and at peak (3 hours after administration).
Results: A two-compartment model with first-order absorption appropriately described the data. A dominant influence of GFR on clearance (CL = 1.36 * GFR) and of body weight on central volume of distribution (V_c = 29.8 * BW/70kg) was observed. Inter-patient variability (CV %) was 25% on CL and 16% on V_c and intra-patient variability was 22% (CV%) and 0.15 mg/L (± SD). No drug interaction was evident. Conclusion: Considering the good

predictability of GCV PK profile after oral VGC, the usefulness of systematic therapeutic drug monitoring in solid organ transplant recipients remains uncertain, in comparison with thorough dosage adjustment to body weight and renal function.

451

Endotoxin-mediated Changes in the Expression of Placental Drug Transporters in Pregnant Rats: Implications on Fetal Drug Exposure

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While the impact of inflammatory stimuli on drug transporters is well characterized in liver, the effect on placental transporters is not well established. We examined the effect of endotoxin-induced inflammation on the placental expression of several important uptake and efflux drug transporters and its impact on fetal drug exposure in rats. Pregnant SD rats were dosed with single 0.1-1.0 mg/kg ip doses of endotoxin on gestational day 17 and sacrificed at 6-24 hr. Control pregnant rats received saline. Expression of transporters was measured via real-time PCR and Western blotting. Biodistribution of glyburide, a hypoglycemic sulfonylurea and substrate of human ABCG2 and OATP2B1, was examined. Significant dose and time-dependent reductions in the mRNA levels of several ABC drug efflux transporters (*Abcb1a*, *Abcb1b*, *Abcc1-3*, *Abcg2*) and organic anion uptake transporters (*Slco1a4*, *Slco2b1*, *Slco4a1)* were observed in placentas of endotoxin-treated rats. Placental protein levels of Abcg2 were significantly decreased at 24h after endotoxin administration. While 1.6 fold higher plasma concentrations of glyburide were seen in endotoxin-treated rats, the tissue: plasma levels of glyburide were decreased in placental and fetal tissues to 45% and 20% of those seen in controls, respectively. Overall, our results demonstrated that endotoxin-induced inflammation imposed a downregulation in the placental expression of several important drug transporters, likely causing altered fetal exposure to glyburide. As glyburide is a substrate of both, influx and efflux transporters, our findings highlight the importance of considering the mutual functioning relationship of placental transporters in predicting the effects of disease on fetal drug exposure.

452

Peroxisome Proliferator-activated Receptor-alpha (PPAR-alpha) Adaptation to Amiodarone Hepatic Exposure

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The risk for adverse hepatic effects with amiodarone administration remains a concern to clinicians. Nevertheless, the use of amiodarone in therapy of atrial fibrillation continues to grow as the prevalence of this condition increases with the aging of the population. Previously, we reported that amiodarone treatment of mice increased the hepatic mRNA levels associated with various PPAR-alpha target genes, in a manner similar to that stimulated by the PPAR-alpha agonist, fenofibrate. Further investigation of amiodarone effects on hepatic mass and serum AST were carried out in wild type and PPAR-alpha knock-out mice. Amiodarone produced increases in hepatic/body mass in wild type, but not PPAR-alpha knock-out mice. Exposure to 80 mg/kg/d of intraperitoneal amiodarone increased ALT more markedly in PPAR-alpha knock-out mice than in wild type. Prior to exposure to amiodarone, half of the mice in each group commenced feeds containing 0.05% fenofibrate (w/w) in their rodent chow. This reduced the elevation in ALT associated with amiodarone treatment in wild type, but not in PPAR-alpha knock-out mice. Amiodarone appears to stimulate an indirect activation of PPAR-alpha that is associated with increased liver mass and PPAR-alpha target gene induction similar to that noted for fenofibrate. As the adverse hepatic effects of amiodarone are exaggerated in PPAR-alpha knock-out mice, we would conclude that hepatic PPAR-alpha may play a pivotal role in adapting to amiodarone exposure. Thus, adaptation to the hepatic effects of amiodarone may be augmented by clinical pre-exposure to fenofibrate which is now being investigated in patients.

453

Safety, Tolerability, Pharmacokinetics (PK), and Pharmacodynamics (PD) of Multiple Ascending Oral Doses of Vabicaserin in Healthy Adult Subjects

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Purpose: Vabicaserin is a 5-HT2$_C$ agonist in clinical development for the treatment of schizophrenia and depression. The purpose of this study was to assess the safety, tolerability, PK, and PD of multiple ascending oral doses of vabicaserin enteric-coated capsules administered to healthy subjects. Methods: This was a double-blind, placebo-controlled, multiple oral, ascending dose study in healthy subjects. Cohorts of 8 subjects (6 vabicaserin, 2 placebo) were assigned to each dose group (25, 50, 75, 100, 150, and 250 mgs capsules every 12hrs for 14 days). Assessments included adverse events (Aes), vital signs, laboratory tests, electrocardiograms (ECGs), and PK. Results: Vabicaserin was absorbed slowly, with median time to achieve peak plasma concentration of 3 to 6 hours. At steady state, vabicaserin had a mean terminal elimination half-life of 6 to 9 hours. Compared to the parent compound, relatively high concentrations of carbamoyl glucuronide were observed. Less than 1% of the administered dose was excreted in the urine. No serious Aes or withdrawals for safety reasons occurred. The most frequently reported Aes were abdominal pain, headache, nausea, diarrhea, and dizziness, which were generally mild in severity. There were no clinically relevant changes in vital signs, laboratory tests or ECG parameters**.** There were no clear effects on cognition, alertness, calmness or contentment for dose studied. Conclusions: Vabicaserin was safe and well tolerated when administered at doses of 25 – 250 mg BID for 14 days. The PK and safety of the drug are acceptable for future clinical development.

454

Comparative Bioavailability Study on Three Methylphenidate Formulations, at Single Oral Doses: 30 mg (Immediate Release Tablets), 20 mg (Modified Release Tablets), and 30 mg (Extended Release Capsules), in Healthy Volunteers

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Objectives: To evaluate the comparative bioavailability of three Methylphenidate formulations: R (one 20 mg + one 10 mg immediate release tablets); T2 (20 mg modified release tablet) and T3 (30 mg extended release capsule), regarding Methylphenidate and Ritalinic acid pharmacokinetic parameters in healthy volunteers. Secondarily the profile or incidences of any secondary effects or adverse events were described. Methodology: The study was developed in the Clinical Pharmacology Study Unit, Hospital Clínico San Carlos, Madrid. 18 subjects (9M/ 9F) were included into a randomized, open-label, 3 periods (wash-out 7 days), cross-over, bioavailability study. 18 plasma samples/ period were collected. Results: Following values for Methylphenidate parameters were obtained, median values (form R; T2; T3) for Tmax (h) were 1.7, 5, 3.5. Mean values (form R; T2; T3) for T1/2 (h) were 3.7, 4.4, 4.6; Cmax/ D (pg/ ml) were 445.4, 304.3, 331; AUClast/ D (pg · h/ ml) were 2315.8, 2213.36, 2295.64 and AUCinf/ D (pg·h/ ml) were 2428.5, 2380.2, 2486.8. Ritalinic acid pharmacokinetic parameters are in accordance to the parent drug findings. Conclusions: The results obtained are consistent with the rate and amount of absorption characteristics of the studied formulations. Statistically significant differences have been found between any of the Test formulations compared to the Reference one regarding rate of absorption (Cmax, Cmax / D, Tmax). The contrast of the parameters AUC last/ D and AUC inf/ D fall well within the interval for bioequivalence (T2 vs R and T3 vs R). There were no relevant findings concerning safety.

455

Impact of *VKORC1* Haplotypes on the Long-term Graft Survival in Kidney Transplantation

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Background: Chronic allograft injury is the major cause of renal allograft loss after the first year of transplantation and is partly related to multi-factorial arterial injury. Recently, vitamin K epoxide reductase complex subunit 1 (*VKORC1)* haplotype combinations were found to be associated to the risk of developing arterial and

venous vascular diseases. We aimed to study the effect of the *VKORC1* haplotypes on the long term survival of renal allograft in a cohort of kidney transplant recipients. Method: A total of 289 renal allograft recipients participated in the study. Long-term graft survival was measured by the estimation of the glomerular filtration rate (GFR). Arterial changes of the graft were obtained from patients' renal graft biopsies. *VKORC1* C+1173T single nucleotide polymorphism (rs9934438) was used as a tagging SNP for *VKORC1*2* haplotype. Results: Patients without or with only one copy of *VKORC1*2* haplotype (*VKORC1* +1173 CC and CT genotypes) had more pronounced deterioration in renal graft function compared to patients homozygous for *VKORC1**2 haplotype (*VKORC1* +1173 TT genotype) (OR: 2.9, 95%CI: 1.2 – 3.9, p=0.02). Among the patients who experienced a renal graft biopsy for renal dysfunction (n=130), those who had *VKORC1*2/*2* haplotype presented a lower degree of moderate to severe arterial changes than the other group (26% vs. 48%, p=0.1) respectively. Conclusion: Our results suggest that *VKORC1* haplotypes play a role on the long-term survival of renal allograft and is associated with vascular lesion of the transplanted kidney. This findings need to be replicated in prospective clinical studies.

456

Germination of *Candida albicans* and Arthrospores of *Trichophyton mentagrophytes* is Inhibited by Dimethyl sulfoxide (DMSO)

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Dimethyl sulfoxide (DMSO) is commonly used as a solvent for antifungal drugs.The author has reported earlier the inhibitory effect of DMSO on the growth of many strains of dermatophytes' colonies in dermasel agar and proposed that this could cause variations in results of different studies on the evaluation of antifungal drugs. The present study aimed to determine the effect of DMSO on the growth of germ tubes of *Candida albicans* and arthrospores of *Trichophyton mentagrophytes* (variant *interdigitale*).*C. albicans* were obtained from clinical samples and grown on glucose peptone agar. *T .mentagrophytes* were also obtained from clinical samples and grown on glucose peptone agar in the presence of 20% CO2 in air for two weeks to produce arthrospores. Candida and arthrospores were removed from the agar plates, filtered, washed and suspended in phosphate buffered saline. The yeast/arthrospore suspension was incubated in glucose peptone broth in the presence of different concentrations of DMSO (1.25 to 10%) and the percentage of germinating yeasts/arthrospores counted. With 10%DMSO there was negligible growth of germ tubes of both the yeast and arthrospores; between 2.5 and 7.5% DMSO there was a linear dose-related inhibitory effect; whereas 1.25% had insignificant effect from controls. The present study shows that besides other factors, variations in the results of the susceptibility tests of antifungal drugs might occur due to the effect of DMSO on the growth of fungi and the differences in the final concentrations of DMSO in the medium.

457

Inhibition of the Central Amygdala GABA-A Receptors Potentiates Morphine-state Dependent Memory

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The central amygdala (CeA) has an important role in mediation of morphine reward and reward-related learning. There is an interaction between opioidergic and GABAergic systems in cognitive functions. Our previous studies also indicate that the CeA is a key brain region which participates in morphine-induced impairment of memory retention. Considering that the GABAergic system is involved in memory and this system in the The central amygdala implicates in morphine reward-related learning, in the present study, we evaluated the effects of bilateral microinjections of GABA-A receptor antagonist, bicucculine into the CeA on morphine state-dependent memory in male Wistar rats. As a model, a step-through passive avoidance task was used. Animals were bilaterally cannulated in the CeA by a stereotaxic instrument, and were allowed to recover 1-week

before behavioral testing. Subcutaneous (s.c.) administration of morphine, immediately after training, dose-dependently induced impairment of memory retention, indicating morphine-induced amnesia. Pre-test administration of the same doses of morphine inhibited the amnesia in the animals under post-training morphine influence. Therefore, the present results show that morphine can produce state-dependent memory. Co-administration of pre-test bilateral microinjection of bicucculine (0.125-1 microgram/rat) into the CeA with a low dose of morphine (0.5 mg/kg) which had no effects by itself, improved retrieval of inhibitory avoidance memory and thus potentiates state-dependent memory. However, pre-test intra-CeA microinjection of the same doses of bicucculine alone did not affect memory retention. Results of the present study thus indicate that the inhibition of the central amygdala GABA-A receptors facilitates morphine-induced memory retrieval.

458

Pharmacological and Biochemical Evidence for the Role of Oxidative Stress in Theophylline Toxicity: An Experimental Study with Clinical Implications

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Theophylline is a methylxanthine bronchodilator, which has reemerged as a useful therapeutic agent in obstructive airway disease and cardiorespiratory critical care. However, its narrow therapeutic index, and the resultant toxicity potential, still restricts its use. The present experiments were designed to evaluate the possible mechanisms involved in theophylline-induced neurobehavioral toxicity, viz. anxiety and seizures, in experimental animals, in an attempt to device strategies for counteracting theophylline toxicity. Theophylline (50-100 mg/kg) induced consistent anxiogenic responses in normal and stressed rats in the elevated plus maze test. No such clearcut pattern was seen with other PDE inhibitors or adenosine agonists. However, pretreatment with antioxidants, alpha tocopherol (20 and 40 mg/kg) and melatonin (50 and 100 mg/kg), attenuated the anxiogenic response of the drug. Biochemical assay of brain homogenates showed that the theophylline-induced anxiogenic behavior was associated with enhanced malondialdehyde (MDA) levels and lowered glutathione (GSH) activity, which were normalized after antioxidant treatments. In mice, theophylline (100 – 250 mg/kg) dose dependently induced convulsions and potentiated pentylenetetrazole seizures, which were not replicated by PDE inhibitors or attenuated by adenosine agonists. Pretreatment with the antioxidants, clearly attenuated the convulsiogenic effects and post-ictal mortality of theophylline. Further, theophylline also enhanced MDA and lowered GSH levels in brain homogenates of these mice, which were reversed by the antioxidants. These results strongly suggest (a) the involvement of oxidative stress during theophylline–induced neurobehavioral toxicity, and (b) a potential role for antioxidants as an antidote for the prevention of the adverse effects of this pharmacoeconomically viable agent.

459

Interindividual Variability in the Glucuronidation of Acetaminophen in Healthy Volunteers

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Reports of the in-vitro glucuronidation of acetaminophen (APAP) using human liver microsomes indicate a ten-fold variability in k_m (6.0 to 54.5 mM) and V_{max} (1.3 to 11.7 nmol/min/mg). Although there are estimates of the metabolic constants of APAP from studies in overdose patients, there are few clinical data indicating the variability in these constants. The aim of the present study was to perform in-vivo estimation for the Michaelis-Menten constants for the glucuronidation of APAP. A two occasion, single dose, crossover trial was performed, using 60 mg/kg and 90 mg/kg doses of APAP, in healthy volunteers undergoing third molar dental extraction. Plasma samples were collected over 24 hours and urine for 8 hours after dosing. Plasma and urine were assayed for APAP and APAP-glucuronide using a HPLC method. Twenty volunteers were enrolled in the study and

data for plasma and urine were available for both doses for 13 volunteers; seven were male, the median (range) age was 22 (19 to 31) years and median (range) weight was 68 (50 to 86) kg. The median and range for k_m was 6.04 (2.01 to 20.4) mM, and for V_{max} was 843 (184 to 1877) micromol/hour/kg. The estimates for the k_m for APAP glucuronidation were lower than those previously reported by in-vitro methods, but in the same order of magnitude. In-vivo methods also demonstrated a ten-fold variability in the range for k_m and V_{max}. The degree of variability in glucuronidation of APAP might explain the inter-individual variability in susceptibility to APAP toxicity.

460

Efficacy of Topical Vimang Cream (1.2 %) in the Treatment of Mild-moderate Acne Vulgaris

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Introduction: Mango stem bark (Vimang) has been traditionally used in many countries for the treatment of menorrhagia, diarrhoea, syphilis, diabetes, scabies, cutaneous infections, anaemia, etc. using an aqueous extract obtained by decoction as reported in the *Napralert Database.* A field study in Primary Health Care (590 patients) was conducted with the *Vimang* cream formulation (1.2%) on skin disorders, mainly related to skin damage, inflammation and pain in several pathologies with good results. Aim: Our objective in this study was to evaluate the efficacy of Vimang cream (1.2 %) in the treatment of mild to moderate acne vulgaris. Methods: This was an open clinical trial. Forty five patients with mild to moderate acne vulgaris were selected to receive either Vimang cream or current treatment for acne. Patients were followed up every 15 days for a period of 3 months. The number of lesions and the acne severity index (ASI) were recorded and compared using Student's *t*-test. Results: Total lesion count was reduced by 60.6% and 20.5 % by Vimang cream and the current therapy respectively (p =0.002). ASI was reduced more with Vimang than the current therapy (p =0.001). No adverse events were found. Conclusion: Vimang cream can be used as an effective treatment in mild to moderate acne vulgaris.

461

The Effect of Lamotrigine on Anxiety in Rats as Measured by the Elevated Plus Maze (EPM) Model

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There is evidence in the literature that the anticonvulsant, lamotrigine, may also be effective in bipolar mood disorder, anxiety disorders and mania. In this study the effect of acute vs. chronic treatment on anxiety in rats, as measured by the elevated plus maze model, was investigated.
Acute study: 24 Male Sprague-Dawley rats were included (12 control and 12 test subjects). The 12 test subjects were subjected to acute severe stress (SPS) on day 1 and a re-stress procedure (swim stress) on day 7. On day 14 post restress, lamotrigine10mg/kg (or saline for controls) was administered and aversive behavior was measured. Chronic study: Experimental procedures were the same as in the acute study with the test subjects receiving lamotrigine 10mg/kg (or saline for controls) daily for 14 days. Previous studies had demonstrated that SRS plus re-stress are anxiogenic in rats. Acute treatment with lamotrigine 10mg/kg resulted in no statistically significant changes in aversive behavior. With chronic treatment, however, a statistically significant increase ($p<0.05$) was found in ratio time spent in open arms at peak drug concentration in the SPS-lamotrigine treated group compared to SPS alone. We conclude that lamotrigine shows anxiolytic activity following chronic but not acute treatment in rats as measured in the EPM.

462

Pharmacokinetic Interaction between Ketoconazole and Praziquantel in Healthy Volunteers

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Praziquantel, a broad-spectrum anthelminthic, has been reported to undergo extensive first-pass metabolism by cytochrome P450 (CYP) enzymes *in vivo*. Ketoconazole, a potent CYP3A4 inhibitor, is known to markedly increase plasma concentrations of many co-administered drugs. However, no data are available on the potential pharmacokinetic drug interaction between ketoconazole and praziquantel in humans. In an open-label, randomized two-phase crossover study, separated by a 2-week period, 10 healthy adult Thai male volunteers ingested a single dose of 20 mg/kg praziquantel alone or with co-administration of 400-mg ketoconazole orally daily for 5 days. Venous blood samples were collected at specific times for a 24-h period. Plasma concentrations of praziquantel were determined using high-performance liquid chromatography. A non-compartment model was applied for pharmacokinetic parameter analysis of praziquantel. Concurrent administration of ketoconazole with praziquantel significantly increased the mean area under the curve from time zero to infinity ($AUC_{0-alpha}$) and maximum plasma concentration (C_{max}) of praziquantel by 93% (955.94 ± 307.74 vs. 1843.10 ± 336.39 ng.h/mL; $P < 0.01$) and 102% (183.38 ± 43.90 vs. 371.31 ± 44.63 ng/mL; $P < 0.01$), respectively, whereas the mean total clearance (*CL/F*) of praziquantel was significantly decreased by 58% (2.65 ± 0.64 vs. 1.11 ± 0.35 mL/h/kg; $P < 0.01$). Ketoconazole co-administration alters the pharmacokinetics of praziquantel in humans, possibly through inhibition of CYP3A, particularly CYP3A4, first-pass metabolism of praziquantel. Our data suggested that when praziquantel is co-administered with ketoconazole, the dose of praziquantel could be reduced to half the standard dose of praziquantel to reduce the cost of therapy.

463

Microvessel Endothelial Cells (hBMVEC) 463 Regulation of P-glycoprotein (P-gp) in Human Brain M by the Human Immunodeficiency Virus Type-1 (HIV-1) Envelope Glycoprotein-120 (gp-120)

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Drug efflux membrane transporters such as P-gp and multidrug resistance associated protein 1 (MRP1) can significantly restrict the permeability of antiretroviral agents at the blood-brain barrier (BBB). HIV-1 viral envelope protein gp-120 permits HIV-1 cellular entry through its interaction with the chemokine receptors i.e., CXCR4 and CCR5. Our laboratory has previously demonstrated that HIV-1 gp-120 can trigger an inflammatory and/or oxidative stress response in cultured rat astrocytes leading to a down-regulation of P-gp and up-regulation of MRP1. In addition, human brain microvessel endothelial cells (hBMVEC) have been shown to exhibit changes in the tightness of their junctions when treated with HIV-1 gp120. We hypothesize that treatment of hBMVECs with HIV-1 gp-120 can regulate the expression of membrane-associated drug efflux transporters and that this regulation is mediated through an inflammatory and/or oxidative stress response. hCMEC/D3, a cell-culture system originally derived from hBMVECs has been well characterized for retaining BBB properties. We have confirmed through immunoblot analysis, endogenous expression of chemokine receptors (CXCR4, CCR5) and drug efflux transporters (P-gp, MRP1) in the cell system. Furthermore, hCMEC/D3 treated with HIV-1 gp120 Bal (R5 tropic) or HIV-1 gp120 MN (x4 Tropic) at various concentrations (0.83 – 1000pM) for 4 hours led to a concentration-dependent increase in P-gp expression up to 4-fold (R5) and up to 2-fold(x4) when compared to untreated cells. Studies are presently ongoing to examine the role that inflammation and/or oxidative stress can play in regulating the observed P-gp response.

464

Allele and Genotype Frequencies of CYP2C9, CYP2C19 and CYP2D6 in a Cuban Population

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Polymorphic cytochrome P450 isoenzymes (CYPs) 2C9, 2C19 and 2D6 metabolize many

drugs. This polymorphism gives rise to interindividual and interethnic variability in the metabolism and disposition of therapeutic agents and may cause differences in their clinical response. Frequencies for the major CYP2C9, CYP2C19 and CYP2D6 mutated alleles and genotypes were evaluated in 140 Cuban unrelated healthy volunteers (73 males and 67 females). Genotyping was performed on peripheral leukocytes DNA by PCR-RFLP method. Genotype frequencies were in Hardy–Weinberg equilibrium. Results showed: 101 subjects (72.1 %) expressed CYP2C9*1/*1, 22 (15.7 %) expressed CYP2C9*1/*2 genotypic and 3 (2.1 %) presented the mutated allele CYP2C9*2/*2 (poor metabolisers). The rest were heterozygous for CYP2C9*1/*3 (8.6 %), no CYP2C9*3/*3 was found. For CYP2C19, 137 volunteers expressed the CYP2C19*1 (75 % homozygous and 22.3 % heterozygous), two subjects were homozygous for CYP2C19*2/*2 (2.1%) (poor metabolisers), no CYP2C19*3 was detected. Genotypes more represented for CYP2D6 were: *1/*1 (59.3 %), CYP2D6*1/*10 (20.7 %) and CYP2D6*1/*17 (10.7 %). CYP2D6 *10/*10 and CYP2D6*17/*17 were found in three volunteers (1.4 and 2.1 % respectively, poor metabolisers). Frequency of apparition of CYP2D6*3 was low, one subject expressed *1/*3 and other *3/*10. Frequencies of the allelic variants of these CYPs in Cubans were similar to those of Spanish and Africans; a high frequency of CYP2D6*17 highly present in Africans but not in Caucasians. For CYP2C9 and CYP2C19, the allelic distribution was similar to Caucasians, especially CYP2C9*3 were not found in the frequencies reported in Spanish or Africans. Results fit to Cuban population origins.

465

Comparison of Resiniferatoxin and Olvanil on Blood Pressure, Temperature and Feeding in the Ferret

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Resiniferatoxin (RTX) is an ultrapotent vanilloid that has potential in the treatment of detrusor hyperreflexia and emesis. However, it is pungent and this limits its clinical use. Olvanil (OLV) is a less pungent vanilloid that also weakly blocks anandamide uptake. In the present investigations, we used the ferret to compare the potential toxicity of the compounds. The studies were done in comparison with the anandamide membrane transport uptake inhibitor, AM404. Male ferrets (1.1-2.4 kg) were implanted with PhysiolTel® C50-PXT transmitters (DSI). 1 week post surgery, the animals were injected s.c. with RTX (0.1 mg/kg), OLV (0.05-5 mg/kg), AM404 (10 mg/kg), or vehicle (10% ethanol, 10 % Tween 80, 80 % saline; 1 ml/kg) and physiological data were acquired via radio-telemetry for 22 h. Basal diastolic and systolic pressures were 105.0±3.9 and 131.1±3.7 mmHg respectively, and heart rate was 224.7±7.9 bpm (n=24). Body temperature was 38.1±0.2 ˚C (n=24). RTX induced transient hypertension and hypothermia (a fall of approximately 5˚C) and reduced generalized locomotor activity (distance travelled) and the number of episodes of drinking and feeding ($P<0.05$); OLV and AM404 were inactive. None of the compounds affected the number of episodes of walking, sleeping, curling-up, or the episodes of defecation/micturition ($P<0.05$). These studies indicate that OLV has a favourable profile compared to RTX following peripheral administration, demonstrating the possibility of using non-pungent vanilloids for the treatment of a wider number of medical conditions. These studies were fully supported by the RGC of Hong Kong (CUHK4527/05M).

466

Pharmacokinetic Profile of Amlodipine Besylate among Indonesians

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Amlodipine is a calcium antagonist of the dihydropyridine class. It widely used as antihypertensive and antiangina. Pharmacokinetic profile in Indonesian people of this drug is limited. The objective of this study was to evaluate the pharmacokinetic profile of amlodipine besylate10mg single dose in Indonesian people. Forty eight healthy Indonesian subjects non-smokers (age in the range 21 – 38

years and BMI within the range of 18 – 25) were enrolled in this study. The subjects were given a single oral 10-mg dose of amlodipine and blood samples taken serially until 144 hour. Amlodipine plasma concentrations were determined with a validated HLPC method. Pharmacokinetic parameters C_{max}, t_{max}, $AUC_{0-\infty,\ and}$ $T_{1/2}$ are calculated and Blood pressure was measured and adverse events monitored.The mean values of pharmacokinetic parameters were as follows (mean ± SEM), C_{max} value is 13.43 ± 0.39 ng/ml, $AUC_{0-\infty}$ value is 432.24 ± 17.883, $t_{1/2}$ value is 47.32 ± 1.34 hour, and t_{max} valueis 5.83 ± 0.16 hour. Headache was the most common side effect, and it was more frequently observed. The results show that the pharmacokinetic profile of amlodipine besylate is within the range of profile that has been reported in the other population.

467

Decreased AMPA GluR2 (but not GluR3) mRNA Expression in the Rat Amygdala and Dorsal Hippocampus Following Morphine-induced Behavioral Sensitization

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Repeated administration of opioid receptor agonists elicits a progressive enhancement of drug-induced behavioral responses, a phenomenon termed behavioral sensitization. These changes in behavior may reflect plastic changes requiring regulation of AMPA receptor function. In the present study, rats were treated for 7 d with saline or morphine (10 mg/kg). After a washout period of either 24 h or 7 d, locomotion, oral stereotypy and state-dependent memory in a passive avoidance test were measured in the presence or absence of CNQX (3 mg/kg), an AMPA receptor antagonist. Meanwhile, real time RT-PCR was used to evaluate mRNA expression of the AMPA receptor subunits GluR2 and GluR3 in the striatum, prefrontal cortex, hippocampus, hypothalamus and amygdala of animals treated repeatedly with morphine. The results indicate that repeated morphine treatment followed by a 7 d (but not 24 h) washout period produces behavioral sensitization. AMPA receptor blockade with CNQX on the test day did not alter these behavioral responses. Additionally, repeated morphine treatment followed by a 7 d (but not 24 h) washout period decreased GluR2 mRNA expression in the ignific (by 50%) and hippocampus (by 35%). Repeated morphine treatment did not alter GluR3 mRNA expression in any brain area assessed. These data imply that AMPA receptors are involved in the development (but not expression) phase of behavioral sensitization. These decreases in GluR2 expression in these two brain regions may result in the formation of calcium permeable AMPA receptors, which are believed to play an important role in behavioral sensitization.

468

Effects of San'o-shashin-to-A Japanese Kampo Medicine, on Mechanical Changes in Perfused Ischemic Hearts of Ovariectomized Rats

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San'o-shashin-to (SST), one of the Japanese traditional medicines called Kampo, has been used to treat hypertension and/or menopause associated symptoms. The aim of this study is to clarify the effects of SST on ischemia/reperfusion-induced cardiac dysfunction in ovariectomized rat hearts. Nine-week-old female Wistar rats were ovariectomized (OVX) and orally administered 750 mg/kg/day of SST suspension (SST group) or water (control group) once a day. After 4 weeks, hearts were isolated to set on a Langendorff apparatus and exposed to 30 min of global ischemia followed by reperfusion for 60 min. Changes in left ventricular (LV) function and coronary flow were observed. Myocardial contents of high-energy phosphates were also measured. In addition, free radical scavenging action of STT was examined by means of 0.1 mM of 1,1-diphenyl-2-picryl-hydrazyl (DPPH). In SST group, ischemic contracture during ischemia was significantly attenuated and cardiac dysfunction after reperfusion tended to be ameliorated. Especially,

LV end-diastolic pressure was significantly improved. Myocardial ATP content in SST group was significantly higher than that in control group, though there were no significant differences in inorganic phosphate and creatine phosphate contents between 2 groups. Meanwhile, SST scavenged the stable free radical DPPH with an IC50 of 4.4 mg/mL.In conclusion, results demonstrated the cardioprotective properties of SST in ovariectomized rats associated with increased ATP production. These ameliorating effects may be linked with the ability of SST to scavenge free radicals.

469

Pharmacokinetics, Pharmacodynamics and Tolerability of Satavaptan, a Selective Vasopressin V_2 Receptor Antagonist: A Single Ascending Dose Study

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Introduction: Satavaptan is a highly selective oral vasopressin V_2 receptor antagonist that promotes free water excretion (aquaresis) and that has potential clinical use in disorders involving water retention and hyponatremia. Methods: Forty-eight healthy male subjects were randomized in a double-blind study to single oral doses of satavaptan from 5 to 200 mg or placebo (6 active/2 placebo for each dose). Standard meals with fixed sodium content (2g/meal) were given, and water intake was standardized. Blood was sampled up to 48 hours. Plasma was analyzed with a validated LC-MS/MS method. Results: Median times to maximum satavaptan plasma concentrations ranged from 1 to 1.5 hours. C_{max} and $AUC_{0–24}$ increased by 2.61 and 2.46 fold, respectively, with doubling dose. Mean terminal half-lives ranged from 6.6 to 13.1 hours. Satavaptan caused dose-proportional increases in urinary volume, urinary flow rate, and free water clearance. Onset of action was within 2 hours of dosing. The increase in urinary volume was associated with a decrease in urine osmolality and was followed by an increase in plasma osmolality. The consequences were increases in serum sodium levels (with mean levels after 4 hours ranging from 139.8 mmol/L [5mg] to 144.5 mmol/L [200mg]), plasma levels of vasopressin, and a sensation of thirst. Urinary excretion of electrolytes was slightly affected, as expected with an aquaretic product. Adverse events included headache, orthostatic decrease in blood pressure, and dizziness. There were no serious adverse events. Conclusions: Satavaptan is well-tolerated and induces dose-dependent aquaretic effects at single doses of up to 200 mg.

470

Time to Onset and Postoperative Pain Control of 4% Articaine and 0.5% Bupivacaine (both with 1:200,000 epinephrine) in Lower Third Molar Removal

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Local anesthetics represent one of the main therapeutical agents to provide intra and postoperative comfort in lower third molar removal. Objective: Comparison of the clinical efficacy of 4% articaine (A200) and 0.5% bupivacaine (B200), both with 1:200,000 epinephrine, for lower third molar removal.
Methods: Fifty patients underwent removal of symmetrically positioned lower third molars, in two separate appointments, under local anesthesia either with A200 or B200, in a double-blind, randomized, and crossover manner. Time to onset, duration of postoperative analgesia, duration of anesthetic action on soft tissues and quality of wound healing were analyzed. Results: A statistically significant difference between the time to onset of A200 (1.66 ± 0.13 min) and B200 (2.51 ± 0.21 min) was found ($P < .05$). There was no statistically difference in the duration of analgesia (3-4 h; $P > .05$). However, when patients received B200 they experienced a statistically significant longer period of anesthesia on the soft tissues as compared with when they had received A200 (around 5 h and 4 h, respectively, $P < .05$). The surgeon's rating of quality of wound healing was similar for both local anesthetics (1.25 ± 0.09 and 1.05 ± 0.05 A200, with and without osteotomy respectively, 1.39 ± 0.11 and 1.14 ± 0.08 B200, with and without osteotomy, $P>.05$). Conclusions: In comparison with 0.5% bupivacaine, 4% articaine (both with 1:200,000 epinephrine) provided a

shorter time to onset and comparable postoperative pain control with a shorter duration of soft tissue anesthesia in lower third molar surgery.

471

Morphine Plasma Monitoring by GC-MS in Patients Controlled Analgesia (PCA) Pump in the Postoperative Period of Cardiac Surgery

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Introduction: Morphine administration using patient controlled analgesia (PCA) for treatment of post surgical and traumatic pain has been a current practice in many hospitals. However, large or repeated doses of this opioid are associated to dose dependent adverse events, including, respiratory depression. Considering patients submitted to thoracic surgery, in addition to the postoperatory analgesia, two other relevant parameters must be considered: regional anesthesia (intrathecal) in the intraoperatory period, which should contribute to the respiratory function improvement and decrease in the extubation time; and the cardiopulmonary bypass (OPCAB), that potentially alters the drugs' kinetics. Objectives: To investigate the influence of intrathecal morphine administration and cardiopulmonary bypass (OPCAB) in the morphine PCA drug requirements, area under the curve of morphine plasma concentration versus time and pain scores in the postoperative period. Consequently, an analytical method GC-MS was developed to quantify morphine in plasma. Methodology: 28 coronary patients of both genders with indication of elective cardiac surgery with (Off pump) and without cardiopulmonary bypass (CPB) were selected and included; the characteristics of patients were: 63 yrs, 65 kg, 165cm, 24.2 kg/m2 BMI, medians. All patients signed the informed consent to participate in the protocol. Morphine PCA was performed by a venous catheter after the orotracheal extubation in the recovery room after surgery. A serial of blood samples were collected from venous catheter of patients at the postoperative period and only 0.5mL of plasma was required for drug solid-phase extraction Bond Elut-Certify®, 50mg followed the quantification of morphine (after derivatization BSTFA) by CG-MS System model HP 6890. Pain scores were monitored during the same period by a visual analogue scale, VAS. PK-PD modelling was applied.Results: GC-MS method was adequate for drug measurements in plasma for pharmacokinetic-pharmacodynamic studies. Validated method presented 2-1000ng/mL linearity, 4.7% e 8.6% intra- inter-day precision, 3.1% systematic error and 95% relative recovery. Drug dose requirements and analgesia were higher in patients of CPB group than off pump group and the PK-PD modelling was an important tool to demonstrate the differences between them. Conclusion: Higher doses of morphine by PCA were required for CPB patients compared to Off pump patients. PK-PD modelling was useful to investigate postoperative analgesia, dose requirements and morphine plasma levels.

472

Evaluation of Cardiac Safety in Pitavastatin, the First Statin to Undergo TQT Testing

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Objective: The statins as a class are one of the most widely prescribed in the US. Most statins were approved for use prior to the adoption of the ICH E14 Guidance therefore none of the statins have undergone repolarization safety testing as part of licensure. This study represents the first TQT testing of a statin. Method: Study data was collected from 176 normal healthy volunteers who were tested in four randomized treatment groups in a double-blind, parallel study design. Groups either received placebo, a therapeutic dose of 4 mg pitavastatin, a supratherapeutic dose of 16 mg pitavastatin, or 400 mg moxifloxicillin. Results: Assay sensitivity was clearly established in the moxifloxicillin group. There was no evidence of QT prolongation in the pitavastatin treated subjects. Conclusion: Pitavastatin, an investigational statin, does not exhibit evidence of QT prolongation. This is the first statin to undergo the scrutiny of a thorough QT trial.

473

Impact of *CYP1A2* Polymorphisms on Perphenazine Clinical Outcome in Schizophrenic Patients

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Perphenazine is a classical antipsychotic drug with a potential for inducing extrapyramidal side effects (EPS). The drug is metabolized, at least partially, by the polymorphic cytochrome P450 (CYP) isoform 1A2. The aim of the present study was to investigate the impact of polymorphisms in the *CYP1A2* coding gene on the steady state plasma levels and the short term clinical effects of perphenazine therapy in schizophrenic patients. Forty-seven Estonian inpatients were evaluated before and after 4-6 weeks of perphenazine treatment (4-48 mg/day) for psychotic symptoms by Positive and Negative Symptom Scale (PANSS), and for the presence of EPS by Simpson-Angus rating scale (SAS) and Barnes scale (BAS). The patients were genotyped for *CYP1A2* by PCR-based methods, and the steady-state plasma levels of perphenazine analyzed by HPLC. Most of the patients (n=37) responded to the treatment (>20% reduction in PANSS score). Homozygousity for an haplotype including alleles coding for normal/enhanced CYP1A2 activity (-3860G/2467T/-739T/-729C/-163A) was significantly associated with good antipsychotic response ($p<0.01$). Subjects carrying this haplotype had lower, though not statistically significant, perphenazine concentration-to-dose (C/D) ratios compared to subjects carrying other haplotypes (median C/D 0.07 vs 0.11, respectively). No significant association was observed between the polymorphisms and EPS occurrence. The results of our study suggest the existence of an association between *CYP1A2* polymorphisms and therapeutic outcome in schizophrenic patients treated with perphenazine monotherapy.

474

Test-retest Reliability of Pharmacodynamic Measures used in Abuse Potential Studies: Evidence from an Immediate Release Formulation of Hydromorphone

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Introduction: Abuse potential studies are typically conducted using a cross-over design since subjective responses to drug effects are susceptible to inter-individual variability. In these studies, subjects receive several doses of the test drug and active comparator, thus reproducibility of the pharmacodynamic (PD) responses is an important factor to consider in interpretation of the subjective measures. The current study, which was part of a larger double-blind, six-period cross-over study, quantitatively examined the test-retest reliability of subjective PD measures using an immediate release formulation of hydromorphone, an opioid agonist with recognized abuse potential. Methods: After completing a qualification session to ensure they could distinguish and like the subjective effects of hydromorphone, healthy subjects with a history of recreational opioid use received single doses of oral 8 mg hydromorphone on two separate occasions separated by a minimum of 14 days. Following each treatment, subjects completed a series of PD assessments, including Visual Analog Scales (VAS) of drug liking and drug effects, Addiction Research Center Inventory with Cole Modification (Cole/ARCI), Subject-rated opiate scale and Subjective Drug Value (SDV) at several time points up to 48 hours post-dosing. Results: VAS scores showed high test-retest reliability ($r=0.63$), as did the Subject-rated opiate scale and SDV. ARCI subscales demonstrated good reproducibility, however Stimulation-Euphoria subscale scores were lower upon retest ($p<0.05$). Discussion: The subjective PD measures assessed in the current study exhibited robust test-retest reliability indicating that the repeated testing typically used in abuse potential studies does not significantly influence subjective drug effects in healthy recreational drug users.

475

Increase of Disulfiram Biodisponibility in a Complexed Formulation with Hydroxypropyl-Beta-Cyclodextrin

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Alcohol dependence, a major problem of Public Health, is multifactorial and for this reason, a strategy of biopsychosocial boarding is required. A pharmacological approach to support the biopsychosocial treatment of the alcohol dependence is the use of disulfiram, a drug that interferes with the metabolism of the alcohol inhibiting the action of aldehidodehidrogenase. The acetaldehyde accumulation produces an aversive reaction to alcohol ingestion in the patient, which helps to reinforce the decision of the abstinence. In the present work we determined the biodisponibility of an inclusion complex of disulfiram hydroxypropyl-beta-cyclodextrin in healthy participants in a cross-over nonreplicated design. Nine participants received a tablet of 500 mg of oral Antabus®; after a washout period of two weeks they received the same dose of complexed formulation. Other 9 subjects were initially treated with the complex and after the washout period they received plain disulfiram. Disulfiram and its metabolites were measured in blood samples by means of HPLC/UV. Blood samples were obtained at 30 min intervals during the first 4 hr after administration and then by two hr intervals until 12 hr. Results indicated that the area under the curve (AUC) and peak concentration (Cmax) of the complex (AUC = 0,617 µgxh/mL and Cmax = 0.131 µg/ml) were statistically higher than the AUC obtained by the same dose of plain disulfiram (AUC = 0,227 µgxh/mL and Cmax =0.035 µg/ml). We conclude that complex of disulfiram hydroxypropyl-beta-cyclodextrin modifies the physicochemical properties of disulfiram, increasing its solubility and bioavailability.

476

Changes in Serum Cytokine Levels in Rats with Experimental Periodontal Disease

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Cytokines are related to the development and progression of inflammation, and can be classified as accelerating or suppressing alveolar bone absorption. It is well-known that after the production of interleukin-1β(IL-β), interleukin-6(IL-6) and tumor necrosis factor-α(TNF-α) are produced, and pathological conditions specific to inflammatory stimulation occur. It has been confirmed that such inflammatory cytokines increase in gingival tissue and exudates from gingival crevices in patients with periodontal disease. In this study, we measured the plasma levels of IL-1β, IL-6 and TNF-α in rats with experimental periodontal disease (ODUS/Odu), an animal model of periodontal disease developed by our laboratory, which has reached the 104th generation as an inbred strain. In the experiment, the plasma cytokine levels were measured at the initiation of the experiment and 1, 3, 6, 9, and 12 months in ODUS/Odu and control rats (plaque-resistant rats:Res) using an ELISA kit. The IL-1β andTNF-α levels were significantly greater in ODUS/Odu than in Res at all examination time points ($p < 0.001$). The IL-6 level was significantly greater in ODUD/Odu than in Res at 6, 9, and 12 months ($p < 0.05$). These findings were similar to those in patients with periodontal disease, suggesting that ODUS/Odu are serve as a useful animal model.

477

Tolvaptan Decreases Pulmonary Capillary Wedge Pressure in Heart Failure Subjects: Lack of Relationship between Pharmacokinetics and Pharmacodynamics

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Tolvaptan (TLV) is a non-peptide V2 receptor antagonist that produces a pronounced aquaresis. The objective was to compare the effect of a single dose of TLV 15, 30, and 60 mg to placebo

on the peak change from baseline in pulmonary capillary wedge pressure (PCWP) from 3 to 8 hours postdose. The study enrolled 181 adult subjects with heart failure (NYHA class 3/4, ejection f¾tion ≤40%, b¾line PCWP>18 mmHg). Maximum peak plasma concentration (Cmax) and the area under the curve from 0-8 hours increased linearly with dose. Mean (SD) Cmax values were 165 (58), 314 (146) and 657 (302) ng/mL for 15, 30 and 60 mg, respectively. The median time of Cmax was 2 h for all TLV doses. Mean peak PCWP was significantly decreased -2.64, -1.82 and -1.91 mmHg for 15, 30 and 60 mg TLV, respectively, when compared to placebo ($p < 0.05$ for all TLV vs placebo). In all TLV groups, decreases in mean PCWP were observed as early as 2 hours postdose and continued to decline through 8 hours. For the placebo group, mean PCWP from 2 to 8 hours seemed to fluctuate around the value of the reduction obtained at 2 hours. All TLV doses produced the same increase in urine excretion rate and decrease in free water clearance (FWC) at 2 hours postdose; for the placebo group, urine excretion rate and FWC were decreased compared to baseline values. There was no apparent relationship between TLV concentrations and changes in PCWP, urine volume and FWC.

478

Abuse Potential Assessment of an Oral Osmotic-controlled Extended Release Hydromorphone vs. Immediate Release Hydromorphone

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Introduction: Hydromorphone (HMO) is a semisynthetic opioid tthat has experienced significant increases in its abuse. A controlled release (CR) formulation of HMO using Osmotic Release Oral System technology has been developed to provide sustained pain relief and potentially reduce its likelihood for abuse by delaying its onset of action. This study assessed the abuse potential of HMO CR compared with HMO immediate release (IR). Methods: In this double-blind, placebo-controlled, randomized, six period crossover study, subjects with a history of recreational opioid use received single oral doses of placebo, HMO IR (8 mg) and HMO CR (16, 32 and 64 mg). Subjects completed a series of assessments, e.g., subjective effects Visual Analog Scales (VAS), Subjective Drug Value (SDV), at several time points up to 48 hours post-dosing. Results: Independent of formulation, maximum Drug Liking was greater for HMO vs. placebo ($p<0.05$). Drug Liking was higher for 8 mg IR vs. 16 mg CR, but similar to 64 mg CR, and occurred earlier (2 vs. 15 hr). Bad drug effects were greater for HMO CR vs. IR (64 mg CR vs. 8 mg IR, $p<0.05$). SDV was higher for 8 mg IR vs. 16 mg and 32 mg CR, but comparable to that of 64 mg CR. Discussion: HMO CR doses of 64 mg were required to produce the same peak drug liking as 8 mg IR HMO. Delayed onset of good effects and prominent bad effects of HMO CR suggest that this formulation may have lower abuse potential than HMO IR.

479

Amantadine Acetylation in Cancer Patients and Utility as a Diagnostic Biomarker

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Background: We hypothesized that amantadine acetylation would serve as a biomarker for malignancy, since it occurs only by spermine/spermidine acetyltransferase, an enzyme upregulated in tumour tissue. Methods: In a phase 2A clinical investigation, we administered a 200 mg dose of amantadine HCl 2 hr after supper to cancer outpatient volunteers, and collected their urine for 12 hr thereafter. Coded specimens were analyzed for their content of acetylamantadine, using a newly developed LC/MS assay. Results: One hundred patients completed this study, age range 30 – 83 yr (median 62 yr). Ingestion was confirmed by quantitation of unchanged amantadine in the urine. Total urinary creatinine was used as an estimate of completeness of the sample. Using a newly developed LC/MS assay, all patients had detectable acetylamantadine in their urine, with a median concentration of 5.9 ng/ml (3.5 – 9.1 ng/ml interquartile range). Patients with lung cancer were the most frequent

in this study and 21/40 excreted urinary acetylamantadine at concentrations higher than the study median. In 6/9 patients with hematological tumours, urinary acetylamantadine concentration was above the study median. In patients with tumours of the gastrointestinal tract, 10/16 excreted acetylamantadine in concentrations greater than the study median. It remains to be determined what the normal excretion of this metabolite is in healthy adults without cancer, and this study is under way. Conclusion: These initial findings provide support for further investigations to determine the utility of this simple intervention as a useful screening test for the presence of cancer in the human population.

480

Methadone Inhibits CYP2D6 Metabolism of Codeine and Tramadol in Humans

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Although methadone is predominantly a CYP3A4 substrate it can inhibit CYP2D6 metabolism of other drugs in human liver microsomes. There is less evidence for this drug-drug interaction in humans. The CYP2D6 prodrugs codeine and tramadol are sometimes given to methadone subjects to relieve their pain, even though they are opioid tolerant. We compared the CYP2D6 O-demethylations of codeine and tramadol in genotypic *CYP2D6* extensive metaboliser methadone (MMT) and buprenorphine (as a control group) (BMT) maintenance therapy subjects. They received a single oral dose of either 60 mg codeine (n=10 MMT; n=9 BMT) or 100 mg tramadol (n=9 MMT; n=7 BMT). Urine was assayed for morphine plus glucuronides and codeine and, tramadol and its M1 metabolite. Urinary metabolic ratio of metabolite to parent drug was calculated. Methadone significantly reduced the metabolic ratio for codeine (BMT 2.46±2.34 versus MMT 0.65±0.38, p = 0.004) and tramadol (BMT 0.28±0.21 versus MMT 0.058±0.026, p=0.0002). Methadone significantly inhibited the CYP2D6-mediated O-demethylations of codeine and tramadol to their respective active metabolites morphine and M1. Thus, methadone is similar to quinidine which inhibits but is not a CYP2D6 substrate *in vivo.* Consequently, additional and unpredicted drug-drug interactions for people in methadone maintenance therapy programs can be expected whereby methadone inhibits the metabolism of other CYP2D6 metabolised drugs such as anti-depressants with the potential to cause toxicity. Notwithstanding opioid-induced tolerance, codeine and tramadol will not be effective analgesics in methadone maintenance therapy subjects.

481

Modeling and Simulation in Cytokine Induction and Physiological Changes from The Human Endotoxin Challenge Model

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Experimental endotoxin administration to healthy volunteers elicits a short-lived, inflammatory, cytokine response (ICR) that results in physiological responses (PR) that can be used as a model in the development of new chemical entities. Hence, ICR and PR modeling after endotoxin challenge (EC) and a compound (CA) effect on ICR and PR was attempted. ICR and PR data after EC were pooled from 4 healthy volunteers clinical trials (n=27). ICR and PR data after CA administration were from the literature (Clin Exp Immunol 2991;124;16-20). TNF-alpha, IL-6 and IL-8 were modeled as changes in Cytokine=K_{in}*[Stimulator]-K_{out}*[Cytokine], while LPS, TNF-alpha, IL-6 are stimulators of TNF-alpha, IL-6 and IL-8, respectively. Heart rate (HR), body temperature (TEMP) and systolic/diastolic blood pressure (SBP/DBP) were modeled as changes in PR=CircadianEffects+Emax*[Cytokine]/(EC_{50}+[Cytokine]). The Model was validated using CA effect on ICR and PR using direct TNF-alpha production inhibition as $K_{in}CA=K_{in}*(1-[CA]\wedge^{gamma}/(IC_{50}\wedge^{gamma}+[CA]\wedge^{gamma}))$. K_{in}s and K_{out}s for TNF-alpha, IL-6 and IL-8 were 4.02, 1.72 and 8.81/hr and 1.15, 1.65 and 7/hr, respectively. IL-8 changed HR, TEMP and SBP, while TNF-

alpha did DBP, Emaxs for HR, TEMP, SBP/DBP were 25.6 beats, 3.48 F, 1.65/1.89 mmHg, respectively. Maximum CircadianEffects for HR, TEMP, SBP/DBP were 18.5 beats, 1.54 F, 11.1/9.5 mmHg, respectively. IC_{50} and gamma of CA against TNF-alpha were 1.02 mg/mL and 0.0734, respectively. This model was able to describe the ICR and PR and CA effect on ICR and PR. Hence, the current model can be used in drug development targeting ICR and PR employing EC model.

482

Pharmacokinetic Characterization of Mycophenolate Mofetil and its Metabolite, Mycophenolic Acid, in Normal Healthy Volunteers

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Mycophenolate mofetil (MMF) is an immunosuppressive used in the prevention of graft rejection. MMF has been reported to be undetectable following oral administration due to its rapid esterification to mycophenolic acid (MA). By developing an analytical method with a previously unachieved lower limit of quantification (20pg/mL), we were able to directly quantify of MMF in plasma. To our knowledge, this is the first description of the pharmacokinetic parameters of MMF. Pooled data from studies conducted with healthy volunteers, using a single oral dose of 500mg MMF (CellCept®) under both fasting and fed conditions, were considered for the purpose of this work. Under fasted conditions, mean C_{max} of 2.5ng/mL and 13.0ug/mL were observed for MMF and MA, respectively. The mean t_{max} for both molecules was observed around 0.9 hours. MMF disappeared quickly from the circulation (mean half-life of 2.3 hours) whereas MA was eliminated more slowly (mean half-life of 15.7 hours). On a molar basis, the relative exposure of MMF (mean AUC_{0-t} 0.0053nmol*h/mL) was found to be ~17 000 fold lower than MA (mean AUC_{0-t} 91.5 nmol*h/mL). It was observed that the presence of food decreased the mean C_{max} to 8.9ug/mL for MA while increasing it to 4.0ng/mL for MMF. Our results showed that MMF pharmacokinetic profiles could be reliably characterized when using a very sensitive assay. However, the relative exposure to the parent MMF is negligible compared to the contribution of the MA active metabolite; the observed variability in the pharmacokinetic parameters was almost doubled for MMF compared to MA.

483

Renin-angiotensin System in Renal Ischemia and Reperfusion in Rats

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Renal ischemia/reperfusion (RIR) injury is associated with microvascular dysfunction. The renin-angiotensin system (RAS) plays an important role in the renal hemodynamic control. The present study investigates the renal profile of RAS components using a RIR model. Male Wistar rats were anesthetized (40 mg/Kg thiopental) and the left kidney was excised. Ischemia (45min) and reperfusion (4h) was performed in the right kidney. Renal and plasma angiotensins (Ang II, Ang-(1-7) were determined by radioimmunoassay. Renal mRNA for Mas and AT1 receptors and for ACE, ACE 2 and CPN was measured by real time PCR and their enzyme activities were determined by fluorimetry. Renal Mas protein expression was analyzed by immunohistochemical and western blot studies. Plasma levels of Ang II and Ang-(1-7) were not changed by RIR. However, RIR increased the content of renal Ang II (5.28±1.49, n=7 vs CTL, 1.46±0.47ρg/mg total protein, n=8). In contrast, renal Ang-(1-7) was decreased by RIR (1.73±1.25, vs CTL, 7.89±2.39 ρg/mg total protein, n=8). RIR also decreased renal expression (in arbitrary units) of AT1 receptors (0.32±0.03 vs CTL,1.03±0.15, n=4), ACE 2 (0.36±0.013 vs CTL, 1.18±0.24, n=4) and CPN (0.65±0.12 vs CTL, 0.19±0.04, n=5). In addition, RIR reduced renal ACE activity from 0.41±0.06 (CTL) to 0.12±0.03 μM.min-1.mg-1(n=8). Surprisingly, RIR increased renal expression of Mas receptor from 1.04±0.22 (CTL) to 5.80±2.05, n=4), which was confirmed by immunohistochemical and

western blot analysis. Increased renal expression of Mas receptor associated with changes in renal RAS related peptidases suggest that the vasodilatory RAS may play an important role in RIR process.

484

Evaluation of Istradefylline Metabolism in *in vitro* Systems

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Introduction: Istradefylline, a selective adenosine A_{2A} receptor antagonist, is in late stage clinical development for the treatment of Parkinson disease. In vitro studies were conducted to identify CYP isozymes involved in istradefylline metabolism and to evaluate istradefylline's inhibitory effect on CYP activities. Method: Metabolite formation was assessed following istradefylline incubation with recombinant CYPs or co-incubation with selective CYP inhibitors in human liver microsomes (HLMs). Istradefylline's inhibitory effects were evaluated in HLMs and hepatocytes using model substrates of individual CYPs with and without pre-incubation. Results: When istradefylline was incubated with HLMs, 4'-*O*-demethyl istradefylline, M1, 1beta-hydroxylated istradefylline, M8, and 3',4'-*O*-didemethyl istradefylline, M3, were formed. Ketoconazole completely inhibited M1, M8, and M3 formation and sulfaphenazole partly inhibited M8 and M3 formation. These three metabolites were also formed in recombinant microsomes expressing CYP3A4, CYP1A1, CYP2C9, and CYP2C18 isozymes. Of CYP selective metabolic activities, testosterone 6beta-hydroxylation and nifedipine oxidation were inhibited by istradefylline and the inhibition was greater following pre-incubation with istradefylline. In addition to irreversible inhibition, radioactivity derived from istradefylline was bound to microsomal protein. However, the inhibition toward testosterone 6beta-hydroxylation activity was weaker and the extent of irreversible binding to microsomal protein was significantly lower when istradefylline was incubated with human hepatocytes. Discussion and conclusion: The primary CYP isoenzyme responsible for istradefylline metabolism was CYP3A4. Other CYP isoenzymes identified as having a minor role included CYP1A1/2 and CYP2C family. Istradefylline inhibited CYP3A4 activity in a mechanism-based manner. The mechanism-based inhibition and irreversible binding were weaker in hepatocytes compared with HLMs.

WEDNESDAY, JULY 30, 2008
STREAM 3:
MEDICINES AND SOCIETY

485

Inappropriate Drug Prescribing in Patients with Chronic Liver Disease

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In patients with chronic liver impairment the kinetics and dynamics of a number of drugs are altered and numerous drugs have to be avoided or their doses must be adjusted. We aimed to determine the frequency of inappropriate prescribing in patients with chronic liver disease (CLD) treated in a hospital providing primary and tertiary care. To identify patients with liver cirrhosis, chronic liver failure, hepatic coma, or liver dysfunction we developed an algorithm based on lab values (MELD parameters) and clinical findings (ICD-10 codes). A prescription was considered inappropriate if its doses exceeded those recommended in the label or its use was contraindicated. Consecutive electronic prescriptions of 28060 patients were analyzed and 562 patients (2%) were identified with CLD. CLD patients were exposed to 841 drugs (375 active ingredients): 38 of these patients (6.8 %) were exposed to contraindicated medication (e.g. sulfonyl ureas, rifampin, acetazolamide) and 61 (10.8 %) received excessive doses (mainly pantoprazole, metoclopramide). On average, one in 24 drugs was not appropriate in these patients. These findings suggest that patients with CLD are a small fraction of all patients in a university hospital but they appear to be a population at risk for medication errors. Therefore physicians should

be supported during the prescription process to efficiently prevent these errors.

486

Comparison of Recommendations for Additional Post-launch Research from National Reimbursement Agencies: Canada and the United Kingdom

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Introduction: There is increased interest in post-launch studies as more jurisdictions require economic data for drug pricing and reimbursement decisions. Methods: We reviewed, categorized and compared final recommendations about post-launch research from the Canadian Common Drug Review (CDR) from May 2004 through January 2007 to those from the National Institute for Health and Clinical Excellence (NICE) in the UK. Decisions were categorised as: listed, listed with criteria or not listed. Recommendations for post-launch research were categorized by type of data requested. Using a comparative framework, we describe the frequency of the different types of data recommended for collection post-launch to highlight trends across jurisdictions.Results: 34 of 64 CDR submissions recommended 'no listing', 17 'list with criteria', and 13 'list or list in similar manner as other drugs in the same category'. 41 of 64 recommended collecting specific items of data (n=28), conducting subgroup analysis (n=13), or collecting data using a more appropriate study design (n=19). The most commonly requested item was long-term adverse events or safety data (16/28), consistent with the fact that many post-launch studies are safety surveillance studies. In addition, 11 of 28 recommended collecting clinically important outcomes, long-term effectiveness (7/28). Similarly, 41 of 48 NICE appraisals recommended further research to collect 'real-world' data, including treatment pathways, effectiveness, and long-term effectiveness. Conclusions: This review suggests that recommendations for post-launch research from CDR and NICE appear to be similar. This highlights the inherent weakness of regulatory trials as a piece of evidence in informing reimbursement decisions.

487

Impact of Cerebrovascular Disease on the Time to Nursing Home Placement and Survival of Alzheimer's Disease Patients Treated with Cholinesterase Inhibitors

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Background/Objectives: Cognitive impairment may be caused by cerebrovascular disease (CVD), Alzheimer's disease (AD), or a combination of both. Cholinesterase inhibitors (ChEIs) have been approved for the treatment of AD, but not for dementia associated with CVD. It is unclear whether ChEIs should be used when AD and CVD co-exist, particularly in light of evidence that survival is reduced in patients with mixed pathology. This study examined the impact of co-existing CVD on survival and nursing home placement (NHP) among AD patients treated with ChEIs. Methods: The Régie de l'Assurance Maladie du Québec (RAMQ) databases were used to identify AD patients aged 66+, with and without CVD, who received a ChEI dispensation. The primary endpoint was time to a composite of NHP or death. Results: 4,428 patients met inclusion criteria for AD with CVD; 13,512 had AD alone. In total, 3.8% of AD with CVD patients and 3.2% of those with AD alone met the composite endpoint. Although time to death and time to NHP or death were significantly shorter for patients with vs. without CVD, absolute differences were very small. No between-group differences were found for time to NHP, and a classification of AD with CVD did not predict time to death or NHP in adjusted regression analyses. Conclusions: The co-existence of CVD among AD patients receiving ChEIs appeared to have no impact on time to death or NHP, after

adjustment for covariates. This suggests that AD patients with CVD should not be denied access to ChEI treatment.

488

Is the Concept of Essential Medicines Still Able to Support Renewed PHC?

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Introduction: There is renewed interest in Primary Health Care (PHC) as a means to promote universal access and achieve the Millennium Development Goals. The provision of essential medicines has always been part of PHC. Is the concept still relevant? Results: The 1978 picture of a paternalistic government is increasingly being replaced by the concept of justice as a right, not as a charity. Emphasis on human rights strengthens the case for PHC and universal access to essential medicines. Selection of essential medicines has become much more systematic. In 1978, financing PHC depended heavily on free services in public facilities. Now the private sector should also receive financial support for delivering PHC services, with priority funding for the poorest. Better medicine price information now proves financial advantages of generic policies. Health insurance schemes will increasingly become guardians of medicine prices. Regulation of medical products is improving but the quality of *systems* should now also be regulated, with a system for licensing private facilities to qualify for public subsidy. Universal access to PHC through public and private channels also depends on perceived quality of care, and convenience to the patient. Approaches to promoting rational use of medicines are much better supported by scientific evidence on effective interventions than in 1978. National programmes should be started to reduce the huge medical and economic waste.
Conclusion: The concept of essential medicines is as relevant for PHC as it was in 1978; but it is now generally accepted and much better supported by scientific evidence.

489

Innovations in the Teaching of Undergraduate Pharmacology in a Philippine Medical School

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The teaching of pharmacology in the UP College of Medicine has undergone numerous changes over the century. It has evolved from traditional discipline-based to its present integrated approach. The INTARMED program is an innovative approach initiated in 1988 but only fully implemented in 2004 as an Organ System Integration. A historical background of the school, its vision, mission, and goals, a profile of the faculty and their scope of teaching and training is presented. Pharmacology in this present curriculum is taught in two core subjects – basic and clinical. Pharmacotherapeutic Conferences are given after these. A wide selection of seven (7) electives in pharmacology are offered as an adjunct to learning to complement essential concepts and principles learned in the core courses. Traditional methods of teaching are combined with innovative tools that feature organ-system integration where core and elective courses have been carefully designed to fit into this scheme. This combination of learning tools allows students to benefit from the strengths offered by both approaches. Students are able to work independently in their search for information. Both horizontal and vertical integration facilitates better synthesis of learning. There is a free flow of ideas from the learners and improved communication skills, under more individualized faculty guidance working with invited experts.

490

Antibiotic Utilization in an Egyptian Hospital

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Introduction: The use of antibiotics has often been mentioned as a target for pedagogic interventions. As resistance to antibiotics is a global problem, also seen in Egypt, we have tried to assess the antibiotic utilization in a major hospital in Cairo.
Methods: We implemented the WHO ATC/DDD methodology to measure antibiotic use in Ahmed Maher hospital. Data on dispensed antibiotics (ATC J01) were

collected from the hospital pharmacy at this tertiary care hospital in downtown Cairo with 600 beds covering most medical and surgical specialities. The data was converted to Defined Daily Doses (DDD) and DDDs/100 bed-days. Cost was measured in Egyptian pounds (100EGP =18.2 US Dollar). We focused on drugs accounting for 90% of the volume – the DU90% profile, and adherence to WHO Essential Medicines List (EML) 2007 within this segment. Results: The hospital use of antibiotics was 59,7 DDD/100 bed-days. Ten of 20 drugs accounted for 90% of the use (DU90%), with a 57% adherence to EML. Penicillins, aminoglycosides and cephalosporins were the most commonly used antibiotics with gentamicin as number one. A significant amount (82%) of the total volume was given by injections. Conclusion: The DU90% profile highlighted the antibiotic utilization in the hospital based on pharmacy data in a clear and easy form and can be applied with routinely available data in any hospital. Combined with resistance data it may serve as an indicator of the quality of antibiotic use.

491

Persistence of Weekly Oral Bisphosphonate Therapy: Wasted Drug and Fracture Costs due to Early Discontinuation

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Introduction: Large proportion of patients initiated with bisphosphonates (BP) therapy stop their treatment within the first year. The objective of this study was to estimate economic impact of the poor persistence to BP in terms of drug dispensed and fractures not saved. Methods: Patients newly started on BP therapy were selected from the RAMQ (Québec) between June 2002 and June 2006. The study was done on primary and secondary prevention cohorts. The concept of the "point of visual divergence" (PVD) was used. The risk of fracture among non compliant patients (< 80%) was estimated using Cox regression models. The hazard ratios (HRs) of compliant patients versus non compliant patients were used to estimate the number of fractures saved in the cohorts. Drug costs were estimated using Québec provincial drug reimbursement data and fracture costs from the literature. Results: Primary and secondary prevention cohorts included 31,467 and 1,337 patients respectively. The PVD was 6 months. The cost of wasted drugs was $34.68 per patient initiated in the primary prevention cohort and $39.23 in the secondary prevention cohort. The HRs for compliant versus non compliant patients derived from Cox regression were 0.51 for the primary prevention cohort, and 0.43 for the secondary prevention cohort. Total cost for the 110 fractures saved with compliance was $62.95 in the primary prevention cohort and $330.84 for the 19 fractures saved the secondary prevention cohort. Conclusions: This study provided further evidence that poor persistence to oral weekly BP therapies lead to important waste of money.

492

The Safety of Quinolones in the First Trimester of Pregnancy: A Meta-analysis

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Background: Quinolones have been avoided in pregnancy due to concerns regarding fetal teratogenicity following first trimester exposure. Joint toxicity has been observed in experimental animals treated with quinolones, mainly with older quinolones. Objective: To determine whether first trimester exposure to quinolones increases the risk of major malformations .We also sought to examine whether quinolones are associated with other pregnancy-related complications, such as preterm births and spontaneous abortions. Methods: The literature search was conducted for studies using Medline (1950-2007) and Embase (1980-2007). Key words used to search were teratogenesis, pregnancy outcomes, quinolones derivate. Included studies were controlled clinical trial, cohort and case-control in any language. Data extraction and quality assessment were performed independently and in duplicate with four studies meeting the inclusion criteria. Odds Ratios were calculated to determine the risk of major malformations,

spontaneous abortions and preterm births, between those exposed and those not exposed to quinolones. Results: Four cohort studies involving 684 women exposed to quinolones and 153,133 women in control group met our inclusion criteria. There was no difference in the rate of major malformations between those exposed and not exposed to quinolones. OR= 0.92 (95%CI 0.59-1.44).Similarly, there was no difference in the rate of preterm births. OR=0.88 (95%CI 0.47-1.65).Women exposed to quinolones during the first trimester did not have a higher rate of spontaneous abortions than women that were not exposed to quinolones. Conclusion: Based on the results of this meta-analysis, first trimester exposure to quinolones does not appear to be associated with a measurable teratogenic effect in human.

493

The Risk of Intrauterine Exposure to Folic Acid Antagonists during the First Trimester of Pregnancy

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Introduction: Exposure to folic acid antagonists during the first trimester of pregnancy has been associated with congenital malformations in case control studies Aim: Investigate the safety of folic acid antagonists use during the first trimester using cohorts of exposed women. Methods: A computerized database for medications dispensed from 1998 to 2007, to women registered in "Clalit" Health Maintenance Organization, southern district of Israel, was linked with computerized databases from the district hospital, containing maternal and infant hospitalization records of the same period. The following known confounders were controlled for in the statistical analysis: parity; ethnic group (Jews, Bedouins); maternal age, diabetes, smoking and fever before and around delivery. Results: A total of 117,960 infants were born; 517 of them were exposed to 1 or more folic acid antagonists in the first trimester (342 to dihydrofolate reductase inhibitors and 176 to other antagonists). Exposure was associated with an increased risk of congenital malformations (OR= 1.54, 95% CI: 1.11 – 2.13), but not with low birth weight or perinatal mortality. Antiepileptics were associated with an increased risk of congenital malformations (OR=2.12, 95% CI: 1.29 – 3.51), and dihydrofolate reductase inhibitors, with urinary tract defects (OR= 3.14, 95% CI: 1.16 – 8.47). Discussion: These findings corroborate the only available case-control study. Conclusion: Intrauterine folic acid antagonists exposure is associated with an increased risk of congenital malformations, but not with low birth weight or perinatal mortality.

494

Omega-3 Fatty Acids and Risk of Dementia: The Canadian Study of Health and Aging

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Omega-3 polyunsaturated fatty acids (n3-PUFAs) may protect against dementia and Alzheimer's disease (AD) according to research on animals. However, results from epidemiological studies, which assessed exposure via food frequency questionnaires or blood n3-PUFA levels, are inconsistent. Early randomized clinical trials on the effect of supplementation with n3-PUFAs were also inconclusive. Contamination of fish with mercury, a neuroxicant, may explain some of these inconsistencies. This study evaluated the association of blood levels of total n3-PUFAs, docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) with incidence of dementia or AD in the Canadian Study of Health and Aging (CSHA), while adjusting for mercury and other confounders. CSHA is a population study of a representative sample of persons aged 65 years or older conducted from 1991 to 2001. A total of 664

subjects, 150 of whom were dementia cases, including 104 with AD, provided blood samples in CSHA-1 (1991-1992) or CSHA-2 (1996-1997) eligible for prospective analyses. N3-PUFAs and mercury were analysed in whole blood. In Cox regression models with age as the time scale and adjustment for sex and education there were no associations between total n3-PUFAs, DHA or EPA and dementia or AD. Additional adjustment for mercury or further confounders did not change these results. In subgroup analyses according to APOE4 status risk ratios were smaller, albeit not significant, among APOE4 carriers when compared to non-carriers. In conclusion, despite careful methodology and adjustment for mercury and numerous confounders this large prospective study showed no evidence for a protective effect of n3-PUFAs against dementia.

495

A Study on Medication Prescribing Errors at Multispeciality Hospital

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Medication error is a serious problem through out the world. These errors have a huge economic impact on healthcare system and patients. The medication errors and adverse drug events occur in all types of patients and in all type of settings at a rate of nearly 1 of every 5 doses in typical hospital. Pharmacists have a long-standing interest in improving medication safety and provide ways and means to reduce medication errors. All the incidents related to medication errors are due to less awareness about the hospital policies. Therefore, hospital pharmacist must assume the mantle of responsibility for development of the proper policies in hospitals. A study protocol was designed which contains name, age, gender, inpatient/outpatient number, date of admission, date of discharge, reason for admission, associated diseases, educational status, social status, occupation, diagnosis and medication. The data was collected by regular ward round participation with healthcare professionals. Amongst 840 prescriptions, 227 (27%) prescriptions were having medication errors, out of which 92% were contributed by Inpatient and 8% errors were contributed by outpatient department. From the study it was concluded that the medication errors can be prevented or minimized by providing proper labeling, awareness about sound alike and look alike drugs, proper writing of prescription, adopting Computer Physicians order entry, Bar code system, proper administration, proper documentation, regular quality assurance check, appropriate Pharmacist's intervention and close monitoring of the Patient. It has been also suggested that pharmacist and doctor's, have huge responsibility to protect the patients from medication errors.

496

Adverse Events due to Roziglitazone Assessed by Number Needed to Harm (NNH)

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A recent meta-analysis by Nissen & Wolski (NEJM 2007;357:28-38) raised concerns about roziglitazone treatment of diabetic patients as a cause of possible adverse events, when it comes to balancing benefits against harm. To appreciate the beneficial value of a given intervention, rather than relative risk reduction (RRR) it is important to consider absolute benefit – commonly expressed as number needed to treat (NNT). Similarly, a negative NNT value can be regarded as a number needed to harm (NNH), which provides a more pragmatic, convenient and meaningful approach to assessing the absolute risk of harm than by recourse to relative risk (RR). The largest trials (DREAM & ADOPT) accounted for 75% of the 27,847 patients in the 42 trial meta-analysis. For roziglitazone treatment, respective point estimates and 95% Cis of RR and NNH/year for myocardial infarction were 125 (58 to 271)%, and 2639 (-1072 to 529), 131 (8 to 215)% and 913 (-1015 to 315), and for all 42 trials 94 (68 to 130)% and 5044 (-788 to 1147). Corresponding values for cardiovascular death were 90 (36 to 226)% and -7875 (-814 to 1026), 80 (15 to 423)% and -11316 (-1412 to 1880), and 139 (82 to 238)% and 2380 (-4334 to 934).

Irrespective of the RRs, these NNH/year values (mostly in the thousands and non-significant) indicate an extremely small or no absolute risk of such events, and should be viewed in the context of the clinical heterogeneity between these trials

497

Impact of a Continuing Education Workshop based on the use of a Toolkit on Fragility Fractures Prevention Offered to Primary Care Family Physicians

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A one-hour workshop on the prevention of fragility fractures was offered to family physicians in private clinics. In a cluster cohort study, the annual rates of bone mineral density (BMD) testing, initiation of osteoporosis pharmacotherapy and reference to an osteoporosis specialist were compared in patients followed-up by physicians exposed and unexposed to the workshop. Using provincial administrative databases, exposed physicians were matched to unexposed physicians based on gender and year of graduation. Patients were included if they were alive and at least 70 years old at the index date; had one medical visit with a study physician one year before and after the index date; had no BMD test and no osteoporosis treatment five years prior to the index date; and had not been institutionalized. Annual rates of osteoporosis medical practices were compared using multivariate and multilevel logistic regression adjusting for risk factors for osteoporosis and bone fractures. In all, 25 exposed physicians (1124 patients) and 209 unexposed physicians (9663 patients) were included. In women, differences were observed in the annual rate of BMD testing (exposed: 11%, unexposed: 6%, $p<0.001$), osteoporosis pharmacotherapy (exposed: 10%, unexposed: 7%, $p=0.01$) and reference to a specialist (exposed: 16%, unexposed: 22%, $p=0.002$). These differences remained significant after adjusting for potential confounders (BMD testing: OR=1.89 (95% CI: 1.15 – 3.09), reference to a specialist: OR=0.60 (0.44 – 0.82)), except for pharmacotherapy. No differences in BMD testing or pharmacotherapy were observed in men. The workshop was associated with slightly higher rates of osteoporosis screening in women but not in men.

498

Prescription of Potentially Inappropriate Medications to the Elderly between 1994 and 2004 in France

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Introduction: Potentially inappropriate medication use (PIM) is frequently encountered in the elderly and associated with a high risk of adverse drug reactions (ADR). Polymedication tends to increase with ageing, increases the iatrogenic risk and PIM prevalence. The aim of this study was to assess the trend of PIM over a 10-year period in very old people (≥90 years). Methods: During three consecutive periods (January 1994 to April 1996, March 1997 to January 1999, and October 2002 to October 2004), a cross sectional study on the use of drugs was conducted in an acute care medical geriatric unit. PIM prevalence was established using 1997 Beers criteria. Consumption was assessed and tested by trend Chi-square. Results: 855 patients, aged 92.9±2.6 years were included. During the three periods, the number of drugs per patient was respectively 5.8±2.9, 6.2±2.9, 6.8±3.2. PIM prevalence was respectively 63.5%, 61.5%, 49.7% (trend $p<0.0001$). The reduction was attributable to a cut in the administration of cerebral vasodilators from 2001. Dextropropoxyphene or anticholinergic antidepressant use remained constant. Discussion: These results suggest a change in the prescribing patterns in very old patients: drug consumption tended to increase while PIM decreased from 1994 to 2004. A few similar studies focused on 65-year-olds and over have similarly identified an

increase of the number of drugs but a stable use of PIM during a 10-year period. In France, PIM is high, and includes medications with an elevated ADR risk. Conclusion: Prescribing in the elderly should take into account frailty, the number and the inappropriateness of drugs.

499

The Risks of Multiple-generic Substitution of Antiepileptic Drugs: The Case of Topiramate

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Background: Multiple-generic substitution of antiepileptic drugs (AEDs) may be problematic. Objective: To investigate consequences of generic substitution of one versus multiple generics of topiramate (Topamax®).Methods: Medical and pharmacy claims data of Régie de l'assurance-maladie du Québec (RAMQ) from 01/2006-10/2007 were used. Patients with epilepsy treated with topiramate were selected. An open-cohort design was used to classify the observation period into periods of brand, single-generic, and multiple-generic use. One-year generic-switch and switchback-to-brand rates were computed using Kaplan-Meier methodology. Medical resource utilization (frequency per person-year (p/y)) was compared among the three periods using multivariate regressions. Results: 948 patients were observed during 1,105 p/y of brand use, 233 p/y of single-generic use, and 91 p/y of multiple-generic use. 38% of brand users switched to generic topiramate, of whom 14% switched back to brand. Generic users received on average 1.4 generic versions, with 23% taking two or more versions. Multiple-generic use was associated with increased utilization of both AEDs and non-AEDs compared to brand (RR=1.27, 95%CI=1.24-1.31) and single-generic use (RR=1.21, 95%CI=1.19-1.23) after covariate adjustment. Compared to brand use, multiple-generic use was associated with higher hospitalization rate (0.48 vs. 0.83 visit/p/y, RR=1.65, 95%CI=1.28-2.13) and longer hospital stays (2.6 vs. 3.9 days/p/y, RR=1.43, 95%CI=1.27-1.60), but the effect was less pronounced in single-generic use (hospitalization: RR=1.08, 95%CI=0.88-1.34, length of stay: RR=1.12, 95%CI=1.03-1.23). The risk of head injury or fracture was 3 times higher (HR=2.84, 95%CI=1.24-6.48) following a generic-to-generic switch compared to brand use. Conclusion: Multiple-generic substitution of topiramate was significantly associated with deleterious outcomes, such as hospitalizations and injuries.

500

Economical Impact of Non-persistence to Antidepressant Treatments: Comparison across Products

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Introduction: Although practice guidelines recommend that antidepressants (Ads) should be used for at least 6 months, rates of premature discontinuation are high in a "real-life" setting. Although previous studies have assessed the economical impact of non-persistance, differences across individual products remain inadequately explored. The objective of our study was to compare treatment persistence across individual SSRIs and associated direct health care costs. Methods: A retrospective cohort using data from the public drug program of Quebec was conducted in which all adults age 18-65 years who initiated an antidepressant treatment in 2003 were identified. Persistance was defined as treatment duration of at least 6 months. Economical impact was assessed through drug costs, medical services costs, hospitalization costs, and total health care costs. Comparisons across products were conducted using incremental cost-persistence ratio

(ICPR). Results: A total of 13,936 patients initiated an SSRI treatment in 2003. The 6-month persistance to treatment ranged from 34.9% (citalopram) to 39.6% (paroxetine). Mean antidepressant costs ranged from CDN$215 (fluvoxamine) to CDN$309 (venlafaxine). Using citalopram as the reference (lowest persistence), ICPR for other SSRIs ranged from CDN$6,571 (venlafaxine) to – CDN$67 (fluvoxamine). The ICPR for total health care costs ranged from – CDN$4,695 (paroxetine) to -CDN$34,479 (sertraline). Conclusion: In our study, sertraline appears to have the most favourable cost-persistence ratio of all SSRIs. Since all estimates were adjusted for main health cost related factors, ICPR differences across products might reflect differences in hospitalisation/medical services rates. These results hence support the use of persistence as a proxy for effectiveness in antidepressants studies.

501

The Impact of Drug Vintage on Patient Survival: A Patient-level Approach using Quebec's Provincial Health Plan Data

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Introduction: There is much controversy about the value of new medications and the substantial spending on R&D associated with new treatments. The current study aimed at evaluating the impact of drug innovation on longevity using patient-level data. Methods: An analysis of health claims from Québec's provincial health plan data from 1997-2006 was conducted. Elderly patients with continuous health plan coverage and ≥1 drug prescription per calendar year were selected. Drug vintage, defined as the ingredient's earliest marketed date, was drawn from Health Canada Drug Product Database. Multivariate analysis was conducted to estimate the impact of drug vintage on patients' probability of dying using time-varying Cox proportional hazard model. The covariates used for adjustment in the regression model were: demographics characteristics, guaranteed income supplement (GIS) status, medical resources utilization, drug utilization, and comorbidities. Results: A total of 102,743 elderly subjects formed the study population, of which 14,154 (14%) died during the observation period. Mean age was 68 years; 59% of subjects were women. After controlling for confounding factors, the use of recent medications (i.e., Post-1990 ingredients) was associated with a statistically significant risk reduction of mortality (hazard ratio: 0.556; 95% CI: 0.504-0.613, p<.0001), relative to older ingredients, suggesting that recent drug innovation had a significant beneficial impact on longevity. Other covariates associated with an increased risk of mortality included age, gender, GIS beneficiaries, hospitalization, and number of comorbidities. Conclusion: This analysis showed that drug innovation, in particular medications launched after 1990, had a significant beneficial impact on longevity of elderly patients.

502

Impact of the Regulation on Methodological and Ethic Quality of Postauthorization Studies in Spain

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Introduction: For many years postauthorization-studies (PAS) have been used as instruments for promotional purposes. Since 2002, PAS were regulated in Spain (through the Royal-Decree 711/2002). This regulation requires a prior authorization by local-health-authorities for all prospective follow-up PAS. The objective of this study is to compare the methodological and ethical quality of the PAS during the period before and after this regulation, in order to evaluate its impact on the quantity and quality of PAS submitted to the Spanish-Agency-for-Medicines and-Healthcare-Products. Methods: We collected information relative to administrative-procedures, methods, follow-up and ethical-issues from the

protocol of PAS submitted in two periods: 2001(P1) and 2002-2007(P2). Results: In P1: 162 PAS with a total of 306,539 patients included; P2: 530 PAS with 1'045,806 patients included and distributed as follows: 2002(21PAS), 2003(101PAS), 2004(101PAS), 2005(108PAS), 2006(102PAS), 2007(97PAS). Main variables: in relation to the type of *design of the study* in P1: "non-controlled prospective follow-up study [NCPF]"(75%), "controlled prospective follow-up study[CPF]"(17%) and P2: NCPF(50%), "transversal-studies"(21%), CPF(14,5%). *Source of information*: P1 (99%: physicians), P2 (60%: physicians and 29%: physicians + clinical records). *Calculation and justification of the sample-size* defined in protocol: P1 (53%), P2 (80.4%). *Presentation of the protocol to an ethics-committee-of-clinical-investigation*: P1 (13%), P2 (98%). *Information to the participants*: P1 (44%), P2(90%). *Procedure of confidentiality*: P1 (60%), P2 (91,4%). Conclusions: The number of the PAS has diminished 45.5% after the regulation, but its methodological and ethical quality improved considerably. The results emphasize the need to regulate this sector in order to eradicate the use of PAS as a promotional practice and support PAS with scientific aims.

503

Maternal use of Antihypertensive Drugs in Early Pregnancy and Delivery Outcome

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Introduction: The possible association between ACE inhibitors or other antihypertensive agents and congenital malformations, notably cardiovascular defects, was investigated. Methods: We used the Swedish Medical Birth Register, containing comprehensive medical information on nearly all infants born in Sweden since 1973. Possible confounders such as diabetes, high maternal age, first infant born, smoking, and high BMI were adjusted for. We calculated odds ratios (OR) or, when the expected number of events was low (<10), risk ratios (RR). Results: We found a generally increased risk for cardiac defects associated to all antihypertensives (OR=2.69), markedly for ventricular or atrium septum defects (OR=2.81). The risk was only slightly higher after ACE inhibitor exposure (RR= 3.11) compared to all other antihypertensives taken together (RR= 2.62), and most dominated by the cases on beta-blockers (OR= 2.86). No significantly increased risk for other congenital malformations connected to antihypertensives was seen. Discussion: Earlier data indicate an increased risk for cardiovascular defects forACE-inhibitors but not for other antihypertensives. We found an increased risk not limited to ACE-inhibitors only. ACE-inhibitors represented about 10% of the antihypertensives as compared to more than 50% of the infants in the earlier study. A possible explanation to the divergent findings could be that we adjusted our data for possible confounders such as diabetes and high BMI. Conclusion: Cardiovascular teratogenic risk does not seem to be a specific characteristic of ACE-inhibitors. It seems rather associated with antihypertensive treatment in general, and was as apparent with beta-blockers in particular. Possibly hypertension itself or disturbances in fetal circulation contribute to the risk.

504

The Safety of H2-Blockers Intrauterine Exposure during the First Trimester of Pregnancy

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Introduction: Little data exist on the safety of H2-blockers use during pregnancy. Aim: Assess the safety of H2-blockers use in the first trimester, by linking computerized databases. Methods: A computerized database for medications dispensed between 1998 and 2007 to women registered in "Clalit" Health Maintenance Organization, southern district of Israel, was linked with

computerized databases containing maternal and infant hospitalization records from the district hospital. The following known confounders were controlled for in the statistical analysis: maternal age, ethnic group (Jews, Bedouins), maternal diabetes and smoking, maternal fever before and around delivery and parity. Results: A total of 117,960 infants were born during the study period; 1148 of them were exposed to H2-blockers during the first trimester of pregnancy. Exposure to H2-blockers was not associated with an increased risk for congenital malformations (OR: 1.077, 95% CI: 0.836 – 1.386), increased risk for perinatal mortality, premature delivery, low birth weight, or APGAR score <7 at the 1st and 5th minutes. Famotidine, when assessed individually, was not associated with an increased risk for congenital malformations (OR=1.263, 95% CI: 0.966 – 1.653). Discussion: This study reports on tenfold more infants exposed to H2-blockers during the first trimester than all other studies combined. Conclusion: These findings suggest that intrauterine exposure to H2-blockers is not associated with an increased risk for congenital malformations, perinatal mortality or morbidity.

505

Parallel Trade and Pharmaceuticals: The Risks of Counterfeit Drugs

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Background: The European Commission and US Food and Drug Administration have identified safety issues associated with parallel trade in pharmaceuticals. Methods: Assessment of parallel trade rules and counterfeit drug incidence in the EU and America and means to protect the populace from fake drugs. Results: Parallel trade, particularly through the Internet, has allowed virtually unfettered patient access to drugs without provider oversight and criminal element introducing fake drugs into the drug supply. Legal efforts have had limited success in stemming this result. These weaknesses create dangers to vulnerable patient populations who may obtain excessive amounts of drugs, be left completely untreated, and/or have their health detrimentally affected by materials used in fakes. Conclusion: Vulnerable patient populations are at risk under the current system of parallel trade and the Internet. Pharmaceutical professionals must educate patients and be aware of the risks associated with the current infrastructure of drug access.

506

Cost-effective of Drug use and Readmission Rates for Schizophrenia Patients

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Schizophrenia is a chronic and serious brain disorder. Complex formation of schizophrenia is making it costly and difficult to cure. Since drug cost represents a major proportion of the expenditure, it is important to find a more cost-effectiveness way of using drugs for the treatment. This study uses a nationwide population-based dataset to explore the association between different drugs use and 30-day readmission rates for hospitalized schizophrenia patients. We performed a Cox proportional hazard regression on a pooled population-based database from the Taiwan National Health Insurance Research Database (NHIRD) after adjusting for hospital, physician and patient characteristics, covering the three-year period 2001-2003, with the study sample comprising of 29,373 first-time hospitalizations under principal ICD-9-CM diagnosis code 295 (schizophrenic disorders). The sample is divided into A-E five drug groups: haldol, dogmatyl, fluanxol tegol, neurotin. The results show that of the total of 29,373 first-time hospitalizations for schizophrenic disorders during the 2001-2003 period, 12,468 (42.5%) were readmitted within a 30-day period. The regression analysis shows that 30-day readmission is reduced statistical significantly for some types of drugs

using groups. We find that different drug use is associated with different 30-day readmission rate, and suggest that there is a need for the development of a more cost-effective use of drugs for treating schizophrenia patients, in order to lower the probability of an early return to hospital for such patients.

507

Identifying the Risk of Liver Injury after Chronic Oral Amiodarone Intake

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Background/Aim: The number of patients receiving amiodarone will increase in years to come. Oral amiodarone has shown to be associated with liver injury, a disease difficult to be foreseen. A new bayesian - developed diagnostic algorithm to help physicians in the diagnosis of amiodarone-induced liver injury is proposed. Methods: 41 published cases of amiodarone-induced hepatotoxicity were identified by a literature search. Incidences of abnormal liver enzymes in patients receiving amiodarone were obtained from placebo controlled clinical trials. Maximum number of expected hepatotoxicity cases in amiodarone and placebo-treated patients were calculated using the Poisson distribution. The calculated odds ratio was used as prior odds to subsequent quantification, using a Bayesian-based approach, of individual amiodarone-induced hepatotoxicity likelihood. One new case of amiodarone-induced hepatotoxicity is described. Results: The prior probability of amiodarone-induced hepatotoxicity is 48%. Forty per cent of published case reports developed an irreversible lesion such as cirrhosis. Latency period and abnormal total bilirubin values were the most relevant likelihood ratios of the Bayesian model to establish a causal relationship between liver injury and amiodarone. Also, the time elapsed between drug discontinuation and normalization of blood tests is valuable to confirm the causality of amiodarone. Conclusions: An early assessment of amiodarone-treated patients that develop liver injury using an algorithm based on liver function test values and latency period information allows an early and fast removal of a potential liver toxic and may be the only option to avoid a severe and irreversible injury.

508

Management of Oral Anticoagulant Therapy: A Survey of Primary Care Physicians

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Background: Little information is available on how primary care (PC) physicians manage patients on oral anticoagulant. Objective: Describe the characteristics of PC physicians' follow-up. Method: In a randomised controlled trial, the quality of international normalised ratio (INR) control of PC physicians' and pharmacist-managed anticoagulation service's (PMAS) patients was compared. Physicians (n=138) were solicited to complete a telephone interview. Logistic regression models were used to identify determinants of INR control below and above the median percent time in range. Results: A total of 121 physicians (88%) completed the questionnaire. Majority were men (63.6%), had graduated before 1990 (79.3%) and were practicing in private clinic (87.6%). For a large proportion of physicians, the clinic secretary is responsible for receiving the INR results (74.4%), searching for missing results (60.3%), and advising patients to maintain (64.5%) or modify

their dosage (30.9%). Physicians are responsible for analysing INR results (97.5%) and adjusting dosage (100%). When needed, advices are solicited from colleagues' physicians (14.9%) and PMAS (14.9%). Most physicians do not use a standardized protocol (72%) and do not have access to a computerized central system for INR results (59.5%) and drug interaction program (92.6%). Availability of drug interaction program (OR 3.5; p=0.0006), referring to medical specialists (OR: 5.9; p=0.01), or colleague's physicians (OR 2.9; p=0.01) were significant predictors of high quality control. Discussion: The management of oral anticoagulant treatment by PC physicians is not supported by other health professionals or by technological tools. Conclusion: Better support may be associated with better INR control.

509

Pharmacoeconomic Study of Low-molecular Weight Heparin as Prophylaxis of Venous Thromboembolism after Total Hip Arthroplasty

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Among all hospitalized patients, those undergoing total hip replacement have a very high risk of venous thromboembolism. Our retrospective study includes all 595 patients with total hip replacement treated in the Orthopaedic Clinic of the Clinical County Hospital Timisoara, Romania, during 4 years of observation (2004-2007). All patients received thromboprophylaxis with unfractionated heparin (UFH) or low molecular weight heparin (LMWH), continued until discharge. Postoperatively, occurrence of deep vein thrombosis or pulmonary embolism was recorded during the first 15 days after surgery. We monitorized the costs of heparin, administration and laboratory tests, the direct nonmedical costs and the lost of working days. The price of UFH is aproximatively five to ten times lower than the price of LMWH. Similar studies from developed countries has shown that treatment with LMWH confer economic advantages over UFH therapy, but including all particular aspects, treatment with UFH is equally cost-effective in our country. The patient often pays from his pocket, and the costs are not always full covered by the national insurance company and so the treatment with UFH preserves its place in the prophylaxis of deep vein thrombosis in patients with total hip arthroplasty.

510

The Safety of Metoclopramide Intrauterine Exposure during the First Trimester of Pregnancy

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Introduction: Metoclopramide is the antiemetic drug of choice in pregnancy in many countries, but insufficient information exists regarding its safety during the first trimester. Aim: Investigate the safety of metoclopramide use during the first trimester in a large cohort, by linking computerized databases. Methods: A computerized database for medications dispensed from 1998 to 2007, to women registered in "Clalit" Health Maintenance Organization, southern district of Israel, was linked with computerized databases containing maternal and infant hospitalization records of the same period from the district hospital. The following known confounders were controlled for in the statistical analysis: parity, maternal age, ethnic group (Jews, Bedouins), maternal diabetes and smoking, and maternal fever before and around delivery. Results: There were 113,612 singleton deliveries; 3458 of them were exposed to metoclopramide in the first trimester. Metoclopramide was associated with a slight increased risk of premature delivery (OR= 1.123, 95% CI: 1.021 – 1.236), but not with an increased risk for congenital malformation (OR=1.099, 95% CI: 0.943 – 1.280), low birth weight (<2500grams) (OR=1.039, 95% CI: 0.920 – 1.174), or perinatal mortality (OR=0.908, 95%

CI: 0.573 – 1.438). Discussion: This study contains a tenfold larger cohort of infants exposed to metoclopramide during the first trimester than all published literature combined Conclusion: Intrauterine metoclopramide exposure is not associated with an increased risk of congenital malformations, low birth weight or perinatal mortality. More research is needed to corroborate the apparent risk for prematurity.

511

Surveillance of Brugada Type ECG for Drug Development at Phase I Clinical Trial Unit by Newly Determined Automatic Diagnosis Protocol

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QT prolongation from Potassium ion handling abnormality is great interest in drug development. Another syndrome that causes sudden cardiac death, Brugada syndrome, relate with other important ion Sodium, but little attention has been paid upon in the drug development. As novel compounds given for the first time in Phase I trials, automated ECG diagnosis of Brugada syndrome will become more important for safe trials. In 2006, Japanese scientific society has established Brugada type ECG diagnosis protocol for automated ECG recorder. We aimed the validation of this protocol on young male healthy volunteers, and also the basic epidemiologic data collection of Brugada syndrome, for possible application on the surveillance in Phase I trail unit. ECG surveillance was performed on the healthy volunteers between 20 to 40 years old who visited Sekino Clinical Pharmacology Clinic for the first registry from Jan to Aug of 2007. ECG was recorded including one upper inter-costal space recording (U-Rec) for Brugada check-ups. ECGs were checked independently by two experts for Brugada Syndrome research using J point determination assistance program on Fukuda Denshi's ECG recorder, and finally combined the results. Consecutive 499 volunteers were enrolled. From the standard 12 leads ECG readings, 5 (1%) were diagnosed as Brugada type ECG. No one had Coved type form, while 31 (6%) showed Brugada type ECG with U-Rec, but Coved type. This appearance rate seemed reasonable for epidemiologic surveillance on healthy volunteers from the former surveillance studies results, and our result will support this diagnosis protocol appropriate.

512

Effectiveness of a Change in Reperfusion Strategy to Primary Percutaneous Coronary Intervention in a Non-selected Population

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Introduction: Treatment of ST-elevation myocardial infarction (STEMI) has evolved in recent years. Randomized controlled trials (RCT) have established the clinical superiority of primary percutaneous coronary intervention (PCI) over fibrinolysis in selected populations. The purpose of this study is to validate results from RCTs in a non-selected population. Methods: We performed a retrospective study of 243 consecutive patients who presented with a STEMI in the emergency room of a large academic center. Baseline characteristics, treatment strategies and in-hospital outcomes of patients treated in 2004-2005 (129 patients) were compared to those of patients treated in 1999-2000 (114 patients). Logistic regression was used to adjust for imbalanced baseline characteristics. Results: Patients in 2004-2005 vs. those in 1999-2000 were older (65±13 vs. 59±13 years old), more likely to be hypertensive (58% vs. 39%) and to present in Killip class 2-4 (33% vs. 23%). All of the patients in 2004-2005 underwent primary PCI strategy (100% vs. 32% in 1999-2000). In the 1999-2000 cohort, 68% received fibrinolysis, of which 18% required rescue PCI. The combined in-hospital incidence of death, re-infarction or stroke was reduced from 21.6% in 1999-2000 to 15.5% in 2004-2005 (OR=0.462; p=0.055), largely due to a reduction in re-infarction (10.3%

to 3.1%, OR=0.275; p=0.041). Mortality (12.1% vs. 10.9%, OR=0.668; p=0.422) and stroke rates (1.7% vs. 2.3%, OR=1.092; p=0.934) did not change significantly. Conclusion: In this non-selected population, the change in reperfusion strategy from fibrinolysis to primary PCI in the treatment of STEMI was associated with an important reduction in adverse in-hospital events. "In press, Canadian Journal of Cardiology"

513

Progress toward Optimal Pediatric Drug Therapy: An International Imperative

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Introduction: The millennium development goals set a standard to reduce child mortality by 2/3 before 2015. Drug manufacturers, regulators, pediatricians, pharmacologists, and pharmacists have, until recently, failed to meet the needs of children for properly validated therapies available in formulations suitable from infancy to adolescence. Nowhere is the lack of evidence to inform optimal therapy more evident than in developing countries where, in many cases, the majority of the population is aged 14 years or less. There are an estimated 5 million deaths annually among children 5 years or younger that could be prevented by effective, affordable, accessible drug therapy. Methods: In 2007 key steps were taken to rectify this situation following passage of resolution 60.20 "Better medicines for children" by the World Health Assembly. Importantly, a group of pharmacologists, pharmacists and pediatricians have created the International Alliance for Better Medicines for Children. The Alliance comprises scientific and clinical organizations, regional leadership groups, and national societies as well as individuals committed to research and knowledge transfer relevant to pediatric therapeutic advances. Discussion: Priority Alliance objectives 2008-2009 are to: 1) pursue international regulatory harmonization, 2) support WHO listing of essential medicines for children, 3) clarify regional needs through formal needs assessments, 4) develop and test appropriate pediatric formulations, and 5) develop guidelines for optimal and safe use of medications for children. The Alliance welcomes expanded participation by pediatric pharmacologists and pharmacists committed to a view of optimal therapy for children as an imminently achievable priority health goal.

514

Patterns for Switching from a Generic to a Brand-name Drug

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Introduction: Substitution of a generic (G) for a brand-name drug (BND) is promoted by health authorities for economical reasons. The aim of this study was to identify G to BND switch reasons using allopurinol as a test drug. Methods: Allopurinol newly treated patients were identified from the Healthcare Insurance Database and followed for 18 months. When a G to BND switch was identified, a questionnaire was sent to the patient, the practitioner and the pharmacist, asking for the reasons for this change. A synthesis was made from the answers received. Results: There were 1,010 women (29.7%) and 2,390 men (70.3%). Patients were 68.7±13.5 years old. Allopurinol was supplied 33,923 times; a G to BND switch occurred 522 times. 2,058 questionnaires were sent. The response rate was 42% from patients, 42% from practitioners, and 58% from pharmacists. The main reasons for switching were the lack of supplies in pharmacies, a change of drugstore, or on request of the practitioners (267 cases). A fear of confusion between treatments, look-alike packaging, and an alleged poor G quality were reported in 115 cases. Adverse effects were reported in 57 G treatments. Discussion: The inconstant availability of G in pharmacies, and changing drugstores, favoured the switch. In the elderly population, a change in the name and appearance of supplied drugs may lead to confusion. Adverse effect reactions were not the main reason of a G to BND switch. Conclusion: With more cautious and careful explaining of substitution, in time, the financial benefits of generic drugs will be safely maintained.

515

Pattern of Narcotic Analgesic Drug Usage among the Hospitalized Patients in Shahid Beheshti Hospital, Kashan, Iran

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To profile the pattern of narcotic analgesics prescribing for hospitalized patients in Shahid Beheshti Hospital, Kashan, Iran this study was carried out. As a national plan, the type and amount of narcotic analgesic drugs prescribed for all the hospitalized patients in the different divisions of the hospital are recorded in the special forms by nursing personnel routinely. In this retrospective cross sectional study, a review on all the filled forms during the six months period from 20th September, 2006 until 20th March, 2007 was done. The data analysis shows all the drugs have been administered as IM or IV injection. The most commonly used agent was pethidine (96.7%) followed in severe decreasing frequency by morphine (1.7%) and methadone (1.6%). Our results indicate that pethidine has been utilized with the highest amount in order to the relief of postoperative pain (84.3%) and after that burning pain (7.7%). Overall the most amounts of opioids have been used in the division of surgery (87.0%) and then ICU (8.1%). In the other departments i.e. internal medicine, infectious diseases care and CCU the next amounts of drugs have been ordered respectively. Also there was no data indicating usage of narcotic analgesics in some of departments such as pediatrics. This study provides a baseline data for monitoring analgesics usage trends but it is recommended the particular recording forms need to be developed to have more adequate data so well-designed controlled trials may be executed to deliver more accurate information on the drug using in pain relief.

516

Pharmacogenomics in Pharmaceutical Development and Regulation

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Pharmacogenomics potential will be realized with the development of personalized medicine, entailing genotype-based diagnostics and prescribing. Pharmacogenomics has been defined as the identification and study of genes and their corresponding products that influence individual variation in the efficacy and/or toxicity of therapeutic products. If pharmacogenomics is truly to become part of personalized medicine, a regulatory framework designed to pick up adverse reactions much earlier in the pharmaceutical lifecycle and that recognizes specific variations in drug response is needed. It is recognized that many pharmacogenomic results are not yet sufficiently established scientifically to be appropriate for regulatory decision making. However, a number of regulatory guidance documents by FDA, HC, EMEA, and ICH have been published since 2004 when FDA published its white paper, *Stagnation or Innovation? –Challenge and Opportunity on the Critical Path to New Medicinal Products*. FDA's 2005 guidance outlines when pharmacogenomic data submissions are required, when to submit them during the development and review process, and what content and format to use; voluntary genomic data submissions (VGDS) are also discussed. Health Canada's 2007 guidance describes how and when to submit pharmacogenomic information for drugs, biologics, devices, or as part of ongoing post marketing activities. Both VGDS to the FDA and joint meetings between agencies and sponsors, including joint agency (FDA/EMEA)-sponsor meetings are outlined in these guidance and encouraged as mutually beneficial in terms of understanding such issues as developing new markers, tests and methods of data analysis. Pharmacogenomics will change the way therapeutics are regulated by agencies worldwide.

517

A Nationwide Survey on Serious Adverse Events in Healthy Volunteer Studies in Japan

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Object: Since a tragic event in England was broken out in March 2006, investigators as well as co-

medical staff at Phase I units have had increasing interests in the safety of subjects. Although most of the observed adverse events were minor in intensity, several serious adverse events were also found. Some reports on adverse events and serious adverse events have been made, but most of them have been based on individual experiences of their own institutes. The Japan Association of Contract Institutes for Clinical Pharmacology (JACIC) was organized by Japanese clinical pharmacology units, in which most of Phase I studies in Japan are performed. Methods: JACIC surveyed serious adverse events found in studies performed from 1993 to 2006 by sending a questionnaire to its member institutes. Results: As the result of the survey, 5,694 protocols and 113,093 subjects were provided with a detailed evaluation. Among them 68 serious adverse events were found, and 30 cases (0.027%) were assessed as side effects, which consist of excessive pharmacological effects, anaphylaxis, abnormal liver function tests, infectious diseases and so on. All of the serious adverse events were reversible, and no subject suffered from sequela. Discussion and conclusion: The low prevalence compared with other reports from other countries may be due to the doses used in the studies, since dosage was not escalated to the maximum tolerated dose in Japan.

518

Effect of Comedications on the Occurrence of Serious Adverse Reactions in Subjects Treated with Cholinesterase Inhibitor Users

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Introduction: Cholinesterase inhibitors (ChIs) are the main drugs used in Alzheimers disease, mostly in subjects aged over 60 years with potential co-morbidities and co-medications. The objective of this study was to identify factors associated with severe adverse drug reactions (SADRs) in patients treated with ChIs. Methods: A search was performed in the French Pharmacovigilance database since the launching of ChI to January 2007 to identify all adverse drug reactions (ADRs) for which donepezil, rivastigmine or galantamine was suspected. Reports of SADRs were defined as reports in which ADRs had led to hospitalisation or prolongation of which, were life threatening, induced incapacity or invalidity, had fatal issue or were medically significant. Multiple logistic regression was used to identify risk factors of SADRs. Results: We identified 773 reports for which a ChI was suspected. Among these, 438 were SADRs. The patients median age was 80 years (IQR: 75-84) and patients were mostly women (65.1%). A significant interaction was found between cardiovascular drug use and psychotropic drug use. After stratification, the occurrence of a SADR was associated with analgesic use (OR 0.4; 95%CI 0.2-0.9) and with other central nervous system drug use (OR 2.5; 95%CI 1.1-5.9) in psychotropic users. In psychotropic drug non-users, it was associated only to cardiovascular drug use (OR 2.3; 95%CI 1.5-3.5). The distribution of effects was similar between psychotropic drug users and non-users. Conclusions: These results suggest that severity of ADR in ChI users may depend on co-prescribed drugs. The need of the co-prescribed psychotropic drugs should be reconsidered.

519

Disease Management Program to Optimize the use of Antidepressants: Results from a Pilot Study

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Introduction: While recommendations state that antidepressant treatments be at least 6 months, there is a high rate of premature discontinuation in a “real-life” setting. We hypothesize that a disease management (DM) program that aims at

empowering the patient will improve treatment persistence and follow-up care. The objective of this pilot study was to develop and assess the feasibility of a DM program to improve treatment persistence among adult patients who initiate an antidepressant treatment with a GP. Methods: The program includes three components: i) *Periodic nurse telephone contacts* to emphasize the importance of persistence, evaluate patient treatment knowledge, and encourage patients to consult his/her GP for any treatment issues; ii) *Knowledge transfer modules* mailed to participants; iii) *Feedback letter to the prescriber* that identifies treatment items that were not well understood by the patient. Study outcomes were: 1) GP participation rate; 2) patient participation rate; 3) representativeness of patients included in the program; 4) health professionnals satisfaction; 5) feasibility of implementation in a public health care system. Results: Of participating GPs, 53% recruited patients. The program was offered to 40 eligible patients with a participation rate of 92.5%. Drop out rate between 1st and 2nd contact was 4 (10,5%). Results of the qualitative assessment of acceptability will be presented. Conclusion: Patients and physicians found the program very beneficial. Patient participation rate was high. The main barrier of implementation was the inclusion of patients through physicians. The efficacy of the program on persistence should formally be evaluated through a randomized trial.

520

Does Ultrafiltration of Plasma Samples Resolve Analytical Interferences in Digoxin Immunoassays?

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Interference from both endogenous and exogenous compounds in commercial digoxin immunoassays has been described for >20 years. One source is digoxin-like immunoreactive-substance(s) (DLIS) that is present in many disease states (including kidney and heart failure). Plasma ultrafiltration has been advocated as a means of removing DLIS interference. We compared 2 ultrafiltration devices from one manufacturer that use the same YM-30 membrane (Centrifree® and Microcon-30®, Amicon) in 250 patient digoxin samples. Digoxin immunoassay (DRI®, Fisher Scientific) ultrafiltrate (unbound) and total digoxin assays gave unbound fraction (Fu%) results that were highly variable (range 25-610%) and a Deming regression: Centrifree = 1.31xMicrocon + 0.042 (95%CI for slope of 1.24-1.39). The reason for the discrepancy between these devices is not known. Hence, the ultrafiltration approach using these devices to remove DLIS did not resolve such interference and appears to have introduced additional sources or error. The additional cost (parts and labour) of performing ultrafiltration routinely is unlikely to be acceptable in high throughput routine laboratories, even if it were reliable, and ultrafiltering 'selected' samples is a flawed strategy. Our study shows that ultrafiltration did not resolve one source of error (DLIS) reliably, and it should not affect a range of other known interferences from lower molecular weight compounds. Clinicians requesting digoxin tests reasonably expect that such assays should deliver reliable results that are free from predictable interferences, and laboratories should not have to undertake corrective measures to remedy such well-known problems.

521

Exposure to Paroxetine in Early Pregnancy and the Risk of Cardiac Malformations: An Updated Meta-analysis

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Background: Recently debate has arisen surrounding paroxetine's (Paxil®) safety in pregnancy, prompted by reports of increased risks for cardiac defects following first trimester exposure. Objective: To determine whether infants with first trimester paroxetine exposure have increased cardiac malformation rates compared to non-exposed infants. Methods: Two reviewers independently searched for case-control and cohort studies published between 1985 – 2007 in

any language on the MEDLINE, EMBASE, REPROTOX, Scopus, and Biological Abstracts databases, using the search terms pregnancy outcome, congenital or fetal AND anomalies, malformations, cardiac/heart defects, AND selective serotonin reuptake inhibitors, paroxetine and Paxil®. Studies reporting cardiac malformations after first trimester exposure to paroxetine that had non-exposed comparison groups were included. A 27-item checklist was used to assess quality of included articles. Random effects models combined odds ratios across case-control studies and rate differences for cohort studies. Heterogeneity of effects was tested with χ^2 and I^2 and publication bias with Begg-Mazumdar test. Results: Nine studies met inclusion criteria. Heterogeneity of effects and publication bias were both absent (P>0.4 in all cases; I^2 =0 for both). Three case-control studies found no increased risk of congenital malformations associated with paroxetine (OR=1.18; CI_{95}:0.88-1.59). Cardiac malformation rates were similar (1.1% each) and within population norms (0.7%–1.2%). Six cohort studies found non-significant weighted average difference of 0.3% (CI_{95}:-0.1%-0.7%; P=0.19). Conclusions: First-trimester exposure to paroxetine does not appear to be associated with increased rates of cardiac malformations. This information should be reassuring to women who require treatment with paroxetine in pregnancy.

522

Anticonvulsant Activity of *Hypoxis hemerocallidea* Fisch. & C. A. Mey. (Hypoxidaceae) Corm ['African Potato'] Aqueous Extract in Mice

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Current biomedical evidence suggests that approximately 80% of South Africans rely on traditional health practitioners and medicinal plants for their daily healthcare needs. Unfortunately, however, very little data exists in the literature on the efficacy, safety and quality of the plant products used in South African traditional medicines. Various extracts of *Hypoxis hemerocallidea* (Hypoxidaceae) corm [popularly known locally as 'African Potato'] are frequently used in South African traditional medicines for the treatment, management and/or control of an array of human ailments, including childhood convulsions and epilepsy. The anticonvulsant effect of the plant's corm aqueous extract (APE, 50–800 mg/kg i. p.) against pentylenetetrazole (PTZ)-, picrotoxin (PCT)- and bicuculline (BCL)-induced seizures has, therefore, been examined in mice. Phenobarbitone and diazepam were used as standard anticonvulsant drugs for comparison. Like the reference antiseizure drugs used, *H. hemerocallidea* corm aqueous extract (APE, 100–800 mg/kg i. p.) significantly delayed (p<0.05–0.001) the onset of, and antagonized, pentylenetetrazole (PTZ)-induced seizures. APE (100–800 mg/kg i. p.) also profoundly antagonized picrotoxin (PCT)-induced seizures, but only weakly antagonized bicuculline (BCL)-induced seizures. Although the data obtained in the present study do not provide conclusive evidence, it would appear that APE produces its antiseizure effect by enhancing GABAergic neurotransmission and/or action in the brain. Taken together, the results of this laboratory animal study indicate that APE possesses anticonvulsant activity, and thus lend pharmacological credence to the suggested folkloric, ethnomedical uses of the herb in the management of childhood convulsions and epilepsy in some rural communities of South Africa.

523

Advertisement of Pharmaceutical Drugs in African and Nigerian Medical Journals: Is the Pharmacological Information Provided Adequate for Safe Prescribing?

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Objective: To evaluate drug advertisements in African and Nigerian medical journals for their adequacy of information.

Methods: African and Nigerian medical journals comprising of West African Medical Journal (WAMJ), East African Medical Journal (EAMJ), South African Medical Journal (SAMJ), Nigerian Medical Practitioner (NMP), Nigerian Quarterly Journal of Hospital Medicine (NQJHM) and

Nigerian Postgraduate Medical Journal (NPMJ) were reviewed. EAMJ, SAMJ and NMP are published monthly, and WAMJ, NQJHM and NPMJ are published quarterly. Monthly journals published between January 2005 and December 2006, and quarterly journals published between January 2001 and December 2006 were evaluated. Drug information with regards to brand/non-proprietary name, pharmacological data, clinical information, pharmaceutical information and legal aspects was evaluated. Results: 41 pharmaceutical companies made 192 advertisements. 112 (58.33%) of these advertisements were made in African medical journals. Pfizer and Swipha advertised most. Four (2.08%) adverts mentioned generic names, 157 (81.77%) mentioned clinical indications. Adults and children dosage (39.58%), use in special situations (36.46%), adverse effects (30.21%), average duration of treatment (26.04%), and potential for interaction with other drugs (18.75%) were less discussed. Pharmaceutical information such as available dosage forms (65.63%), and product and package description (50%) were fairly mentioned. The product and package description provided in the Nigerian medical journals was significantly higher than that provided in the African medical journals (P <0.001). Conclusions**:** Almost none of the drug advertisements in the journals adequately provided the basic information required for appropriate prescribing. It is therefore necessary to provide detailed prescribing information on advertised drugs in medical journals.

524

Comparative Study of Student Performances in Multiple Choice Questions versus Theory Papers of Pharmacology Formative Examinations with Focus on Gender Differences

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Introduction: Assessment is a powerful driver of innovation and change in education. In this study, methods of assessments included: theory short essays and multiple choice questions (MCQ); (viva voce and practicals were excluded). The study was designed to compare: student performances in theory and MCQ of three formative examinations in Pharmacology and explore gender differences. Materials and methods: A total of 208 second year medical students (male: female 107:101) with records of total scores in theory and MCQ papers were analyzed. Performances of students scoring > 60% in theory papers were compared with that of students scoring > 60% in MCQ papers and vice versa. Differences in performances with respect to their genders were also studied. Statistical analysis was done using McNemar, Chi-Square and Pearson's co-efficient of correlation. $P < 0.05$ was considered significant. Results: Analysis showed probability of students scoring > 60% in theory papers was higher when they have scored > 60% in MCQ papers ($P < 0.001$).Theory paper performance positively correlates with MCQ performance ($r = 0.779$, $r = 0.747$ and $r = 0.739$ for first, second, third tests respectively).Girls scored higher than boys in all tests ($P < 0.001$). Discussion and conclusion: Literature indicates that MCQ as a tool of assessment is debatable. This study indicates that MCQ performance is an effective predictable tool to assess theory paper performance. This study correlates with data across the globe on gender differences in student performances. Population studied included a single batch to enable uniformity of question papers.

525

Economic Impact of Generic Substitution of Lamotrigine: Projected Costs in the United States using Findings in a Canadian Setting

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Introduction: Generic substitution may not always save healthcare costs for antiepileptic drugs (AED). The objectives were to examine the economic impacts of generic substitution of lamotrigine in Canada; and to convert observed Canadian costs to a United States (US) setting. Methods: Health claims from Québec's health plan (RAMQ) between 08/2002-07/2006 were

analyzed. Patients with at least 1 epilepsy, claim and treated with branded lamotrigine (Lamictal®) before generic entry were selected. Healthcare costs ($/person-year) were compared during periods of branded and generic use of lamotrigine. Two cost-conversion methods were employed: one using purchasing power parities, US/Canada service use ratios, and exchange rate, and another employing Canadian healthcare utilization and US unit costs. Results: 671 patients were observed during 1,650.9 and 291.2 person-years of branded and generic use of lamotrigine, respectively. The generic-use period was associated with an increase in overall costs (2006 constant Canadian dollars) relative to brand use (CA$7,902 vs. CA$6,419/person-year; cost ratio (CR) =1.22; p=0.05), despite the lower cost of generic lamotrigine. Non-lamotrigine costs were 33% higher in the generic period (p=0.013). Both conversion methods yielded increases in total projected health care costs excluding lamotrigine (2006 constant US dollars) during the generic period (Method 1: cost difference: US$1,758/person-year, cost ratio (CR)=1.33, p=0.01); Method 2: cost difference: US$2,516, CR=1.39, p=0.004). Conclusion: Use of generic lamotrigine in Canada was significantly associated with increased overall medical costs compared to brand use. Projected overall US healthcare costs would likely increase as well. Study limitations pertain to treatment differences, conversion parameters and possible claim inaccuracies.

526

Latent Opportunities for Errors in Medication Orders in Four Canadian Health Authorities

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Introduction: One of the main latent opportunities for medication prescribing errors is the use of potentially dangerous abbreviations and dose designations. Little is known about frequency and potential consequences of errors related to misinterpretation of medication orders in Canada. Methods: Five medication-use safety indicators, selected from a list of 20 such indicators derived using the Delphi technique, were prospectively tested for feasibility, reliability and validity in four health authorities. Medications and abbreviations chosen for testing were based on the Institute of Safe Medication Practices' (ISMP) lists of high-alert medications, error-prone abbreviations and dose designation. Results: Over the three-month data collection period, 7113 medication orders were reviewed in each participating health authority. Seventy-seven percent of medication orders had at least one latent opportunity for error according to the composite indicator. Most latent opportunities were related to route of administration and dose unit. Clinical clerks (95%) and nurses (88%) generated the most latent opportunities, while pharmacists (23%) and nurse practitioners (55%) generated the least and physicians (77%) were somewhere in between. Discussion: The percentage of medication orders containing at least one latent opportunity for error was high for all sites. Latent opportunities for error were mostly due to use of "U or u" for units and "SC or SQ" for subcutaneously. Conclusion: These performance measures will allow organizations to evaluate the frequency and types of potentially dangerous medication abbreviations and dose designations used in medication orders and to target selected healthcare providers for further education.

527

Real Patient Videotapes Help Teach Principles of Clinical Pharmacology and Rational Therapeutics

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Introduction: Medical students face a dwindling pharmacology curriculum. Most pharmacology teaching comes from residents or specialists, many of whom know little more than their students. EBM concepts derive from populations, whereas a doctor's prescription involves a therapeutic experiment where N=1; the important outcomes are for that individual patient. Drug benefits (e.g. analgesia) are often underutilized, whereas harms may not be recognized, are seldom well documented, and almost never reported to regulatory authorities. How can we improve

clinical pharmacology training? Methods: I have produced teaching videotapes/DVD's which illustrate important pharmacologic concepts. I use them in the undergraduate medical curriculum and for continuing medical education. Results: Delegates to CPT 2008 may view examples such as the rapid onset and efficacy of IV morphine for severe pain, use of propofol to sedate for cardioversion, the insidious onset and slow offset of lithium neurotoxicity, paroxetine-induced tremor and its rapid suppression by metoprolol, and the human and financial costs of irrational polypharmacy for pain or depression. Discussion: Videotaping patients who provide informed consent allows each patient to teach many health professionals about adverse drug reactions which (while frequent in a large population) may be unrecognized or infrequently encountered by individual physicians. Acquiring video images is easy with modern videocameras. It still requires astute recognition of drug effects and consent for recording from the patient. Informal consent to acquisition of a video and sound recording can later be formalized, or rescinded. The educational potential of such resources could be expanded if patients provide informed consent to use of their video images over the internet.

528

Prescribing Metformin in Type 2 Diabetes with a Contraindication: Prevalence and Outcomes

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Metformin is the only antidiabetic agent proven to reduce diabetes-related and total mortalities in type 2 diabetes. Contraindications to its use, which are concerned with conditions predisposing to lactic acidosis, have not been well defined. Its prescribing in patients at risks has been observed in practice. This study determined the prevalence and the patient outcomes of metformin prescribed for those with and without a risk factor. 1,630 ambulatory Type 2 diabetic patients at the General Hospital were identified through patient database. The majority of them (1458/1630, 89%) had taken metformin as part of antidiabetic regimens. Among these, 23% (336/1458) had at least one risk factor, with renal impairment being the most frequent risk. Compare the following data of those with (N = 336) and those without risks (N = 1122), respectively: age (66.4 ± 11.59 vs. 59.5 ± 11.1, p = 0.00), hospitalization (1.42% [95%CI 1.16-1.69] vs. 0.53% [0.46-0.59], p = 0.00), and all-cause mortality (3% [1-5] vs. 0% [0-1], p = 0.00) were higher for the former than for the latter. Metformin dose (1409.4 ± 782.4 vs. 1553.03 ± 820.9 mg/d, p = 0.005) was lower for those with a risk factor. The durations of metformin use (years) were similar in both groups (4.2 ± 3.5 vs. 3.9 ± 2.9, p = 0.187). No case of lactic acidosis was evidenced. Metformin prescribing against its contraindications remained prevalent but no lactic acidosis occurred. However, more hospitalization and death cases were found among those posing a risk factor.

529

Prescription Medication Utilization in British Columbia's Pediatric Population

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Introduction: Although an increasing variety of medications developed for adults are being prescribed to children, comprehensive assessments of pediatric pharmaceutical utilization are scarce. A recent Canada-wide study by Khaled et al. (2003) provides a model for developing a population-based utilization profile. Our objective was to corroborate and extend this work by analyzing utilization in the entire population of British Columbia children within 11 therapeutic classes, drawing upon the province's PharmaNet database. This database records all prescription dispensings in the province's community pharmacies regardless of the payer, enabling us to address a limitation of the Khaled et al. study, namely the restriction of their study population to children in families with private drug coverage. Methods: We identified a cohort aged 0-17 years with 1+ prescriptions filled in 1999-2000 (N=517,000) and computed person- and prescription-based utilization rates for individual drugs and therapeutic classes, using provincial population estimates and cohort / subgroup counts as denominators. Results: 60% of the province's children were medication users with utilization highest among the youngest and

oldest subgroups. Prevalences and rankings of the classes were similar in the 2 studies. Antibiotics, the most prevalent class, were used by 81% of cohort members, while antidiabetics were the least prevalent (0.3%). Our estimate of antidepressant prevalence was double that of Khaled et al. Discussion: Agreement in a wide range of findings is reassuring considering the important difference in the data sources. Conclusion: Important issues are highlighted through these analyses concerning prescribing of particular drug classes, notably antidepressants in younger children.

530

Diclofenac and Ibuprofen Increase the Risk of Myocardial Infarction in Males, Smokers and Patients with Higher Cardiovascular Risk

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The increased cardiovascular risk associated with long term non-steroidal anti-inflammatory use has received significant media attention recently. In 2004, Vioxx was withdrawn from the market after it was found rofecoxib (selective COX-2 inhibitor) increased the risk of cardiovascular events. The cardiovascular risks posed by non-selective COX inhibitors are not fully elucidated. We performed a systematic review of the literature to investigate the cardiovascular risks associated with ibuprofen and diclofenac. We searched the Medline, EMBASE and BioMedCentral databases with the keywords "non-steroidal anti inflammatory drugs", "ibuprofen", "diclofenac" and "myocardial infarction". We only included randomised controlled trials, case control studies, case crossover studies in our review. Only studies which used data collected in Europe and USA were reviewed. We excluded existing reviews, comments, letters and meta-analyses. 10 studies showed that ibuprofen users had an increased risk of myocardial infarction. One particular study showed that ibuprofen users had a relative risk equalling 1.5 of having a myocardial infarction, compared to non-users. The relative risk of myocardial infarction associated with ibuprofen becomes statistically significant in the studies using large populations. Diclofenac is also associated with an increased risk of myocardial infarction in all the studies reviewed. We conclude that there is a significantly increased risk of myocardial infarction associated with diclofenac and ibuprofen. This risk is predominantly increased in males, smokers and patients with initial higher cardiovascular risk. Based on these results, physicians should be more cautious in prescribing ibuprofen and diclofenac to patients who are considered to be at high risk.

531

Testosterone Induces the Simultaneous Production of Nitric Oxide and Superoxide and Forms Peroxynitrite in Rodent Mesenteric Vessel

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The increased incidence of cardiovascular disease in men compared with premenopausal women suggests an unfavourable effect of male sex hormone testosterone (T2) on the cardiovascular system. However, numerous clinical and epidemiological studies reported a controversial relationship between T2 and cardiovascular disease. Increasing amounts of evidence indicate that T2 can exert acute vasorelaxing effects, *via* non-genomic mechanisms. These effects involve primarily the vascular smooth muscle (VSM), without requiring the presence of endothelium, although an endothelial contribution is apparent in some studies. An important point of the previous studies was that high concentrations of T2 in micromolar ranges were required to elicit vasodilation. To date, the mechanism behind the vasodilatory action of testosterone is still under debate and might be through either activation of K^+ channels or blockade of Ca^{2+} channels in VSM cells. Our preliminary studies show that in addition to inducing the production of nitric oxide, testosterone also stimulates the production of superoxide and these two reactive molecules combine to form peroxynitrite. Based on our preliminary studies we hypothesize that testosterone exerts its vascular effect through peroxynitrite.

532

The Biological Role of Medications in Epidemiological Research

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The increasing availability of biomarker data from population-based surveys have provided a better understanding of the complex physiological mechanisms that mediate health and mortality. Little is known, however, about the potential effects of medication use on these biological indicators. Considering older adults in the United States consume a disproportionately large and increasing share of medications, there is a growing need to understand and account for the biological role of medications in analyses pertinent to older adult health. This is especially important in the aging population as they are more vulnerable to the physiological and pharmacological effects of medications. Thus, the inclusion of medication data in statistical models warrants further attention in health-related analyses. Using data from a national population-based study of older adults this paper provides evidence of the modifying and mediating effects of medication use on the associations between biomarker data and physical and mental health.

533

Antigenotoxic Effect of *Gymnema montanum* against Oxidative Stress Induced DNA Damage in Human Peripheral Blood Lymphocytes and HL-60

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Introduction: *Gymnema montanum* Hook (Asclepiadaceae), an endemic plant species found mainly in Western Ghats, India, was found to have antihyperglycemic and antihyperlipidemic effect in experimental diabetes as demonstrated in our earlier studies. Objective: This study evaluated the antigenotoxic activity of ethanol extract of *G. montanum* leaves (GLEt) in human peripheral blood lymphocytes and HL-60 cell line *in vitro* using the comet assay. Methods: To explore the potential mechanism of action, two approaches were performed: DNA damage caused by H_2O_2 alone and by other DNA damaging agents with different mode of action such as alkylation and methyl methan sulfonate (MMS). The effect of GLEt on repair capacity of H_2O_2 /MMS induced DNA damage was assessed using alkaline comet assay. Results: GLEt treatment effectively protected from H_2O_2 induced oxidative DNA damage in dose dependent manner whereas the extract was not effective against DNA damage caused by MMS. At 200 μg/ml level, its repair capacity against H_2O_2 induced DNA damage was comparable to that of vit C (100μM). Furthermore, exposure to GLEt reduced the formation of apoptotic cells induced by H_2O_2, which was demonstrated by the decreased Sub-G1- DNA content in cell cycle analysis and apoptotic frequencies of lymphocytes in annexin-V binding assay using flow cytometer. In addition, GLEt was found to have effective H_2O_2 scavenging ability *in vitro* model system. The screening of active components using GC-MS analyses showed the presence of gallic acid, resveratrol, clavatol, and quercetin in the extract. Conclusion: The biological implications of these findings could be important not only for the understanding the antioxidant property but also in understanding the cytoprotective effect of GLEt.

WEDNESDAY, JULY 30, 2008
STREAM 4: CLINICAL PHARMACOLOGY IN SPECIAL POPULATIONS

534

Potentially Inappropriate Drug Prescription in Hospitalized Geriatric Patients

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Beers criteria have been widely used to identify potentially inappropriate prescription (PIP). In Chile, these criteria are not used routinely. The purpose of this study was to assess PIP frequency in patients hospitalized in the Geriatric Unit (GU) of the Clinical Hospital of the Universidad de Chile. Clinical Pharmacists carried out a prospective follow-up study in patients

hospitalized in the GU between June 2006 and January 2007. Selection criteria were ages 65 years or older, hospitalization of at least 3 days, with more than 3 months of life expectancy. Inappropriate drug prescription was defined by Beer's criteria published in 2003. Outcomes of the study were PIP frequency; Beers estimated severity of PIP and mean length of hospital stay. A total of 119 patients were hospitalized during the study period, 78(65%) of them met selection criteria. The mean age of patients was 79.1±9.7 years and 54(69%) were women. During hospital stay, 799 medications were prescribed; mean number of medication per patient was 10.2±4.1. Twenty five (32%) patients received at least 1 PIP. Of the 799 prescribed medications, 32(4%) corresponded to PIP and 26(81.3%) of these corresponded to Beers estimated high severity. Use of inappropriate drugs was significantly associated (p=0.01) with higher mean length of hospital stay, 10.4±7 and 7±5.4 days, respectively. This study showed a longer mean length of hospital stay among patients with PIP. Other analyses must be performed to clarify this association. Further studies using Beers criteria, and conducted in different Chilean settings by interdisciplinary team are required.

535

Benzodiazepines and SIADH

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Introduction: The use of psychotropic drugs has been associated with the syndrome of inappropriate antidiuretic hormone secretion (SIADH) in a number of case reports. SIADH is characterised by the sustained release of antidiuretic hormone (ADH) from the posterior pituitary. Many conditions have been associated with SIADH, which may be classified into four major groups: neoplasia, neurological disorders, lung disease (non-malignant) and an increasing variety of drugs. The patients have a reduced ability to excrete diluted urine, ingested fluid is retained, and the extracellular fluid expands and becomes hypo-osmolar. The cardinal signs are hyponatraemia, serum hypoosmolality and a less than maximally diluted urine. Common symptoms include weakness, lethargy, headache, anorexia and weight gain. These symptoms may be followed by confusion, convulsions, coma and death. The early symptoms are vague and nonspecific, and they may even mimic the symptoms of the psychiatric disorder itself. Patients and methods: During last year we had 10 patients (7F:3M) in our Nephrology Department who were admitted to Hospital with laboratory data of severe hyponatremia. They were diagnosed as having syndrome of inappropriate secretion of antidiuretic hormone, associated with oxazepam; this drug was discontinued immediately after admission. The hyponatremia was treated with saline infusion, water restriction, and furosemide. Discussion and conclusion: Oxazepam is being increasingly used for depression and insomnia, however, we should be alert to hyponatremia in patients on oxazepam by carrying out periodic monitoring of serum electrolytes, especially in elderly patients.

536

Therapeutic Drug Monitoring of Gentamicin in Neonates Critically Ill at the 1st Week of Life

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Background: Gentamicin (Ge) is an aminoglycoside with antimicrobial activity more dependent on plasma concentration (Cpl) than on the dose, characterized by the narrow therapeutic window determined by risks of toxicity. In neonates, the risk is further enhanced by maturational processes and/or pathological conditions modifying drug disposition. Therefore, individual dosage prediction based on Cpl is justified. Methods: This prospective study enrolles 45 neonates of 24-42 weeks´ gestational age (GA), and 0.8-4.56 kg birth weight, admitted at the Neonatal Intensive Care Unit at first week of life and given Ge for suspected or proven sepsis. Two initial Ge doses (i.v. 30-min infusion) were predicted according to b.w. and GA (2-4 mg/kg). Following the first initial dose, Cpl of Ge were detected (TDx, Abbott Lab.) and individual pharmacokinetic parameters (PK) estimated using MW Pharm version 3.15A MediWare, NE). Fluctuation of Cpl was simulated and Ge dosage

was assessed and adjusted according to individual PK if steady-state Cpl were out of the target range (0.5-2.0 mg/L Ctroughss and 6-9 mg/L Cpeakss). PK guided dosage prediction was verified by detection of Ctroughss and Cpeakss related to dose 4. Results: Target Ctroughss and Cpeakss predicted were compared to that measured. The difference was assessed by Bland-Altman analysis. There was a good agreement in 14/45 cases. In remaining ones we identified two main covariates strongly influencing body fluids and pharmacokinetics of Ge: oedema and/or capillary leak syndrome, and furosemide given as a cumulative dose > 1mg/kg b.w, either by a single dose or continuous i.v. infusion. Discussion and Conclusion: In neonates during the first week of life, gentamicin Cpl after the first dose seem to be predictive of steady state Cpl in absence of covariates significantly modifying pharmacokinetics of Ge such as body fluid retention (systemic capillary leak syndrome) and furosemide.

537

Remifentanil for Tracheal Intubation in Infants and Children

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Introduction: Propofol combined with the ultra-short acting opioid, remifentanil, provides good conditions for tracheal intubation without the use of muscle relaxants. This study aimed to determine the optimal dose of remifentanil to facilitate tracheal intubation. Methods: With REB approval and parental consent, 64 subjects undergoing elective procedures requiring tracheal intubation were recruited into three groups: 0-4 months (group I), 4-12 months (group II), and 1-3 years (group III). Anesthesia was induced with 5mg/kg propofol, followed by a bolus dose of remifentanil. A sequential up-and-down design determined allocation of the bolus dose, starting at 3 mcg/kg, with 1 mcg/kg increments (range 1-6 mcg/kg). Tracheal intubation was performed 60 seconds after remifentanil administration by a study investigator, blinded to the remifentanil dose, who also graded intubating conditions using the Good Clinical Practice Scoring system. The time to tracheal intubation, duration of apnea, SpO2, HR and NIBP were recorded. Logistic regression was used to determine ED95 of remifentanil. Results: Tracheal intubation was successful in all subjects. ED95 (95% confidence interval) for all subjects was 6.8 (4.3-9.3) mcg/kg. There was no significant difference in ED95 estimates between groups, although Group II showed a marked variability in dose response. Discussion: ED95 estimates were higher than previously reported, likely due to sequence and timing of drug administration. Conclusion: Doses of 4-6 mcg/kg was effective and without adverse effects. Further investigation of the variability in dose response in infants and assessment of the safety profile of higher remifentanil doses is warranted.

538

One of the Problems with Traditional Medicines: Interactions with Prescription Drugs

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Traditional medicines are still widely used in South Africa as well as most other African countries. As affordable and accessible herbal remedies, their use is encouraged by Government and WHO. Worldwide, their popularity is increasing. While consumers generally regard them as natural and therefore safe, they may have serious adverse effects as well interactions when used alongside conventional medicines. The present study was undertaken to search the literature for documented adverse events of this nature in order to establish guidelines for the safe use of traditional health care. Traditional/herbal medicines as part of complementary/alternative medicine (CAM) are increasing in popularity. This applies to South Africa as well as the international community. However, traditional remedies may be associated with serious adverse events. These relate mainly to quality, safety and efficacy. Of growing concern is their interaction with prescription drugs. In the South African context, antidiabetics, anticancer drugs, anticoagulants and ARVs seem to pose a particular problem if used concomitantly with traditional medicines. Doctors are usually not aware of the risks since patients often do not

inform their practitioners of concurrently taken self-medication. Guidelines are suggested for the safe use of all medicines available on the market. Awareness, education and suitable legislation are the relevant cornerstones. In this way, the smooth cooperation between the two health care systems will be guaranteed and protected.

539

The Assessment of the use of Artesunate-Amodiaquine Combination in the Treatment of Uncomplicated Malaria in Kinshasa, Democratic Republic of Congo

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Introduction: Since 2005, the D.R.CONGO government has adopted a new malaria treatment policy using the Artesunate-Amodiquine combination as the first line medicine for the treatment of uncomplicated malaria. Studies have reported his efficacy against Plasmodium falcifarum malaria. This study aim at the valuation of the use of the combination in the community, the medical centres and the pharmacies, two years since this policy has been applied. Methods: This study is a transverse survey in 1445 households, 447 medical centres and 556 pharmacies. The interview throught standardized questionnaires interrelated to a documentary review were the method to collect datas. Results: 71.97 % of households heads didn't have knowledge of the current national policy. Among those who pretended to know it, 13.49 % presented erroneous knowledge. Quinine was the most prescribed antimalaria medicine (41.39 %) in medical centres while Artesunate-Amodiquine combination was prescribed only by 4.92 % of prescribers. In the pharmacies, artesunate in monotherapy treatment was the most counseled antimalaria medicine by the pharmacy sellers, while quinine remained the most sold antimalaria medicine (42.4 %). Discussion and Conclusion: This study shows that the knowledge level of the new malaria treatment policy remained low two years since the policy has been adopted. It's urgent for the health authorities to set up appropriate strategy. The abusive use of quinine as first line medicine can step up the plasmodium resistance emergence to this effective medicine yet available.

540

Outcomes with Antiepileptic Drug Treatment in Pregnancy in Malta

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The aim of the study was the evaluation of the efficiency of antiepileptic drug treatment in pregnant women with epilepsy in Malta from 1996 – 2005. Pregnancy in woman with epilepsy can give rise to several serious medical problems and always belongs to the group of high obstetric risks. The data obtained was analysed with student t-tests at the 5 % significant level. In total, 58 birth outcomes in 46 PEW were documented i.e 0.1% of total births during that time. The epilepsy was idiopathic in 42 cases. In 5 cases, the PEW were not taking any AEDs. All PEW were on folic acid 5 mg daily. CBZ (n = 29) and PHT (n = 21) were the two most widely prescribed AEDs in this group: 60% were on monotherapy, while 22% on two drugs, 9% on 3 drugs and 9% were on no treatment. Birth weight (3.29 $\pm$ 1.25 kg; $p > 0.05$), AGPAR scores (8.2 vs 8.25; $p>0.05$) and incidence of congenital anomalies fitted in with the general population, but there was a higher incidence of obstetric intervention in PWE (normal vaginal delivery 50 % vs 74.8 %); $P <0.05$). T Further studies to assess the level of risk of adverse outcomes over a longer follow up period are necessary. It is only with the emergence of solid evidence of the difference in the teratogenicity of AEDs, which may change current practice in the management of epilepsy in women of childbearing potential.

541

Determining the Rate, Nature and Predictors of Adverse Drug Reactions Associated with the use of Highly Active Antiretroviral Therapy (HAART) in a Resource Limited Setting (Zimbabwe)

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Introduction: The study aims at determining the rate, nature and predictors of toxicities associated with HAART in a Zimbabwean population. Methods: The study is a retrospective-descriptive study at Parirenyatwa Hospital, Zimbabwe. Using a standardized questionnaire, 400 HIV positive adults stabilized on HAART will be studied. A sub-study was conducted in 130 patients to find out whether toxicity profiles of six selected generic fixed dose combinations (FDCs), with the same active ingredients (Stavudine 30 or 40mg, Lamivudine 150mg and Nevirapine 200mg), from different manufacturers, would have statistically significant differences. Results: Using Pearson Chi-square test, a significant difference in the incidence of peripheral neuropathy (p=0.039) between the FDCs was observed. The FDC, Triomune® (Stavudine 30 or 40mg, Lamivudine 150mg and Nevirapine 200mg) caused headache, dizziness and urinary incontinence, not caused by other FDCs. No statistically significant differences were noted for incidence of other adverse drug reactions (p>0.05). Discussion: Some FDCs had uncharacteristic adverse events and some had greater incidences of peripheral neuropathy. Since different excipients are used in different formulations, these could be the cause of these differences. One hypothesis questioned the use of bioequivalence testing as the basis for formulating generic FDCs. Bioequivalence studies are conducted as single dose studies in healthy individuals whereas antiretrovirals are taken chronically, in HIV positive patients. The steady-state pharmacokinetics, of 'bioequivalent' FDCs might lead to accumulation of the drug. Conclusion: Equivalent antiretroviral FDCs from different manufacturers exhibited different toxicity profiles in a Zimbabwean population. Several factors might also cause adverse events in patients on HAART.

542

Nevirapine Pharmacokinetics in Premature Infants

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Introduction: The purpose of this study was 4-fold. Firstly, to determine if premature infants (PI's) whose HIV-positive mothers (HPM) received single dose nevirapine (sdNVP) during labour (group 1) and those whose HPM missed NVP treatment during labour, but received sdNVP at birth, require a sdNVP at 48-72 hrs after birth to maintain NVP therapeutic plasma concentrations above 100ng/ml through the 1st week of life. Secondly, to describe NVP pharmacokinetics (PK). Thirdly, to determine the NVP dosing regimen required to maintain plasma concentrations (PC's) above 100ng/ml throughout the first week of life in both groups of PI's. Fourthly, to determine the mother to child HIV transmission (MTCHT) rate at 4 weeks after birth. Methods: After informed consent, PI's born before 37 weeks of gestation were prospectively enrolled. Blood was collected for NVP level determination immediately after birth, then 1, 2, 4, 6, 8, 14 and 21 days later. PC's of NVP were determined by liquid chromatography mass spectrometry. HIV-DNA-PCR test was done at day 28 after birth. The study was approved by the ethic committees of the Universities of the Western Cape and Stellenbosch. Results and discussion: We report results from 81 infants including 58 born to mothers who received sdNVP and 23 whose mothers missed NVP. The mean (±SD) gestational age (GA) and birth weight (BW) are 33.1±2.8 weeks and 2035.5±697.8g for Group 1 and 34.0±2.9 weeks and 2058.0±675.8g for Group 2. Cmax, AUC, VD, Cl, T1/2 and Ke are respectively 1630.1±838.6ng/ml, 173761.6±104942.5hr x ng/ml, 176.2±128.4ml, 1.909±1.983L/hr,

76.1±32.4 hr and 0.011±0.004 for Group 1 and 1802.2±1038.0ng/ml, 173182.3±97984.4 hr x ng/ml, 72.4±34.4 ml, 3.172±1.857L/hr, 0.043±0.058ml/hr, 72.4±34.4hr, and 0.012±0.006 for Group 2. PC's exceeding 100ng/ml (10 times the in vitro IC50) are achieved over 11 days in both groups of neonates. No correlation is found between the GA and T1/2. There is a small but significant correlation between BW and T1/2 (r=-0.265, p=0.0166). The estimate for proportion of HIV (+) based on 4 HIV (+) and 79 HIV (-): 4/83=0.048 = 4.8%, 95% confidence interval estimate for proportion of HIV (+) is (0.013, .119) or (1.3%, 11.9%). Conclusion: It is unnecessary to administer an additional dose of NVP at 48-72 hrs after birth in order to maintain therapeutic plasma concentrations above 100ng/ml through the 1st week of life. According to our results, the MTCHT rate is 4.8%.

543

Quality of Drug Prescribing among Elderly Residents in Nursing Home

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Introduction: Polypharmacotherapy is a common problem in elderly patients in nursing homes and it has been associated with an increased risk of drug-related problems (DRP) and adverse drug reactions. Objective: We aimed to analyze drug prescribing in elderly NH residents (NHRs), using patients' age, indications of prescribing and frequency of some predefined inappropriate drug prescription and DRPs. Methods: After approval the local ethic committee the study were conducted in the largest Geriatric Center in the city of Banja Luka with 320 NHRs, over a period of one year (2007), by gerontologist and clinical pharmacologists. Medical records were examined, drugs were coded using WHO ATC classification, the prevalence of potentially inappropriate medication use in the elderly aged 70 years or over was evaluated using Beers' criteria. Inappropriate co-prescribing was evaluated using a list of ten potentially harmful drug combinations, assessment of DRPs were performed using explicit criteria. Results: Mean age of was 77.8 ± 5.6 years, 67.5% were females, 90.1% suffered from chronic conditions. A total of 67% of NHRs received >6 drugs /day. The prevalence of inappropriate medication was 28.2%, mostly long-acting benzodiazepine diazepam, antibiotics in viral aetiology, amitriptyline and antipsychotic for nonpsychotic diagnoses. The inadequate co-prescribing was 15.9%, mostly used harmful combinations were a non-steroidal anti-inflammatory drug (NSAID) co-prescribed with a diuretic or acenocoumarol, two benzodiazepines co-prescribed. DRP were found in 18%, associated with antipsychotic, antihypertensives and NSAID. Conclusion: We found high rates of potentially inappropriate prescribing, therefore, further steps need to be implemented to prevent this occurrence.

544

Milrinone Plasma Levels after Inhalation in Cardiac Patients

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Background: Milrinone is a vasoactive drug administered intravenously or through inhalation to patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) for the treatment and prevention of pulmonary hypertension (PH) associated with difficult separation from bypass (DSB). Targeted steady-state concentrations during intravenous administration are considered to be 100-300 ng/ml. However, when given intravenously milrinone is associated with a high occurrence of systemic hypotension while this is not the case after inhalation. The blood concentrations of milrinone administered through inhalation have never been measured but the absence of hypotension could be secondary to lower systemic concentrations. Our objective was to confirm that the safer profile of inhaled

milrinone could be secondary to lower systemic exposure. Method: A pilot observational study was carried out in patients preoperative PH scheduled for elective cardiac surgery with CPB and for whom administration of inhaled milrinone was indicated. Milrinone (5 mg) was administered before CPB by nebulization (conventional or ultrasonic) over 15 min. Arterial blood samples were obtained before starting inhalation (time zero), at 20, 25, 30 min thereafter, and immediately after CPB. Milrinone concentrations were determined by HPLC with UV detection (LLOQ 1.25 ng/ml). Results: In all patients, systemic levels of milrinone are below 80 ng/ml. Discussion: Because the first sample was drawn 5 min after stopping inhalation, peak concentrations could have been underestimated. However, these concentrations remain significantly below those measured after intravenous administration of milrinone. Conclusion: Our pilot study suggests that a negligible systemic availability may explain the higher therapeutic index of inhaled milrinone.

545

Pregnancy Outcome and Cognitive Development of Children Exposed *in utero* to Cyclosporine Following Maternal Renal Transplant

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Introduction: Cyclosporine is among the most common immunosuppressive therapies used to prevent organ transplant rejection. Its reproductive safety, including long-term neurodevelopmental effects on exposed children, must be assessed. The present objective was to evaluate perinatal outcomes and children's long-term neurocognitive development following exposure to cyclosporine for maternal renal transplant. Methods: This cohort study with matched controls used a prospectively collected database and maternal interviews to document pregnancy outcomes. Validated standardized psychological tests assessed the neurocognitive achievements of exposed and unexposed children. Results: 39 exposed children were assessed (15 single pregnancies; 11 repeated pregnancies with two or three children) and compared to 38 matched unexposed children. Renal transplant children were exposed to immunosuppressive (cyclosporine, azathioprine, prednisone), anti-hypertensive and anti-bacterial substances. 33.3% of exposed children were born prematurely with low birth weights, high rates of perinatal complications and instrumental deliveries versus 0.5% in unexposed controls. There were no significant differences in Full-Scale IQ between exposed and unexposed groups (104.46 vs 109.00, p=0.157) or between children of single and repeated pregnancies (108.27 vs 102.08, p=0.162). Premature-low birth weight children had statistically and clinically significant lower scores than controls (98.46 vs 109.00, p=0.49). Conclusions: Although cyclosporine does not appear to affect children's long-term cognitive abilities, maternal renal transplant, associated comorbidity, and perinatal complications may adversely impact child outcome. Proper management of maternal morbidity and improved obstetric care may significantly improve child's neurocognitive performance.

546

Third Trimester Fetal Heart Rate and Doppler Blood Flow Characteristics Following Prenatal SRI Antidepressant Exposure

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Introduction: Prenatal exposure to serotonin reuptake inhibitor (SRIs) antidepressants may increase risks for persistent pulmonary hypertension and poor neonatal adaptation. The fetal antecedents to these outcomes remain unclear. This study investigated the impact of prenatal SRI exposure on third trimester fetal vascular flow/indices and heart rate characteristics. Methods: At 36 weeks gestation fetal heart rate (FHR) (50 min) (Sonicaid 8002), and Doppler middle cerebral artery (MCA) blood flow velocity waveforms (Aloka 5500) were obtained from SRI exposed (EXP) (n=14) and nonexposed (NEXP) (n=27) fetuses during a morning (AM) (0800 hrs) and post SRI dose afternoon (PM) (12:30 hrs) (3.2 ± .4 hrs after SRI

dose) session. Maternal mood was assessed at 36 weeks. Results: FHR accelerations were significantly less frequent in EXP fetuses compared with NEXP fetuses in AM (11.6 ± 3.8 vs 15.5 ± 5.7) and PM (12.7± 3.7 versus 18.3±6.0) sessions. MCA resistance indices (PI, RI and S/D ratio) were significantly lower in the EXP in the AM, but not in the PM ($p<0.05$). EXP fetuses had decreased number of short and long term variations and episodes of high HR variability in PM compared with AM sessions ($p<0.05$). Group differences remained controlling for maternal mood. Conclusion: Prenatal SRI exposure reduces 3rd trimester FHR variability and MCA resistance or impedance, even when accounting for maternal mood. Acute SRI pharmacologic and metabolic factors, as well as SRI-related neurological and vascular alterations remain to be studied.

547

Elastic Properties of Arteries and Endothelin-1 Levels in Angiotensin Converting Enzyme Inhibitor Therapy in Black Hypertensive Patients

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Increased arterial stiffness and endothelial dysfunction are associated with end-organ damage in hypertensive patients. In this study we investigated the effect of a 9-month treatment with perindopril on arterial elasticity and endothelin-1 levels in black hypertensive patients. Newly diagnosed hypertensive patients (N=44, diastolic pressure > 85 mmHg and/or a systolic pressure > 135 mmHg) were recruited for the study. They received 4mg perindopril daily for 9 months. Pulse wave velocity (PWV) was used as an indicator of arterial elasticity and was measured non-invasively. PWV was measured along the carotid-femoral artery segment and along the brachial-ulnar artery segments. PWV was calculated using the distance between sensor placement and the time of travel of the pulse wave. Blood pressure was measured non-invasively using calibrated equipment. Venous endothelin-1 was measured using an I^{125} immuno-assay radioactive ligand system. Systolic pressure decreased significantly from 145 ± 23 to 133 ± 26 mmHg, diastolic pressure from 89 ± 15 to 79 ± 15 mmHg, mean arterial pressure from 110 ± 7 to 99 ± 12 mmHg and pulse wave velocity in the aorta-femoral segment from 11.5 ± 6 to 7.9 ± 3 m/s after 9 months. There was no change in the PWV of the brachial-ulnar segment and in the endothelin-1 levels. No correlation was found between endothelin-1 and PWV. Arterial elasticity did improve and it was independent of endothelin-1 level and of the lowering of blood pressure. However, PWV in the brachial-ulnar segment is not a good indicator of arterial elasticity.

548

Topical Retinoids Exposure during the First Trimester of Pregnancy

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Concerns have been raised with the topical use of retinoids since the publication of occasional cases associated with characteristic retinoid embryopathy, originally described after oral use. Epidemiological data are still scant. A collaborative study was carried out to evaluate the rate of congenital malformations following 1st trimester topical retinoid exposure. Using a standardized protocol exposed pregnancies and non exposed controls were prospectively recorded by the European Network of Teratology Information Services (ENTIS). A population of 222 pregnant women exposed to topical retinoids (median age [range]: 30 [21 – 42] years; gestational week at call: 7 [3 – 35]) were compared to 444 women not exposed (median age [range]: 32 [17 – 48] years; gestational week at call: 8 [2 – 39]). The following retinoids were identified: adapalene: 22; retinoic acid: 10; tretinoin: 135; isotretinoin:49, others: 6. The exposed and non-exposed groups did not differ in maternal alcohol and tobacco use, gestational duration, birth weight and length. There were no

significant differences between groups in the rate of pregnancies ending in spontaneous abortion (8.7% in exposed vs. 5.9% in unexposed; P=0.18) or in infants with minor malformations (3.7% in exposed vs. 2.9% in unexposed; P=0.61) and major malformations (3.7% in exposed vs. 2.2% in unexposed; P=0.29). No child showed features of retinoid embryopathy. In conclusion, these results bring reassurance in cases of fortuitous topical retinoid exposure. However, according to the current knowledge, topical retinoids can not be recommended for use during pregnancy, as the risk/benefit ratio remains questionable.

549

Evaluation of Medication Prescribing Patterns and Dosage Adjustment in Patients with Chronic Kidney Disease in a Renal Unit of Bogotá

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Introduction: The main purpose of Drug Utilization studies (DU) is to facilitate rational use of medications in general population. Chronic Kidney Disease (CKD) is an important and costly health problem. For many drugs is necessary dosage adjustment according to renal function in order to avoid overdosage and iatrogenic risk. The purpose of this study was to evaluate prescribing patterns and quality prescription for the first three comorbidities associate to CKD in a renal unit of a university hospital in Bogotá. Methods: Cross-sectional. Quantitative/qualitative DU. Medication prescribing data of all ambulatory haemodialysis patients was recorded. Patient, diagnosis, comorbidities and prescription variables were recorded. Appropriateness of dosage adjustment criteria were taken according to dug database (Micromedex ®). Results: 75 patient's medication prescriptions were surveyed. Average time of management of CRD in the institution was 27 months. The most frequent drugs prescribed were in their order the iron, complex B and eritropoyetina. The medications more frequently prescribed for three first comorbidities were calcium antagonists (nifedipine and amlodipine) for hypertension, insulin NPH for diabetes and levotiroxin for hypothyroidism. For hypertension, twelve different antihypertensive were prescribed, between they enalapril, fosinopril, furosemide and minoxidil those require dose adjustment. Concerning to dosage adjustment 29.9% prescribed medications of a total of 75 revised formats requires dose adjustment. Discussion and Conclusion: According to similar studies the revision and analysis of medication profiles in patients with CKD is a useful tool for identification drug-related problems. This can rebounds on improvement of the therapy.

550

The Role of Placental Breast Cancer Resistance Protein in the Efflux of Glyburide across the Human Placenta

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Introduction: Gestational diabetes mellitus is a common medical complication in pregnancy. Recent findings demonstrate that glyburide is effluxed against a concentration gradient from the fetal to the maternal circulation. However, the transport systems involved in the active efflux of glyburide in the human placenta have not yet been identified. The ATP-binding cassette transporter, breast cancer resistance protein (BCRP), is highly expressed in placental syncytiotrophoblast suggesting it may play a role in protecting the fetus from drug toxicity. The objective of the present study was to determine whether BCRP participates in the transport of glyburide across the human placenta. Methods: The placental transfer of glyburide in the presence of specific BCRP inhibitor, nicardipine, was investigated using the ex vivo dual perfusion system of isolated human placental lobules. In a closed experiment, glyburide was added (200ng/mL) to the maternal and fetal circulations and the BCRP inhibitor (20uM) was added to the maternal circulation. Samples were taken during pre-

control, experimental, and post-control periods for measurement of glyburide and markers of tissue viability. Results: Results obtained from perfusions (n=4) in the presence of the BCRP inhibitor show a significant increase in the mean fetal-to-maternal concentration ratio of glyburide determined at 180 minutes, 0.56 ± 0.06, when compared to the mean ratio obtained in the absence of inhibitor, 0.32 ± 0.06 (p=0.04). Discussion: These data indicate that nicardipine partially blocked the transfer of glyburide across the whole placenta through its inhibition of BCRP. This is the first ex vivo evidence that BCRP actively transports glyburide.

551

Mangifera indica L. (Vimang) Protection against Serum Oxidative Stress in Elderly Humans

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Background: We searched for the protective effect of a natural extract from stem bark of *Mangifera indica* L. extract (Vimang) on age-related oxidative stress. Methods: Healthy subjects were classified in two groups, elderly (>65 years) and young group (<26 years). The elderly group received a daily dose of 900 mg of extract (three coated Vimang tablets, 300 mg each, before meals) for 60 days. Serum concentration of lipid peroxides, serum peroxidation potential, extracellular superoxide dismutase activity (EC-SOD), glutathione status (GSH, GSSG, GSSG/GSH ratio)) and total antioxidant status (TAS) were determined before (both experimental groups) and 15, 30, and 60 days after treatment (only elderly group). We confirmed the existence of an age-associated oxidative stress in human serum as documented by an age-related increase in serum lipoperoxides and GSSG and a decrease in serum antioxidant capacity and EC-SOD activity. Results: Vimang tablet supplementation increased EC-SOD activity ($p<0.01$) and serum TAS ($p<0.01$). It also decreased serum thiobarbituric reactive substances ($p<0.01$) and GSSG levels ($p<0.05$). We suggested that the antioxidant components of the extract could have been utilized by the cells (especially blood and endothelial cells), sparing the intra- and extracellular antioxidant system and increasing serum peroxil scavenging capacity, thus preventing age-associated increase in GSH oxidation and lipoperoxidation. Conclusions: Vimang tablets prevent age-associated oxidative stress in elderly humans, which could retard the onset of age-associated disease, improving the quality of life for elderly persons.

552

Paediatric Clinical Pharmacology In-patient Consultation Service: A One Year Experience at a Regional Children's Hospital

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Background: Teaching in pediatric clinical pharmacology includes education of fellows. It is unclear as to what fellowship clinical goals should be, as the spectrum of patients to be seen is not well understood, notably for in-patients. This study reviewed a mid-size Children's Hospital to determine type and scope of consultations in pediatric clinical pharmacology. Methods: The study was conducted at Children's Hospital of Western Ontario, a 120 bed Hospital serving 1.5 million people. All consultations for in-patients from July 1st, 2005 to June 30, 2006 were prospectively collected. The consultation service was available working hours Monday to Friday. Data were analyzed using Microsoft Excell. Results: Over the year there were 36 consultations for in-patients, evenly distributed except for September (19%) and December (3%). 30% of consults were on Friday and only 11% on Monday. Consultations came primarily from General Pediatric Wards (50%), with Pediatric Sub-Specialty services the next commonest (22%) and the ICU, Surgical Services and others accounting for the remainder. There was an equal distribution of ages from newborn to adolescents; 60% of were male. The commonest consultation was for assessment of a possible adverse drug event (64%, $p>0.05$), with neonatal drug exposure being next commonest (28%). A change in therapy was recommended in 41% of cases; in 38% of cases drug-related causes were ruled out. Conclusions: The commonest reason by far for consultation on a pediatric in-patient is assessment of a possible adverse drug event. Fellowship curriculum need to have a robust component for

teaching this and in assessment of drug exposure during pregnancy and lactation. A consultation service can be developed at a pediatric care facility providing exposure to a wide range of patients without an overwhelming clinical burden, with the caveat – common to many services – that consultations may group themselves on Fridays.

553

Comparative Pharmacokinetics of Once-daily Tramadol in Healthy Young and Elderly Subjects

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A two-way crossover study was conducted to compare the single-dose pharmacokinetics of a once-daily formulation of tramadol (Tramadol Contramid® OAD; Labopharm Inc., Laval, Qc., Canada) in healthy young adult and elderly human volunteers. Elderly subjects with mild hepatic or renal impairment were eligible to participate. Twenty subjects aged 18-40 years and 15 subjects aged 75 years or older were enrolled. One subject discontinued for personal reasons. Subjects were administered a single oral 200 mg tablet dose. Plasma and urine samples were collected at pre-defined timepoints and tramadol and O-desmethyltramadol levels were determined using validated enantioselective chromatographic methods. Primary pharmacokinetic parameters in plasma [AUC_{0-t}, AUC_{0-24}, AUC_{0-48}, AUC_{inf}, $AUC/AUC_{inf,}$ C_{max}, t_{max}, k_{el}, $t_{½}$, CL/F, V_{area}/F] and in urine [$Ae_{t'-t''}$:, Ae $_{0-48}$, R_{max}, CL_r] in the two populations were compared using ANOVA. A similar rate and extent of tramadol exposure was demonstrated in the elderly and young populations. The apparent clearance was also similar between the age groups. The apparent volume of distribution was approximately 30% higher in the elderly, likely reflecting age-related physiologic differences. This resulted in a prolongation in half-life of 3 hours in the elderly subjects. Elderly subjects reported fewer adverse events. These results suggest that in the absence of significant hepatic or renal impairment, no dosage adjustment is required in elderly subjects.

554

Estimation of Bilastine Dose in Paediatrics

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Introduction: Bilastine is a nonsedating, histamine H_1-receptor antagonist currently under clinical development. The pharmacokinetics (PK) and pharmacodynamics (PD) of bilastine have been successfully described in adults with a recommended oral dose of 20 mg/day. However, no dosing regimen has been yet established for the paediatric population. Aim: Develop a predictive model in order to recommend the optimal dose of bilastine to be used in paediatrics, employing M&S techniques. Methods: In order to predict the PK parameters in paediatrics, two approaches were used. The first approach was based on the principles of allometry. Scaling of the PK parameters (Vc, CL, Vp and Q) was performed based on the preclinical data of the drug obtained from rats and dogs as well as in vitro data from humans. The second strategy was based on the knowledge of the PK principles and physiology. Simulations of time evolution of plasma concentrations of bilastine in 1500 virtual children were carried out after four consecutive oral doses of: 5, 10 and 20mg of the drug. These simulations, performed with NONMEM, were developed in order to reach the therapeutic endpoint (wheal/flare inhibition) described for adults. Results and Discussion: After 4 consecutive doses of 10 mg/day in children (2, 6 and 12 years), bilastine concentrations remained always above IC_{50} value for wheal inhibition effect. For flare, the antihistaminic activity lasts nearly all the dosing interval. Therefore, this dose can be considered as a safe and effective dose in children (2-12 years). For infants (age=1 year) the dose of 5 mg/day is considered an appropriate dose, as the antihistaminic activity remains during the whole treatment period.

555

Proteomic Analysis of the Cellular Responses to Bioactive Triterpenoid Saponin Astragaloside IV from *Radix Astragalus* in Human Liver Cancer HepG2 Cells

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Chemoprevention of oncogenic transformation is recognized as a cost-effective strategy to reduce the incidence of various cancers. Most of the chemoprevetive agents are derived from the natural resources. *Radix Astragalus* is one of the most important chemopreventive medicinal herbs in traditional Chinese medicine. This study was designed to investigate the biological effects of astragaloside IV, a major active triterpenoid glycoside in the herb, on the protein expression in human liver cancer HepG2 cells. Following the treatment with astragaloside IV, the protein extracts of HepG2 cells were resolved by two-dimensional electrophoresis (2-DE) and visualized by silver staining. The protein spots were quantitatively compared and ranked according to the changes induced by astragaloside IV. Fifteen top up-regulated and 13 top down-regulated spots were excised from the gels and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Of the astragaloside IV-regulated proteins, oncogene Vav3b was suppressed up to 1.70-fold and in a dose dependent manner. Vav3 protein as the third member of oncogene Vav family is known to be involved in the regulation of several key signal transduction pathways leading to cell malignant transformation. Vav3b is only composed of the C-terminal two SH3 domains and one SH2 domain of Vav protein, suggesting an uncharacterized role in regulating cell signal transduction. Astragaloside IV-mediated regulation of Vav3b protein expression was verified by Western blot analysis using specific antibody. Thus, our results suggest that downregulation of Vav3b expression by astragaloside IV may represent a novel strategy for cancer therapy.

556

Association of Antiepileptic Drugs with Risk of Development of Fracture-Case Control Study

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Introduction: Epilepsy is a major public health problem affecting nearly 50 million people worldwide. Treatment with antiepileptic drugs (AEDs) is generally chronic, and may be associated with significant metabolic effects including decreased bone mass and increased fractures. Methods: This case-control study was conducted at M.S. Ramaiah Memorial Hospital, Bangalore, India. The information was obtained from epileptic patients attending Neurology OPD and around 80 patients and also 20 controls were enrolled for the study. Patients were grouped on the basis of type of AEDs, duration of treatment and also mono/polytherapy. Blood and urine samples were collected for analysing bone biochemical markers after overnight fasting. Results: In group A, serum calcium, phosphorous and 25(OH)D levels were reduced significantly , ($p<0.001$ and $p<0.003$ respectively). Bone formation and resorption markers were significantly elevated ($p<0.001$). Similar reduction in serum calcium, phosphorous and 25(OH) D levels was found in patients on polytherapy ($p<0.03$). Discussion: In the present study it was observed that use of enzyme inducing AEDs was associated with altered bone metabolism (52.5%, n=80). The patients who were on non-enzyme inducing drugs did not show significant alteration in indices of bone metabolism (20%, n=80), though bone resorption markers were elevated. Linear regression analysis has revealed that there is 0.269mg/dl and 1.82ng/ml reduction in serum calcium and 25(OH)D level respectively every year in patients on enzyme inducing AEDs continuously. Patients who were on polytherapy were also with a higher risk of bone metabolism abnormalities. Conclusion: The present study shows that there is correlation between the

severity of bone biochemical abnormalities with duration and number of AEDs used. So it is necessary that patients receiving AEDs who are at particular risk for bone disease should have routine examination of bone density using DEXA scan and routinely receive calcium and vitamin D supplementation.

THURSDAY JULY 31, 2008
STREAM 1:
NEW THERAPEUTIC APPORACHES

557

The Prophylactic Effect of an Herbal Preparation, STW5, in an Acute Model of Reflux Esophagitis in Rats

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Gastro-esophageal reflux is commonly associated with functional disorders of the stomach, including functional dyspepsia, where STW5 (Iberogast®), a multicomponent herbal medicinal preparation, has been successfully used to alleviate symptoms including heartburn. The present study aimed at investigating the effect of this drug in an experimental model of reflux esophagitis, with a view of providing further pharmacological evidence for its therapeutic usefulness. Male Wistar rats were fasted for 18 h before operating them under ether anesthesia and a ligature was made between the fore-stomach and corpus and between stomach and pylorus to induce esophageal reflux. Four hours later, the rats were sacrificed. The gastric ligations led to ulcerative inflammation of the esophageal mucosa. To test the activity of STW5, rats were treated with the drug daily for 5 successive days at dose levels ranging from 0.2 to 2 ml/kg by oral gavage. On day 5, animals were anesthetized 3 h after the last dose, and esophagitis was induced as described above. STW 5 led to a significant dose-dependent reduction of the ulcerative area. Measurement of myeloperoxidase activity and lipid peroxidation as well as mediators, including TNFα, and histopathological examination confirmed the anti-inflammatory activity of the drug and correlated well with its overall effects. Pantoprazole (5 mg/kg) was used as a reference standard. The results indicate that the beneficial effect of STW 5 in heartburn as a symptom of functional dyspepsia could in part result from its anti-inflammatory effect on the esophageal mucosa.

558

Study of Serotonin Receptors Expression and Role of Serotonin in Colorectal Cancer HT29 Cell Line

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Introduction: Colorectal cancer is the second most common cause of cancer death in men and women. Worldwide colorectal cancer affects 1 million people every year and is responsible for .5 million cancer-related annual deaths. Serotonin has been described as a mitogen in variety of carcinomas. it exerts its mitogenic effect by interacting with a wide range of its receprors.in Methodology: In this present study we have determined expression f serotonin receptors on colorectal cancer HT29 cell line by western blotting and immunohystochemistry. The effect of serotonin and its agonists and antagonists on cell growth was studied using MTT assay; 5Florouracil and leucovorin were used as controls. Results and discussion: MTT proliferation assay revealed that 5HT at concentration more than 50 um had antiproliferation effect and below 35 um had proliferative effect. Also antagonist of serotonin receptors especially 1b and then 1a, 3, 2a ,and 4 subsequently had more antiproliferative effect at concentration more than 50 um. Also we have demonstrated that 5HT1a,1b,2a,3,4 receptors have been expressed clearly in cell line and tumor tissues but the expression of 2b and 2c were not detectable. Thus we can describe that serotonin at high concentration can have antimitogenic and at low concentration mitogenic effect and because of antiproliferation effect of some specific antagonists of serotonin receptors, it can open a new approach for further researchs in chemotherapy of colon cancer.

559

Inhibition of 5-Lipoxygenase and Leukotriene C_4 Synthase in Human Blood Cells by Thymoquinone: A Possible Clinical Relevance

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Thymoquinone (TQ), the main constituent of volatile oil from *Nigella sativa L*. has been subjected to a range of pharmacological investigations in recent years. Since leukotrienes (LTs) are important mediators in inflammatory process, therefore, the effect of TQ on LTs formation by human blood cells were investigated. Preincubation of human granulocyte cells with different doses of TQ (1,3,10 and 100 µM) for 15 min before being subjected to calcium ionophore A23187 stimulation provoked a significant inhibition of both leukotriene C_4 (LTC_4) and leukotriene B_4 (LTB_4) generation in a concentration-dependent manner as compared to diluent-treated control cells. Testing the effect of TQ on the enzmyes involved in LTs biosynthsis in human granulocyte cells; arachidonate 5-lipoxygenase (EC:1.13.11. 34), LTC_4 synthase (EC: 2.5.1.37) and LTA_4 hydrolase (EC: 3.3.2.6) revealed that different doses of TQ induced a marked inhibition of 5-lipoxygenase as evidenced by suppressed conversion of exogenous arachidonic acid into 5-hydroxy eicosatetraenoic acid (5-HETE). In addition, higher doses of TQ (10 and 100 µM) induced a significant inhibition of LTC_4 synthase activity as judged by suppressed transformation of exogenous LTA_4 into LTC_4. In contrast, the drug was without an inhibitory effect on LTA_4 hydrolase. Since LTA_4 is the substrate for both LTC_4 and LTB_4, thus in the presence of TQ, shunting of LTA_4 from synthesis of LTC_4 was directed to LTB_4 formation. Human platelets possess a specific membrane-bound LTC_4 synthase. A similar inhibition of LTC_4 formation was observed when exogenous LTA_4 was added to platelet suspension preincubated with TQ. The inhibitory effect of TQ increased considerably at longer preincubation times. LTC_4 synthase activity can be controlled via phosphoregulatory mechanism, protein kinase C activation induced suppression of LTC_4 synthase enzyme. The unselective protein kinase inhibitor, stoursporine failed to prevent the inhibition of LTC_4 synthase enzyme induced by TQ. In addition, TQ displayed a similar inhibition of LTC_4 synthase in sonicated platelets suspension, incubated with exogenous LTA_4 and glutathione.

560

Imidazole–Dioxolane Compounds as Selective Heme Oxygenase Inhibitors: Effect of Modifications to the Appendage in the 4-position of the Dioxolane Ring

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Introduction: The heme oxygenase (HO) system is comprised of two active isozymes, namely, HO-1 (inducible) and HO-2 (constitutive), which are involved in the biotransformation of heme into biliverdin and releasing carbon monoxide (CO) and ferrous iron (Fe2+). The cellular regulatory actions of CO have been acknowledged. The use of HO inhibitors as pharmacological tools may prove to be indispensable in the investigation of the CO/HO system and related physiological pathways. Several imidazole–dioxolane compounds were synthesized and evaluated as novel inhibitors of HO. These compounds comprise a series of derivatives of 2-[2-(4-chlorophenyl)ethyl]-2-[1H-imidazol-1-yl)methyl]-1,3-dioxolane hydrochloride substituted at position 4 of the dioxolane ring by methyl(thiophenol) or methyl(phenol) groups, and also a series of smaller functionalized derivatives. Methods: Several imidazole–dioxolane compounds were synthesized in a novel manner. Rat microsomes (spleen, HO-1; brain, HO-2) were used to determine HO activity by measuring the formation of CO. Results: One of the compounds studied, namely, (2R,4S)-1-{2-[2-(4-chlorophenyl)ethyl]-4-(naphthalen-2 ylsulfanylmethyl)-[1,3]dioxolan-2-ylmethyl}-1H-imidazole hydrochloride exhibits an IC50 value of 0.9 ± 0.1 micromolar for HO-1 (rat spleen microsomes) and an IC50 value of 30 ± 4 micromolar for HO-2 (rat brain microsomes), with a selectivity index of 33. Discussion: Structure–activity relationships of various analogues with respect to the inhibition of HO will be presented.

Conclusion: In vitro, most of the compounds were found to be highly potent inhibitors of both of the stress-induced isozyme HO-1 and the constitutive isozyme HO-2, showing only moderate selectivity for HO-1. However, a few of the compounds displayed higher selectivity towards HO-1.

561

Challenges in the Testing of Clinical Safety and Efficacy of Lessertia Frutescens (Sutherlandia) in HIV Infection

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Clinical testing of South African traditional plant medicines (TPM) poses several challenges. The International Center for Indigenous Phytotherapy Studies (TICIPS) on HIV/AIDS Secondary Infections, and Immune Modulation are testing the safety of Sutherlandia in two Phase I studies in South Africa. One (completed) was a randomized double–blind placebo controlled trial of Sutherlandia leaf powder in healthy adults, the second (ongoing) a RCT in HIV-infected adults. To identify and address the particular challenges that clinical testing of indigenous plant medicines' with claimed use in HIV infections poses the study designs, strategies and procedures employed, the regulatory and ethical review processes and outcomes, experiences gained and the time and cost requirements of the 2 trials were reviewed and compared to that for a typical phase 1 trial of a new pharmaceutical. The analysis showed that the cost and implementation time requirements of the Sutherlandia studies, once approved, were comparable to that of the typical Phase I trial; limited input from traditional healers can be obtained and used, and most of the ICH – GCP guidelines could be implemented. The time to secure regulatory and ethical approval for study 2 was however long (1.83 yrs) and the lack and poor quality of appropriate pre-study pharmacological data and data on the quality and consistency of the TPM product, significant factors. While the approval process is expected to improve as more traditional medicine trials are done, the lack of pharmacokinetic and pharmaceutical quality data on the trial product will remain significant challenges.

562

AZD6140, the First Reversible Oral Platelet P2Y12 Receptor Antagonist, Exhibits Linear Pharmacokinetics Following Multiple Doses in Healthy Subjects, with Greater And Less Variable Inhibition of Platelet Aggregation Compared with Clopidogrel

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Introduction: AZD6140, the first reversible oral platelet P2Y12 receptor antagonist, is in Phase III development for the reduction of clinical atherothrombotic events. This study assesses pharmacokinetics and pharmacodynamics of multiple doses of AZD6140 in healthy subjects. Methods: In this 14-day study, healthy volunteers (N=48) were randomized to receive oral doses of AZD6140 (AZD6140/placebo = 7/2): 50, 100, 200, 300, 400, and 600mg qd or bid to steady-state, and 14 subjects received a clopidogrel loading dose of 300mg and then 75mg qd. Plasma concentrations of AZD6140 and active metabolite AR-C124910XX were analyzed. Inhibition of ADP-induced platelet aggregation (IPA) and drug tolerability were also evaluated. Results: AZD6140 was rapidly absorbed, with a median T_{max} of 1.5-3 hrs across doses. Mean $AUC_{0\text{-}inf}$ and C_{max} of AZD6140 and AR-C124910XX increased approximately dose-proportionally over the 50-600mg range, with mean accumulation ratios of 1.2-1.8. Mean final extent of IPA was 93-99% with AZD6140 bid doses $\geq$100mg at trough plasma concentrations (with lower variability throughout the dosing interval), compared to 77% with clopidogrel qd. No safety or tolerability issues arose up to 600mg bid. Discussion: IPA was greater and better sustained at high levels over the dosing interval with twice-daily regimens of AZD6140 than with once daily. Conclusion: Pharmacokinetics of AZD6140 were linear following multiple doses of 50-600mg, with >90% IPA at 100mg and higher doses. Greater

and more consistent IPA with AZD6140 at doses >100mg bid and >300mg qd was observed compared to clopidogrel. AZD6140 was well tolerated up to multiple daily doses of 600mg.

563

Heart Rate Reduction by Ivabradine Increases Electrical Threshold of Ventricular Fibrillation in a Dose-dependent Manner during Experimental Myocardial Ischaemia in the Pig

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Background: The ischaemic ventricular fibrillation (VF) onset is often facilitated by tachycardia. The present study assessed the impact of heart-rate (HR) reduction induced by ivabradine (IVA), a selective inhibitor of the cardiac pacemaker current I_f, on the propensity toward ischaemia-induced VF. Methods: Short-term (1 min) complete occlusions of the anterior interventricular artery were produced in anesthetized pigs. VF was triggered by electrical stimuli of increasing intensity under spontaneous HR. Three doses of IVA were tested: 0.25, 0.5 and 0.625 mg/kg, bolus iv (n=5-6/dose). One group of pigs received saline as control (n=6). HR and VF threshold (VFT) were determined during ischemia. The correlation between HR and VFT was studied. Additionally, the size of the hypoxic area (% of LV mass) was measured histologically at the end of the experiment. Results: IVA induced a dose-dependent HR reduction, –13% ($p<0.05$), -23% ($p<0.01$) and –27% vs saline at 0.25, 0.5 and 0.625 mg/kg respectively. IVA increased VFT in a dose-dependent manner by 2, 3 ($p<0.01$) and 4-fold at 0.25, 0.5 and 0.625 $mg.kg^{-1}$ vs saline, respectively. A significant inverse correlation between HR and VFT was found ($r = -0.62$, $p<0.0001$). The size of the hypoxic area was significantly ($p<0.001$) reduced, 18.7%, 19.8% and 21% vs saline (29.8%) in 0.25, 0.5 and 0.625 mg/kg IVA –treated pigs, respectively. Conclusion: Dose-dependent reduction of HR induced by IVA protects against ischaemia-induced ventricular fibrillation by increasing fibrillation threshold and provides cardioprotection by decreasing myocardial damage. This effect could result in the prevention of ischaemia-induced sudden death.

564

Reduction of PAI-1 Levels in Diabetic Patients with IMD-1041, A Novel IKKbeta Inhibitor

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Introduction: IMD-1041 is a novel pro-drug of the IKKbeta inhibitor, IMD-0354, discovered using rational drug design. Inhibitors of IKKbeta are likely to have anti-inflammatory effects via inhibition of NF-kappaB activation. The transcription of plasminogen activator inhibitor-1 (PAI-1) is dependent upon NF-kappaB and PAI-1 appears to be involved in wide variety of disease processes including thrombosis and tissue remodeling (fibrosis). Method & Result: An initial small scale open POC study was conducted with 12-week administration of IMD-1041 capsules 200 mg twice daily after food to 21 patients with type 2 diabetes, aged 56.6 ± 1.7 years who were mainly on treatment with sulphonylueas with baseline HbA1c 7.6 ± 0.2%. Most diabetic parameters showed no significant change with this dosage regimen and period, but there was marked reduction in plasma PAI-1 (mean ± SE: 43.4 ± 7.3 to 12.1 ±1.5 ng/mL, at baseline and after 12 weeks, respectively, $p < 0.0001$ by U-test). Only abnormally elevated PAI-1 levels were decreased while no significant changes were seen in subjects with normal baseline levels suggesting IMD-1041 is acting as a PAI-1 normalizer. IMD-1041 was well tolerated and safe with no serious drug-related adverse events or significant changes in laboratory safety parameters including coagulation tests, which were also not affected in Phase 1 Study. Conclusion: IMD-1041, a novel orally available IKKbeta inhibitor, resulted in normalization of elevated PAI-1 levels in diabetic patients. This suggests that the compound can be developed as a

promising drug to prevent thrombotic events and potentially tissue remodeling.

565

Transient Receptor Potential Channels as a Therapeutic Target for Protecting the Endothelium against Hyperglycaemia-induced Calcium Overload

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Introduction: The transient receptor channel (TRP) family consists of six subfamilies. TRPC1, a member of the canonical family, is upregulated in neointimal injury in human vascular tissue and experimental vascular injury models and hypothesized to contribute to calcium overload and vascular disease. We explored whether exposure of bovine aorta endothelial cells (BAECs) to high glucose [HG] also affected TRPC expression and calcium signalling. Methods: BAECs were cultured in either normal [NG, 5.5mM] or HG [25mM] media and expression levels of TRPC1, 3, 4 and 6 mRNA and protein were determined by RT-PCR and Western blotting. P2Y receptor-mediated ATP-induced increases in calcium entry and intracellular calcium [Ca_i] release were determined using the fura-2 and 340/380 ratio technique. Antisense was used to assess the contribution of TRPCs to changes in Ca_i. Results: TRPC1, but not TRPC3, 4, or 6, was significantly increased in HG, but not NG, after 72 h. HG did not change basal Ca_i or ATP-induced Ca_i but significantly increased the amplitude of sustained Ca_i which was reduced by the TRPC blockers gadolinium and SKF96365. Treatment with TRPC1 antisense reduced TRPC1 protein (but not other TRPCs) and ATP-induced calcium entry. Discussion: These data indicate that selective inhibition of TRPC1 in endothelial cells reduces the enhanced Ca_i that is seen in HG following endothelial cell activation thus reducing the deleterious effects of elevated Ca_i, such as apoptosis, that may trigger hyperglycaemia-induced endothelial cell dysfunction. Conclusion: Targeting TRPC1 may reduce the development of both endothelial dysfunction and vascular smooth muscle proliferation.

566

Cloning and Transport Properties of a Novel Gene Encoding Breast Cancer Transport Peptide 1, BCTP1, from a Human Breast Cancer cDNA Library

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We isolated a novel gene encoding breast cancer transport peptide 1 (BCTP1) from a human breast cancer cDNA library. Isolated BCTP1 cDNA consisted of 246 base pairs that encoded an 82-amino acid protein with a 3 putative membrane spanning domain. By RT-PCR analysis, BCTP1 mRNA was detected in various human tumor tissues such as the lung, liver, stomach, small intestine, ovary, and colon as well as the breast. To determine the functional characterization of BCTP1, we employed a *Xenopus laevis* oocyte expression system. When expressed in *X.* oocytes, BCTP1 mediated the high affinity transport of [^{3}H]5-fluorouracil (5-FU) with a K_m value of 69.2 ± 24.5 nM in time- and pH-dependent, and Na^+-independent manners. A *cis*-inhibition experiment revealed that BCTP1 mediated transport of [^{3}H]5-FU is strongly inhibited by antineoplastic agents (cisplatin and etoposide) and nucleic acids (pyrimidine, uracil, uridine, guanosine, inosine, thymidine, adenosine, cytidine and purine) suggesting that BCTP1 may be involved in the membrane transport of these drugs and endogenous substrates. Immunohistochemical analysis revealed that the BCTP1 protein is localized in the lactiferous duct epithelium. Thus, the present results indicate that a newly isolated cDNA clone, BCTP1, is a key molecule for the breast handling of 5-FU in humans.

567

Effect of Endogenous Adenosine on Adenosine A_1 and A_3 Receptor Subtypes during Simulated Ischemia

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Endogenous adenosine (EA) is released from cardiomyocytes during ischemia/reperfusion injury, and therefore any exogenous agonist must work in the context of significant local agonist concentrations. We assessed the hypothesis that EA conceivably activates A_1 and A_3 receptor-mediated ischemic defence. Adenosine deaminase (ADA) can be used to remove EA in a simulated ischemia (SI) model, and thus we evaluated the effects of A_1 and A_3 agonists in the presence and absence of ADA. Simulated ischemia was induced by incubating 60-70 % confluent H9c2(2-1) cardiomyocytes in SI buffer for 12 hrs in 100 % N_2 gas at 37^0C then incubated with propidium iodide (5μM) for assessment of necrotic cell death followed by lactate dehydrogenase assay. Annexin-V and Caspase-3 flow-cytometry performed for apoptosis detection. Cells were treated with the A_1-CPA, A_3-IB-MECA agonist and a series of novel compounds designed to act as A_1 (VCP-28), A_1/A_3 (VCP-102) and A_3 agonists (VCP-103; 0.1-1000nM) alone ± DPCPX and MRS 1523 (100 nM) respective antagonists ± ADA (1 U/ml). The percentage reductions of cell death (non-viable cells) in the presence of CPA, IB-MECA, VCP-102, VCP-28 and VCP-103 (10nM) were 70.79±12.87%, 57.46±10.89%, 76.24±7.67%, 67.95±6.29% and 53.01±22.50% and reduced with ADA to 13.22±4.81%, 55.17±8.78%, 55.27±6.97%, 51.35±4.35% and 47.35±3.66% (Mean ± SEM n=3, $P<0.05$, vs. SI, SI+ADA) respectively. Cell death increased in presence of respective antagonists for both A_1 and A_3 agonists. In conclusion A_1 and A_3 agonists exhibit protection against ischemic cell death; however in absence of endogenous adenosine A_3 agonists show significantly ($P<0.01$, CPA+ADA vs. IB-MECA+ADA) greater protection.

568

Effects of Rheum ribes Shoot and Root Ethanol Extracts on Proliferation and Apoptosis in HL-60 Cells

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This study was conducted to demonstrate the effects of ethanolic shoot and root extracts of *Rheum ribes* L. on the proliferation and apoptosis of HL-60 cells for the first time in literature. Dried and pulverized plant samples were extracted by ethanol at a ratio of 1:12 (w/v) at 50 °C for 24 hours. Human Myeloid Leukemia (HL – 60) cells were cultured in the presence of various concentrations of extracts upto 72 hr. The percentage of cell viability was determined by metabolism of the tetrazolium salt XTT (2,3 – bis(2-methoxy-4-nitro-5-sulfophenyl) -5-[(phenylamino) –carbonyl] -2H-tetrazolium hydroxide). *R. Ribes* shoot and root extracts were found to inhibit the survival of HL-60 cells in a concentration- and time-dependent manner. ED50 values of ethanolic shoot and root extracts were calculated as 120.01 ± 0.65 μg/ml and 104.15±0.54 μg/ml, respectively. For further examinations, HL-60 cells were plated overnight in T75 flasks at a density of $1x10^5$ cells/ml and treated with root and shoot extracts at a final concentration of 100 μg/ml of growth medium for 16 hours. Isolated RNAs of both treated and non-treated cells were then reversely transcribed to cDNAs using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV-RT). The expression of Bax and Bcl-2 genes were examined by real time PCR. The apoptosis caused by treatments were also demonstrated by cytoplasmic cytochrome c release, DNA ladder formation and TUNEL. The antiapoptotic effects of extracts were correlated by comparing the levels of Cyp1B1 and Cyp1A1 expressions separately.

569

Selective Contribution of Cyclooxygenase-2 to Vascular Reactivity is Dependent on the Presence of Testosterone in Fructose-fed Rats

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The presence of testosterone is an important factor in the development of endothelial dysfunction and subsequent increase in blood pressure secondary to insulin resistance. Testosterone regulates the

expression of vascular cyclooxygenase-2 (COX-2), a key vasoactive mediator. However, its role in vascular reactivity is unclear in insulin resistance. We used fructose-fed male Wistar rats to study whether the inhibition of COX-2 affects vasodilatation or vasoconstriction and how insulin resistance and testosterone influence these responses. Blood pressure in intact and gonadectomized fructose-fed rats was measured prior to and after 9 weeks of fructose feeding. Following an oral glucose tolerance test, the superior mesenteric arteries (SMA) were evaluated for responses to phenylephrine prior to and post selective COX-2 inhibition using NS-398 (10^{-6}M) and non-selective inhibition using indomethacin. Relaxation responses to acetylcholine were determined subsequent to incubation with indomethacin (10^{-5}M). While gonadectomy did not ameliorate insulin resistance, it prevented the diet-induced increase in blood pressure. In comparison with untreated tissues, NS-398 and indomethacin decreased PE-evoked vasoconstriction in the SMA of both control and fructose-fed rats but not in gonadectomized rats. Further, insulin resistance impaired endothelium-dependent relaxation in intact but not gonadectomized rats. Inhibiting COX (indomethacin; 10^{-5}M) improved endothelium-dependent relaxation in intact rats suggesting a vasoconstrictor role for COX-2. In conclusion, COX-2 selectively contributes to vasoconstriction in the SMA, which is dependent on the presence of testosterone.

570

Evaluation of Herbal Medicine for the Management of Sickle Cell Anemia

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About 70% of all sickle cell anemia (SCA) subjects in the world reside in Africa. Infant mortality is about 8% and survival rate of SCA babies in rural areas by five years of age is about 20%. Thus, SCA is probably the most neglected serious public health disorder in Africa. Pre-clinical and clinical assessments of a herbal extract *vis-à-vis* management of SCA using Good Laboratory Practice and Good Clinical Practice principles respectively. In Africa, there is no standard treatment for SCA. Consequently, most SCA subjects use herbal medicines. NIPRISAN is a standardized extract from four medicinal/food plants. Short term toxicity study indicated that NIPRISAN was safe in laboratory animals. Bio-activity guided fractionation show that vanillin and aromatic aldehydes may be the bioactive moieties. NIPRISAN reversed sickled red blood cells and protected them from being sickled when exposed to low oxygen tension. NIPRISAN dose-dependently delayed polymer formation of hemoglobin S. NIPRISAN induced 85% increased solubility of deoxy hemoglobin S. Histological examination of lungs of control Tg transgenic mice carrying human sickle hemoglobin showed entrapment of massive numbers of sickled cells in alveolar capillaries. NIPRISAN significantly cleared the lungs of sickled cells. Furthermore, NIPRISAN induced profound effect on the survival time of Tg mice under hypoxic conditions ($p<0.0001$). The phase II clinical data indicated that about 80% of the participants did not experience any crisis during the study while attendance at school profoundly increased. NIPRISAN has been patented, licensed to an American company, registered and being manufactured in Nigeria for global market.

571

Effect of Changtong Oral Liquid on Serum Cytokine Concentrations in Rats with Postoperative Intestinal Adhesion

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Peritoneal adhesion is a common complication, especially after abdominal operations. Currently only few herbal preparations for such purpose are available. We investigated the effects of Changtong oral liquid (CTOL) on serum levels of tumor necrosis factor-alpha (TNFα), transforming growth factor (TGF)-beta1, interleukin-1beta (IL-1), IL-4, IL-6 and IL-10 in rats with postoperative intestinal adhesions. Methods: Fifty-four male Sprague-Dawley rats were randomized into six groups: Control placebo, model, Simo decoction (SMD) groups and three CTOL groups. Intestinal adhesion was induced by Ellis' method in rats of the groups other than the control group. The rats in the control placebo and model groups received intragastric administration of distilled water

(10ml/kg), and those in the treatment groups had SMD (10ml/kg) and CTOL at 4.3, 8.6 and 17.2 g/kg for low, moderate, and high dose groups, respectively. On day 7 after surgery, blood samples were taken for measuring serum cytokine levels with enzyme-linked immunosorbent assay followed by adhesion grading according to a 5-grade scale. Results: Model adhesions grew so dense that it was difficult to separate the involved organs. The four experimental groups only developed a membranous adhesion or even no adhesions at all. CTOL reduced the severity of postoperative adhesions and decreased serum levels of proinflammatory cytokines TNFα, IL-1, TGF-beta1 and IL-6 by 35%, 30% and 22%, respectively. However, it had no significant impact on serum levels of anti-inflammatory cytokines IL-4 and IL-10. Conclusion: Significant indices for postoperative adhesion assessment have been established, which provides the experimental basis for evaluating clinical therapeutic effects of postoperative adhesions as well as for developing new therapeutic drugs.

572

Characterization of YM-244769, A Novel NCX Inhibitor in Isolated Cardiac Cells

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Recently, it was reported that YM-244769 (N-(3-aminobenzyl)-6-{4-[(3 fluorobenzyl)oxy]phenoxy} nicotinamide) is a new selective Na+/Ca2+ exchange (NCX) inhibitor (Iwamoto and Kita, 2006). We examined the effect of YM-244769 on the NCX current in isolated guinea pig ventricular myocytes with the whole-cell voltage-clamp technique. YM-244769 suppressed the bi-directional NCX current in a concentration-dependent manner. The IC50 values of YM-244769 were 0.12μM and 0.1μM for the bi-directional outward and inward NCX current, respectively, with a Hill coefficient of 0.7 and 0.8 for the bi-directional outward and inward NCX current, respectively. However, YM-244769 suppressed the unidirectional outward NCX current more potently than inward NCX current with the IC50 value of 0.05 μM (Hill coefficient=1.2). YM-244769 at 10 μM inhibited the unidirectional inward NCX current by only about 50%. YM-244769 at 1 μM suppressed INCX more potentially when intracellular Na+ concentration became higher. Next, Intracellular application of trypsin via the pipette solution did not change the blocking effect of YM-244769, implicating that YM-244769 may not affect the exchanger from the cytoplasmic side. YM244769 is a trypsin-insensitive NCX inhibitor. These results indicate that YM244769 inhibits unidirectional outward NCX current more potently than unidirectional inward or bidirectional NCX currents. YM-244769 inhibits NCX in a similar manner to those of KB-R7943 and SN-6, another relatively selective inhibitor of NCX. In single guinea pig cardiac ventricular myocytes, YM-244769 has potency similarly to SEA0400, another selective NCX inhibitor, and YM-244769 affects more potently than KB-R7943 and SN-6.

573

Preclinical Evidence Supporting Clinical Development of a Liposomal Formulation of Irinotecan

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Introduction: A novel formulation (Irinophore C™) of irinotecan has been developed; maintaining CPT-11 in its therapeutically-active lactone conformation. The PK profile and efficacy of this preparation against a panel of human cancer xenografts in mice were compared to irinotecan (Camptosar®). Methods: CPT-11 was encapsulated into DSPC/Chol (55:45 mol%) liposomes with entrapped $CuSO_4$ in the presence of the ionophore A23187, which exchanges Cu^{2+} from the liposome interior for $2H^+$ from the external buffer thereby generating and maintaining a transmembrane pH gradient. Pharmacokinetic studies were completed in mice. RAG2-M mice with established subcutaneous xenograft tumours (NSCLC H460; colorectal LS180 and HT-29; pancreatic carcinoma Capan-1; prostate carcinoma PC-3) were treated with either

a single dose, 3 doses q4d, or 3 doses q7d of Irinophore C™ or Camptosar®. Results: Irinophore C™ mediated an 8-fold increase in $t_{½}$, a 100-fold increase in Cmax and 1,000-fold increases in AUC and Cl, for the active lactone form, as compared to Camptosar®. Further, continuous plasma levels of SN-38 lactone were achieved for at least 24 hours after administration. Irinophore C™ significantly delayed the time required for tumors to increase in size 4-fold relative to the time required for the same fold increase in control animals. Efficacy data, available stability data and product attributes will be presented as well as proposed GLP toxicology and clinical trial protocol synopses.Conclusions: Irinophore C™ engenders substantial increases in therapeutic activity against a panel of 5 human xenografts when compared to Camptosar® and is a promising candidate for clinical studies.

574

Minimal Protective Effects of Baicalein on *t*-Butyl Hydroperoxide-mediated Oxidative Stress in Recombinant HEK 293 Cells Over-expressing Choline Acetyltransferase

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Choline acetyltransferase (ChAT) produces the neurotransmitter acetylcholine in cholinergic neurons. Previous studies showed that ChAT activity is decreased under oxidizing conditions. The objective of this study was to evaluate the protective effects of the flavonoid, Baicalein (BE) in recombinant HEK 293 cells over-expressing ChAT, following exposure to the cell permeant oxidant, t-butyl hydroperoxide (t-BHP). Redox-sensitive green fluorescent protein 2 (roGFP2) was transiently transfected into these cells, facilitating assessment of their redox status in real time by confocal microscopy. Treatment with t-BHP (10-500 micromole) resulted in concentration- and time-dependent LDH leakage. With 500 micromole t-BHP LDH leakage was 6.2% at 1 h. t-BHP (10-500 micromole) also caused a rapid concentration-dependent depletion of GSH. Neither t-BHP-dependent LDH leakage, GSH depletion nor the amount of oxidized roGFP2 formed was significantly attenuated by BE pre-treatment. Immunoblot analysis using specific anti-GSH or anti-ChAT antibodies in non-reducing SDS-PAGE showed disulfide bond formation between cysteine thiol residues of ChAT and between ChAT and GSH in cytoplasmic extracts of cells treated with 1minimole t-BHP for 60 min. These data show that ChAT, when oxidized, forms one or more GSH-ChAT disulfides, as well as oligomeric forms of ChAT that also exist as GSH-ChAT ignifi or trimers. Although BE did not significantly attenuate intracellular oxidative stress in HEK cells over-expressing ChAT, confocal microscopy showed that 10 micromole BE pretreatment for 30 min significantly attenuated the blebbing induced by treatment with 50 micromole t-BHP, and maintained the integrity of the cell membrane under these specific conditions.

575

PKC-beta2 Activation Plays a Major Role In TNF-alpha-induced Human Vascular Endothelial Cell Apoptosis

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Introduction: The circulatory inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) is increased in pathological conditions, such as diabetes, which initiate or exacerbate vascular endothelial injury. Protein kinase C (PKC) has been shown to play a critical role in TNF-alpha – induced human endothelial cell apoptosis. However, the relative roles played by specific isoforms of PKC in TNF-alpha-induced human endothelial cell apoptosis have not been addressed. We investigated the effects of the selective PKC-beta2 inhibitor CGP53353 and selective PKC-delta inhibitor rottlerin,

respectively, on TNF-α-induced apoptosis in human vascular endothelial cells, and their effects on reactive oxygen species and nitric oxide production. Methods: Cultured human vascular endothelial cells (ECV304) were either not treated (Control), or treated with TNF-alpha (40 ng/ml) alone or TNF-alpha in the presence of 10 μM rottlerin, 1 μM CGP53353, respectively, for 24 h. Cell viability was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Cell apoptosis was assessed by flow cytometry. Results: TNF-alpha-induced endothelial cell apoptosis was associated with dramatic increases in intracellular hydrogen peroxide (~20-fold of control) and superoxide (~16-fold of control) production as measured by dichlorofluorescein and dihydroethidium fluorescent staining, respectively, accompanied with reduced superoxide dismutase and glutathione peroxidase activities and subsequently an increase in the lipid peroxidation product malondialdehyde. CGP53353, but not rottlerin, abolished or attenuated all these changes. However, both substances could attenuate the TNF-alpha-induced increase in nitric oxide production in the culture medium. Discussion and Conclusion: It is concluded that PKC-beta2, but not PKC-delta, plays a major role in TNF-α-induced human vascular endothelial cell apoptosis.

576

Increased Macrophage Migration Inhibitory Factor Expression in Early Diabetic Cardiomyopathy Patients

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Diabetes is regarded as a wild inflammation condition and there is a prevalence of early diabetic cardiomyopathy, left ventricular diastolic dysfunction (LVDD), in diabetes. This study was to evaluate the role of macrophage migration inhibitory factor (MIF) in early diabetes cardiomyopathy patients. Total 47 patients with type 2 diabetes were recruited, who were aged 30-60 years without evidence of hypertension, coronary artery disease, congestive heart failure, other diabetic complications. Left ventricular diastolic function was evaluated by cardiac Doppler echocardiography. Pseudo-normal pattern of ventricular filling and E'/A'<1 was regarded as LVDD. 30 age-sex matched health men were used as controls. In addition, H9c2 cardiomyoblasts cell were exposed to glucose at 5.5- 33 mM, adjusted the same osmotic pressure with mannitol. The results showed that plasma MIF was significantly increased in diabetic patients compared with health subjects, and plasma MIF level was significantly higher in diabetes patients with LVDD than those patients without LVDD (p<0.05). Plasma MIF level in diabetes was correlated with plasma glucose, glycosylated hemoglobin and urine albumin levels, but not with body mass index, HOMA-IR1, LDL cholesterol, HDL cholesterol and total cholesterol levels. In H9c2 cardiomyoblast cells, coincided with the increase of ignific-NF-κB P65 (ser536) protein level, *MIF* mRNA and protein expression is significantly increased in a glucose concentration-dependant manner. These findings have provided further insights into the role of inflammation in the development of diabetic cardiomyopathy which has a therapeutic implication.

577

Palmvitee Decreased Plasma Total Bilirubin and Hepatic UDP-Glucuronyltransferase Activity in Hyperbilirubinemic Suckling Rat

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Introduction: Hyperbilirubinemia or jaundice is common during the first few days of postnatal life. Our previous study has shown that maternal administration of palmvitee reduced serum total bilirubin in hyperbilirubinemic rat neonates. Therefore, this study was conducted to investigate the effect of palmvitee on hyperbilirubinemia induced by δ-aminolevulinic acid (ALA) in rat neonates. Materials and methods: Wistar rat neonates were divided into three groups. They were given 30 (PV30) or 60 (PV60) mg palmvitee/kg body weight intraperitoneally once daily starting on day 1 postnatal through day 14 postnatal, while the other group was given olive oil (control). At day 14 postnatal, the sucklings

were induced with hyperbilirubinemia, while the rest were given vehicle. Twenty-four hours after the induction, the neonates were sacrificed. Plasma total bilirubin, hepatic thiobarbituric acid reactive substance (TBARS), UDP-glucuronyltransferase (UGT) activity and vitamin E content were determined. Results: ALA administration increased plasma total bilirubin. In the palmvitee-treated groups, plasma total bilirubin was significantly lower than in the control. ALA administration did not affect the hepatic UGT activity, but the enzyme activity was reduced in the palmvitee-treated groups. ALA did not influence hepatic TBARS level, but pretreatment with 30 mg/kg palmvitee reduced this parameter significantly. However, in PV60 groups, there were increases in TBARS content. Palmvitee pretreatment at both doses increased the hepatic vitamin E content in the neonates dose-dependently. Conclusion: The administration of palmvitee showed protective effect on hyperbilirubinemia. However, this administration could lead to a decreased hepatic glucuronidation activity in the rat neonates.

578

Melatonin: An Endogenous Mediator of Esophageal Mucosal Defence and a Therapeutic Target

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After 50 years ago of discovery melatonin (MT) as pineal gland hormone is known that this indolamine endogenously produced by entero-endocrine cells make a wide range of biological actions on gastrointestinal neuromodulation, motility, mucosal defence, inflammation and secretory function. The precise etiology of erosive esophagitis (EE), one major form of gastroesophageal reflux disease is still unclear. To elucidate the role of MT in esophageal defence and analyze the relationship between MT and EE in rats were induced acute esophageal injury against acid-pepsin bile intraesophageal perfusion; several experimental series were used without (vehicle only) and with pretreatment with graded doses of MT; without and with inhibition of COX/PG and NOS/NO systems or sensory denervation by neutotoxic dose of capsaicin (125 mg). Lesions area was determined by esophageal macroscopic score system, histology activity index and lectin histochemistry. Esophageal blood flow was assessed using electrolytic regional blood flowmeter, plasma MT, NO and mucosal PG levels by immunoassays. Results: Inhibition of COX/PG and NOS/NO systems manifested in increased esophageal injury. Tested MT dose-dependly afforded esophagoprotection against acidic damage and this was accompanied by an increase in PG content and esophageal microcirculation, both changes being reversed by pretreatment with neurotoxic dose of capsaicin. Conclusions: MT plays a critical role in esophagotroprotection, because involves the mechanism of esophageal hyperemia and increase in PG and decrease reactive nitrogen species generation due to sensory neuropeptides interaction.

THURSDAY, JULY 31, 2008
STREAM 2:
FROM FUNDAMENTAL TO CLINICAL PHARMACOLOGY

579

Safety, Tolerability, Pharmacodynamics and Pharmacokinetics of BAY 60-5521 - A New CETP Inhibitor

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BAY 60-5521 is a potent, novel CETP-inhibitor intended for the treatment of dyslipidemia. Method: The First-in-Man (FIM) study investigated the safety, tolerability, pharmacodynamics and pharmacokinetics in healthy male subjects following administration of single oral doses. The study was performed in a randomized, single-blind, placebo-controlled, single dose-escalation design. 38 young healthy male subjects (aged 18-45 years) received an oral of dose of 5, 12.5, 25, or 50 mg BAY 60-5521 (n=28) or were treated with a placebo (n=10). Results: In all 4 dose steps, only 1 adverse event (25 mg; macular erythema) was considered drug related. Clinical laboratory parameters showed no clinically relevant changes. A clear dose-

dependent CETP-inhibition could be demonstrated starting at a dose of 5 mg. At a dose of 25 mg, a CETP-inhibition > 40% over 24 h was observed. After 50 mg, CEPT inhibition >40% lasted more than 48 h. Mean HDL values showed a nearly dose-proportional increase. After 50 mg, a significant HDL increase by about 30% relative to base-line values was found. BAY 60-5521 was slowly absorbed reaching maximum concentrations in plasma after 4 to 6 h. The disposition in plasma was multi-exponential with an estimated mean half-life of 87 to 142 h. Conclusion: BAY 60-5521 was clinically safe and well tolerated and devoid of effects on heart rate, blood pressure and ECG recordings. A clear pharmacodynamic effect on CETP-inhibition and HDL could be demonstrated.

580

Pharmacokinetics, Safety, and Tolerability of a Wide Range of Doses of the Novel Cannabinoid CB_1 Receptor Antagonist, CP-945,598, in Healthy Volunteers

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Our objective was to establish the pharmacokinetics, safety, and tolerability of CP-945,598, a novel cannabinoid CB_1 receptor antagonist in development for weight management, in healthy subjects. Adults (BMI 18−30 mg/kg^2) received single oral doses of CP-945,598 in 2 randomized, double-blind, placebo-controlled ascending-dose studies (5, 25, 75, 125 mg; cross-over, within-subject design; 2 cohorts of 6 subjects each; and 125, 250, 375, 500 mg; parallel-group design; 5 cohorts of 8 subjects each, respectively). Subjects returned for safety assessments and pharmacokinetic blood samples for analysis of CP-945,598 and its primary N-desethyl metabolite up to 21 days post-dose. Over 5−75 mg, greater than dose-proportional increases in mean systemic exposure (C_{max} and $AUC_{(0\text{-}tlast)}$) were observed. Approximately dose-proportional increases in mean systemic exposure were observed over 75−375 mg. The 375 and 500 mg doses yielded similar mean exposures. At 25−500 mg, CP-945,598 was absorbed at a moderate rate, with median t_{max} ranging from 4−10 hours. Generally, the concentration-time profiles declined in a bi-exponential manner, with estimates of mean half-life of the terminal phase ranging from 107−139 hours. CP-945,598 was generally well tolerated at 5−375 mg with adverse events mild to moderate in intensity. The most commonly observed adverse events were nausea, hiccups, reduced appetite, vomiting, and dizziness, consistent with the pharmacologic mechanism of action. In this healthy population, the maximum tolerated dose of CP-945,598 was 375 mg. CP-945,598 had a long terminal half-life and was well tolerated over a wide dose range. These attributes support once-daily dosing of CP-945,598 and its development for weight management.

581

***In vitro* and *in vivo* Evaluation of a Novel Injectable Polymer-lipid Drug Implant System**

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Introduction: We have developed an injectable formulation for sustained, intraperitoneal (ip) delivery of chemotherapeutics. The biocompatibility and drug release profile of this novel polymer-lipid blend implant ($PoLi_{gel}$) was evaluated. Methods: L929 cells were treated with $PoLi_{gel}$ (1μL/mL–10μL/mL) for 24 hours and cell viability assessed via MTT. Drug free $PoLi_{gel}$ was injected into CD-1 mice (50μL-100μL ip). Subgroups (n=4) were sacrificed weekly for 4 weeks. Plasma ALT and IL-6 levels, capsid formation, and organ morphology were examined post-mortem. Docetaxel (DTX) release from DTX loaded $PoLi_{gel}$ was monitored in vitro. Preliminary efficacy of DTX-$PoLi_{gel}$ (19.2mg/kg–48.0mg/kg ip) was evaluated in a SKOV-3 xenograft mouse model of human ovarian cancer. Discussion: $PoLi_{gel}$ was well tolerated *in vitro* and *in vivo*. $PoLi_{gel}$-treated mice demonstrated no evidence of toxicity nor weight loss. No inflammation was observed upon sacrifice, and histological examinations revealed normal organ morphology. Limited or no fibrous capsid formation was detected. Plasma ALT and IL-6 concentrations were within levels of healthy mice. Sustained in vitro release of DTX from DTX-$PoLi_{gel}$ was observed, with 2.14 +/- 0.25% released per day

over prolonged periods. DTX-PoLi$_{gel}$ resulted in 100.0% and 84.5% inhibition of tumour burden at doses of 19.2 mg/kg and 28.8 mg/kg, respectively, over a three-week period. Physical wasting, ascites fluid accumulation and abdominal distension were not observed in mice treated with DTX-PoLi$_{gel}$ but were present in controls. Conclusion: The PoLi$_{gel}$ injectable implant formulation is biocompatible *in vitro* and *in vivo*, and has shown promising efficacy potential in the treatment of ovarian cancer.

582

Effects of Vimang® on Oxidative Stress and Marker of Disease Progression in HIV/AIDS Patients

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Since antiretroviral therapies do not completely eliminate HIV, it is likely that the final outcomes of treatment will depend not only on the efficacy of treatments in reducing viral load, but also on the immune system's ability to recover and control residual virus. Eighty-two HIV-positive patients naives of antiretroviral therapy and no receiving any vitamin supplements were randomized in a double-blind study to receive supplements of VIMANG® (1,2 mg daily) or placebo for 6 months. Plasma antioxidants status (TAS), peroxidation potential (PP) gluthatione (GSH), malondialdehyde (MDA), plasma total hydroperoxides (TH), superoxide disumutase (SOD), gluthatione peroxidase (GPx), percent of DNA fragmentation (%DNA) and CD4, CD38, CD95 lymphocytes subsets were measured at baseline and at 6 months. Nutritional index and clinical outcome were carried out too. 68 subjects were completed the study. At baseline not difference was detected between placebo and VIMANG® supplemented groups. The supplemented group (n=36) had an increase antioxidant status. No significant differences were detected in CD4 and CD8/CD38 between groups. There was also a trend towards a reduction in CD95 receptor. No significant differences were detected in nutritional index and during clinical ephrecto. No adverse reaction was detected. It is known that HIV population is oxidative stressed and deficient in antioxidant system. Since *in vitro* replication of HIV and depletion of CD4+ T lymphocytes are increased with apoptosis induced by oxidative stress, this study show evidences that an antioxidant supplementation on HIV patients can improve antioxidant status and reduced oxidative damage.

583

Dialyzability of Faropenem Sodium (FRPM) in Hemodialysis (HD) Patients

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Background: FRPM is the widely used antibiotics with broad spectrum, however, date are limited about its dialyzability. Aim: To determine the dialyzer clearance (CL_{HD}) and elimination fraction (EF) of FRPM in HD patients. Methods: Six renal failure patients under HD program with polysulfone membrane dilalyzer were enrolled during FRPM 200 mg twice daliy. Blood samples were taken from arterial and venous sides of the hemodialyzer at 0, 2, 4h after the initiation of dialysis and plasma FRPM concentration and hematocrit were measured. The values CL_{HD} and EF of FRPM were determined by the arterial-venous difference method. Results: The CL_{HD} and EF of FRPM were 15.0 ± 8.7mL/min/m^2 and 17.4 ± 7.0% (mean ± SD), respectively. Plasma FRPM concentration at the beginning of the hemodialysis session was between 2.0 to 7.6mcg/mL (at 74 to 301min after dosing). One patient complained mild diarrhea during the study, which was improved later. Discussion and Conclusion: This is the first study evaluating the dialyzability of FRPM in HD patients. Plasma FRPM concentration before hemodialysis was comparable to the maximum plasma concentration in the previous multiple-dose study (200mg three times) in healthy volunteers. Thus, we think that a dose of FRPM 200 mg twice daily is safe and

enough to achieve effective plasma FRPM concentration. Additional dose after dialysis is not needed. We are now evaluating some factors which may affect the FRPM clearance (such as pH, protein concentration, etc.) by in vitro system.

584

Systemaric Review of Infliximab for the Treatment of Psoriatic Arthritis

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Introduction: Psoriatic arthritis (PsA) is classified as a spondyloarthropathy. The objective of this review is to assess whether infliximab provides a therapeutic advantage over placebo or other treatments in adult patients with PsA. Methods: Databases searched – MEDLINE (1966-May 2007), EMBASE (1988-May 2007), and the Cochrane Database of Systematic Reviews Issue 1 2007. Only double blind ignifican controlled trials (DB RCTs) were critically appraised. Harm data was gathered from RCT and non-RCT sources. Results: No trials compared infliximab to other treatments. There were 2 RCTs of 14 weeks (N = 200) and 16 weeks (N = 104) comparing infliximab to placebo in patients who had failed 2 or more immuno-modulator therapies. The RCTs found no advantage in terms of mortality or serious morbidity compared to placebo. Patients in the infliximab group showed significant improvement in SF 36 QoL score (ARR= 18% and 6%, NNT=6 and 17 for PCS and MCS scores), HAQ disability score(ARR= 67% to 51%, NNT=2), ACR 50 (ARR= 33% to 42%, NNT= 3 to 2), ACR 70(ARR= 29% to 14%, NNT= 4 to 7) and PsARC scores(ARR= 50% to 54%, NNT= 2). Discussion and Conclusion: There is insufficient evidence that infliximab provides a therapeutic advantage over other active comparators in the treatment of adult patients with PsA. There is insufficient evidence that infliximab provides a therapeutic advantage in terms of mortality or non-fatal SAEs compared to placebo. Patients in infliximab group showed significant improvement in SF 36 QoL score, HAQ disability score; ACR 50, ACR 70 and PsARC scores.

585

Multiple Model Dosage Design and a New Sequential Bayesian Method for Updating Individual Patient PK/PD Models

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Introduction: Parametric population PK/PD models and maximum aposteriori probability (MAP) Bayesian individualization provide no means to maximize the expected precision of target achievement with a dosage regimen. However, the multiple support points in nonparametric (NP) population models provide multiple serum concentrations predictions. One then can compute the regimen hitting the target with the least expected weighted squared error. This is "multiple model" (MM) dosage design. MM control is widespread in aerospace. The MM-USCPACK software uses this new approach. Individualization of these NP population models computes the Bayesian posterior probability of each support point in the population model. Those points fitting the patient's data well become more likely, and vice versa. However, the patient must be represented by support points.Unstable patients have changing parameter values with changing status. All current Bayesian updating procedures assume that there is only one set of fixed parameter values that best fit the data. Methods: We have implemented a Sequential Interacting MM (IMM) Bayesian procedure, widely used in aerospace. It permits parameter values to change during the fitting procedure. Results: IMM tracks drug behavior in a simulated changing patient with less than half the error of the MAP or MM procedures. It has been incorporated into the MM-USCPACK clinical software. It now has tracked the behavior of gentamicin and vancomycin significantly better than MAP and MM methods in over 130 unstable cardiac surgical patients. Discussion and Conclusion: This new tool shows great promise in optimizing patient care. Supported by NIH grants GM068968 and EB005803.

586

Comparison of Nimesulide Dosage Forms in Turkey

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Nimesulide (NIM) is a COX-2 selective NSAID for oral, rectal and topical use. In this presentation, results of a comparative in-vitro study on the diffusion profiles of the 1% Nimesulide patented formulation (Sulidin Gel, Embil Pharmaceutical, Turkey) and two commercially gel formulations containing 1% (Nimulid, Panacea, India) and 3% NIM (Aulin, Helsinn, Switzerland), as well as two clinical studies, investigating pharmacokinetics and efficacy of topical NIM gel are given. The diffusion studies were carried out using Franz-type diffusion cells and concentrations were determined by a HPLC method. Diffusion experiments (n=6) were carried out for a period of 6 h. The cumulative release of patented formulation was 1.5*($p<0.001$)* and 1.7 *($p<0.03$)* - fold higher than 3% and other 1% NIM gel formulations, respectively. These results show that NIM diffused significantly faster and to a higher extent from the patented gel formulation as compared to other 1% and 3% gels. We compared synovial and plasma NIM concentrations after topical (3x1, 0,4 mg/10 cm^2) and oral (2x100 mg) application of NIM during 4-7 days before arthroscopy to 34 patients with knee osteoarthritis, it was found that NIM passes into the synovial fluid after topical use. In addition, in an open-label study investigating the efficacy and safety of 1-week administration of NIM gel to 63 patients with knee osteoarthritis, patients showed a significant improvement on 3 main parameters of WOMAC (physical functioning, pain, joint stiffness).In conclusion, these studies reveal that topical application of NIM 1% gel (Sulidin® Gel) may have potential benefits in knee osteoarthritis treatment.

587

Domperidone and Verapamil are Metabolized by Human Heart Microsomal Fractions

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Backgroud: Verapamil and domperidone block ion channels found in human heart (ICa-L and IKr). Both drugs undergo metabolic inactivation by various CYP450 isozymes, some of which are found in the heart. Our objective was to develop a method for the preparation of active microsomes from human heart ventricular samples and to determine the extent of CYP450 activity obtained using verapamil and domperidone as probe drugs. Methods: Frozen tissues were obtained from human heart ventricles (n=3). In vitro incubations were performed with microsomes prepared from left ventricular sections (5-6g) and domperidone (200 μM) or verapamil (100-400 μM) were used as substrates. Formation rate of metabolites of domperidone and verapamil were monitored by HPLC with fluorescence and UV detection, respectively. Metabolites were also identified by LC-MS-MS and monitored during immunoinhibition studies (antibodies raised against CYP2C and CYP2C8). Results: Human heart microsomes showed metabolic activity towards domperidone and verapamil. An inter-heart variability was observed in the pattern of formation of various metabolites amongst the three hearts tested. The addition of CYP2C and CYP2C8 antibodies showed a decrease >75% in the formation rate of verapamil metabolites. In contrast, no inhibition was observed for domperidone. Conclusions: Our data indicate that the human heart has the capacity to metabolize domperidone and verapamil. Immunoinhibition studies suggest that CYP2C8 is an important isoform involved in the metabolism of verapamil in the human heart. We suggest that the metabolic activity present in the heart may regulate intra-cardiac concentrations of some drugs and consequently, their actions on various cardiac effectors.

588

Short Chain Fatty Acids as Drug Absorption Enhancers in the Colon of Rats

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Short chain fatty acids (SCFA) are endogenous acids of the colon that increase colonic circulation and increase water and ion uptake. For these reasons it was hypothesised that these acids may be used as absorption enhancers for peptide drugs at the colon. A C5a antagonist, AcF-[OP(D-Cha)WR] (10mg/ml) was formulated in either a 0.1 M solution of butyric acid, acetic acid, sodium acetate or water. AcF-[OP(D-Cha)WR] (10 mg/kg) formulated in the SCFA solutions or water was administered to the colon of anaesthetised female Wistar rats and blood samples were taken periodically from systemic and portal veins and bile was collected. Plasma and bile AcF-[OP(D-Cha)WR] concentrations were measured by LC-MS. Systemic plasma AcF-[OP(D-Cha)WR] concentrations were significantly higher at early time points when administered with butyric acid. When AcF-[OP(D-Cha)WR] was formulated in acetic acid, prolonged elevated portal and systemic drug concentrations were observed. Biliary elimination of AcF-[OP(D-Cha)WR] was greatest when the drug was administered with butyrate. In a separate set of experiments porcine insulin (20 IU/ml) formulated in the SCFA solutions or phosphate-buffered saline was administered (20 IU/kg) to the colon of the rats. Blood samples were taken periodically and blood glucose levels were measured with a glucometer, indicating the extent of insulin absorption. No significant blood glucose level differences were seen between formulations. These results suggest that for smaller peptide drugs SCFA may be useful as an absorption enhancer at the colon, but for larger peptides they have a limited effect.

589

Characterization of Cytochrome P450 mRNA Expression Pattern in Human Small Intestine of Japanese

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The cytochrome P450 (CYP), major drug metabolizing enzyme, is mainly expressed in the liver, and consists of a number of isozymes. Particularly, many studies about expression or role of CYP isozymes in the liver have been reported. On the other hand, the current studies indicate CYP3A4 locates in small intestine either, and contributes to the first pass metabolism (Bioavailability). However, the expression pattern of CYP isozymes in the small intestine of Japanese is unknown. Thus, this study aimed to characterize the expression pattern of CYP isozymes mRNA (1A2, 2C9, 2C19, 2D6, 3A4 and 3A5) in small intestine of Japanese. After the informed consent, small intestines were obtained from 21 patients on duodectomy by the therapeutical reason in St. Marianna university hospital. After the mRNA was isolated from the samples using commercial kit, reverse transcribed to cDNA, CYP mRNA expressions were determined by quantitative real time PCR. From these results, the expression of CYP1A2, 2C9, 2C19, 2D6, 3A4 and 3A5 mRNA in small intestine of Japanese, were shown respectively. CYP3A4 mRNA was most expressed in small intestine: subsequently, it was expressed in order of CYP 2C9, 2C19, 2D6, 3A5 and 1A2. In conclusion, we evaluated the expression pattern of CYPs mRNA in the small intestine of Japanese.

590

Sulfhydryl Modifiers have Biphasic Effects on Human Equilibrative Nucleoside Transporter 1 (hENT1) Translocation Activity and Ligand Binding

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ENT1 mediates nucleoside flux across cell membranes and is required for cellular entry of nucleoside analogue drugs. Studies using N-ethylmaleimide showed cysteine residues to be important in ENT1 function. We have tested several methanethiosulphonate (MTS) reagents of varying sizes, charges, and permeabilities to better characterize the environment of these cysteine

residues. PK15-NTD cells, expressing hENT1 as the sole nucleoside transporter, were incubated for 10 min at ~22°C with MTS reagents, washed extensively, then assessed for changes in the binding of the ENT1 probe, [3H]nitrobenzyl-thioinosine (NBMPR), and the uptake of [3H]2-chloroadenosine (2-CADO). The large positively-charged MTSET (5 mM) reduced NBMPR Bmax (31 ± 4%) and 2-CADO Vmax (39%), with no change in ligand affinity. The smaller positively-charged MTSEA (5 mM) decreased both Bmax and Kd by 63 ± 7% and 82 ± 4%, respectively. In contrast, the negatively-charged MTSES (10 mM) had no effect on hENT1. The neutral, membrane-permeable, MMTS had biphasic effects on both NBMPR binding and 2-CADO uptake; low concentrations (1 mM) increased binding Bmax by 48 ± 5% and enhanced uptake Vmax by almost 2-fold, while high concentrations (10 mM) decreased both binding and uptake relative to control. These results suggest that the effects of MTS reagents on hENT1 function involve two cysteine residues, one in a negatively-charged environment accessible to the extracellular space, and the other in a hydrophobic or intracellular region accessible only to neutral reagents.

591

Enantioselective Pharmacokinetics of Carvedilol in Patients with Chronic Chagas Disease

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Carvedilol is an anti-hypertensive drug with non-selective beta and alpha$_1$-adrenergic receptor blocking activities approved for the treatment of congestive heart failure (CHF). It is administered clinically as a racemic mixture. The beta-receptor blocking activity of the S-(-)carvedilol is about 200 times higher than that of R-(+)-carvedilol, whereas both enantiomers are equipotent alpha-blockers. Chagas' disease (CD) is a major public health problem in South America, and a frequent cause of chronic cardiomyopathy, cardiac arrhythmias and the late manifestation of CD is congestive heart failure. Infectious diseases like CD can affect hepatic CYP enzymes and alter pharmacokinetics of drugs. The aim of this study was investigate the enantioselectivity on the carvedilol pharmacokinetics in patients with chronic CD. Patients with chronic Chagas cardiomyopathy (n=6) received a single dose of 25mg of racemic carvedilol and blood samples were collected from zero to 36 hours after drug administration. The enantiomers of carvedilol were separated on a teicoplanin macrocyclic antibiotic chiral column (Chirobiotic T), and analysed by LC-MS-MS. The enantiomeric ratios different from one were evaluated by the Wilcoxon test and the results are reported as medians. The following differences ($p<0.05$) were observed between the R-(+) and S-(-)-carvedilol: C_{max} 80.54 vs 33.28 ng/mL; $AUC^{0\text{-infinity}}$ 525.01 vs 188.83 ng/h/mL and Cl/F 24.12 vs 66.53L/h. The results showed plasma accumulation of the pharmacologically less active R-(+)-carvedilol enantiomer in chronic Chagas cardiomyopathy patients.

592

Vitamin D Receptor (VDR) Gene Polymorphism BsmI Influences Blood Pressure

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Introduction: Seasonal and geographical differences in blood pressure and in the prevalence of hypertension are well documented. UV exposure and vitamin D status are discussed as risk factors. The aim of this study was to find a linkage between hypertension and the BsmI VDR gene polymorphism. Methods: BsmI VDR polymorphism was analysed in 310 hypertensive patients of both sexes (197 patients with essential hypertension, 113 dialysis patients) and in 221

normotensive controls. Blood pressure was measured using the Riva-Rocci method. Results: Genotype frequencies of the VDR BsmI polymorphism differed between hypertensive patients and controls. Genotype frequencies were 20% and 21% (BB), 35% and 37% (Bb), and 45% and 42% (bb), respectively, in the two hypertensive groups, and 10% (BB), 51% (Bb), and 39% (bb) in controls. The group differences BB vs. bb, BB vs Bb, and BB vs. Bb+bb were all statistically significant ($p<0.05$) with odds ratios of 2.0 in each case. Discussion and conclusion: The VDR gene polymorphism seems to be an additional risk factor for hypertension. People with low vitamin D levels need adequate substitution, especially in winter and when living far from the equator. Therefore, screening and normalization of 25-hydroxy-vitamin D serum levels should be included into the recommendations on prevention of essential hypertension.

593

Effect of Alpha-lipoic Acid on Arsenic Trioxide Induced Renal Toxicity

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The renal toxicity of arsenic trioxide limits its use as a leukemia therapy. We found previously that reactive oxygen species (ROS) were strongly associated with arsenic trioxide induced renal toxicity using DNA microarray technique. Therefore, we investigated potential treatments to reduce renal toxicity of arsenic trioxide using alpha-lipoic acid known as an antioxidant agent. Arsenic trioxide and/or alpha-lipoic acid were exposed to human primary renal cortical cells (PRCC), HEK 293, HL60, NB4, KMS1213M and U266 cells and then cell viability was measured. Moreover, superoxide anion was measured in HEK293 cells treated with arsenic trioxide and/or alpha-lipoic acid. The cell viability of PRCC and HEK293 cells was regained by alpha-lipoic acid addition, while that of HL60, NB4, KMS and 1213M cells was not changed. The production of superoxide anion from arsenic trioxide exposed HEK293 cells was suppressed by addition of alpha-lipoic acid. Alpha-lipoic acid demonstrated cytoprotecitive effect to PRCC and HEK293 cells and this effect may be mediated by its inhibition of ROS production. On the other hand, alpha-lipoic acid had no cytoprotective effect on human promyelocytic leukemia cell lines (NB4 and HL60 cells) and multiple myeloma cell lines (KMS12BM and U266 cells). These results suggest that alpha-lipoic acid may be a suitable agent for prevention or treatment of arsenic trioxide induced renal toxicity.

594

Caffeine Reverses Antinociception by Amitriptyline in Wild Type Mice but not in those Lacking Adenosine A1 Receptors

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The tricyclic antidepressant amitriptyline is used to treat neuropathic pain. It produces antinociception in several preclinical models, and this effect is blocked by adenosine receptor antagonists, which implicates adenosine it such actions. Adenosine produces antinociception in several pain models, and spinal adenosine A1 receptors (A1Rs) are believed particularly important. In this study, we used A1R knockout (-/-) mice to examine: (a) the ability of amitriptyline to produce antinociception in a model involving intraplantar formalin, and (b) the ability of caffeine to reverse this action. Amitriptyline produced dose-related suppression of flinching behaviours in wild type (+/+) mice following both systemic and intraplantar drug administration; both of these effects were unaltered in A1R -/- mice. Following systemic administration, caffeine reversed the systemic effect of amitriptyline in A1R +/+, but not -/- mice; +/- mice exhibited an intermediate effect. Intraplantar administration of caffeine also reversed the effect of intraplantar amitriptyline in A1R +/+, but not in -/- or +/- mice. These results indicate that adenosine A1 receptors are required in order to see caffeine reversal of the antinociceptive actions of amitriptyline, but they do not seem to directly mediate the actions of amitriptyline. The dose of caffeine used in the present study is similar to human intake. There is no clinical data on effects of caffeine on pain relief by antidepressants, but the present data suggest that this is important to examine.

595

Hyperammonemia due to Valproic Acid (VPA) - Some Facts and Recommendations

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VPA is a frequently prescribed drug for epilepsy, bipolar disorder and migraine prevention. Hyperammonemia is a very rare complication of VPA treatment. We present a case of 59 years old bipolar inpatient woman who exhibited impaired consciousness after 7 days treatment with VPA (1000 mg/day) and olanzapine (5 mg/day). Soon after the stupor began all drug treatment was stopped. Immediately she was moved to ICU and received flumazenil (0.5 mg IV). She finally recovered with well evolution. The only significant finding was ammonemia 9.1 mcg/dl with VPA serum concentration in normal range (48 mcg/ml). The etiology of this adverse reaction is unclear. Risk factors that contribute to its development are adulthood, female gender, polypharmacy, subclinical inborn errors of urea metabolism and vegetarian diet or low carnitine intake. Many authors believe that VPA causes a carnitine deficiency and a lack in acetyl-CoA synthesis that impair the Carbamoyl Phosphate Synthase-I (CPS-I) activity. Besides those ideas, we suggest that VPA might antagonize the natural alosteric CPS-I modulator, N-acetylglutamate (NAG). Thus, urea synthesis is inhibited and ammonemia rises. Conclusions: VPA is a valuable and useful drug. It has been approved to use in major psychiatric and neurologic disorders. It is noteworthy the similarity in three-dimensional structure between VPA and NAG. It could be the basis of an antagonism between them at CPS-I level. The rule in VPA use to avoid hyperammonemia should be the search of risk factors prior to start any treatment and the installation of routine laboratory control.

596

Effect of Bradykinin Antagonist on Beneficial Effect of Captopril on Duration of Survival after Acute Coronary Artery Ligation in Hypertensive Rats

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Angiotensin converting enzyme inhibiters (ACEIs) might be mediating cardioprtection via bradykinin (BK) release. In the present investigation, we evaluated the potential effects of captopril, an ACEI, and BK-2 receptor antagonist, D-Arg-[Hyp3-D-Phe7]-BK, on the duration of survival after acute coronary artery ligation for 15 min in spontaneously hypertensive rats (SHR). The captopril treatment (16ug/kg and 32ug/kg; i.v.) resulted in a significant ($p<0.05$) increase in survival time of SHR as compared with saline-treated control SHR. BK-2 receptors antagonist (4ug/kg; i.v.) pretreatment abolished ($p>0.05$) the beneficial effect of captopril on survival time as compared to saline-treated control SHR. Both the ligation of coronary artery and captopril treatment resulted in a significant ($p<0.001$) fall in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) of SHR as compared to the saline-treated control SHR. In addition, captopril administration caused a significant ($p<0.05$) fall in the SBP, DBP and HR of SHR before ligation of the coronary artery (preligation). However, there was no significant ($p>0.05$) in SBP, DBP and HR between saline – and BK -2 receptor antagonist plus captopril-treated SHR during preligation. These finding might indicate that captopril possesses a cardioprotective property as demonstrated by increased in survival time of SHR. This beneficial effect of captopril is mediated via the BK-2 receptor pathway because BK-2 receptor antagonist pretreatment blocked the captopril-induced increase in survival time of SHR.

597

Investigation on the Behavioural and Neurochemical Effects of Deltamethrin in Rats Subjected to Footshock Stress

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Deltamethrin (DM), a pyrethroid pesticide has been reported to cause neurological deficits in farmers and industrial workers. The effect of Deltamethrin on regional brain key enzymes and their correlation with behavioural responses were examined. Male Wister rats were divided in four groups consisting six rats each group. First group was control, second group received footshock stress(40-45V for 1 second/shock/min for 1 hour/rat) ,third group received Deltamethrin(5mg/kg B.W) orally and Coexposd group received deltamethrin and footshock stress for 45 days. Our results indicate that deltamethrin markedly impaired locomotor activity and learning ability, while aggressive behaviour significantly increased in footshock and deltamethrin group. An overall estimation of Lipid Peroxidation (LPO) showed significantly increase in footshock stress and deltamethrin group whereas coexposed group did not show significant increase in corpus striatum region. Whereas. Significant increase in LPO region was observed in DM treated group while no significant increase was observed in stress and coexposed group in frontal cortex region. No significant change of LPO in Hippocampus region was observed in any group. In addition, there was significantly decrease in glutathione (GSH) level of corpus striatum and frontal cortex in all groups as compared to control. In hippocampus region DM and Stress exposed group showed significant GSH decrease while no significant change was there of coexposed group to control. Therefore, this study aimed to demonstrate the brain region specific effect of deltamethrin in adult male rats by examining levels of key enzymes of the neurotransmitter pathway, spontaneous locomotor activity, aggressive behaviour, and learning and memory functions.

598

Clinical Pharmacology of HX575, The First Approved Biosimilar Epoetin alfa in Europe

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In agreement with European guidance (EMEA/CHMP/94526/2005), the clinical pharmacokinetics (PK) and pharmacodynamics (PD) of HX575 were compared to the reference product Erypo(r) in two pivotal trials in healthy volunteers following intravenous (i.v.) and subcutaneous (s.c.) administration. Both studies had a prospective, randomised, paral andomizeddesign with 37-39 subjects per group. Treatment duration was 4 weeks. The dose administered was 100 IU/kg BW 3 times per week. For both trials, the primary PD endpoint was the absolute haemoglobin response AUEC [g/dLxh]. Biosimilarity was assumed if the 90% CI of the AUEC ratio was within a range of 97% to 103%. The primary PK endpoint was the extent of exposure AUCTAU [mIUxh/mL]; from administration to TAU = 0-48 h after repeated administration. I.V. results: AUEC was 10055.5+/-353.7 (arithmetic mean+/-SD) for HX575 vs. 10070.8+/-365.1 for the comparator; [99.9; 90% CI, 98.5-101.2]. AUCTAU was 8422+/-2419 (arithmetic mean±SD) for HX575 vs. 9224+/-1850 for the comparator; [89.2; 90% CI, 82.5-96.2]. s.c. results: AUEC was 10248.4+/-493.6 for HX575 vs. 10469.3+/-495.3 for the comparator; [98.9; 90% CI, 97.7-100.2]. AUCTAU was 2045+/-587.9 for HX575 vs. 2095+/-486.4 for the comparator; [96.9; 90% CI, 88.2-106.5]. For haemoglobin, the 90% CI for the AUEC ratio was included in the acceptance range and thus met the predefined biosimilarity criteria following both routes of administration. For AUCTAU, the point estimate and 90% CI were within conventional bioequivalence acceptance criteria indicating a similar relative bioavailability as the reference product. The trials support a key role of clinical pharmacology in the assessment of human recombinant biosimilar erythropoietins.

599

Phase I and Early Clinical Studies to Determine Intravenous Pharmacokinetics and Absolute Bioavailability using Tracer Intravenous Doses of 14C-Radiolabelled Drug

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Introduction: The introduction of Accelerator Mass Spectrometry (AMS) into early clinical development has enabled determination of

intravenous (IV) pharmacokinetics and absolute bioavailability using tracer IV doses of 14C-drug administered at the same time as an oral therapeutic dose. This "piggy-back" approach has been applied to assessing the contribution of formulation performance, absorption, first pass metabolism and systemic clearance on drugs with low and highly variable oral bioavailability. This information has proved to be invaluable in distinguishing between pharmaceutical and physiological drivers of oral bioavailability. Methods: Healthy subjects (8) were administered an NCE at an oral therapeutic dose (100mg non-radiolabelled) and with a concomitant IV tracer dose of 14C-drug (100microg /200nanoCi) given at the time of the maximum concentration of the oral dose. Plasma samples were collected over 96 hours, intravenous pharmacokinetics were defined using AMS determination of the IV administered 14C-drug, whereas the oral pharmacokinetics were defined using LC-MS/MS. Results: Plasma concentrations for the IV dose were approximately 600 to 1000-fold lower than for the oral dose although in both cases the drug half-life was approximately 40-45 hours. Variability in the AUC (Coefficient of variation = 57%) after oral dosing (100mg) was much greater than after the IV tracer dose (100microg) (Coefficient of Variation = 21%). The individual absolute oral bioavailability varied seven-fold from 8 to 53% (mean 33%). Discussion and Conclusions: This study clearly demonstrated that variability in the oral bioavailability was not due inter-subject variation in clearance but to variability in absorption and/or performance of the oral formulation.

600

Moxifloxacin-induced QT Prolongation in Healthy Human Volunteers: Analysis of Gender Differences

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Moxifloxacin is the most widely used positive reference agent in clinical cardiac repolarization (Thorough QT/QTc) studies, but its QT-prolonging properties have not been well compared between genders. In order to clarify the difference, we evaluated the effects of a single oral therapeutic dose (400 mg) of moxifloxacin on the QT/QTc interval of healthy adult subjects between 20 and 60 years of age (n=69; 35 males, 34 females) using a 12-lead resting ECG, according to the ICH-E14 guideline. Digital ECG information was processed using a fully automatic data analysis system (Wreport, Physio-Tech, Tokyo, Japan), and each result was confirmed and/or corrected by 2 expert technicians and 1 certified cardiologist on a monitor in series. Moxifloxacin prolonged the time-matched, placebo-subtracted QTcF (delta-delta-QTcF) in both genders, but to different extents. Maximum delta-delta-QTcF (mean±SE) in males was +12.7±2.2 ms at 2 hours after administration, whereas it was +16.9±1.7 ms in females at 3 hours. In other words, the QT-prolonging effect was slower in onset and longer-lasting in females than in males. C_{max} (micro-g/mL), T_{max} (h) and $AUC_{0\text{-}23h}$ (micro-g h/mL) were 2.9±0.1, 2.6±0.1 and 36.1±0.7 in males, whereas they were 4.2±0.1, 2.7±0.1 and 49.6±1.2 in females, respectively. These results indicate that moxifloxacin can prolong the QT interval in each gender, and suggest that the slow pharmacodynamic property of QT prolongation in females may be explained by gender differences seen in the pharmacokinetic profiles.

601

Opposite Effects of Methanolic Leaf Extract Compounds of Dialium Guineense on Blood Glucose Level in Normoglycaemic Rat Model

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The leaves of *Dialium guineense* are used in Senegalese traditional medicine as a remedy for the treatment of diabetes. Preliminary studies showed that the higher doses of aqueous leaf extract of *Dialium guineense* had no effect in glycaemia in normoglycaemic rats. This study aimed at investigating the effect of methanolic leaf extract and its fractions on blood glucose in

normoglycaemic rats. Air-dried and powdered leaves of *Dialium guineense* were boiled in methanol and evaporated. The compounds of methanolic extract were separated by exclusion-diffusion chromatography in sephadex gel. Five fractions were obtained. Experiments were performed in normoglycaemic rat model. The methanolic extract and its fractions were administered per os. The total methanolic leaf extract (900 mg/kg, per os) did not significantly decrease the blood glucose level. The F1 and F2 fractions had a hyperglycaemic effect in normoglycaemic rats. However, pretreatment with F1 fraction did not prevent at all the hypoglycaemic effect of glibenclamide. The F3 and F4 fractions did not modify significantly the basal glycaemia in normoglycaemic rats. Such as glibenclamide, the F5 fraction (300 mg/kg, per os) induced significantly a hypoglycaemia (0.65±0.03 vs 0.80±0.03 g/l) ($p<0.05$, n=5). It also significantly prevented hyperglycaemia in glucose tolerance test. These results suggest that the methanolic leaf extract of *Dialium guineense* contains hyper- and hypoglycaemic compounds which induced an opposite effects in glucose level. This may explain the absence of hypoglycaemic effect when the methanolic leaf extract of *Dialium guineense* was administered in normoglycaemic rats.

602

Arrhythmogenic Property of the Pulmonary Vein Myocardium in Guinea Pigs as a Source of Atrial Fibrillation

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Introduction: The pulmonary vein has been suggested to be a source of atrial fibrillation based on clinical observations. However, spontaneous activity of the pulmonary vein varies among species. In this study, we assessed the pulmonary vein automaticity in guinea pigs and analyzed its arrhythmogenic activity. Methods: The pulmonary veins and left atria were isolated from Hartley guinea pigs. The action potential signals were recorded using the standard microelectrode method. Results: Spontaneous activity was observed in 16 out of 119 of pulmonary vein preparations (13.4%), whereas no spontaneous activity was detected in the left atria. The incidence in the left superior pulmonary vein was relatively higher (20.7%) than that in the other veins. Ryanodine (0.1 microM) decreased frequency of the spontaneous action potentials with a fast phase-0 upstroke. In pulmonary vein preparations with no spontaneous activity, delayed afterdepolarizations were induced after the termination of burst pacing in 58 out of 71 preparations (92.9%), which was higher than that in the left atria (23.1%). Also, spontaneous activity appeared in the pulmonary veins after the treatment with norepinephrine (10 microM) or ouabain (1 microM). Conclusion: The pulmonary veins have more arrhythmogenic abilities than the left atrium through spontaneous activity or triggered activity. Intracellular Ca^{2+} overload may be associated with spontaneous activity of the pulmonary vein.

603

A Novel Brain-specific Transporter with Receptor Function for Iron-binding Lipocalin

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Introduction: NGAL (Neutrophil Gelatinase-Associated Lipocalin) is a 25kD secretory protein in the lipocalin family, which is expressed in a variety of tissues including immune cells, inflamed intestinal cells and airway epithelia. Its novel role is being revealed as an iron binding/uptake protein in the extracellular environment to counteract pathologic growth of iron-requiring bacteria, and to maintain cellular iron homeostasis. Recently, NGAL receptor (Ngal-R) has been identified, which turned out to be identical to a putative transporter gene in the solute carrier (SLC22) family. In this study, we characterized genomic organization and tissue distribution of the NGAL receptor, focusing on its brain specific expression. Methods: In silico bioinformatics approach for human and mouse genome, Northern blotting on mouse and human tissue panels, in situ hybridization and immunehistochemistry on mouse brain tissues

(C57BL/6J) were performed. Results and Discussion: The cloned Ngal-R cDNA was 1560-bp in both species, and the estimated mouse and human Ngal-R amino acid sequences were 98% identical with a typical transporter configuration. Northern blot indicated strong brain expression in both mice and humans. In situ hybridization showed its global brain expression in the neurons and choroid plexus, except thalamus neurons. Immunohistochemistry confirmed the distribution, and in particular, choroids plexus expression was localized to the luminal side. Conclusion: Ngal-R is expressed predominantly in the brain, except for thalamus. Its cellular localization is confined to neurons and choroids plexus epithelia. In addition to its receptor function for NGAL, whether it has a specific transporter function remains unknown.

604

Impact of Transdermal Absorption and Genetic Polymorphisms of CYP3A5 and MDR1 on Fentanyl Pharmacokinetics in Patients with Cancer Pain

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Introduction: Clinical pharmacokinetics of fentanyl is characterized by a large interindividual variation in cancer patients using transdermal fentanyl reservoir patch (TFRP). Recently, genetic polymorphisms in CYP3A5 and P-glycoprotein have been reported to alter fentanyl pharmacokinetics and response. The aim of this study is to evaluate the influence of fentanyl transdermal absorption rate and genetic polymorphisms of CYP3A5 and MDR1 on fentanyl pharmacokinetics. Methods: This study enrolled 39 cancer pain patients treated with TFRP. Fentanyl in spent TFRP and in human plasma was determined with HPLC-UV and coupled LC-MS/MS method, respectively. Polymorphisms of *3 (6986A>G) of CYP3A5 and 1236C>T, 2677G>A/T and 3435C>T of MDR1 gene were detected by PCR-RFLP assay. Results: A large interindividual variation was observed in plasma concentration of fentanyl in cancer patients. Plasma fentanyl concentrations were significantly correlated with its theoretical delivery rate as well as measured absorption rate (P<0.01, r=0.61 and P<0.01, r=0.62, respectively). The plasma fentanyl concentration adjusted with its theoretical delivery rate or measured absorption rate was significantly higher in *3/*3 patients than in *1/*3 patients (P=0.02 and P<0.01, respectively). There was no significant relationship between the plasma fentanyl concentration and MDR1 genetic polymorphisms. Conclusion: Interindividual variation of fentanyl pharmacokinetics depended on CYP3A5 metabolism rather than absorption from TFRP. This finding suggests that genetic analysis of CYP3A5*3 was useful to individualize fentanyl dosage in case of opioid switching related to TFRP in patients with cancer pain.

605

Establishment of Novel Assay for Human CYP3A Activity by Measuring of Lansoprazole Metabolite

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Objective: CYP3A4 is responsible for the metabolism of many drugs of more than 60% of clinical used drugs. Because individual differences have been shown in CYP3A activities of human liver, it is important to know its activity for efficacies and adverse events of drug therapy in individual patients. Lansoprazole, proton pump inhibitor is extensively metabolized to in the liver, respectively. We investigated that novel assay for human CYP3A activity by measuring of lansoprazol sulfoxidation.LS.activity, *in vivo* and *in vitro*. Methods: Testosterone 6β-hydroxytestosterone (T6β-H) activity, already known as a marker of CYP3A activity and LS activity of human liver microsomes were measured *in vitro* using a high performance liquid chromatography (HPLC). Furthermore, Plasma levels of lansoprazol and its metabolites as well as 6β-hydroxycortisol.6β-OHF. in blood collected for 24h after intake of single oral 30 mg lansoprazole in healthy subjects were analyzed *in vivo* using HPLC. Results: The LS activity was correlated significantly with the T6β-OH activity *in vitro*. In addition, plasma concentrations of

lansoprazol sulfone at 2 h later were closely correlated *in vivo* with 6β-OHF concentrations. Conclusions: These results suggest that LS activity may be a new indicator of human liver CYP3A activity.

606

Preliminary Experimental Data Regarding the Antinociceptive Effect of Systemically Administered Cadmium

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Introduction: Cadmium (Cd) is an essential divalent trace element. Cadmium is chemically similar to zinc and because of the well known influence of zinc on the nociceptive processes, we looked upon the possible modulator effect in nociception after systemically administered cadmium. Methods: Groups of 7 mice were treated with various doses of cadmium, administered intraperitoneal. Different tests were utilized to evaluate the antinociceptive effect on thermal (hot plate test, tail flick test) and chemical nociception (writhing test), or the influence on spontaneous behavior (activity cage assay) of the substances tested. Results: Our preliminary data for response latencies for hot-plate and tail-flick tests suggests that cadmium administered systemically produces a slight analgesic effect under thermal nociceptive stimulation. Discussion: Pain inhibition is even more significant in conditions of chemical nociceptive stimulation, in a model of visceral pain. Conclusion: The mechanism throughout which cadmium exerts its analgesic effect is still unclear, and will require more investigations, including dose-efect analysys, though it may be related to superoxide dismutase enzymes on pain mediation and hyperalgesia.

607

Influence of the Disease and Immunosuppressive Therapy on the P-gp Function in Peripheral Lymphocytes of Systemic Lupus Erythematosus Patients

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by autoantigen-reactive T cells and autoantibody production. Prednisolone is the mainstay of treatment but some patients have been found to acquire a resistance to prednisolone therapy after an extended period of time. The MDR-1 gene codes for a drug efflux pump, P-glycoprotein (P-gp), expressed on the surface of lymphocytes and actively transports prednisolone out of target cells, thereby reducing their efficacy. We evaluated the P-gp function in peripheral lymphocytes obtained from 13 SLE patients by detecting the fluorescence of Rh123, which is a P-gp substrate. We identified that the percentage of CD8 positive T cells in peripheral lymphocytes obtained from these SLE patients was higher than those obtained from 21 healthy subjects. There was a significant correlation between P-gp function in CD8+ T cells and the dose of prednisolone for treatment ($r=0.68$, $p=0.040$). No significant correlation between P-gp function in lymphocytes and SLEDAI (SLE disease activity index) was observed. We also found a significant correlation between P-gp function in CD8+ T cells and plasma cholesterol levels ($r= -0.67$, $p=0.049$), or between P-gp function in CD4+ T cells and plasma albumin concentrations ($r=0.71$, $p=0.015$). These findings raise the possibility that P-gp function in peripheral lymphocytes of SLE patients was up-regulated during the improvement of the disease by immunosuppressive treatment.

608

Population Plasma Pharmacokinetics of Tazarotenic Acid Following Once-daily Dosing of Tazarotene 4.5 mg Capsules for up to 24 Weeks in Patients with Moderate to Very Severe Psoriasis

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Study Objectives: To characterize the population pharmacokinetics (PK) of tazarotenic acid (TA) in patients with moderate to very severe psoriasis following oral dosing of tazarotene 4.5 mg once

daily. Methods: PK samples were collected at Weeks 4, 8, 12, 16, and 24 from 485 patients in three Phase 3 studies. Nonlinear mixed effects modeling using NONMEM was performed on the TA plasma concentration-time data to estimate the population PK parameters of TA as well as the associated inter- and intra-patient variability. The estimated population PK parameters included the apparent oral clearance (CL), the apparent volumes of distribution of the central and peripheral compartments (V2 and V3), and the apparent absorption rate constant, Ka. In addition, potential effects of demographic variables (age, weight, height, gender, smoking, and alcohol drinking status), serum albumin concentration, and creatinine clearance on these parameters of TA were examined. Results: A 2-compartment model with first-order absorption characterized the PK of TA well. The apparent clearance of TA in alcohol drinkers was found to be 12.1% less than that in nondrinkers which is well within the typical variability of 30% in AUC observed from a Phase 1 PK study of TA. No other demographic variables were found to have any significant effects on the PK of TA. Oral clearance values were similar to those found in the multiple-dose phase 1 PK studies. Conclusions: Systemic drug exposure is expected to be similar between psoriasis patients and healthy subjects.

609

Analysis of Tamsulosin in Human EDTA K_2 Plasma by LC/MS/MS

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Purpose: Tamsulosin is an alpha-blocker that is used to improve symptoms associated with an enlarged prostate (benign prostatic hypertrophy). It works by relaxing muscles in the bladder and prostate. This may improve urine flow rates and decrease urinary hesitancy/urgency. The purpose of this work was to develop and validate a specific and robust method for the determination of tamsulosin in human EDTA K_2 plasma. Methods: Tamsulosin and its internal standard tamsulosin-d_4 were extracted from human EDTA K_2 plasma by liquid-liquid extraction using methyl-ter-butyl ether. Analysis was performed on a MDS Sciex API 3000 tandem mass spectrometer with TurboIonSpray interface. Positive ions were measured with m/z 408.8 $\rightarrow$ 228.1 for tamsulosin and 413.0 $\rightarrow$ 228.1 for IS. The chromatographic run time was 2.2 minutes on a Gemini C18 50 X 4.6 mm column. The mobile phase was a mixture of methanol and Milli-Q type water (35/65) with 5mM ammonium acetate and 0.25% ammonium hydroxide. Results: This assay was validated over a nominal range of 100 to 20000 pg/mL. Linearity over the calibration range was $\geq$ 0.9991. The between-run accuracy ranged from 101.34 to 102.51% with precision ranging from 2.16 to 5.38%. The within-run accuracy ranged from 96.08 to 109.64% with precision ranging from 0.79 to 6.07%. The recovery of tamsulosin and its internal standard was greater than 83%. No matrix effect on quantitation was observed. Tamsulosin was found to be stable in human EDTA K_2 plasma after 46 hours at room temperature for short term stability, after 96 days at -20°C for long term stability, after 127 hours at room temperature for post-preparative stability and after 4 freeze and thaw cycles at -20°C and -80°C. Dilution integrity and matrix selectivity were also demonstrated. Hemolysis effect was evaluated. Conclusions: This method is sensitive, accurate, reproducible and was successfully applied for the analysis of clinical samples. Over 900 study samples were analysed with accuracy ranging from 96.55 to 103.18% and precision ranging from 4.93 to 6.94%.

610

The ADME Transcriptome in Human Duodenum – There are Enormous Amounts of Information about the Gut Still to Digest

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Human expression of ADME genes is highly variable and can lead to altered drug response. Expression of all ADME genes, particularly forms that have been more recently identified or that is expressed at a lower level in intestine, is not well described in earlier reports. The objective of this study was to use Affymetrix gene expression

arrays to profile the expression of ADME genes. Interaction maps and gene networks were developed from the final list of genes whose expression changed significantly. Our results allow us to begin inferring master regulators of co-regulated ADME genes to begin building expression networks. We selectively studied the distinct pattern of the basal intestinal ADME gene expression of ABC transporters, CYPs and NHRs. The Inter vs. Intra person variation in intestinal gene expression showed much greater variation between persons vs. within, suggesting a strong genetic component. Conversely, genes that showed high within person variability were largely genes known to have a strong environmental regulatory influence. Data was also used to identify hub genes significantly associated with other genes in expression. Genes with similar expression patterns to 3A4, VDR and HNF genes related to liver homeostasis and bile salt transport were correlated with 3A4. Our study is intended to be a resource for drug metabolism researchers that defines and compares the variability of basal ADME intestinal gene expression to discern co-regulated genes and potential biomarkers of inducible gene expression. Future analysis would further significantly enhance our understanding of NHRs by identifying PXR sensitive intestinal drug detoxification genes influencing drug-interactions and identifying novel biological pathways regulated by these NHRs in human intestine.

611

Hair Testosterone Levels in Hypogonadal Men Receiving Testosterone Therapy

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Hypogonadism in men is due to insufficient testosterone secretion, and is commonly treated with testosterone therapy. Serum testosterone levels are used for diagnosis and monitoring therapy, but as body mass increases, serum levels become less reliable due to changes in the bioavailable testosterone. We attempted to determine if testosterone could be recovered from human hair and to examine any relationship between hair testosterone levels, gonadal status and testosterone therapy. In this study we recruited eugonadal men (n=17), and hypogonadal men both on (n=13) and off (n=10) treatment. Serum testosterone values were collected from charts and hair samples were collected for testosterone analysis using a modified commercial salivary EIA kit (Alpco Diagnostics). In hypogonadal patients on testosterone replacement we found a significantly higher hair testosterone level (4.4pg/mg) than in the eugonadal group (1.8 pg/mg, $P<0.05$ Independent T-Test). Hair testosterone levels were also significantly higher in the hypogonadal group receiving hormone replacement than in the no therapy group (4.4 compared to 1.2pg/mg, $P<0.05$ Mann-Whitney U). The hypogonadal group not on therapy had a significantly lower serum testosterone than the eugonadal group (19.8 compared to 5.1 nmol/L, respectively; $P<0.01$ Independent T-Test). Hemoglobin and hematocrit correlated significantly with hair testosterone in the entire hypogonadal group ($r2=0.63$, $P<0.05$ and $r2=0.49$, $P<0.05$ Spearman). This work demonstrates that testosterone levels measured in hair reflect gonadal status. The increased hair testosterone levels in treated hypogonadal men compared to healthy controls suggest that hypogonadal men may be over-treated and this could potentially result in additional risk.

612

AcMPAG Levels and *UGT* Genetic Variations are Associated with Occurrence of Side Effects in Thoracic Transplant Recipients Treated with Mycophenolate Mofetil

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Background: Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), is a frequently used immunosuppressive drug in solid organ transplantation. MPA is metabolized by UDP-glucuronosyltransferases (UGTs) to the

major phenolic glucuronide (MPAG, 95%) and the minor acyl glucuronide (AcMPAG), which is pharmacologically active and potentially toxic. We studied whether polymorphisms in the *UGT* genes, and MPA and AcMPAG levels were associated with occurrence of side effects related to MMF, namely rejection, infections, anemia, leucopenia, thrombocytopenia and diarrhea. Methods: 25 Heart and 27 lung transplant recipients in British Columbia, Canada, were recruited and blood samples were obtained over a 12-hour period after MMF administration. Concentrations of MPA, MPAG and AcMPAG were determined by a validated high-performance liquid chromatography method with ultraviolet detection. Over 40 Genetic polymorphisms in the *UGT1A8*, *UGT1A9*, *UGT2B7*, and *ABCC2* genes were assessed by direct sequencing. Association of side effects with variables (MPA and AcMPAG levels, polymorphisms, gender, comedication) was performed by Fisher's exact test. Results: Women had higher occurrence of infections. Patients with infections showed a tendency towards increased AcMPAG levels; anemic patients tended to have higher MPA free fraction (>0.04). AcMPAG levels >50 micrograms*h/mL and use of cyclosporine (vs. tacrolimus or sirolimus) as comedication were significantly associated with increased risk of rejection ($p<0.05$). *UGT2B7* -79G>A polymorphism correlated with an increased risk for occurrence of anemia ($n=4$, $p<0.05$). Conclusions: Results indicate that AcMPAG levels, gender, comedication and certain *UGT* polymorphisms are associated with the occurrence of side effects. Findings warrant additional studies with larger sample size.

613

The Effect of Dosing Interval on the Gemfibrozil-repaglinide Interaction: Evidence for Mechanism-based Inhibition of CYP2C8 *In Vivo*

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Repaglinide is metabolized by cytochrome P450 (CYP) 2C8 and 3A4. Gemfibrozil, a strong in vivo inhibitor of CYP2C8, has raised the area under concentration-time curve (AUC) of repaglinide 8-fold, when repaglinide was administered 1 hour after gemfibrozil.This interaction has been suggested to be caused by mechanism-based inhibition of CYP2C8 by a metabolite of gemfibrozil, gemfibrozil 1-O-beta-glucuronide. Thus, we studied the time-dependency of the gemfibrozil-repaglinide interaction. In a randomized 5-phase crossover study, 10 healthy volunteers ingested a single oral dose of 0.25 mg repaglinide either alone or 0, 3, 6 or 12 hours after the last dose of 600 mg gemfibrozil twice daily. Plasma drug and blood glucose concentrations were measured. Gemfibrozil increased the total AUC of repaglinide 7.0-, 6.5-, 6.2-, and 5.0-fold, when repaglinide was taken either simultaneously with, or 3, 6 or 12 hours after the last dose of the gemfibrozil treatment, respectively ($P<0.001$). Peak repaglinide concentration was increased about 2-fold ($P<0.001$), and the half-life was prolonged from 1.2 hours to 2-3 hours ($P<0.001$) during all gemfibrozil phases. The metabolite- to-repaglinide AUC ratios were decreased by gemfibrozil in all gemfibrozil phases, in particular that of the CYP2C8 specific metabolite M4. The pharmacodynamic effects of repaglinide were also increased by gemfibrozil treatment. In conclusion, gemfibrozil strongly interacts with repaglinide even when repaglinide is administered 12 hours after gemfibrozil, i.e. when the concentrations of both gemfibrozil and its 1-O-beta-glucuronide are only about 5-10% of their peak. The long-lasting interaction is likely due to mechanism-based inhibition of CYP2C8 by gemfibrozil 1-O-beta-glucuronide.

614

Pharmacokinetics of Valacyclovir under Fasting and Fed Conditions in Humans

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Purpose: Valacyclovir is the L-valyl ester prodrug of acyclovir, an effective anti-herpetic drug. Systemic availability of acyclovir in humans is three to five times higher when administered orally as the prodrug. To date detection of valacyclovir in plasma has been challenging and its pharmacokinetic (PK) profile has not been adequately characterized. The purpose of this

study was to describe the bioavailability of valacyclovir and acyclovir under fasting and fed conditions using a sensitive and specific method developed at Anapharm. Methods: Data from two separate bioavailability studies have been used, one under fasting conditions and another under fed conditions. In each study 34 healthy adult males and females have been enrolled. A 1-gram single oral dose of valacyclovir was administered in each study. Plasma concentrations of valacyclovir and acyclovir were determined by LC/MS/MS (LLOQ of 10 and 20 ng/mL for valacyclovir and acyclovir, respectively). Non-compartmental pharmacokinetics and statistical analysis were performed using SAS. Results: The absorption and elimination processes of valacyclovir were found to be rapid with a T_{max} of approximately 1.5 hrs and $T_{1/2\ el}$ of 0.8 hrs. The mean extent of valacyclovir exposure was 308.78 and 554.53 ng·h/mL under fasting or fed conditions, respectively. Food increased the bioavailability of valacyclovir (fast/fed ratio of 55% and 53% for AUC and C_{max}, respectively). As stated in the product monograph, acyclovir AUC was not altered significantly by food. Conclusion: The pharmacokinetics of valacyclovir was well characterized using LC/MS/MS methodology under both fasting and fed conditions.

615

CAST-ELISA Test in the Diagnosis of Allergy to Additives

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Allergy to additives can be in combination with food and drug allergy serious diagnostic and therapeutic problem. The mechanisms by which food additives affect the immune mechanisms are poorly understood. These reactions are described as intolerance or pseudoallergy and are not IgE-mediated. Concomitant drug allergy to vehicles must be diagnosed too. At present, detection of food additives allergy is still difficult and requires an adequate clinical examination and specific in vivo and in vitro diagnostic tests. The aim of the study was to investigate the diagnostic value of cellular antigen stimulation test (CAST) in detection of hypersensitivity reaction to additives. We tested 47 patients with positive history of hypersensitivity reactions to food additives (sodium salicylate, sodium benzoate, potassium metabisulfite and tartrazine). Clinical symptoms included urticaria/angioedema (25 patients), medicamentous exanthema (6 patients) and eczematous dermatitis (16 patients). Sulfidoleukotrienes (sLT) quantification was carried out by CAST-ELISA after in vitro peripheral blood leukocytes stimulation with food additives. The results higher than the cut-off range value (sodium salicylate 120 pg/mL, sodium benzoate 90 pg/mL, potassium metabisulfite 40 pg/mL and tartrazine 120 pg/ml) were considered positive. Our results demonstrate that CAST was positive in 8% (4/45) of patients with allergy to sodium salicylate, 26% (12/45) of patients to sodium benzoate, 28% (13/37) of patients to potassium metabisulfite and 28 % (13/45) of patients to tartrazine. Our results show that increased sulfidoleukotriene production was observed with different frequency by any off the food additive tested. In vitro measurement of sLT production may be helpful or identifying some patients with allergy to food additive and drug allergy.

616

Translational Medicine: Benefits of Computerized Cognitive Function Assessment in Routine Phase I Safety and Tolerability Trials of Cognition Enhancers

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The aim of Translational Medicine in clinical trials is to speed up drug development and reduce costs by implementing study designs and procedures to disqualify "defective" new compounds early in development and identify potentially more effective compounds for further development. Computerized cognitive function testing can easily be incorporated into routine Phase I safety and tolerability trials to identify cognitive toxicity or unwanted CNS side-effects which would otherwise not be detected until later

in development, and can also reveal signals for cognitive enhancement. Data from the Cognitive Drug Research (CDR) computerized assessment system have contributed to go/no-go decisions in numerous clinical trial programs. The laptop-based system assesses various aspects of attention, episodic memory, working memory and subjective mood, and is suitable for healthy volunteers and patient populations. A case study will be described in which the cognitive effects of S-12024 were tested in a multiple-dosing safety and tolerability trial in elderly volunteers. Dose-dependent cognitive benefits were seen on measures of attention and memory, the largest at 50mg and 100mg. A follow-up bridging study in Alzheimer's patients identified cognition enhancements on the same cognitive tests at 100mg and 200mg. Further case studies will be presented demonstrating how positive signals in Phase I predicted subsequent positive outcome in clinical trials in Alzheimer's disease (MEM3454), Schizophrenia (GTS-21), MCI and AAMI (TC-1734/AZD3480), and ADHD (NS2359/GSK372475). Computerized cognitive testing in Phase I trials can provide valuable information for critical go/no-go decisions without the need to increase sample size, maintaining minimal exposure and providing potential resource savings.

617

Tolerability, Pharmacokinetics and Pharmacodynamics of the Selective Vasopressin V_2 Receptor Antagonist, Satavaptan: A Multiple-dose, Placebo-controlled Study in Healthy Male Subjects

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Introduction: Properties of satavaptan, a highly selective oral vasopressin V_2 receptor antagonist, were assessed in a randomized, ascending, multiple-dose study. Methods: Forty healthy male subjects (18-35 years) were randomized in this double-blind study to 7-day treatment with satavaptan 1, 10, 50, or 100 mg once daily, or 25 mg twice daily, or placebo (6 active/2 placebo per group). Subjects received meals with fixed sodium content (2g/meal), and water intake was standardized on Day 0, 1 and 7. Blood was sampled on Day 1 and 7 for full pharmacokinetic profiles and at pre-dose on specified days. Plasma was analyzed with a validated LC-MS/MS method. Results: Median times to maximum satavaptan plasma concentrations ranged from 1.5 to 3 hours. C_{max} and AUC_{0-24} increased in more than a dose-proportional manner (3.50- and 3.61-fold, respectively). Mean terminal half-lives ranged from 13.4 to 17.4 hours. Steady state was reached by 4 to 6 days, with up to 4-fold accumulation. A dose-dependant increase in urinary volume and corresponding decrease in urine osmolality were observed with maximal effects 1–2 hours after dosing. Consequently, plasma osmolality, plasma vasopressin concentrations, serum sodium levels and thirst increased. There were no significant changes in plasma renin or aldosterone activities. Repeated oral administrations were well-tolerated. Adverse events were mainly observed from 50 mg upwards, included dry mouth, abdominal pain, headache and fatigue, and most were potentially related to the pharmacodynamic activity of satavaptan. Conclusions: Satavaptan is well-tolerated and shows dose-dependent aquaretic effect with repeated administration up to 100 mg/day.

618

The Homogeneity of 24-Hour Blood Pressure Control of Once-daily Ramipril in Essential Hypertensive Thai Patients

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Ramipril is a long-acting angiotensin converting enzyme inhibitor which is indicated in the treatment of hypertension, congestive heart failure, and nephropathy. Albeit the established evidence of the 24-hour blood pressure reduction, the homogeneity data for blood pressure control of once-daily ramipril is still limited. Employing the 24-hour ambulatory blood pressure (24-h ABP) measurement, this study aims to evaluate the degree and homogeneity of 24-h ABP reduction of ramipril 2.5 and 5 mg once daily in

essential hypertensive Thai patients. The 24-h ABP data were evaluated in terms of systolic and diastolic blood pressure loads (SBP/DBP loads), trough to peak ratio (T/P ratio), and smoothness index (SI). A 1-week placebo run-in, open study design was conducted and followed by at least 2 week period of the treatment. Of nineteen hypertensive male subjects, there were twelve subjects whose blood pressure was responded and/or normalized with once-daily ramipril. The office and 24-h ABP of these subjects were decreased significantly from baseline ($p<0.01$) without significant changes of the heart rate. The percentage and magnitude of 24-h SBP/DBP loads after treatment were significantly decreased from 92% ± 9.7/91% ± 15.9 to 67% ± 23.8/65% ± 27.6 ($p<0.01$) and from 23 ± 10.6/16 ± 5.3 mmHg to 17 ± 10.3/10 ± 4.8 mmHg ($p<0.05$), respectively. T/P ratio for SBP/DBP were 0.59/0.52 (overall-estimated) and 0.68 ± 0.23/0.52 ± 0.22 (individual-estimated). The SI for SBP/DBP was 0.89/1.03. In conclusion, ramipril 2.5 and 5 mg once daily exerted the smooth 24-hour blood pressure reduction in essential hypertensive Thai patients.

619

Influence of Mu-opioid Receptor Polymorphism on Adverse Drug Effect of Oxycodone and Fentanyl in Japanese Lung Cancer Patients

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Objective: Oxycodone (Oxy) and fentanyl (Fen) are widely used opioid analgesics to treat cancer pain. Mutations in the human mu-opioid receptor, which is coded by the OPRM1 gene, are thought as a primary candidate for the variability of clinical effect of Oxy and Fen. The aim of this study was to clarify the influence of mu-opioid receptor polymorphism on analgestic and adverse effects of Oxy and Fen in Japanese lung cancer patients. Methods: Forty-three Japanese lung cancer patients with adequately controlled pain by Oxy or Fen were enrolled in this study. Genotype of OPRM1 (G118A) was determined. Adverse events (somnolence, nausea or vomiting, constipation) were defined as above Grade 2 according to Common Terminology Criteria for Adverse Events (CTCAE). Results: There were no significant differences of doses of Oxy and Fen among patients carrying OPRM1 118A/A, A/G and G/G genotype. Genotype of OPRM1 (G118A) was significantly associated with incidence of patient with nausea or vomiting after the administration of Oxy and Fen. No significant association with incidences of patients with somnolence and constipation was shown among OPRM1 genotypes. Conclusion: These results suggest that OPRM1 118G/G increased incidence of nausea/vomiting in the treatment of Oxy and Fen in Japanese lung cancer patients, although requirements of Oxy and Fen were not changed among OPRM1 genotypes.

620

Non-response to Antiepileptic Pharmacotherapy is Associated with the ABCC2 -24C>T Promoter Polymorphism in Young Patients with Epilepsy

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Introduction/Objective: To evaluate the association of non-response to antiepileptic pharmacotherapy with the variant allele frequency of drug metabolizing enzyme and transporter genes in a study cohort of young epileptic patients and an independent cohort of adult Caucasians

with refractory epilepsy. Methods: 221 Caucasian patients (116 male; aged 14.5 ±6.54 years) were recruited from two large German epilepsy centres in Kiel and genotyped for eight putatively functionally relevant polymorphisms in the ABCB1, ABCC2, CYP2C8, CYP2C9 and CYP2C19 genes. In addition, an independent cohort of 70 adult patients (35 male; aged 41.9 ±11.5 years) with drug-refractory epilepsy that had earlier undergone neurosurgical therapy and a control cohort of 242 healthy volunteers were genotyped.Results**:** The cohort of young epilepsy patients consisted of 103 (46.6%) responsive and 118 (53.4%) patients not responsive to the first anticonvulsant. Among the latter, heterozygous carriers of the ABCC2 -24T variant allele were significantly overrepresented (OR 3.06 (1.46-6.41); p=0.003). In contrast, hetero- or homozygous carriers of the ABCB1 3435T allele (OR 0.33 (0.13-0.83); p=0.019) as well as heterozygous CYP2C9*3 variant allele carriers (OR 0.39 (0.16-0.96); p=0.041) were significantly underrepresented among non-responders. The significant overrepresentation of ABCC2 -24T variant allele carriers among non-responders was confirmed in the cohort of adult drug-refractory patients for hetero- (OR 3.84 (1.62-9.11); p=0.002) and homozygous subjects (OR 7.11 (1.05-48.0); p=0.044). Discussion/Conclusions: These data argue for a higher risk of non-response to antiepileptic therapy in patients bearing the low-functional ABCC2 -24T variant allele, while ABCB1 and CYP2C9 polymorphisms are associated with increased response rates.

621

Intestinal Expression and Function of the Newly Identified Human Proton Coupled Folate Transporter hPCFT

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Folates are essential vitamins necessary for purine/pyrimidine synthesis and DNA methylation. The human proton coupled folate transporter (hPCFT) was recently identified through genetic association studies where mutations in this transporter were linked to hereditary folate deficiency and confirmed using in vitro studies in terms of its ability to mediate the pH-dependent uptake of folate. Given its central role in folate absorption/homeostasis, we carried out a study to determine the extent of intersubject variation in intestinal hPCFT expression and then create a model cell system to better assess the transport activity of this transporter. We obtained pinch biopsies from patients undergoing routine diagnostic endoscopy procedures to determine hPCFT protein and mRNA expression. We also stably expressed human PCFT in the polarized epithelial cell line, MDCK-II. Analysis of biopsy samples suggested that intestinal expression of this transporter is highly variable. hPCFT protein varied 9-fold whereas mRNA expression varied over 100-fold between subjects. MDCKII-hPCFT cells were able to mediate robust, pH dependent transport of [^{3}H]-folate compared to the parental MDCKII cells. In addition, we are now able to show hPCFT can transport methotrexate, a widely used dihydrofolate reductase (DHFR) inhibitor used for the treatment of cancers and chronic inflammatory conditions. Furthermore, drugs such as sulfasalazine, known to elicit folate deficiency in humans, appeared to be a significant inhibitor of hPCFT. In summary, our data suggest that intersubject hPCFT intestinal expression is highly variable and may be a heretofore underappreciated contributor to variable of intestinal folate absorption and the risk for drug-induced folate deficiency.

622

Interaction between Cranberry Juice and Medications Metabolized by CYP2C9

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Recently, several case reports showing that the patients on warfarin therapy suffered from a profound hypoprothrombinemia after the ingestion of cranberry juice (CrJ) were published. Since (S)-warfarin is metabolized by CYP2C9 in

human, it is important to clarify a potential interaction of CrJ with other medications metabolized by CYP2C9. The purpose of this study was to determine an interaction between CrJ and phenytoin (PHT). Wistar rats were ingested with CrJ for 7 days, and PHT (20 mg/kg, iv) was injected. Plasma PHT concentrations were measured by FPIA method. In addition, the influence of CrJ on PHT metabolism was investigated in rat and human liver microsome systems. In results, CrJ did not influence the PHT pharmacokinetics in rats. PHT metabolism by rat liver microsomes was only inhibited by its higher concentration (6%). However, in human liver microsomes, PHT metabolism was decreased significantly by 1% of CrJ, and IC50 was calculated to be 0.7%. We did not perform any clinical trials in patients or healthy volunteers, because of the safety concern about PHT. Based on the present findings, we think that it is needed to pay attentions to patients taking PHT with cranberry juice by monitoring carefully the symptoms of drug-associated adverse reactions. Now we are carrying out a human study to examine whether daily ingestion of CrJ causes pharmacokinetic and pharmacodynamic alterations of CYP2C9 substrates.

623

Genetic Variation of *Thiopurine S-Methyltransferase* on Azathioprine Induced Myelosuppression in Thai Kidney Transplant Recipients

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Introduction: Azathioprine (AZA) is an immunosuppressive administered to prevent graft rejection after transplantation. Thiopurine- S-methyltransferase (TPMT) is a key enzyme metabolizing AZA to inactive metabolites. Recent data showed that patients with *TPMT* mutant allele have low activity of TPMT than those with wild type and therefore, are at high risk of myelosuppression. Objective: To investigate correlation between TPMT genetic polymorphism and AZA-induced myelosuppression in Thai kidney transplant (KT) recipients. Materials and Methods: Medical records and clinical data of KT recipients were retrospectively evaluated during AZA therapy. *TPMT*3C* and *TPMT*6* mutation were analyzed by real-time PCR and PCR-RFLP assays, respectively. TPMT activity in RBC was measured using HPLC techniques. Patients who had lower TPMT activity but were not identified as *TPMT*3C* or *TPMT*6*, would be further investigated by ORF direct sequencing. Results: Nine out of 142 (6.33%) KT recipients were identified as *TPMT*1/*3C*. However, TPMT activity was determine only in 122 patients and heterozygous mutant exhibited significant reduced TPMT activity when compared to wild type group (median 21.37 vs 36.93 nmol 6-MTG. g^{-1}Hb. Hr^{-1}, $p < 0.001$). Heterozygous mutant of *TPMT*1/*3C* was at 14.17 folds higher risk for AZA-induced hematotoxicity after receiving standard dose of AZA (90% CI 2.22 – 313.19, $p < 0.01$). The positive and negative predictive values of *TPMT* genotyping for AZA-induced myelosuppression were 88.89% and 63.91%, respectively. In addition, a novel SNP located in exon 5 at position 319 was identified. Discussion and Conclusion: The presence of *TPMT*3C* in KT recipients was correlated well with reduced TPMT activity in RBC and AZA-induced myelosuppression. Determination of TPMT status prior to AZA therapy in KT is recommended to reduce adverse drug reactions.

624

Inhibition of Human Organic Anion Transporter 1 (hOAT1, SLC22A6) as the Mechanistic Basis of Drug-adefovir (ADF) Interactions during Highly Active Antiretroviral Therapy (HAART)

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Emergence of viral resistance and unexpected drug-drug interactions remain a major obstacle to safe and effective HAART in our patients infected

with HIV. Further complicating the treatment option is that many with HIV are also co-infected with other chronic viral diseases such as hepatitis-B (HBV). One common drug interaction during HIV therapy relates to drug-ADF interactions. ADF is an acyclic nucleoside phosphonate analogue that is actively secreted by the kidney via renal hOAT1 mediated uptake transport. Since hOAT1 can interact with drugs such as nonsteroidal anti-inflammatory drugs, cephalosporine, and tenofovir, we hypothesized that the clinically observed drug interactions during ADF therapy occur via modulation of hOAT1. Accordingly, to better understand the mechanism of hOAT1-associated renal drug-drug interactions, we created a polarized kidney epithelial cell line (MDCKII) stably transfected with hOAT1. Targeted stable expression of hOAT1 in MDCKII-OAT1 cells was demonstrated by western-blot analysis. We then assessed the inhibitory effects of known OAT1 inhibitors and an array of HIV protease inhibitors (Pis) using [^{3}H] ADF as model substrate in this cell line. Interestingly, most of the tested HIV-Pis exhibited minimal inhibitory effect on hOAT1-mediated ADF transport with the exception of tipranavir (TPV) and lopinavir (LPV) (TPV>>LPV > atazanavir (ATZ) > ritonavir (RTV)). Known hOAT1 transport modulators such as mycophenolic acid, tetracycline, probenecid and furosemide significantly inhibited hOAT1-mediated ADF transport in our model system. These results suggest that inhibition of hOAT1 likely contributes to the observed ADF-drug interactions during HAART in our HIV patients.

625

Pharmacogenomics of Severe ADRs in Children: Identification of Novel Genetic Markers of Anthracycline-induced Cardiotoxicity in Children

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Severe adverse drug reactions (ADRs) are a major health problem in the developed world, claiming thousands of lives and costing billions of dollars annually. Children are especially at risk for ADRs, since most drugs are never tested in pediatric populations. Anthracylines, such as doxorubicin, are commonly being used to treat adult and childhood leukemia and solid tumors, and are highly effective. However, their usefulness is limited by the occurrence of cardiotoxicity, potentially leading to death or requirement of heart transplantation or life-long treatment. High cumulative dose has previously been associated with greater risk, but some patients get cardiotoxicity even at low doses. We aimed to identify genetic markers predictive of anthracycline-induced cardiotoxicity (ACT) in children. DNA-samples and detailed clinical information were obtained from 52 cases and 222 drug-matched controls through our nationwide GATC ADR-surveillance network. DNA samples were genotyped for a panel of 3072 SNPs in 220 ADME genes. One variant in a key drug-metabolizing enzyme was found to be highly associated with anthracycline-induced cardiotoxicity (P-value 4.96×10^{-5}, OR=5.3). In addition, variants in two drug transporters were also highly associated (P-value 0.00057, OR=2.2 and P-value 0.0035, OR=2.7) and one in another drug-metabolizing enzyme (P-value 0.003, OR=4.4). These results confirm that genetic variation significantly influences susceptibility for ACT. Identification of these genetic markers is essential for developing a diagnostic test to predict who is at risk for ACT. Ultimately, this will reduce this potential lethal ADR and make treatment safer and more effective.

626

Effects of Tanshinones from Danshen (*Salvia miltiorrhiza*) on Human CYP1A2, CYP2C9 and CYP3A4 Activities *in vitro*

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Tanshinones are abietane type-diterpene quinones isolated from the roots of Danshen (*Salvia*

miltiorrhiza), a well-known traditional Chinese medicine in the treatment of cardiovascular and cerebrovascular diseases. However, exaggerated anticoagulation and bleeding complications have also been observed during concurrent use of Danshen and warfarin in patients, although the mechanism(s) of the herb-drug interaction remained uncertain. The present study investigated the effects of tanshinones on the metabolism of probe substrates of specific CYP isoforms including CYP1A2, CYP2C9 and CYP3A4, the major isoforms that are responsible for the metabolism of warfarin to assess the potential interactions of Danshen with drugs that utilize these isoforms for their biotransformation. The effects of tanshinones on human CYP1A2 (phenacetin *O*-deethylation), CYP2C9 (tolbutamide 4-hydroxylation), and CYP3A4 (testosterone 6beta-hydroxylation) activities were investigated *in vitro* using pooled human liver microsomes and human CYP isoforms. Tanshinones inhibited human CYP1A2, 2C9 and 3A4 activities. Enzyme kinetic studies showed that tanshinone I, tanshinone IIA, and cryptotanshinone were competitive inhibitors of CYP1A2, 2C9 and 3A4 with varying effectiveness. Dihydrotanshinone was not only a competitive inhibitor of CYP1A2 and 2C9, but also a noncompetitive CYP3A4 inhibitor. In conclusion, these results confirmed that Danshen-inhibited CYP activity, especially CYP1A2, then CYP2C9 in human *in vitro.* Given that CYP1A2, 2C9 and 3A4 are responsible for the metabolism and disposition of a large number of drugs currently used in man, the concomitant use of Danshen with drugs which are substrates of CYP1A2, 2C9 and 3A4, especially CYP1A2, must be met with great caution.

627

Anti-Xa is Better Suited than PT Ratio or INR for Monitoring of Factor Xa Inhibitors

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Introduction: Apixaban and other Fxa inhibitors are in late stage clinical development as anticoagulant agents. Although monitoring will likely not be required, in certain situations (e.g., surgery, over-dose) Fxa inhibitor levels may need to be evaluated. This study was performed to assess if the current coagulation assays (PT/INR and anti-Xa) are suitable for measuring Fxa inhibitor levels in patients. Methods: Twelve PT/INR (ISI 0.89-2) and seven anti-Xa assays were evaluated *in vitro* using human plasma spiked with three Fxa inhibitors. Assay variability and assay correlations with Fxa inhibitor plasma concentration were analyzed in apixaban clinical samples. Results: Fxa inhibitors prolonged PT regardless of the reagents used. PT sensitivity of a given Fxa inhibitor varied greatly among reagents and conversion to INR using ISI increased variability. Standard deviations across reagents were 0.59, 0.46 and 0.65 for PT ratios and 0.93, 0.77 and 1.77 for INR in samples containing apixaban, razaxaban and BMS-645068, respectively. Fxa inhibitors interacted differentially with PT reagents. The difference in PT ratio between apixaban and razaxaban was +0.15 and -1.02 for NPCI+ and PT-FIB-Recombinant, respectively. Evaluation of anti-Fxa assays revealed that Rotachrom had the best sensitivity and dynamic range (LOQ=10ng/mL, range=10-1000ng/mL for apixaban). In clinical samples, anti-Fxa showed a strong positive correlation with PK (R^2 = 0.898), while INR did not (R^2 = 0.397). Conclusion: Commercial PT/INR assays may not be appropriate for assessing Fxa inhibitors as they are highly variable and inconsistent. The Rotachrom anti-Fxa assay is preferred with much lower individual variability and strong concordance with PK.

628

Effects of Hesperidin, a Component of Orange Juice, on the Pharmacokinetics of Pravastatin and on the Expression of Pravastatin Transporters in Rat Small Intestine and Liver

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Multidrug resistance-associated protein 2 (MRP2) and organic anion transporter 2 (OATP2) are concerned in the transport of pravastatin (PRV). We already reported that orange juice (OJ) ingestion increased the bioavailability of PRV and affected the expression of pravastatin transporters in rat small intestine and liver. In this study we investigate the effect of hesperidin, which is one

of the components of OJ, on the pharmacokinetics of PRV and on the expression of MRP2 and OATP2 in rat small intestine and liver. Hesperidin suspension (0.079%) or tap water as a control was given to the 8-week-old male Sprague-Dawley rats in the same schedule. A single dose of PRV at 100 mg/kg p.o. was administered 1 hr after the last ingestion of hesperidin or tap water. Intravenous blood samples were collected successively from the tail. Plasma PRV levels were measured by the HPLC methods. Hesperidin ingestion significantly increased the AUC, the Cmax and the t1/2 value of PRV compared with the controls. MRP2 protein levels in both tissues were significantly decreased by hesperidin ingestion, respectively. OATP2 protein level in small intestine was significantly increased by hesperidin ingestion but the level in liver tissue was not significantly changed. These results were same as those obtained from the former experiment with OJ ingestion. Therefore, it is suggested that the bioavailability of PRV increased by OJ and the expression of both MRP2 and OATP2 protein changed by OJ are based on hesperidin in the juice.

629

Efficient Gene Transfer into Human Intenstinal Epithelial Model with Adenovirus Vector

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A number of drug-metabolizing enzymes are low expressed in the Caco-2 cell monolayer, a model of useful tool for evaluating human intestinal permeability, compared with human intestinal epithelium. Development of efficient gene transfer systems into the Caco-2 cell monolayer appears to be crucial to the basic research of drug intestinal permeability. On the other hand, recombinant adenovirus (Ad) type 5 vectors are potentially useful for gene transfer to a wide variety of cells. However, Kesisoglou *et al.* suggested that Caco-2 cell monolayer are resistant to transduction by Ad vector. The aim of the present study was to develop the simple method for efficient gene transfer into Caco-2 cell monolayer by Ad vector. The primary receptor, the coxsackievirus and adenovirus receptor (CAR), and the secondary receptor, integrins and andomi sulfate glycosaminoglycans, are the tropism determinants of Ad type 5. Cohen *et al.* reported that CAR is one of the component protein in the tight junction, and Ad vector is hardly accessible to CAR sequestered within tight junction in the epithelial cells. Therefore, we tried to modulate tight junction with sodium caprate (C10), which can be open the intestinal epithelial tight junction, for efficient transduction of Ad vector to the Caco-2 cell monolayer. The co-transfection with Ad vector and C10 mediated higher transgene expression into the Caco-2 cells monolayer than Ad vector alone. Our system could be a powerful tool for gene transfer into Caco-2 cell monolayer in studies of gene function as well as drug intestinal permeability.

630

Basic and Clinical Study of Oral Chicken Type II Collagen on Rheumatoid Arthritis

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The preclinical study was performed to explore the effects and mechanisms of chicken type II collagen (CCII) for inducing immune tolerance in experimental arthritis. Furthermore, a clinical trial was performed to determine the efficacy and safety of oral CCII in comparison with methotrexate (MTX) on patients with rheumatoid arthritis (RA). In animal experiment, arthritis model was established and the relative parameters were observed. In clinical trial, five hundreds and three patients, aged 18-65 years, with active RA were randomized to receive CCII at 45 μg twice daily or MTX at 10 mg once weekly, respectively. CCII had a therapeutic effect on experimental arthritis, which was related to regulate balance of cytokines. Both CCII and MTX could improve the symptoms and signs of RA. In the CCII group, the ACR20 responder rates were 30.7% at week 12 and 41.6% at week 24. ACR50 responder rates were 8.8% at week 12 and 16.9% at week 24 with

CCII. In the MTX group, ACR20 responder rates were 44.7% at week 12 and 57.9% at week 24. ACR50 responder rates were 12.6% at week 12 and 30.8% at week 24 with MTX. After 24 weeks treatment, the response rates to treatment of the CCII group, based on both ACR20 and ACR50 responder rates, were lower than that of the MTX group, and this difference was statistically significant. The incidence of adverse events between the CCII group and MTX group was not statistically significant. The CCII has clinical efficacy and safety in treatment of RA.

631

Validation of a LC-MS/MS Method for Quantitation of the Furosemide in Pharmacokinetic Study

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Introduction: Furosemide is a potent and widely used loop diuretic in the treatment of edematous states and/or associated with chronic renal failure, hypertension, congestive heart failure, or cirrhosis of the liver. Objectives: The objective of the present study was to develop and validate a LC-MS/MS method for quantitation of furosemide in human plasma to support human clinical studies. Methodology: The analyses were carried out using a C_{18} column at 40 ºC with a mobile phase of acetonitrile:0.1% acetic acid:methanol (80:10:10) at a flow rate of 0.7 mL/min (split 1:5). The mass spectrometer equipped with an electrospray source in negative mode, was set up in multiple reaction monitoring, monitoring the transitions 328.4>284.9 (furosemide) and 336.4>190.0 (chlortalidone, internal standard). Results and Discussion: The chromatographic separation was obtained within 2.0 min and it was linear in the concentration range of 10-2000 ng/mL. Good precision and accuracy were obtained for the LC-MS/MS method. The stability studies of furosemide in plasma showed results within the acceptable range. An excellent recovery of furosemide at low, medium and high quality control samples by liquid-liquid extraction was obtained. Conclusions**:** A fast LC-MS/MS method for the determination of furosemide in human plasma was developed and validated. The data obtained for the optimized LC-MS/MS method showed specificity, linearity, precision and accuracy. Moreover, the proposed method was successfully applied to a pharmacokinetic study in healthy human volunteers, and results showed that the two furosemide formulations are bioequivalent in their rate and extent of absorption.

632

An Oral, Rising Single-dose Tolerance, Pharmacokinetic and Pharmacodynamic Study of Rivoglitazone (CS-011) in Healthy Volunteers

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Rivoglitazone is a novel thiazolidinedione (TZD) that has demonstrated more potent antidiabetic effects than other TZDs in preclinical models of diabetes. The objectives of this study were to assess the safety/tolerability and pharmacokinetics-pharmacodynamics of rivoglitazone over a range of single, oral doses in healthy volunteers. In this randomized, placebo-controlled, 2-period, crossover study, 23 volunteers received either an oral dose [1mg (n=3), 2.5mg, 5mg, 10mg, 20mg, or 30mg (all n=4)] rivoglitazone oral solution or placebo in the first period followed by the opposite treatment in the second period. Following administration, blood samples were collected over a 96-hour period for pharmacokinetic profiling, and analysis of insulin and glucose levels. Rivoglitazone was generally well tolerated. Eighteen adverse events (Aes; mild=13, moderate=5) were reported by 9 volunteers. No serious Aes were reported. Aes did not increase in frequency or intensity with dose escalation. After each dose, C_{max} of rivoglitazone occurred within 2 hours. Plasma concentrations declined monoexponentially with a T½ of 9.2–14.9 hours. C_{max} (mean [%CV] = 0.5[5]µg/mL–1.6[5]µg/mL) and $AUC_{0-\infty}$(mean [%CV] = 0.7[18] µg·hr/mL–19.5[9] µg·hr/mL) increased proportionally over the dose range. Pharmacokinetic variability for single-dose rivoglitazone was low. Renal excretion of unchanged rivoglitazone was minimal (Ae% = 0.00–0.02). Normal glucose and insulin

concentrations were maintained in healthy volunteers. In summary, single doses of rivoglitazone up to 30mg were safe and well-tolerated. Mean peak and total plasma exposures increased proportionally with dose, and pharmacokinetic variability was low. These favorable safety and pharmacokinetic data support further clinical evaluation of rivoglitazone.

633

SNAC Co-formulation Produces Significant Enhancement of Oral Vitamin B12 Bioavailability in Rats

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Gastrointestinal absorption of Vitamin B12 (B12) is limited by an active receptor mediated transport process of B12 bound to intrinsic factor (IF). B12 deficiency leads to a wide spectrum of hematologic and neuropsychiatric conditions. The prevalence of vitamin B12 deficiency is high, particularly in older adults, and many are currently treated with parenteral therapy due to problems with absorption through oral routes. This study evaluated the oral bioavailability of the co-formulation of B12 (cyanocobalamin) with Eligen carrier SNAC (Sodium N-[8-(2-hydroxybenzoyl) amino]caprylate) in comparison to intravenous and unenhanced oral B12 tablets.
Male Sprague-Dawley rats (325-350g) were dosed with B12 intravenously (0.5 mg/kg) alone, or orally (50 mg/kg) alone or in combination with SNAC (200 mg/kg). Blood samples were collected at 0, 3, 10, 20, 30, 60, 120, 240 and 360 minutes post dosing. Serum samples were analyzed for B12 by radioimmunoassay (RIA). The model independent PK metrics obtained following B12-SNAC combination were compared to those obtained following B12 alone (t-test analysis). The extent of B12 absorption, measured as AUC and Cmax, was significantly enhanced by the administration of the combination. Absolute bioavailability was ~18 fold greater for drug-SNAC combination compared to B12 alone. Peak B12 concentrations following drug-carrier combination were reached significantly earlier than those following B12 alone. These data demonstrated that SNAC co-formulated with B12 enhance GI absorption through a mechanism other than the saturable receptor mediated uptake mechanism. Therefore, the oral formulation of SNAC with B12 is potentially therapeutic for patients lacking IF and /or receptor.

634

KMUP-1 Prevention of Monocrotaline-induced Pulmonary Hypertension via Modulation of Rho-kinase and K^+-Channel Dysfunctions

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Pulmonary artery hypertension (PAH) is characterized by a sustained increase in pulmonary artery pressure (PAP) leading to progressive right ventricle failure and death. KMUP-1, a xanthine-based derivative, has been demonstrated to stimulate BK_{Ca} currents in basilar artery myocytes. This study is to investigate the actions of KMUP-1 on the K^+-channel and Rho-kinase (ROCK) in PAH rats. Sprague-Dawley rats were divided into three groups including the control, PAH, and KMUP-1-treated. PAH rats were induced by single intraperitoneal injection (i.p.) of monocrotaline (MCT, 60 mg/kg), then KMUP-1 (5 mg/kg, i.p.) was administrated once daily for 21 days. All rats were sacrificed at the 21 day after treating MCT. In PAH rats, increased PAP and right ventricle hypertrophy were markedly induced. The increase of PAP was almost fully prevented by KMUP-1. In isolated Pas, KCl (80 mM) or phenylephrine (10^{-5} M)-induced contraction was weaker in PAH than in control rats, but the action was reversed in KMUP-1-treated rats. Moreover, the ROCK inhibitor Y-27632 (10^{-6} M)-induced relaxation was stronger in PAH than in control rats, and the action was also reversed in KMUP-1-treated rats. These results suggested that KMUP-1 could improve the vessel dysfunction in PAH rats. The BK_{Ca} current measured using whole cell patch-clamp techniques. BK_{Ca} current attenuated in PAH rats compared with control rats. KMUP-1 reversed PAH rats caused BK_{Ca} channel inhibition. In light of these results suggested that prevention of PAH and improvement of PA tone

by KMUP-1 could be due to the alteration of ROCK and K^+-channel functions.

635

Phase 1 Safety and Pharmacokinetic Study of Monoclonal Antibody against Hemorrhagic Fever with Renal Syndrome Virus for Injection in Chinese Healthy Volunteers

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Hemorrhagic fever with renal syndrome (HFRS), an infectious disease, is an important public health concern throughout China. A monoclonal antibody against HFRS virus (HFRS mAb) is thus far the most efficient remedy for this disease. To determine the safety and pharmacokinetics of HFRS mAb in Chinese population, we applied single-site, open-label and randomized trials with different doses of HFRS mAb to 50 healthy Chinese volunteers (20 in tolerance trial and 30 in pharmacokinetic trial). In the tolerance trial the volunteers were treated intravenously with four escalating doses of HFRS mAb: 2.5, 5, 10, 20 mg. And in the pharmacokinetic trial the subjects received the last three dosages described above. For the kinetic analysis of HFRS mAb in the serum, we established a highly sensitive, rapid, accurate, and precise immunoassay. Our results showed HFRS mAb was well tolerated and the most frequent adverse events associated with its administration were dizziness and skin rash. No other side effects were noted during treatment regardless of the doses used in this study. A one-compartment model best described the serum distribution of HFRS mAb. Its serum levels were dose-dependent and showed linear pharmacokinetics after intravenously infusion at 5-20 mg. The elimination rate of HFRS mAb was relatively slow and nearly 20 days were needed for a complete clearance of HFRS mAb from systemic circulation.

636

Gargling with Tea Catechin Extracts for the Prevention of Influenza Infection

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Experimental studies revealed that tea catechin extracts have preventive effects against influenza infection; however, clinical effects have been inconclusive. We conducted two clinical studies on the effects of gargling with tea catechin extracts on the prevention of influenza infection. In a prospective cohort study of nursing home residents, 76 elderly residents gargled three times daily with tea catechin extracts (total catechin concentration; 200 microgram/ml) in winter season, and were compared with 48 residents who used a gargle without the extracts. In the catechin group, one resident [1.3%] got the influenza infection, while five residents [10%] of the control group got with the influenza infection. Also we conducted a succeeding study of randomized, double-blind manner in the 395 healthy adult volunteers, inoculated with influenza vaccine. In the catechin group, two participants [1.0%] got the influenza infection, while four participants [2.0%] of the control group got with the influenza infection. Though the latter study could not find significant effects of gargling with tea catechin, it might be probably because of the small incidence of influenza infection. In conclusion, further studies are needed to clarify the effects of tea catechin extracts in the more susceptible populations such as adults without inoculated with influenza vaccine, the elderly, immune suppressants, or children.

637

A Placebo-controlled, Ascending Multiple-dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Apixaban in Japanese Healthy Adult Male Subjects

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Apixaban is an oral, selective, direct, reversible inhibitor of coagulation factor Xa inclinical development for prevention and treatment of thromboembolic diseases. This randomized, placebo-controlled, double-blind, sequential, ascending multiple-dose study examined apixaban safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) in healthy male Japanese subjects. Eight subjects were randomized in a 3:1 ratio to receive apixaban or matched placebo in each of 3 dose panels, 2.5, 5 and 10 mg twice daily (BID) for 7 days. Apixaban was safe and well tolerated; few adverse events were reported, none were bleeding-related and all were mild. Peak apixaban plasma concentrations were achieved approximately 3 hours post-dose. Terminal half life ranged from 8 to 10 hours. Exposure increased in a dose proportional manner on both Days 1 and 7 with minimal accumulation to steady state (accumulation index (AI) $\leq$ 2). AI and renal clearance were consistent across doses. M1 (BMS-730823), an inactive circulating apixaban metabolite, represented about 24-26% of steady state apixaban AUC (TAU). Dose-and exposure-dependent prolongation of clotting times (INR, APTT and mPT) and anti-Xa activity were observed. Changes in these parameters closely followed the apixaban plasma concentration-time profile. In conclusion, apixaban was safe and well tolerated in healthy Japanese subjects at doses up to 10 mg BID for 7 days. A linear PK profile was observed with little accumulation and no time dependent changes in elimination. Observed changes in clotting times and anti-Xa activity were consistent with direct reversible Fxa inhibition. Further evaluation of apixaban in the Japanese population is warranted.

638

Influence of Clinical and Genetic Factors on Warfarin Dose Requirement among Japanese Patients

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Frequent monitoring is required to achieve an adequate dose regimen of warfarin because of its narrow therapeutic index and wide individual variability in response to this drug. In this study, we investigated the clinical and genetic factors that influenced anticoagulant effect of warfarin. To identify the genetic factors, 108 Japanese patients on stable warfarin anticoagulant therapy (international normalized ratio: 1.5-3.0) were genotyped by PCR based method. Genetic polymorphisms we analyzed were as follows: CYP2C9*3, vitamin K epoxide reductase complex subunit 1 (VKORC1) -1639G>A, gamma-glutamyl carboxylase ((CAA) repeats in intron 6 and R325Q), and coagulation factor 7 (-402G>A, -401G>T, -323p0/10, and R353Q). Genetic polymorphisms significantly associated with mean warfarin dose were CYP2C9*3 (*1/*1: 2.64±1.10 mg/day vs *1/*3: 1.75±0.79 mg/day, P=0.05) and VKORC1 -1639G>A (AA: 2.41±0.94 mg/day vs GA: 3.81±1.53 mg/day, P<0.001). Only one patient was homozygous for CYP2C9*3, whose maintenance dose was 0.5 mg/day. The simple linear regression analysis has revealed that warfarin dose is significantly associated with sex (r2=0.039, P<0.05), age (r2=0.15, P<0.001), body surface area (r2=0.17, P<0.001), CYP2C9*3 (r2=0.067, P<0.05), and VKORC1 -1639G>A (r2=0.17, P<0.001). In the multiple linear regression model, combination of these factors explained 40.1% of the variance in warfarin dose requirement, proposing the warfarin dosing regimen for Japanese patients. This regimen might be useful for the precise prediction of the maintenance dose of warfarin, contributing to the establishment of individualized warfarin therapy.

639

Individual Differences in Voriconazole Oxidation Independent on Cytochrome *P450 2C19* Genotypes

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Voriconazole is a novel azole antifungal agent metabolized mainly by cytochrome P450 (P450 or CYP) 2C19 into voriconazole *N*-oxide. To investigate voriconazole clearance in individuals,

the oxidative metabolism of voriconazole by human liver microsomes and therapeutic monitoring in patients treated with voriconazole between 2005 and 2007 in NIH were studied. CYP3A4 produced a new methyl hydroxylated metabolite and *N*-oxide from voriconazole. The voriconazole 4-hydroxylation to *N*-oxidation metabolic ratios in liver microsomes from the wild-type *CYP2C19*1/*1* individuals (0.07) were lower than those observed in other genotypes (0.20-0.27) at a clinical substrate level (25 μM). There was a correlation of serum *N*-oxide of voriconazole levels and voriconazole dose (r = 0.26, $p < 0.01$) in a total of 171 serum samples in 62 predominantly Caucasian patients (range 1-17 samples/patient). There was an inverse correlation of the voriconazole metabolic ratio (voriconazole *N*-oxide/voriconazole) and voriconazole dose adjusted for weight (r = 0.48, $p < 0.001$), implying saturation of voriconazole metabolism. There was no difference between median voriconazole and median *N*-oxide voriconazole serum levels in patients heterozygous for *CYP2C19* mutations and the wild type. These results suggested that voriconazole was efficiently catalyzed by both polymorphic CYP2C19 and abundant CYP3A4 in livers to form voriconazole *N*-oxide and that CYP3A4 could also mediate the methyl hydroxylation to yield the polar 4-hydroxyvoriconazole. The effect of *CYP2C19* genotype on the levels of voriconazole and *N*-oxide of voriconazole was modest and probably not clinically relevant in our Caucasian population, while voriconazole levels did not correlate with the given dose.

640

Rapid and Sensitive HPLC-MS/MS Method for Measuring Ribavirin and its Application in Human Pharmacokinetics

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Ribavirin is a synthetic purine nucleoside analog with a broad spectrum of antiviral activity. There are no detailed pharmacokinetic data have been reported for this drug in Chinese until now, it is necessary to assess ribavirin pharmacokinetics in Chinese and develop a rapid and sensitive method for the measurement of ribavirin in human plasma. The analytical method involves protein precipitation, dilution of the supernatant, HPLC separation, and quantification by MS/MS system using positive electrospray ionization in the multiple reaction monitoring mode (MRM). Twenty healthy volunteers received a 300-mg oral dose of ribavirin. Blood samples were then collected up to 120 h postdosing. The results showed the calibration curve for ribavirin was linear over a concentration range of 1–1500 ng/mL. The lower limit of quantification (LLOQ) was 1 ng/mL ribavirin. The single dose of ribavirin was well tolerated and no serious adverse effects occurred. The mean time to maximum concentration was about 1.25 h. The mean maximum concentration of drug in plasma for oral ribavirin was 250 ng/mL. The mean elimination half-life was 43.6 h. The present study describes a simple, specific, sensitive LC–MS/MS method to measure plasma drug concentration and analyze human pharmacokinetics of ribavirin. Following a single dose of 300mg, the plasma pharmacokinetics of ribavirin in healthy Chinese volunteers is similar to previous reports in Western population.

641

Phenotypical Characterization of CYP1A2, CYP2A6, N-Acetyltransferase 2 and Xanthine Oxidase by using Caffeine in Turkish Population

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Caffeine is metabolized by drug metabolizing enzymes cytochrome P450 (CYP) 1A2, 2A6, N-acetyltransferase 2 (NAT2) and xanthine oxidase (XO). In the present study caffeine was used as a phenotyping agent in order to assess the activities of these enzymes. Healthy subjects (n=253) participated in the study. The subjects drank a cup of instant coffee that contained 60-100 mg of

caffeine. The fifth hour spot urine samples were used for the analysis of caffeine metabolites. 1,7-dimethylxanthine (17X), 1,7-dimethyluric acid (17U), 1-methylxanthine (1X), 1-methyluric acid (1U), and 5-acethylamino-6-formylamino-3-methyluracil (AFMU) were analyzed with high pressure liquid chromatography. For the assessments of enzyme activities of CYP1A2, CYP2A6, NAT2 and XO the following ratios were used: (AFMU+1X+1U)/17U; 17U/(17U+17X+1U+1X+AFMU); AFMU/1X and 1U/1X+1U, respectively. The mean (range) ratios calculated from the above metabolite proportions were 4.32 (0.34 – 82.17), 0.70 (0.01 – 111.00), 0.26 (0.02 – 0.86), and 0.58 (0.15 – 0.98), respectively. A large variation was observed among the individuals. Further studies are warranted to clarify the genetic and environmental factors affecting these large inter-individual differences.

642

Alternation of OAT3 Expression Induced by Angiogenic Factors

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Endothelial cells (Ecs) have important functions such as blood-tissue permeability, vascular tonus, and angiogenesis. Especially the angiogenesis is an intrinsic feature of Ecs. It has been known that vascular endothelial cell growth factor (VEGF) and prostaglandin E2 (PGE2) are angiogenic factors. Expression of some transporters which transport physiological or synthetical substances has been reported in various tupes of Ecs. Especially, transport of neurotransmitter metabolites by organic anion transporter 3 (OAT3) is important in endothelial cells of blood brain barrier. However, it is not clear whether angiogenic factors influence cell permeability in Ecs. In the present study, we confirmed the expression of hOAT3 in the plasma membrane and cytoplasm of human brain microvascular Ecs (BMECs) and human umbilical vein Ecs (HUVECs), and then the alteration of the hOAT3 expression induced by VEGF and PGE2 was investigated. VEGF increased the protein level of hOAT3 in Ecs during 1-48 h. The uptake of [3H] methotrexate in HUVECs increased in a time-dependent manner by the stimulation of VEGF. Immunohistochemical analysis revealed that VEGF stimulation amplified the hOAT3 expression in the cytoplasm and membrane of Ecs. PGE2 increased a transient expression of the hOAT3 at 1 h, however, its expression returned to the basal level. Our results suggested that VEGF and PGE2 affect the expression of hOAT3, and the alteration of hOAT3 induced by angiogenic factors might affect the kinetics of endogenous or exogenous substrates under some pathological conditions.

643

Influence of *Momordica charantia* and *Eugenia jombolina* on Intestinal Uptake of l-tyrosine and D-glucose Across the Intestinal Sac - *in vitro*

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Momordica charantia (MC) and *Eugenia jambolina* (EJ) are well known plants with identified antidiabetic, laxative, anti-inflammatory and antimicrobial properties. This study investigates the effects of aqueous fruit extract of MC and aqueous bark extract of EJ on the transport of d-glucose, l-tyrosine across rat-everted intestine in vitro. Krebs–Henseleit bicarbonate buffer was used to incubate everted intestinal sacs from rats. Graded concentrations up to 12 mg/ml of MC and EJ extract were incubated in the mucosal solution. d-glucose and l-tyrosine absorptive capacity of the intestine were significantly inhibited by MC and EJ extracts in combination and individually. The aqueous extract of the plants was found to inhibit primarily the uptake of glucose in a dose-dependent manner. Additionally absorption of electrolyte (Na and K) was also studied to find out the influence on absorptive capacity. It is assumed that bioactive phytochemicals such as saponins in MC fruit extract and tannins from EJ inhibit the active transport of d-glucose, *l*-tyrosine across rat intestine. It is to be expected that MC and EJ can be a potential alternative drug therapy of

postprandial hyperglycemia via inhibition of glucose uptake across the small intestine and could involve a washout of glucose from the blood stream.

644

Cytochrome P450 2B6 (CYP2B6) Catalyzes the Formation of Pharmacologically Active Sibutramine Metabolites in Human Liver Microsomes

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We identified CYP isozymes that are involved in the formation of two active sibutramine metabolites (M1 and M2) in humans using a combination chemical inhibition, correlation analyses in human liver microsomes (HLMs), and activity assays using recombinant CYPs. Mechanism-based CYP2B6 inhibitors (i.e., clopidogrel, ticlopidine, and thio-TEPA) significantly inhibited the formation of M1 from sibutramine and M2 from M1, respectively; in contrast, no effect was observed when using potent inhibitors of 8 CYP isozymes (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A). In addition, the formations of M1 and M2 from sibutramine ($r = 727$, $p = 0.0029$) and M2 from M1 ($r = 0.834$, $p < 0.0001$) were strongly correlated with CYP2B6-catalyzed bupropion hydroxylation in 16 different HLM panels. Furthermore, recombinant CYP2B6 catalyzed M1 and/or M2 formation at the highest rate among 10 CYPs. Although recombinant CYP2C19, 3A4, and 3A5 also catalyzed, to a less extent, M1 formation at high substrate concentrations (> 5 μM), those contribution might be minor considering usual concentrations of sibutramine and M1 in the clinical setting. The kinetics of M1 and/or M2 formation from sibutramine in HLMs were fitted by a two-enzyme model, and the mean apparent Km values (4.79 μM) for high-affinity component was similar to that observed in recombinant CYP2B6 (8.02 μM). In conclusion, CYP2B6 is the primary catalyst for the formation of sibutramine two active metabolites, which may suggest that pharmacogenetics and drug interactions of sibutramine in relation to CYP2B6 activity should be considered in the pharmacotherapy of sibutramine.

645

Hemodynamic Effects of Diltiazem, Losartan and Amlodipine in a Rat Model Following Multiple Doses *in vivo*

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Introduction: The hemodynamic effects of diltiazem, losartan and amlodipine are compared in a rat model following repeated subcutaneous injections. Methods: Male SD rats (n = 6 – 14 per group) weighing between 300 – 450 g were used. Each rat received either saline (control) or 5 mg/kg of one of these anti-ischemic agents s.c. bid for 5 dose. Hemodynamic measurements were recorded continuously for each animal before and following treatment for up to 6 h. Data were analysed by ANOVA following by multiple comparison using the Dunnett's or the Turkey's test and differences considered significance when $p < 0.05$. Results and Discussion: The basal SBP, DBP and HR in the control SD rats measured over 6 hours were 131 +/- 14 mmHg, 102 +/- 17 mmHg, and 460 +/- 30 bpm, respectively. Diltiazem decreased the SBP to 128 +/- 22 ($p > 0.05$), DBP, 99 +/- 21 mmHg ($p < 0.05$), and HR to 433 +/- 73 bpm ($p < 0.05$). The blood pressure lowering effects measured over the 6 hours were greatest for amlodipine (-27%), followed by losartan (-17%) and then diltiazem (-4%) ($p < 0.05$). The effects on heart rate were similar between amlodipine (-5%) and diltiazem (-6%) ($p > 0.05$). Losartan increased heart rate to 488 +/- 25 bpm (+6%) ($p< 0.05$). Conclusion: Amlodipine has the greatest and most sustained blood pressure lowering effect. The effects on reducing heart rate were similar between amlodipine and diltiazem. Losartan increased heart rate after the same dosage

646

Negative Feedback Regulation of NO Production in LPS-Stimulated Microglia

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The overproduction of nitric oxide (NO) by activated microglia has been proposed to play a pathogenetic role in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. In this study, we investigated the effect of NO preconditioning on lipopolysaccharide (LPS)-induced NO production in microglial cell line BV-2. The production of NO was assessed as the accumulation of nitrite in the culture supernatants, using a colorimetric reaction with Griess reagent. The level of inducible NO synthase (iNOS) protein was estimated by Western blot analysis. In BV-2 cells, LPS increased the levels of NO and iNOS protein in a time- and concentration-dependent manner. Pretreatment with NOC18 or sodium nitroprusside (SNP), NO donors, for 24 h concentration dependently attenuated the LPS-induced increase in the levels of NO and iNOS protein. LY83583, a soluble guanylate cyclase inhibitor, inhibited the attenuation of NO production by NOC18 and SNP. Pretreatment with dibutyryl-cGMP, a cell-permeable cGMP analogue, for 24 h attenuated the LPS-induced increase in the levels of NO and iNOS protein. These results suggest that the production of NO by activated microglia is regulated by negative feedback mechanisms via cGMP signaling pathway.

647

Single Dose Apixaban Pharmacokinetics and Pharmacodynamics in Healthy Male Japanese and Caucasian Subjects

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Apixaban is an oral, selective, direct, reversible inhibitor of coagulation factor Xa in clinical development for prevention and treatment of thromboembolic diseases. Intrinsic and extrinsic differences across races may influence pharmacokinetics (PK) and pharmacodynamics (PD) of drugs. This 4-treatment, 4-period, randomized, double-blind, placebo-controlled, sequential intrasubject dose escalation study evaluated apixaban PK and PD in young healthy male Japanese and Caucasian subjects. Sixteen Japanese subjects were randomized 3:1 to single dose apixaban (2.5, 10, 25 and 50 mg) or placebo. Caucasian subjects were matched 1:1 to Japanese based on weight, age and smoking status, and assigned to the same treatment (apixaban or placebo). Serial blood samples were collected for apixaban concentration and clotting time (INR, aPTT, and modified PT) determination. Safety assessments were performed regularly. Apixaban was safe and well tolerated in both groups. At the 2.5 mg dose, exposure was comparable between Japanese and Caucasian subjects. Slightly lower exposure was observed in Japanese subjects at doses greater than or equal to 10 mg (10-20% lower based on AUC); these differences are not considered to be clinical relevant. Ratios of Cmax and AUC (INF) for the 2.5 and 10 mg doses were approximately 4, indicating dose-proportionality, beyond which increases were less than proportional. Intra-subject variability in Cmax and AUC was low (23 and 16%, respectively). Apixaban caused comparable dose-dependent prolongation of clotting time in both groups. In conclusion, ethnicity does not have a clinically relevant effect on single-dose apixaban pharmacokinetics or pharmacodynamics. Apixaban exposure increases proportionally across the anticipated therapeutic dose range.

648

No Drug-drug Interaction between Ketorolac and Ofloxacin Following Ocular Dosing of a Ketorolac/Ofloxacin Combination Solution to Healthy Subjects

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Study Objectives: To compare the pharmacokinetics (PK) of 1) ketorolac after ocular dosing of the ketorolac tromethamine 0.5% (Keto)/ofloxacin 0.3% (Oflox) combo with Keto alone; 2) ofloxacin after ocular dosing of Keto/Oflox combo with Oflox alone. Methods**:** 36 subjects (12 in each group) received either the Keto/Oflox combo, ACULAR, or OCUFLOX eye drops. Eye drops were applied to the right eye only every 30 minutes for 12 hours a day on the

first two days and hourly for 12 hours a day on the next three days. Serial blood samples were collected on day 0 and day 4 after the last daily instillation. Serial tear samples were collected throughout the study period to evaluate the kinetic profiles of drugs in the tears. Plasma drug PK parameters included Cmax and AUC (0-4) on days 0 and 4, and Thalf on day 4. A population PK approach was used to model tears ketorolac and ofloxacin concentration data using NONMEM. Results: Plasma and tear ketorolac PK profiles were similar between the Keto Alone and the Keto/Oflox Combo dosing groups. Plasma and tear ofloxacin PK profiles were also similar between the Oflox Alone and the Keto/Oflox Combo dosing groups. Conclusions: There is no drug-drug interaction between ketorolac and ofloxacin in the eye and in the systemic circulation after ocular dosing.

649

Gradually Releasing and Liver Targeting Methotrexate Erythrocytes Carriers by a Hypertonic Method

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Aim**:** To distinguish the gradually releasing and liver targeting characteristics of methotrexate-loaded red blood cells (MTX-RBCs), pharmacokinetics and tissue distributions of intravenous injected MTX-RBCs and free-MTX were compared.Methods**:** MTX-RBCs were made of rats' erythrocytes followed a hypertonic method firstly developed by our group. After intravenous injection of MTX-RBCs or free-MTX to SD rats, both plasma and tissue homogenate samples at each time points were collected and analysis was performed on RP-HPLC, then pharmacokinetics and tissue distributions of MTX-RBCs or free-MTX after venous administration could be demonstrated. Results: MTX-RBCs were successfully produced by the hypertonic method with an envelopment efficiency reached to 60%, few obvious morphological changes were observed. These MTX-RBCs got more than 3 times long terminal half-life and MRT against free-MTX and made the velocity of MTX cleared from plasma much more slowly after intravenous injection. The ratio of AUC_{tissue} to AUC_{plasma} of liver was obviously higher than that of other organs after MTX-RBCs administration, which also leaded a longer MRT in liver. Conclusions: The present study demonstrated that the hypertonic method making MTX-RBCs showed gradually releasing and liver targeting characteristics in rats, which offered a great potential for the treatment of tumors in liver and deserved much more investigation.

650

Cytochrome P450 2D6 and 2C19 Genotype Distributions in Forensic Cases

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In Sweden about 500 individuals die every year due to intoxications with drugs. Many of these drugs are metabolized by CYP-enzymes such as CYP2D6 and CYP2C19. Lack of these enzymes, resulting in a poor metabolism, can lead to adverse reactions even with fatal outcome. On the other hand, so-called ultra-rapid metabolism can lead to insufficient plasma concentration and with that failed treatment. The aim of this work was to study different CYP2D6 and CYP2C19 alleles associated with poor metabolism, and to identify CYP2D6 gene multiplications in fatal intoxication cases (n=242), suicide cases (n=262), natural death cases (n=212) and Swedish blood donors (n=282). We have developed applications based on the Pyrosequencing technology for analysis of CYP2D6 and CYP2C19 single nucleotide polymorphisms (SNPs), for determination of CYP2D6 copy number of variations (CNVs) and for identification of CYP2D6 multiple alleles. We found a statistical significant difference between the four materials when the individuals were grouped according to the different number of active CYP2D6 alleles. That indicates differences in CYP2D6 distribution in the four materials. In the material with suicide cases we found surprisingly many ultra-rapid metabolisers (n=12). On the other hand, in the material with natural death cases we found only one ultra-rapid metaboliser. No difference was found for

CYP2C19 polymorphisms. The reason for why there are differences in the CYP2D6 genotype distribution between the four materials is so far not known and further studies have to reveal this.

651

Steady-state Population Pharmacokinetics of Saquinavir Boosted by Ritonavir in Healthy Subjects and HIV-infected Patients

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Introduction: Saquinavir boosted with ritonavir (SQV/RTV) is a protease inhibitor combination used in the treatment of HIV-1 infection at an approved dosage of 1000/100 mg BID. The present analysis was performed to characterize SQV steady-state population pharmacokinetics (PK) in healthy volunteers (HVs) and in HIV-patients (HIV-pts).Methods: Three studies in HVs (N=32) and 1 study in HIV-pts (N=12) were included. SQV/RTV was dosed up to 14 days in HVs or for at least 2 weeks with 2 NRTIs in HIV-pts under fed conditions. PK samples were collected at steady-state. NONMEM software was used. Covariates tested were age, body weight, height and RTV concentrations. Individual SQV estimates of AUC_{12h} and C_{trough} were calculated from individual PK parameters derived from the final model.Results: A total of 575 (HVs) and 132 (HIV-pts) observations were included. The mean age was 30.3 (HVs) and 41.3 (HIV-pts) years. The majority of participants were male (42/44) and white (31/32 HVs). Boosted SQV PK profiles were described by a 1-compartment model with first order absorption and elimination. Adding covariates to the model did not affect CL/F, V/F or K_a. The population PK parameters (inter CV%) in HVs and HIV-pts were 36.8 (42%) and 81.2 (69%) L/hr for CL/F, 56.9 (22%) and 190 (50%) L for V/F, 0.155 (13%) and 0.225 (31%) hr^{-1} for K_a, respectively. SQV AUC_{12h} in HVs and in HIV-pts were 29.4 ± 11.4 and 15.0 ± 9.0 μg·hr/mL and C_{trough} were 1.16 ± 0.58 and 0.56±0.61μg/mL, respectively. Conclusions: Age, body weight, height, and RTV concentrations did not affect SQV PK parameters. Lower SQV exposures were observed in HIV-pts than in HVs.

652

Population Pharmacokinetic Modeling of Ustekinumab, a Human Anti-IL-12/23p40 Monoclonal Antibody (mAb), in Two Phase 3 Studies in Patients with Moderate to Severe Plaque Psoriasis

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Introduction/Aims: Ustekinumab (CNTO1275), a human IgG1-kappa mAb against IL-12/23p40, demonstrated significant efficacy in treating patients with moderate-to-severe plaque psoriasis in two Phase 3 studies (PHOENIX 1 and PHOENIX 2). This analysis was conducted to assess typical pharmacokinetic (PK) parameters of ustekinumab and associated variability, and to evaluate the influences of intrinsic/extrinsic factors on the pharmacokinetics of ustekinumab in psoriatic population. Methods: Ustekinumab was administered as two 45 or 90 mg SC doses 4 weeks apart followed by 45 or 90 mg every 12 weeks. A total of 9938 serum concentrations from 1937 patients were included in a population PK analysis using non-linear mixed effect modeling (NONMEM) approach. A one-compartment model with first-order absorption and first-order elimination was selected as the structural model. The final model was validated using both bootstrap and visual predictive check approaches.
Results: The apparent clearance (CL/F), apparent volume of distribution (V/F), and absorption rate constant (k_a) estimates were 0.465 L/d, 15.7 L, and 0.354 1/d, respectively. Patient body weight, diabetic comorbidity, and positive immune response to ustekinumab were the only factors that associated with a >20% change in CL/F and/or V/F of ustekinumab. There were no significant changes in CL/F or V/F in patients aged >=65 years; with past use of methotrexate, cyclosporine or therapeutic biologics for psoriasis; or receiving any of the frequently used concomitant medications. Conclusion: A robust population PK model was developed to evaluate the pharmacokinetics of ustekinumab in psoriatic population. The clinical relevance of these covariate findings needs to be evaluated concurrently with efficacy and safety data.

653

Structural Determinants of Inhibitor Interaction with the Human Organic Cation Transporter OCT2

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Organic cation transporters (OCTs) provide an important pathway for the uptake of cationic compounds in the liver and the kidney, which are the essential steps in their elimination from the organism. Although many drugs have been identified which interact with human OCT2, which is predominatly expressed in the kidney, structural elements required for an interaction with OCT2 are not well defined. To address this issue, HEK293 cells stably expressing hOCT2 were generated. Uptake of the prototypical OCT2 substrate [^{3}H]MPP$^+$ was inhibited to varying extents by 48 structurally unrelated drugs. A subset of 25 of these molecules was used to determine IC_{50} values for inhibition of [^{3}H]MPP$^+$ uptake and to correlate these inhibition data with physicochemical descriptors such as molecular weight, logP, pKa, volume, solvent accessible surface area (SASA), and topological polar surface area (TPSA). The most potent inhibitors were imipramine, fenfluramine, doxepine, amitryptiline, chlorpromazine, ipratropium bromide, clonidine, diphenhydramine, propafenone, and sibutramine with IC_{50} values for the inhibition of OCT2-mediated [^{3}H]MPP$^+$ (10 µM) uptake in the range 6.0 to 29.1 µM. Interestingly, we found a significant correlation between IC_{50} and TPSA values (r=0.66, p=0.0004). Structural alignment of these compounds was used to construct a two point pharmacophore which consists of an ion pair interaction feature and a hydrophobic aromatic feature at a distance of 4.95 Å. Moreover, imipramine, clonidine, verapamil and quinidine potently inhibited uptake of the antidiabetic drug metformin (IC_{50} 0.4, 1.5, 4.8, 7.6 µM, repectively). Taken together, our data identify structural determinants for inhibitor interactions with human OCT2.

THURSDAY, JULY 31, 2008
STREAM 3:
MEDICINES AND SOCIETY

654

Outpatient Utilization of Cardiovascular Drugs in Croatia, 2001- 2005

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Objective: The purpose was to investigate the outpatient utilisation of cardiovascular drugs in Croatia, during the 2001-2005 period using the ATC/DDDs methodology and to investigate the relationship between the utilisation of particular drug groups and the number of hospital admissions. Method: Data on outpatient drug utilization were obtained from Zagreb Municipal Pharmacy to calculate the number of DDD, and DDD per 1000 inhabitants per day (DDD/TID). Drug Utilization 90%(DU90%) method was used on the drug prescribing quality assessment. Data on hospital admissions were collected from the inpatient base kept at Zagreb Institute of Public Health. Results: Total utilisation of cardiovascular drugs (group C), was between 402.9 DDDs/TID and 362.9 DDDs/TID) in Croatia during the 2001-2005 period. Agents acting on the renin-angiotensin system (C09) (104.2 DDDs/TID) and calcium channel blockers (C08) (80.5 DDDs/TID) accounted for more than 50% of drugs used for the treatment of hypertension in 2005. The great increase in the utilization was observed for statins (78.3%). A markedly increasing utilization was recorded for ACE inhibitors in combination with hydrochlorothiazide (HCTZ) (40.5%) and angiotensin II antagonists (278%). Comparison of DU90% segment between 2001 and 2005 revealed pentoxifylline and amiodarone to be absent, whereas cilazapril and ramipril in combination with HCTZ, bisoprolol, valsartan and losartan alone or in combination with HCTZ were added in 2004 and 2005. DU90% segment still contained doxazosin and propafenone, which had no grounds in therapeutic guidelines. Total rate of hospital admissions for major cardiovascular events were decreased by 17.2%. Conclusion: The utilization pattern was improved in 2005, showing

a decrease in the number of hospital admissions for major cardiovascular events.

655

Home Medicines Review – Best Practice in a Pharmacy Setting

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The Home Medicines Review (HMR) was introduced into the Australian Medical Benefits Scheme in October 2001, to increase the awareness and appropriate use of medication and reduce the incidence of medication misuse, leading to better patient medication management [(1).] HMR is a patient-focused, structured and collaborative health care service provided in the community setting to optimize patient understanding and quality use of medicines [(1)]. A HMR, ideally conducted by an Accredited Pharmacist in a patient's home, provides an overall picture of the patient's medication use, both over the counter and prescribed. This process allows the General Practitioner (GP) to make informed decisions concerning the patient's medication. Since the introduction of HMR, the uptake has been steady but slow. Common barriers for GPs such as lack of time to conduct a HMR, lack of information, timeliness of report, and the turnaround time have been hindering the process within our Division. Lack of communication between the GPs and pharmacists is also a barrier in completing the HMR process.
In order to reduce delay, increase the timeliness of the report and to increase the communication between the Clinics and the Pharmacies, the Knox Division is introducing the HMR Pharmacy Helper concept into our catchment. The purpose of a HMR Pharmacy helper is to facilitate the HMR process in the pharmacy and to coordinate with a similar helper role in GP clinics. The expected outcome of this is a quality systematic approach to track HMR requests, reduce the turn-around time and better medication management for patients.A pilot study will be conducted in the next 6 to 8 months whereby three to five pharmacies will be selected to participate. Quantitative and qualitative data will be collated after the study period and all data will be presented at future conferences. Reference: 1. The Pharmacy Guild of Australia website. http://www.guild.org.au/mmr/content.asp?id=53

656

Use of Inhaled Corticosteroids in Children with Symptomatic Persistent Asthma

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Recent Canadian surveys report that many asthmatic children continue to experience asthma attacks. In our study, we examined whether compliance with the Canadian Asthma Consensus guidelines recommended inhaled corticosteroid (ICS) therapy was one of the obstacles faced by children living with this disease. A cohort of 2 355 children aged 5 to 15 years with symptomatic persistent asthma was reconstructed by linking two administrative databases of Québec (Canada), between 1997 and 2005. Children needed to be ICS therapy naïve and having had received more than 3 doses of short-acting beta$_2$-agonists per week during a 12-month period prior to cohort entry. Patients with adjunctive controller therapies were excluded. Both the prescribing pattern and patient adherence were estimated. Twenty percent of the children received only one prescription of ICS during the 12-month follow-up period. The mean number of supplies prescribed (new prescriptions and prescribed refills) from all physicians a patient could have consulted during the 12-month follow-up period was 5.0 (95% CI: 4.9-5.2) corresponding to 152.0 days' of supply prescribed (95% CI: 146.5-157.4). The mean percent patient adherence defined as the ratio of the total days' supply dispensed to the total days' supply prescribed was 62.4% (95% CI:61.1-63.7) and dropped to 36.8% (95%CI: 35.1-38.6) among children with 7 or more supplies prescribed. A large percentage of children with persistent symptomatic asthma were not prescribed ICS for prophylactic use and patient adherence was suboptimal. Under utilization of ICS might suggest that physicians, parents and patients fail to treat asthma as a chronic disease.

657

The Impact of Biologic Drugs on the Psoriasis Market: Patients Retention

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Introduction: Psoriasis is estimated to affect more than 600,000 Canadians. Introduction of biologic products indicated for Psoriasis have changed significantly the trends in this market. Since then, the average total cost of Psoriasis treatment has increased considerably, provoking additional studies on the cost-efficiency, efficacy and safety as well as patient behavior treated with these new products. Objective: To measure the retention of patients on biologic products recently indicated for Psoriasis as a proxy for the success of therapy. Methodology: Patients receiving treatment for psoriasis were identified using claims level data extracted from the Brogan Inc. Public and Private Drug Plan Databases. Aggregate data from this cohort was subsequently employed to determine retention rates. Biologic drugs considered for this study included Amevive, Raptiva, Enbrel, Humira and Remicade. Results: Initial findings show that retention rates differ significantly between the target biologic products. Discussion and Conclusions: Analysts quantifying retention rates of patients using biologics in the treatment of Psoriasis must adjust their measures to account for the use of atypical gaps in treatment. An accurate estimation of retention rates will provide certain insight about the efficacy of biologics for policy-makers. Further investigation is required to determine why poor retention rates are observed for some of these products.

658

Interactions between Herbal Remedies and Medicinal Drugs. Considerations about Cuba

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Introduction: The use of herbal products to treat a wide range of conditions is rising rapidly, leading to increased intake of phytochemicals. This is one of the main reasons for reinforcing the surveillance of the safety, efficacy and quality control of traditional and complementary medicine. Recent studies have revealed some interactions between herbal remedies and traditional drugs. Herbal preparations can interact with a drug at pharmacokinetic and pharmacodinamic level. Material and Methods: Cuba has a Center in charge of Pharmacovigilance of Drugs. This Center offers the data about the adverse effects of conventional and traditional medicine. Results: In this work we show the theoretical and real interaction between herbal products and conventional medicine and we present the reports about side effects of traditional medicine and main interactions between herbal medicines and conventional drugs in Cuba. Conclusions: Herbal products are currently not subject to the rigorous testing indispensable for conventional drugs. However, if potential drug interactions are to be predicted, it is essential that the ability of herbal products to interfere with drug-metabolizing enzyme systems is fully established.

659

Implementation of the Clinical Pharmacy at the Franco Vietnamese Hospital (FVH) in Ho Chi Minh City, Vietnam

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Introduction: In Vietnam, Clinical Pharmacy is a recently taught discipline. Upon government decision, hospitals must implement this activity in a context of poor communication between physicians and pharmacists. Actually, pharmacist social mission was only limited to drug dispensing so far. The Franco-Vietnamese Hospital (FVH) located in Ho Chi Minh City, Vietnam is a 220-bed private hospital opened in March 2003 offering a comprehensive range of medical, surgical and obstetric activities that has been accredited by French "The Haute Autorite de Sante" in July 2007. Methods: In order to enhance the safety of drug use and promote pharmaceutical practice, the Clinical Pharmacy activity was set up in February 2007 with approval of the FVH's Committee on Drugs and medical devices (COMEDIMS). The objective was to assess the impact of this activity on drug prescription 8 months after its implementation in 3 In-patient-departments (medicine, surgery and obstetrics-

gynecology). A "pharmaceutical advice" form was drawn up and approved by the COMEDIMS before being presented to doctors. Results: Between February and September 2007, 7.945 drug prescriptions were analyzed of which 3% had been issued a pharmaceutical advice. The main problems identified were related to warning and precautions (33%), inappropriate administration route (19%), drug interaction (18%), associations discouraged (10%) or contraindications (4%). Conclusion: Despite a sense of intrusion expressed by some doctors the activity of clinical pharmacy has become a fully-fledged mission of Pharmacists at FVHOSPITAL. The accepted pharmaceutical advice rate was evaluated twice in May and September 2007 and rose from 18 to 28.5%.

660

Abstract of the Lipid Management of OAG Patients

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Introduction: Diabetes patients have been shown to exhibit an increased risk of high cholesterol. As a result, the Canadian Diabetes Association recommends aggressive lipid management be pursued. The objective of this study was to determine the extent to which diabetes patients in Canada are concurrently receiving treatment with lipid lowering products. Methods: The analysis focuses exclusively on private sector, non-senior patients who were new to therapy and were 100% persistent with their OAG treatment. Patients were tracked for one year beginning with their first claim in 2005 for a lipid lowering product. Results: Fewer than half the patients starting OAG therapy continued treatment for one year. Of these, less than 20% used a lipid lowering product. The combined results of this preliminary analysis show that 9% of patients claimed medication in accordance with the Canadian Diabetes Association Guidelines. Discussion and Conclusions: Much is made of the over prescribing of medications. This is a case where there is severe underutilization both of OAG products and lipid lowering medication in Diabetes patients. The consequence of this result could have implications for the long-term health of these patients, possibly leading to unnecessarily high prescription drug costs and the overall deterioration of health.

661

Trends of Psychotropic Consumption in Chile

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Due to an increasing consumption of psychotropics, in 1995 Chilean Ministry of Health reinforced the control of its dispensing in community pharmacy. However, changes produced by this action on consumption of psychotropics with the exception of that of antidepressants, has not been studied yet. The purpose of this study was to determine mean level of psychotropic consumption in Chile between 2000 and 2006 and to compare it with data coming from the period 1990-1994. A retrospective observational study was performed using data of psychotropics (anxiolytics/hypnotics/neuroleptics/antipsychotics) sold in community pharmacies. After data were converted to the WHO recommended units, i.e. Defined Daily Doses (DDDs)/1,000 inhabitants/day. Across the period 2000-2006 the mean total consumption of psychotropics was of 14.19DDDs/1,000 inhabitants/day. Consumption of anxyolitics represented the 69.3% of the mean total psychotropics consumption, followed by that of hypnotics (29.32%), neuroleptics (3.07%), and antipsychotics (2.02%). Anxiolytics consumption remained stable, with an annual mean value of 9.48±0.4DDDs/1.000 inhabitants/days. When mean consumption of psychotropics during the period 2000-2006 was compared to that in 1990-1994 it was found a decrease of 55.6%. Between 1990 and 1994 the most consumed anxiolytic agent was diazepam(10.66DDDs/1,000 inhabitants/day), while in the period 2000-2006 was alprazolam(6.11DDDs/1,000 inhabitants/day). Found data show that action taken by the Chilean Ministry of Health in 1995 was effective as it made decrease the consumption of psychotropics, which remained almost without changes through the period 2000-2006. It would be interesting to explore if consumption of OTC drugs with CNS actions has been increasing during the study period.

662

Pitavastatin Improves Glucose Intolerance and Lipid Deposition of Aorta in Metabolic Syndrome Model Rats

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Background: Hypertension, hyperlipidemia and hyperglycemia are frequent causes of cardiovascular events. As sucrose (Suc) loading on our metabolic syndrome model rat, spontaneously hypertensive hyperlipidemic rats (SHHR) which is produce by ourselves, induce hyperglycemia, we used this rat for the studies on these risk factors. (Purpose) We intended to clarify the effect of HMG-CoA reductase inhibitor on such complicated situation as metabolic syndrome. Method: We studied the effect of pitavastatin (Pit) on the glucose intolerance and lipid deposition in aorta of SHHR rats and SD rats treated by 15% Suc and high fat diet (HFD). Suc, HFD and Pit (0.3 mg/kg/day, p.o.) were administered for 2 months on male SHHR and SD rats, then Intraperitoneal glucose tolerance test (IPGTT) was performed under the fast condition. Other related parameters were measured by blood test as well. Results: Plasma glucose and cholesterol of Suc+HFD-treated SHHR was significantly increased than those of the control (Cont) SHHR. Pit significantly decreased the plasma glucose response to IPGTT. Plasma total cholesterol and visceral fatty tissue weight of Suc+HFD-treated SHHR were significantly increased than that of Cont SHHR. Pit significantly suppressed these increases of plasma cholesterol and visceral fat gain of SHHR, but not of SD. Further, Pit improved the increased lipid deposition in aorta of Suc+HFD treated SHHR. Conclusion: These results may have suggested that Pit improved glucose intolerance and lipid deposition in aorta through the improvement of dyslipidemia of metabolic syndrome.

663

Revolving Medicines Fund System (Rmfs) in Primary Health Care: Risk or Benefit?

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Background Revolving Medicine Fund System (RMFS) is a system that a sum money is used to purchase an initial stock of essential and commonly used medicines to be sold, ideally at a price sufficient to replace the stock of medicines and ensure a continous supply. The objective of these systems is to maximized the access to essential medicines. Some districts in Indonesia had difficulty to guarantee the availability of medicine, health equipment and other medicinal stuff. This study was determined the result of a pilot of RMFS in Primary Health Care of Balikpapan City, East Kalimantan. Material and Method: quasi experimental pre and post design before and after RMFS implementation in Primary Health Care – Balikpapan City, East Kalimantan. Result: Availability of medicines improved: Number of medicine items: before 113 after 253; out of stock after RMFS is no more observed. Rational Use of Medicine indicators decreased : percentages of medicine listed in generic name before 99%, after 60%; percent of essensal medicines before 84%, after 49%; generic prescription was before 99%, after 84% and prescription of essensial medicine before 94%, after 75%. Antibiotic prescription before 32% after 50%. Item of medicines in prescription before 2.80 after 3.46. Cost of medicine and revenue increased. Prescription prices before 3463.00 IDR after 8169.00 IDR; Income (General District Hospital) before 4.5 billion IDR after 7.0 billion IDR. Conclusion: RMFS improved the medicine availability in Primary Health Care and District General Hospital and increase the income health institution, but decreasing the Rational Use of Medicine.

664

Cardiovascular Diseases (CVD) Risk Factors Control Among Patients in a Canadian Multidisciplinary Predialysis Clinic

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Introduction: Cardiovascular diseases (CVD) risk factors are common among chronic kidney disease (CKD) patients and contribute to progression towards end stage kidney disease. Treatment guidelines recommend maintaining systolic/diastolic blood pressure (BP) below 130/80 mmHg, glycemia below 7.0 mmol/L, total cholesterol (TC)/HDL-C below 4.0 and LDL-C below 2.5 mmol/L. Methods: In a 6-month cluster randomised controlled trial, CVD risk factors were described at baseline in patients with moderate (glomerular filtration rate (GFR): 30-59 ml/min/1.73m^2) and severe (GFR<30 ml/min/1.73m^2) renal insufficiency followed in a multidisciplinary team. Results: A total of 32 moderate and 57 severe CKD patients were recruited. The mean (SD) age was 72.5 years and 57% were diabetic. A majority of patients were below the therapeutic target for glycemia (moderate: 72%, severe: 63%; p=0.42), TC/HDL-C (moderate: 68%, severe: 63%; p=0.69), and LDL-C (moderate: 73%, severe: 57%, p=0.15). Lower proportions of patients had BP below the target (moderate: 25%, severe: 32%; p=0.52). Mean BP was 141(22)/68(12) mmHg. Antihypertensive treatment was prescribed to all patients except one. Severe CKD patients were taking a mean of 3.7 antihypertensive drugs compared to 3.1 in moderate CKD patients (p=0.04). Discussion: Based on the guidelines recommendations, a substantial proportion of patients followed-up in a predialysis clinic by a multidisciplinary team of health professionals do not reach the recommended targets for CVD risk factors. In particular, BP is poorly controlled despite aggressive treatment. Conclusion: Optimizing CVD risk factors control in CKD patients remains a priority. Better understanding of factors influencing the effectiveness of treatment is warranted.

665

Effect on Knowledge of Pharmacology in Hybrid Curiculum at Phramongkutklao College of Medicine

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Phramongkutklao College of Medicine has changed the curriculum from discipline oriented and lecture-based (conventional) to integration and problem-based (hybrid) courses since 2004. It was not known whether this change would affect the learning ability in pharmacology topics or not. The essential knowledge in pharmacology between student in conventional class and hybrid class during the year 2004-2005 were retrospectively compared in one hundred multiple choice examination questions. The questions were selected to cover all the topics in pharmacology teaching. Each of these questions; which was used to evaluate the knowledge in both classes, was either the same or paralleled question. All (100) pairs of questions were classified into 3 sets. They are recall (51), interpretation (25) and application (24). The index of difficulties (p) of each pair of questions was analyzed. It was found that the average p for either each set or all sets of the questions was not significantly different between the two student groups (p for all sets of the questions was 0.67± 0.21 and 0.72 ± 0.20, respectively. P- value = 0.059, independent sample T test), indicating non-difference in pharmacology knowledge The students attending hybrid course had better performance in some topics: i.e., antimicrobial agents (p was 0.55 ± 0.18 for conventional class and 0.68 ± 0.18 for hybrid class. P- value = 0.018 or < 0.05). This may be due to better understanding in the hybrid course which emphasized on more clinical applications. In this study, hybrid curriculum tends to have a positive effect on students' learning abilities in pharmacology as does problem-based learning (PBL). Thus hybrid curriculum may be used instead of PBL in some circumstances. As not all faculty at medical schools are in a position to adopt PBL on a larger scale. However, several factors should be concerned, as well.

666

Near-peer Teaching for Undergraduates by Junior Doctors: A Popular Modern Adjunct to Core Teaching, but does it Improve Safe Prescribing?

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Introduction: Peer-assisted learning has advantages for students and tutors. We have set up and delivered a novel "near-peer" tutoring scheme run by newly-qualified foundation doctors for final-year medical students. Methods: In 2007, tutorials covering clinical examination and prescribing were provided throughout south-east Scotland. More than half of the sessions involved practical prescribing. In 2008, the scheme was further developed to focus on safe prescribing. We recently ran a "Training the Trainers" symposium to prepare new tutors, and conducted a trial to assess the impact of tutorials on prescribing skills. Twenty volunteers were randomized to receive a 30-minute Foundation doctor-led tutorial on prescribing or no teaching. All students completed mock prescribing exercises using a different clinical scenario. Two blinded assessors scored each chart using pre-agreed criteria incorporating clinical knowledge base and generic prescribing technique; the number of dosing errors was also recorded. Results: In 2007, 271 students attended 73 tutorials; in an anonymised questionnaire, 99% of students expressed interest in attending more sessions. In the assessment study, the overall score was not significantly different between groups (13.9 vs. 12.15, $p = 0.242$). However, the tutorial group made significantly fewer dosing errors (mean 9 vs. 22, $p = 0.049$). Discussion & Conclusion: We have shown "near-peer" tutoring in prescribing is a popular adjunct to core teaching. Furthermore, the overall scores were comparable between groups, suggesting a similar knowledge base. Our tutorials aim to improve generic prescribing skills; the reduced error rate in the tutorial group may be consistent with this being achieved.

667

Prescription Writing Skill and Prescribing Errors: The Gap between Medical School and Residency Program

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Background: A nationwide survey in Bahrain revealed that in primary care a considerable proportion of prescriptions had errors. We reported that drug-related prescribing errors were common among pre-clerkship phase medical students following a problem-based learning (PBL) curriculum. Improving prescribing skills as a core competency is an important strategy to minimize errors in both medical school training and during the residency programs. Objective: To evaluate prescription writing skill of PBL medical graduates in family practice residency program. Subjects and Methods: Prescriptions issued by residents were prospectively collected over a period of one month. Prescribing errors were classified as errors of omission (major and minor), commission (incorrect information), and integration (drug-interactions). Results: In 1088 prescriptions by 12 fourth-year residents the mean number of drugs per prescription was 2.13 ± 1.1, and a third had polypharmacy ($\geq$ 3 drugs/prescription). Of 2312 drugs prescribed, 1742 (75.4%) had errors: 68.5% with errors of omission, 26% with errors of commission, and 5.4 with errors of integration. A propensity for *prn* prescribing, preference for brands rather than generics, and non-standard abbreviations and dosage units was observed with medications prescribed for acute / chronic conditions either by systemic or topical routes. Discussion and Conclusion: Prescribing skill of residents is suboptimal suggesting an attrition of prescription writing skill acquired in medical school during the intervening period. Structured training in prescribing skill and rational pharmacotherapy needs to be emphasized during the residency program to minimize errors and to improve the quality of primary care.

668

Meta-analysis: Effects of Probiotic Supplementation on Serum Lipid Levels in Humans

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Introduction: The results of recent experimental and clinical studies suggest that probiotic supplementation has beneficial effects on serum lipid profiles. We conducted a meta-analysis of intervention studies to evaluate the effects of probiotics on serum lipid levels. Methods: Eligible reports were obtained by searches of the electronic database, Medline, Cochrane Central Register of Controlled Trials and Igaku Chuo Zasshi. We included all randomized clinical trials comparing probiotic supplementation with placebo or no treatment (control). Statistical analysis was performed with Review Manager 5. Subanalysis/sensitivity analysis was also performed. Results: Eight randomized clinical trials were eligible for inclusion in the meta-analysis. No participant had received any cholesterol-lowering agents. Probiotic interventions produced changes in total cholesterol (TC) (mean difference –0.09 mmol/L, 95%CI: –0.15 to –0.03) and LDL-cholesterol (mean difference –0.16 mmol/L, 95%CI: –0.23 to –0.08). HDL-cholesterol and triglycerides did not differ significantly between probiotic groups and control groups. In subanalysis, long-term (>4-week) probiotic intervention was more effective in decreasing TC and LDL-cholesterol than short-term (≤4-week) intervention. Discussion and Conclusion: This meta-analysis shows that supplementation with probiotics could be effective in decreasing TC and LDL-cholesterol levels. Therefore, probiotic supplementation may lead to reductions in risk factors for cardiovascular disease and could be useful in the primary prevention of hyperlipidemia.

669

Canadian Survey to Assess Regional Differences in the Diagnosis and Management of Movement Disorders Responsive to Botulinum Toxin Type-A (BoNTA)

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Objective: To assess diagnostic and treatment pathways of movement disorders responsive to BoNTA by geographic region and to review their relative frequency. Methods: Patients with BoNTA responsive movement disorders completed a 19-question survey developed by the Canadian Movement Disorder Survey Group in 13 Canadian centres. The survey included demographics, time to diagnosis, number of physicians seen and wait times. Results: This interim analysis includes 698 patients. 72% were female. The average age was 57 yrs, 29% being over 65. Average travel was 74 kms one-way, ranging from 44 (Quebec) to 99 kms (Atlantic provinces). Common diagnoses included cervical dystonia (53%), hemifacial spasm (20%), and blepharospasm (10%). The average number of physicians seen prior to diagnosis was 3.2, ranging from 3.0 (Ontario) to 4.0 (Atlantic provinces). Average time from onset of symptoms to diagnosis was 4.7 yrs, ranging from 2.7 (Western provinces) to 6.2 yrs (Quebec). 94% of patients were treated with BoNTA following diagnosis. Average waiting time to treatment was 3.2 months. Common reasons for delay were physician waiting lists (50%) or insurance paper work (20%). Conclusion: Patient population and treatment centers across Canada contribute to the regional differences with time to diagnosis and BoNTA treatment waiting times.

670

Comparisons of Mycophenolate Usage in Northern European and Australian Transplant Populations

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Introduction: Mycophenolate is an immunosuppressant drug used to prevent rejection in renal, cardiac and hepatic transplantation. The aim of this study was to determine Australia's current usage of mycophenolate; to project future usage; and to compare usage with Danish, Finish and Netherlands populations. Methods: Data on mycophenolate usage were obtained from Australian Pharmaceutical Benefits Scheme, Finish and Danish medicines agencies, and the

Netherlands Healthcare insurance board databases. Utilisation was compared 2003-2006 (defined daily dose/1000 population/day). Data on number of transplant recipients each year was sought from national transplant registers, Eurotransplant and Scandiatransplant databases. Results: Utilisation of mycophenolate increased in all countries from 2003-2006.Whilst Australia had the largest increase over the 4-year period, utilisation remained 5-20 times less than the European countries. Transplant rates were similar in all study populations. Discussion: Large differences in the rate of mycophenolate prescribing between Northern Europe and Australia may be due differences in approved indications between countries, differences in prescribing habits (e.g. use of azathioprine) or perhaps because of a more mature market in Europe. Conclusion: If mycophenolate usage in Australia increases to that of the Northern European market its annual cost to the government could increase to AUD 20-80 million per year. This will possibly propel immunosuppressant drugs from their current ranking of 15th highest cost to the Australian government into the top 10, with national financial implications, as mycophenolate is publically ignifican in Australia. Mycophenolate is a high cost drug, increasing worldwide usage will have an impact on international healthcare budgets.

671

Assessment and Quantification of the Benefit Risk Ratio of Rosuvastatin and Simvastatin from a Meta-analysis of Head to Head Randomised Controlled Trials

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Introduction: Statin therapy is effective and is commonly used for the treatment and prevention of cardiovascular disease. Serious side-effects are infrequent, but the wide scale use of statins justifies scrutiny of the relationship between the therapeutic effect and risk of side-effects for competing statin treatments. Meta-analysis of data from head-to-head studies is a robust and statistically powerful method and is utilized here to investigate the benefit and risk of rosuvastatin I, in comparison to simvastatin (S). Methods: A systematic literature review identified 4 comparisons of 1:1 dose ratios (R10mg vs. S10mg etc), 10 comparisons of 1:2 dose ratios (R5mg vs. S10mg etc) and 4 comparisons of 1:4 dose ratios (R5mg vs. S20mg etc). Treatment difference in benefit (% Low Density Lipoprotein-cholesterol [LDL-c] reduction) and risk (odds ratios for myalgia, and withdrawals due to adverse events, difference in means for mean % change in GFR from baseline), were estimated by meta-analysis (random effects) and presented in benefit risk planes. Results: Analysis of 8 studies (~9,000 pts) demonstrated rosuvastatin to be significantly more efficacious than simvastatin, for LDL-c reduction, at 1:1, 1:2 and 1:4 dosage ratios. There were no significant differences between rosuvastatin and simvastatin, at any dose ratio, for i) withdrawals due to adverse events, ii) myalgia, iii) mean % change in GFR. There were significant improvements in GFR with both statins. Conclusion: At 1:1, 1:2 and 1:4 dose ratios, significant additional reductions in LDL-c are obtained by rosuvastatin at a comparable risk of the adverse events investigated.

672

Resistance to the Treatment of Hepatitis B Virus with Lamivudine and Adefovir

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Introduction: Lamivudina and Adefovir are used as first-line treatment of chronic hepatitis B (CHB) hower, both may develop long-term resistance. We analyse the appearance of resistance to the same. Method: Observational retrospective study of patients treated with Lamivudine or Adefovir from January 2002 to December 2007. Developing of viral resistance was evaluated by the increase of viral DNA values and/or elevation in ALT. Risk factors analysed were: age, high HBV-DNA level pre-treatment and after 24 weeks, high ALT levels, treatment length. Results: Twenty patients were included with a median age of 52.5 (18-76) years; seventeen had compensated liver disease and three cirrhosis. Lamivudine: seventeen patients had

VHB-DNA and ALT (mean 194±117 U/L) levels high at baseline. After 6 months the viral charge was undetectable and ALT were normalized. Median length of treatment was 20 (9-48) months. Treatment was discontinued in 3 patients due to resistance, two of them had cirrhosis. Adefovir: nine patients, median length treatment 15 (6-36) months, seven previously treated with Lamivudine three of them because of resintance. Only one patient (cirrhotic and resistant to Lamivudine) developed resistance to Adefovir after 41 months of treatment. Discussion: 20% develop resistance to Lamivudine after one year and 70% after 4 years and to Adefovir 29% after 5 years. In our study the higher percentage (18%) appears between 2nd and 3th years to Lamivudine and only one case was resistant to Adefovir 11%. Conclusion: Antiviral resistance percentage is low. It would be interesting to perform a resistance test before withdrawal of drug to improve its effectiveness.

673

Assessing Utilities at the Canadian Level: Which Instrument to Favor in the Field of Oncology?

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Introduction: Cost-utility analysis uses the QALY to estimate the effect of a treatment on both the quality and length of life. Several indirect instruments exist to assess people's preferences for different health states; however for a same situation these instruments often provide different utilities. Objective: To determine the optimal instrument for assessing utilities in oncology clinical trials. Methods: A systematic literature review was conducted to determine which indirect instruments have been used in oncology. Medline, Embase and PsycInfo were searched to find original studies carried out from 1997 through 2007 using the following key words: EQ-5D, HUI3, AqoL, breast neoplasm, lung neoplasm and skin neoplasm. Results and Discussion: Based on criteria such as time to complete, ease of administration, cost and conceptual framework, out of seven indirect instruments, only the EQ-5D, HUI3 and AqoL were retained. The systematic literature search identified nine studies using these instruments; seven with the EQ-5D, one with the HUI3 and one with the AqoL. A comparison of the results obtained from the EQ-5D studies demonstrates its capacity to detect change over time and between different treatments. These results also support previous findings suggesting a ceiling effect. Unfortunately, no comparison could be made for the HUI3 and the AqoL due to the scarcity of eligible studies. Conclusion: Based on the criteria selected here, the EQ-5D presented the best profile and the literature review confirms that it is the most studied in oncology. A head-to-head comparison of the EQ-5D, HUI3 and AqoL should be considered to determine the most optimal instrument.

674

Three Year Pharmacovigilance Experiences from a Regional Pharmacovigilance Center in Nepal

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The present study analyzed the pattern of ADRs reported to the regional pharmacovigilance center, Western Nepal since its inception (3 years ago). ADRs were collected in the prescribed format from physicians, nurses and pharmacists. Causality (Naranjo scale), severity (Modified Hartwig and Siegel scale) and preventability assessments (Modified Shumock and Thornton scale) were carried out. Cost of drugs used for managing the ADRs was also calculated. Altogether 151 ADRs reports were studied. Mean ± SD age of patients was 34.22 ± 20.54 years. Females had a higher incidence [n= 89 (58.94%)]. More than half [58.94% (n= 89)] of the ADR reports were from Dermatology. Antibiotics accounted for highest number of ADRs [n= 40 (26.49%)]. Maculopapular rash was the most common ADR [13.25% (n= 20)]. Majority of the ADRs [68.21% (n=103)] were 'Probably' related to the suspected drugs and more ADRs [57.62% (n=87)] were of 'Mild (Level 3)' type. We found 9.93% (n=15) of the ADRs were 'definitely preventable'. Corticosteroids were used in 26.77% or (n=102) of the cases in managing the ADRs. The median (interquartile range) of Nepalese

rupees spent by patients on drugs used to manage the ADRs was 188.95 (100.62 – 330.42) (1 US D= 61. 00 Nepalese rupees). Dermatological reactions were the commonest. Antibiotics were responsible for causing the highest number of ADRs. Nearly one tenth of the ADRs were definitely preventable. Although our study had several limitations, it was successful in identifying the pattern of ADRs in Western region of Nepal.

675

Effect of Polyherbal Hair Oil in the Treatment of Dandruff

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Dandruff a major cosmetic problem caused by a lipophilic, dimorphic and yeast-like fungus Pityrosporum ovale. In this present study Polyherbal hair oil (PHO) prepared from six plant extracts (Albizia amara, Achyranthes aspera, Cassia fistula, Cassia auriculata, Datura stramoniuum, and Azadirachta indica) was studied for antidandruff activity using microbiological and clinical tests. There was a clear symptomatic relief from dandruff after 10 days usage of PHO by volunteers. The MIC of P.ovale & C.albicans was found to be 1.0mg/ml and 5.0mg/ml respectively. The zone of inhibition of PHO was observed as 15 mm diameter for P.ovale and 8 mm for C.albicans. Methylene blue reductase test was employed to conform the antidandruff efficacy of the oil. The excellent antidandruff action of “Polyherbal hair oil” might have been due to the synergistic antifungal due to its active ingredients. Therefore, it may be concluded that, “Polyherbal hair oil” is effective and safe in the management of dandruff.

676

Comparison of Non-steroidal Anti-inflammatory Drugs and COX-2 Inhibitors use in Australia and Nova Scotia (Canada)

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Introduction: COX-2 inhibitors prescribing may lead to both safety concerns and budgetary challenges. In Canada and Australia attention has been drawn to the need to improve the safe, appropriate, cost-effective prescribing of drugs. The objective of this study was to compare use of COX-2 inhibitors and non-selective (ns) NSAIDs in Nova Scotia (Canada) and Australia over the period 2001-2006 to identify lessons learned from the two jurisdictions. Methods: Australian data, for concession beneficiaries, for ns-NSAIDs and COX-2 inhibitors were downloaded from the Medicare Australia website. Similar data were obtained for Nova Scotia (for seniors and those receiving community services). Data were collected over the period 2001-2006. Defined daily dose/1000 beneficiaries/day were calculated. COX-2 inhibitors/all NSAIDs ratios were calculated for the two jurisdictions. Ns-NSAIDs were divided into low-, moderate-, and high-risk for gastrointestinal side effect. Drug Utilization 90% was also calculated for all NSAIDs. Results: Overall NSAID use was different in Australia and Nova Scotia. However, ns-NSAIDs use was similar. COX-2 inhibitor and high-risk NSAIDs dispensing was higher in Australia. Low-risk NSAIDs prescribing increased over the study period in Nova Scotia. While the low-risk/high-risk ratio was constant over the study period in Australia, in Nova Scotia, the low-risk/high-risk ratio increased over time. Discussion: There are significant differences in Australia and Nova Scotia in use of NSAIDs, mainly due to COX-2 prescribing. The healthcare systems in the two jurisdictions contributed to the divergent prescribing patterns. Further studies to understand and influence adherence to prescribing policies should be considered.

677

Therapeutic Index of Methotrexate Depends on Circadian Cycling of Tumor Necrosis Factor-Alpha in MRL/Lpr and Collagen-Induced Arthritis Mice

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Objective: Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology. Morning stiffness, a characteristic feature of RA, shows a 24 hour rhythm. Noticing the 24 hour rhythm of RA, we hypothesized the presence of a similar rhythm for a RA indicator, in addition to dosing time dependency of the antirheumatic effect of methotrexate (MTX) in collagen-induced arthritis (CIA) and MRL/lpr mice, which both reflect the symptomatology of RA patients. Methods: To measure cytokines, serum amyloid A (SAA), and IgG rheumatoid factor (IgG-RF), blood was taken at different times (2, 6, 10, 14, 18, or 22 hours after the light was turned on (HALO)) in CIA and MRL/lpr mice. MTX was administered at two different dosing times based on these findings to estimate arthritis and inflammation. Results: In both mouse models, plasma tumor necrosis factor (TNF)-alpha concentrations showed obvious 24hour rhythms with higher levels at light phase and lower levels at dark phase after RA crisis. The arthritis score was significantly lower in the 22 HALO group than in the control and 10 HALO groups in CIA mice. The SAA concentration in the 18 HALO group was significantly lower than that in the control and 6 HALO groups in MRL/lpr mice. Conclusion: Arthritis and inflammation were relieved after administration of MTX during the dark phase in synchronization with the 24 hour rhythm. Our findings suggest that choosing an optimal dosing time associated with the 24 hour cycling of TNF-alpha could lead to effective and safe treatment of RA by MTX.

678

Funding Medicines for Pulmonary Arterial Hypertension - The New Zealand Experience

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Pulmonary arterial hypertension (PAH) is a life-threatening disorder of the lung arterial vasculature. Iloprost, bosentan and sildenafil are available for use in New Zealand and have recently gained registration status. Access to funding of these medications is covered by the Exceptional Circumstances (EC) scheme. Initially considered rare (prevalence less than 10), applications are now considered under the Hospital EC scheme where drug acquisition costs are offset against other public hospital expenditure. The EC Panel requires pulmonary artery pressure, six minute walk test, NYHA class and transplant suitability data to be included with each application. Since 2002, 118 patients have had funding, with 73 currently funded and 28 patients deceased. The prevalence is 17 per million. Eleven patients have had lung transplantation, 3 remain alive. At present 52 patients are taking monotherapy, (47 sildenafil, 4 bosentan and 1 iloprost) and 21 are on combination therapy (10 sildenafil/bosentan, 10 sildenafil/iloprost, 1 iloprost/bosentan). Of these, 16 patients have connective tissue diseases. The median duration on sildenafil monotherapy is 7 months (range 1-29 months). There has been a recent widening of criteria for funding sildenafil to include congenital heart disease and neonatal PAH (4 patients). The EC Panel has witnessed rapid growth in applications for PAH treatment. The EC scheme has allowed funding of PAH treatments, especially sildenafil, prior to registration. Sildenafil is the most cost effective initial therapy. Initially a bridge to transplantation, treatment is now becoming chronic therapy. Combination therapy is increasingly being requested. PAH treatments appear to prolong survival.

679

Using Vignettes to Evaluate Cost Utility with EQ-5D for Osteoarthritis of the Knee in Turkish Hospitals

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Objectives: Although the popularity of the QALY approach has been constantly increasing, Turkish policy makers still use simple epidemiological data for decision making. The objective of this study was to assess the impact of osteoarthritis on patients' health-related quality of life (HRQL) with the EQ-5D instrument before and after treatment and evaluate cost per QALYs following treatment. Methods: A total of 175 respondents completed the EQ-5D. The average age of the respondents was 59 years-old, 39% of them were male and 61% female, 96% of the respondents had health insurance. The unit cost was acquired by the step down allocation methodology from 29 hospitals which represented the various Turkish health systems. The incremental cost-effectiveness ratio (ICER) of adding treatment was analyzed. Costs and quality-adjusted life years (QALYs) gained were used to calculate the ICER for osteoarthritis of the knee (cost/QALY). Quality weights were obtained from published UK data. Quality-adjusted life years (QALYs) were calculated using the utility data and the expected remaining life years of the patients. Results: The mean HRQOL score (scale, 0-1) increased after medical treatment from 0.31 pretreatment, to 0.54 after treatment. The cost per QALY gained was 77.32$. Conclusions: Osteoarthritis effects quality of life. Our results indicate that treatments for osteoarthritis are associated with significant improvement in the EQ-5D index scores after 3 months. The respondent evaluations are important outcomes for cost-utility analysis of the treatments for osteoarthritis, and the present study provides important evidence for future economic evaluations.

680

Behavioral Sensitization after Repeated Exposure to Modafinil and Cross-sensitization with Apomorphine in Rats

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A full characterization of the abuse potential of a wake-promoting agent, modafinil, will become important as the use of this drug increases. A phenomenon, commonly found in rodent following repeated exposure to classical psychostimulants, termed behavioral sensitization, is known to be involved in certain aspects of drug addiction. So far no evidence has been provided that this phenomenon also appears to modafinil. In the present study the possibility of modafinil-induced behavioral sensitization and the existence of cross-sensitization between modafinil and apomorphine were explored. Either modafinil (64 mg/kg) or apomorphine (0.5 mg/kg or 1.0 mg/kg) compared with vehicle was pretreated for repeated 10 consecutive days, followed by a withdrawal phase (3 days or 21 days), and subsequently re-challenged with the respective dose of modafinil or apomorphine to induce behavioral sensitization in male Sprague-Dawley rats. The results indicated that after both 3- and 21-days withdrawal, modafinil induced sensitization to its locomotion (LM) and stereotyped behavior (SB). On the other hand, at 0.5 mg/kg, apomorphine induced sensitization to its LM after the 21-days withdrawal and, at 1.0 mg/kg, also to its SB after both 3- and 21-days withdrawal. When rats were sensitized to modafinil, and then challenged with apomorphine, there was no evidence for cross-sensitization. However, under the reciprocal conditions, there was evidence for cross-sensitization. Our results indicated that behavioral sensitization could be induced in rats exposed to modafinil, and that changes in dopamine receptor activities could be involved in the cross-sensitization of apomorphine to modafinil but not modafinil to apomorphine.

681

The Sources of Drug Information for General Practitioners in Turkey

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Introduction: Information sources for drugs are various but their influence on the physician's attitudes while prescribing is not well known. The aim of the study was to define the importance of the sources of drug information on the general practitioners' prescribing attitudes in Turkey. Methods: 45 general practitioners (GPs) were asked to sort the principles of rational prescribing (efficacy, safety, appropriateness and cost) and information sources according to their importance for getting information in theory and prescribing the old and new drugs. Additionally they were asked to emphasize how frequent they were using these sources. The trainings they have attended were also documented. They were also asked if they were aware of the principles of evidence based medicine. Results: GP's mentioned the drug guide as the most common source for the information in theory and prescription for old drugs. However the congresses and the pharmaceutical representatives were the most common sources of information in theory and prescription for new drugs. GP's think that other colleagues see pharmaceutical representatives as the most common source of information. Meta analysis results are accepted as the most important evidence. Discussion: For GP's sources of information vary for the new and old drugs. The importance of pharmaceutical representatives is clear. Conclusion: The preliminary results show that personal contact plays an important role for the decision for prescription of new drugs for GPs.

682

Clinical Epidemiological Study about Factors Affecting QT Interval

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Introduction: The importance of assessment QT interval of EKG is determined because its prolongation (e.g., in the long congenital QT syndrome (LQTS)) could be associated to severe cardiac arrhythmias that induce sudden cardiac death. Our objective was to study the influence of different disease groups, drugs and the personal or family history of syncopal reactions, or sudden death on QT interval and related parameters. Methods: Prospective study on a sample of 350 patients selected of consecutive mode. Results: A total of 141 men and 209 women, aged 18 to 87 years with a mean Body Mass Index (BMI) of 27.99 Kg/m^2, were evaluated. QT interval corrected for heart rate (QTc) values >=440 ms were in 15.14% of patients and dispersion QTc (DQTc) >=60 ms in 16.9% of them. QTc interval was more prolonged in women, patients >=60 years and BMI>=25 Kg/m^2 ($p<0.001$). Patients who belonged to the reference group without any study factors had shorter QTc interval duration ($p<0.001$). The multivariate analysis showed that age, IMC, personal history of syncopal reactions *versus* belonged to reference group (APPS, p=0.035), family history of sudden death *versus* belonged to reference group (APMS, p=0.058) and have drug-induced LQTS *versus* belonged to reference group (AFQT, p=0.011) were identified as independent factors of QTc interval duration.
Conclusion: The influence of the family (sudden death) and personal history (syncopal reactions) on QT interval and related parameters suggests the existence of a hereditary cause that raises the necessity to make a genotyping study of the candidate genes of this effect.

683

Statins Usage in Croatia: The Effects of Introducing Generics

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The treatment of hypercholesterolemia with statins (or HMG-CoA reductase inhibitors) has an important place in the prevention of atherosclerosis. The aim of this study was to detect changes in statins usage and the effects of generic statins introduction on the national reimbursement list. Drug utilization and financial expenditure data were analyzed for statins prescribed by GPs. The data were obtained from

the Croatian National Health Insurance Institute for the six-year period (2001-2006). Drug utilization data are presented in defined daily doses/1000 inhabitants/day (DDD/1000), while financial expenditure data are presented in Euros. In the period 2001-2006, the usage of statins increased 4.48 times (from 10.01 to 44.86 DDD/1000) while the related expenditure increased 3.16 times. The highest growth of 109.38% was in 2003 (in comparison to 2002) i.e. from 9.82 to 20.56 DDD/1000, and the related expenditure increased 1.8 times. The share of generic drugs increased from 1.01% to 45.38%. For the entire period simvastatin and atorvastatin remained not only the most frequently prescribed statins (ranging from 86.30% in 2001 to 95.97% in 2004), but also had the highest financial share (83.96% in 2001 to 90.27% in 2006). Share of generic simvastatin and atorvastatin increased from 1.17% to 49.64%. The usage of statins in Croatia increased considerably during the investigated period, especially in 2003 as a result of a legal change (the new Insurance Act) with the introduction of supplementary health insurance. The introduction of generic statins and restrictive measures on Croatian drug market slowed down the increase of financial expenditure.

684

Cost of Treating Mild Diabetic Foot Infections in Patients Admitted to Hospital in Canada

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Introduction: Diabetic foot infections (DFIs) account for the majority of inpatient days for diabetics in North America. Little information is available describing costs of DFI treatment in Canada. Methods: A prescription database was retrospectively reviewed to quantify drug treatment and associated costs for patients admitted to acute care hospitals, initially prescribed oral antibiotics for DFI during 2005 to 2007 (mild DFI). Costs were reported in 2007 Canadian dollars and included 1) antibiotics, narcotic analgesics; 2) hospitalization, 3) medical (physician, surgery), and 4) laboratory. Outcome (success/failure) was based on whether initial antibiotic(s) were modified. Results**:** Sixty-eight patients with mild DFI were identified (66% male, mean age 70±13.2 years). Mean total cost/patient was $10,599 (SD=$11,581, range: $674-$54,245). Total cost of antibiotic therapies ranged from $1 to $3151; the majority were <$200. Outcome was success in 49%. Switch to a regimen including intravenous antibiotics was the reason for failure in 40% (27/68). Total costs for patients with a successful outcome were significantly lower than those for failure ($5924 versus $15,706, $p<0.001$). Discussion: Hospitalization accounted for 90% of total costs. Patients not requiring modification to initial antibiotic(s) had shorter hospitalization stays, and thus lower costs. Initial antibiotic(s) with high efficacy would facilitate cost-effective management of mild DFI, thus easing economic burden on the healthcare system. Too few data were available to compare costs among different therapies. Conclusion: Total costs of treating mild DFIs in hospitalized patients in Canada during 2005 to 2007 are considerable. Prospective research is needed to aid in developing cost-effective management strategies.

685

Cross-sectional Prevalence Study of QT-Prolonging Antipsychotic Drug Regimens in the Female vs. Male Inpatient Population of a Teaching Psychiatric Hospital

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Background: Female gender is an acknowledged risk factor for drug-induced QT-interval prolongation and torsades de pointes (TdP). The aim of the study was to compare the prevalence of QT-prolonging antipsychotic (QT/AP) therapy and QT/AP-containing drug combinations (DCs) at increased risk of QT prolongation and TdP in the female vs. male adult inpatient population of a 410-bed teaching psychiatric hospital. Methods: A 1-day cross-sectional review of all the ongoing drug regimens in the inpatient population of the hospital was performed. The screening/rating tool was the knowledge base of the French Agency for Health Products. Hazardous/contraindicated DCs or DCs requiring precaution for use were considered. Results: 287 inpatients (110 women, 177 men) receiving antipsychotic therapy were included: 62% (n=68) of women and 53% (n=105) of men (p=0.84) received QT/Aps. In these QT/AP-receiving patients, prevalence of

QT/AP-containing DCs at increased risk of QT prolongation was similar in women and in men, respectively 47% and 34% (p=0.10), but frequency distribution of these DCs was different: QT/AP polypharmacy and combinations of QT/Aps with bradycardia- or hypokalemia-inducing drugs accounted for, respectively, 31.2% and 68.8% of these DCs in women vs. 63.9% and 36.1% in men (p=0.007). Proportion of QT/AP-containing hazardous/contraindicated DCs was 28.1% in women vs. 55.5% in men (p=0.02). Conclusion: In this study, prevalence of QT-prolonging antipsychotic drug regimens in antipsychotic-receiving women was not minimized as compared with the data recorded in men. Yet women were less exposed than men to antipsychotic-containing DCs that carried the higher risk of QT-interval prolongation or TdP.

686

Non-steroidal Anti-inflammatory use and Risk of Parkinson's Disease: A Meta-analysis of Observational Studies

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Introduction: Oxidative stress and inflammation may contribute to development of neurodegenerative diseases such as Parkinson's disease (PD). Observational studies have examined the possible preventative effects of NSAIDs on the development of PD, but results are conflicting. The relation between NSAIDs and the development of PD was explored through the technique of meta-analysis. Methods: MEDLINE, EMBASE and bibliographies of retrieved articles were searched. Databases were systematically searched from the earliest date to December, 2007. We included case control (CC) and cohort studies presenting relative risks (RR) and 95% confidence intervals. Inclusion was limited to studies in which both the exposure of interest (NSAID) and outcome (PD) were explicitly defined. Data were extracted independently by two investigators onto a computer worksheet. Pooled estimates with 95% confidence intervals were computed using HepiMA software with random effects modeling. To quantify heterogeneity, the Q statistic was calculated. Results: Eleven studies were identified: four cohort and seven case-control. The pooled RR of NSAID use (including ASA) for all studies was 0.91 (0.78-1.07). When CC studies were pooled RR was 0.90 (0.74-1.10) and when cohort studies were examined the RR was 0.92 (0.67-1.27). When only those studies examining effects of ASA were pooled the RR was determined to be 1.08 (0.93-1.27). Heterogeneity was substantial in all analyses. Discussion: Although positive effect of NSAIDS was not observed we cannot exclude potential benefit. Studies were heterogeneous suggesting that further well controlled prospective studies are necessary to elucidate potential benefits. Conclusions: NSAIDs do not appear to prevent development of PD.

687

Exceptional Circumstances and PHARMAC – New Zealand's Pharmaceutical Management Agency

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PHARMAC manages a schedule of medications funded by the New Zealand government. This paper describes the process for assessing applications for unfunded medicines, according to established "Exceptional Circumstances" (EC) criteria, for use within the community. The EC scheme is overseen by a Panel of 6 practicing clinicians from a variety of disciplines, with administrative support. Rare and unusual clinical circumstances (prevalence of less than 10 annually) are considered under Community EC within a capped budget. Applications are assessed at fortnightly teleconferences with 15% overall approval (1000 applications annually). High cost medicines are also subject to Cost Utility Analysis. Applications that are not considered to be rare and unusual can receive government funding through a public hospital (Hospital EC) where there are net savings to the health sector and all other alternative funded treatment options have been exhausted. Such applications may prevent a hospital admission or allow an early

discharge, and are assessed by the Panel within 48 hours. There are a median of 6 applications daily (range 0-11), 85% being approved. Applications for cancer treatments are assessed under different criteria through an automated process with exceptions being assessed by the Panel within 72 hours. The approval rate is 80%.The advantages of the EC scheme are funding of unusual treatments without the requirement of schedule listing, rapid response to the applicant, cost containment, awareness of "the cutting edge" of medicine and appraisal of new technology before more formal assessment and consideration by PHARMAC.

688

Specific Design Issues in a Protocol for Assessing the Effectiveness of Traditional Chinese Herbal Medicine and Tai Chi in Cancer Patients

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Background: Millions of people are exposed every day to traditional Chinese medicine (TCM). Developing a double blind RCT for assessing TCM efficacy faces several challenges to respect the practice of TCM that requires adapting the treatment to the patients' changing status. Objective: to assess the efficacy and safety of Chinese medicine to improve the quality of life (QoL) of cancer patients. Methods: 80 participants with lung cancer who are experiencing at the end of their chemotherapy fatigue, uncontrolled nausea/vomiting or anorexia are randomly assigned to one of 4 treatment groups: herbal treatment or placebo and TaiChi or minimum exercise. Each group is treated for 3 months and observed for 6 months during which time their QoL is measured periodically. Herbal treatment is made of 58 different herbs (approved by NHPD) that are combined in different ways by the TCM doctors, according to TCM principles. Complete dissociation between prescription and decoction preparation had to be made and 6 different types of herbal placebo had to be created to keep practitioners and patients blind, even when the prescription changed. Discussion: The following issues will be presented and discussed: (a) regulatory approval for using Chinese herbs and the importance of MayWay Inc., (b) ethical considerations, (c) the quality control strategy, (d) the strategies of placebo preparation and dispensing, (e) logistic (f) centralized recruitment and group information (g) web based data management. Conclusion: Conducting trials for assessing the practice of traditional medicine is complex and difficult; we stress the utmost importance of communication.

689

Study of Combination Paclitaxel-carboplatin Induced Peripheral Neuropathy using NCI-CTC

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Introduction: Paclitaxel in combination with carboplatin, a standard therapy in advanced ovarian and non-small cell lung cancers produce a more convenient and less toxic therapy. Both drugs in this combination are capable of producing peripheral neuropathy (PN). In this study we tried to evaluate the incidence, severity, dose dependency, and reversibility of PN. Methods: We studied 27 patients with ovarian and lung cancers, treated with paclitaxel (range 240-300 mg/m2) plus carboplatin (range 450-600 mg/m2) intravenous infusion every 3 weeks. We used National Cancer Institute-Common Toxicity Criteria to evaluate peripheral neuropathy. The severity of symptoms was graded. Incidence and reversibility of neuropathy was measured in an interview with the patient. Results: Paresthesias appeared in 23 (85.2%) patients after an average cumulative dose of 624.7 mg/m2 of paclitaxel and 1256.5 mg/m2 of carboplatin. In most patients, PN (65.2%) was seen after the first or second dose of paclitaxel – carboplatin with more grade 3 than 2. Of the patients who suffer from paresthesias 41.0% were stabilized, 5.9% improved, 47.1% resolved completely and 5.9% progressed. In none

of patients, treatment had to be discontinued due to PN. Discussion: The PN appears to be a major side effect of this therapy. Our results are similar to other reports although we did not use the maximum tolerated doses. In nearly half of patients this side effect is reversible. Fortunately it is not severe enough to discontinue the treatment. Conclusion: Patients need to be educated about this side effect in the early course of their therapy.

690

The Effect of Intravenous Furosemide on Renal Function in Hospitalized Patients with Congestive Heart Failure

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Introduction: Congestive heart failure and renal failure frequently coexist and patients with renal dysfunction are known to have a worse prognosis. The aims of this study were to estimate the incidence of renal impairment in the hospitalized CHF patient treated with intravenous diuretics and to identify risk factors for diuretic-related renal impairment. Methods: This was a prospective observational study involving 109 consecutive patients admitted with congestive heart failure exacerbation. All the patients were treated with intravenous furosemide. End points of the study were new onset renal failure or deterioration of a previously diagnosed stable renal failure. Comparisons between the two groups of patients were performed using parametric and non-parametric tests. Results: Of the 109 patients, 24% developed renal impairment. The average length of hospitalization was 15 ± 11 days in the patients who developed renal impairment versus 9 ± 4days in those who did not ($p< 0.001$). The only independent risk factor for the development of renal impairment was previous renal failure (46% vs 25%; p 0.048). Hypertension (73% vs 59%) and treatment with low dose dopamine (58% vs 23%) were of borderline significance. BUN/creatinine ratio was similar among the two groups at the baseline and the end point. Conclusions: A history of renal failure was an independent risk factor for the development of renal impairment during hospitalization. Neither the dose of furosemide nor the route of administration (intermittent versus continuous dosing) had a significant effect on the renal outcome. The renal impairment in our study was not preceded by pre-renal azotemia.

691

Changing Patterns of Asthma Patients' Medication Regimens and Health Service Utilization: A Population-based Study

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Introduction: Asthma patients with suboptimal drug therapy are more likely to require high use of concomitant general practitioner and hospital services than patients with optimal therapy. This study quantifies the optimality of patients' asthma medication regimens and characterizes patients who are persistent high service users. Methods: A cohort of 48, 464 asthma patients was identified using provincial health administrative data (including all prescription medications, physician and hospital visits) for 1996-2000. Canadian Asthma Guideline recommendations were used to categorize patient-specific regimens of inhaled short-acting beta-agonists and corticosteroids into optimal, suboptimal and indeterminate optimality. High service use thresholds were determined from service cost distributions. We examined patterns of regimen and service utilization changes across years. Results: Proportions of patients with optimal or suboptimal therapy declined by 15% and 3%, respectively over 5 years. 20% of patients who had suboptimal therapy $\geq$3 years were frequent high service users. Adolescents were least likely to be prolonged high health service users. 4% of patients had optimal therapy and 6% had suboptimal therapy every year. 8% of patients did not receive asthma drug therapy in any year. Adults were most likely, while children 11-17 years were the least likely to have suboptimal therapy over any year. Discussion: Our guideline-based approach to identify patient-specific regimen optimality is useful in identifying patients with persistent suboptimal asthma regimens. Conclusion: Quantifying optimality of asthma therapy will allow health planners to design and

implement more patient-focused interventions for those who are high-users of health services with suboptimal therapy.

THURSDAY, JULY 31, 2008
STREAM 4:
CLINICAL PHARMACOLOGY IN SPECIAL POPULATIONS

692

Obesity is more Prevalent among Aboriginals with Diabetes as Compared to all Canadians with Diabetes

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Background: The rate of heart disease is 1.5-fold greater among Aboriginals compared to the general Canadian population. Type 2 diabetes is also 3.6-5.3 times more prevalent among Aboriginals. Obesity is relatively common in diabetes and may contribute to poorer outcomes. It is of interest to compare the prevalence of obesity among Aboriginals with diabetes to all Canadians with diabetes. Methods: A MEDLINE search was conducted (1966-2007) using the following MeSH terms: Indians- North American, Canada, diabetes mellitus, obesity and body mass index (BMI). Population-based studies reporting the proportion of Aboriginals with diabetes that were overweight (BMI: women $\geq$ 25, men $\geq$ 27) or obese (BMI $\geq$ 30) were retrieved. Prevalence estimates of elevated BMIs for Canadians with diabetes were obtained from the LCDC National Population Survey (1996-1997) and the Ontario Diabetes Database (1996-1997; Ontario Health Survey). Results: Six studies were identified. 76.2-86.5% of Aboriginals with diabetes were overweight compared to 59.4% of Canadians with diabetes. Furthermore, 44.1-69.6% of Aboriginals with diabetes were obese compared to 28.6% of Ontarians with diabetes. Discussion and Conclusions: When compared with the general Canadian population with diabetes, elevated BMI is more commonly observed among Aboriginals with diabetes. These findings suggest that obesity, as a risk factor for poor diabetes control and heart disease, is not optimally managed within the Aboriginal diabetes population and may pose a great burden to the Aboriginal community. The precise impact of obesity and diabetes on the health of Aboriginals warrants further research.

693

Prevalence of Cardiovascular Disease Risk Factors among Aboriginals

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Background: Cardiovascular disease (CVD) is on the rise among Aboriginals in Canada. The rate of heart disease among First Nations people and Inuit is 1.5 times higher than the general Canadian population, and circulatory diseases are the second leading cause of death in First Nations people. Several CVD risk factors may contribute to the increased prevalence of CVD among Aboriginals. The aim of this review was to compare the prevalence of such factors among Aboriginals and the general Canadian population.
Methods: A literature search using MEDLINE and Google search engines was conducted using the following terms: North American Indians/Aboriginal, Canada, smoking, hypertension, obesity, metabolic syndrome. Studies reporting risk factor prevalence were used for data extraction. Results: The reported prevalence of each risk factor was greater among Aboriginals than the general Canadian population, with the exception of hypertension: Smoking: 40-74% vs. 16-25%; Obesity: 36-58% vs. 18-36%; Metabolic Syndrome: 28-52% vs. 21-26%; Diabetes: 13-23% vs. 2.5-5.6%; Hypertension (measured/on treatment): 16-49% vs. 9-45%. Discussion and Conclusions: CVD is a growing problem among Aboriginals and this increased prevalence of CVD risk factors may further heighten this health burden. More research into risk reduction and treatment programs is needed.

694

Population Pharmacokinetics of Valsartan in Children

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Purpose: The objective of this work was to develop a population pharmacokinetic model for valsartan, an angiotensin receptor blocker, in children to assess the relationship between age, body weight and pharmacokinetics of valsartan.Methods: Pharmacokinetic data were collected from a single dose pharmacokinetic study conducted in 26 hypertensive children ages 1 to 16. All subjects received 2 mg/kg of valsartan suspension under fasting conditions and the maximum dose was 80 mg. Several structural pharmacokinetic models were evaluated for appropriateness. Allometric scaling and standard covariate analyses were performed to explain interindividual variabilities. Objective function values and goodness of fit plots were used for model selection. Posterior predictive check and nonparametric bootstrap methods were used for final model evaluation. Results: A linear 2-compartment first-order elimination model with zero order absorption and a lag time best described the plasma concentration versus time data of valsartan. Allometric scaling and standard covariate analysis revealed age is competitive to body weight as a single predictor of clearance in children; however, after adjustment for body weight, the effect of increasing age is estimated to have insignificant influence on valsartan clearance (2% per year relative to a 30 kg, 8 year old). The final clearance model was summarized as follows: CL/F (L/hr)= $4.64 \cdot (WTKG/70)^{0.75} + 0.0469 \cdot (Age-7.8)$. Conclusions: A population pharmacokinetic model was developed using the plasma concentration data of valsartan in children. The model reveals that the increase in age has minimal influence on the body weight dependent clearance of valsartan in children.

695

Analysis of Adherence and Persistence with Once Daily Extended Release Methylphenidate Compared to Immediate Release Methylphenidate in Pediatric Subjects with Attention Deficit Hyperactivity Disorder

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Attention deficit hyperactivity disorder (ADHD) affects approximately 4-12% of children, and is often treated with the stimulant methylphenidate (MPH). Immediate release methylphenidate (IR MPH) requires twice or thrice daily dosing, which may negatively affect adherence and persistence resulting in poor control and increased healthcare utilization. CONCERTA® (extended release MPH) is indicated for ADHD, and, because of its once daily formulation, may improve adherence and persistence. Pediatric subjects (aged 6–18 years) with a first prescription for CONCERTA (N=1,273) or IR MPH (N=2,788) during the period June 1, 2005 to November 30, 2005 were identified from the Régie de l'Assurance Maladie du Québec (RAMQ) databases. Prescription drug and selected healthcare utilization and costs were obtained for one year following their first prescription. The groups were compared using statistical models controlling for sex, age, and cost of selected healthcare resources in the prior six months. Adherence to therapy over one year was calculated using the medication refill adherence (MRA) measure, the total days' supply of medication divided by 365 days. The adjusted mean adherence rate was approximately twice as high in the CONCERTA group (72% vs. 38%, $p<0.0001$). The majority of the CONCERTA group (57%) were adherent (MRA ≥80%) compared to only 12.6% in the IR MPH group. CONCERTA subjects were persistent on their initial therapy for an additional 115 days over one year ($p<0.0001$; unadjusted means: 224 vs. 109 days).Adherence and persistence rates are significantly higher for subjects in the CONCERTA group compared to the IR MPH group.

696

The Burden of Non-steroidal Anti-inflammatory Drug (NSAID) Utilization for Musculoskeletal Disorders in Blue-collar Workers: A Population-based Cohort Study

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The purpose of this study was to describe the patterns of utilization of NSAIDs by occupational groupings in a general employed population. Method: This was a secondary analysis of the CADEUS cohort study on the 5,651 actively employed patients, who submitted at least one claim for the reimbursement of a NSAID dispensation for a musculoskeletal disorder (MSD) between August 2003 and July 2004, in the French National Healthcare Insurance database. Questionnaires were sent to prescribing physicians to obtain diagnoses and the five-year medical history, and to patients for their occupation, weight and smoking status. Multivariate logistic regression was used to study the determinants of a heavy use of NSAIDs described as over four dispensations in one year with less than two months between any two. Results: Factors associated with heavy use of NSAIDs were age (Odds ratio (OR): 1.8 (ten years), 95% confidence interval (CI): 1.6-1.9), osteoarthritis (versus back pain) (OR: 1.8, 95% CI: 1.5-2.1), body mass index (superior to 30) (OR: 1.8, 95% CI: 1.5-2.2), and occupation (blue collar versus white collar workers) (OR: 1.4, 95% CI: 1.2-1.6). Blue collar workers also had a 20% higher prevalence of 5-year history of dyspepsia. No difference was observed between sexes or in the use of COX-2 selective inhibitors between occupations. Conclusion: Factors associated with occupational constraints that contribute to the severity of MSDs, may explain the heavier use of NSAIDs among blue collar workers in spite of a concurrent and past medical history of adverse reactions to this type of medication.

697

Population Pharmacokinetics of Doxorubicin Using Plasma Post-infusion Drug Concentrations in Infants and Children with Malignant Diseases: A Potential Identifier for Cardiotoxicity Risk

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Introduction: Doxorubicin is an anthracycline glycoside, commonly used in the treatment of pediatric malignancies, which has significant cardiac toxicity. Methods: We performed a population pharmacokinetic (PK) analysis of doxorubicin, in eleven children receiving intravenous doxorubicin. Blood samples were drawn before the initiation of the doxorubicin infusion and at 0, 4, 18, 36 and 48 hours after the end of the infusion. Plasma drug concentrations of doxorubicin were measured by solid-phase extraction and high performance liquid chromatography (HPLC) with fluorescent detection. Data were analyzed by the compartmental module of SAAM II. Results: Of 11 patients, plasma doxorubicin concentration-time courses of 9 children best fitted a 2-compartment intravenous PK model and were included in the population PK analysis. The resulting population parameters were k_{12} 0.78 ± 0.46 1/h, k_{21} 0.17 ± 0.088 1/h, k_{el} 0.22 ± 0.10 1/h, Vd 9.6 ± 6.8 ml/kg, Cl 1,297.5 ± 893.1 ml/kg/h, and half-life of 7.7 ± 11.3 h (ranging from 2.1 to 39.8 h). Discussion and Conclusions: A 2-compartment PK model was successfully used to describe the plasma levels of doxorubicin in pediatric cancer patients.

698

Systematic Review of Analgesics for Immunization Pain in Infants and Children

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Background: Immunization is the most common cause of iatrogenic pain in childhood. We previously demonstrated that analgesics are rarely used. Objective: To assess the effectiveness of topical local anesthetics, sweeteners and vapocoolants on pain using a systematic approach.
Design/Methods: MEDLINE, EMBASE, CINHAL and The Cochrane Library were searched for randomized controlled trials. Data were analyzed using RevMan 4.28; when data could be combined, the weighted mean difference (WMD) and 95% confidence intervals (CI) was calculated. Results: Seven trials (N=950) evaluated lidocaine-prilocaine and 1 trial (N=120) evaluated amethocaine in children from birth to 15 years. Pain was reduced by 6-46%. Using the modified behavioural pain scale (0-10), the WMD in pain was -0.73, (95% CI -0.77, -0.681; p <0.00001). Using VAS (0-100), the WMD was -19.23 (95% CI -25.05, -13.41; p <0.00001). Antibody response to the vaccine was unaffected (N=282; p=NS). Five trials (N=743) evaluated sucrose or glucose (12-75% concentration) in infants (2-12 months). Pain was reduced by 13-

73%.Vapocoolants were evaluated in 3 trials (N=190). Pain was reduced by 8-32%. Conclusions: Topical local anesthetics, sweetening agents and vapocoolants all reduce pain during immunization. Topical local anesthetics did not interfere with the immune response to the vaccine. Knowledge translation strategies are needed to improve utilization of analgesics in clinical practice.

699

Utility of Serum Cystatin C Concentration as a Marker of Renal Function for Dose Adjustment of Antibiotics in Critically Ill Patients

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Serum creatinine concentration (Scr) is often used to predict the glomerular filtration rate (GFR) for adjustment dosage in clinical practice. But in critically ill patients, sudden changes in GFR are not instantly followed by parallel changes in Scr. In recent years, it has suggested that cystatin C (CysC) is a good real-time marker of GFR in these patients. The aim of this study was to estimate the clinical utility of serum CysC (S-cys) values for dose adjustment of antibiotics by the population mean method with use of S-cys as a marker of renal function in clitically ill patients. 15 patients were treated with teicoplanin by intravenous infusion at the intensive care unit in Tokushima University Hospital from January 2005 to December 2007. They were not undergoing dialysis. S-cys was determined by immunonephelometry. GFR from S-cys (GFRcys) and from Scr (GFRscr) were calculated by Hoek's formula and Cockcroft&Gault's formula, respectively. Twenty-four-hour creatinine clearance (GFRucr) was calculated by using urine samples. The correlation coefficients for GFRucr and the reciprocal of S-cys (r=0.871) was significantly greater than that for GFRucr and the reciprocal of Scr (r=0.646, $p<0.05$). GFRcys have good correlate with clearance of teicoplanin (Clteic) (r=0.782). These results suggest that CysC may be a better marker of Clteic than creatinine in critically ill patients. We plan to evaluate in patients treated with vancomycin in the same way.

700

Trends in Drug Abuse Associated with Heavy Alcohol use in Pregnancy

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Fetal alcohol spectrum disorder (FASD) encompasses a range of physical, behavioral, and cognitive disabilities that result from prenatal ethanol exposure. In North America, FASD affects up to 1% of the pediatric population. Many alcohol dependent women use other drugs of abuse that may affect pregnancy outcomes. This study determined the trends in drugs of abuse that are present with heavy alcohol use in pregnancy. Methods: We identified neonates tested for intrauterine ethanol exposure between June 1997 and December 2007, using meconium fatty acid ethyl ester measurements. A total of 756 neonates were identified out of 21,715 cases. Results: A 14.5% positive-rate for intrauterine ethanol exposure was detected. Neonates with heavy intrauterine ethanol exposure were twice as likely to be exposed to opiates (OR=2.11; 95%CI 1.14-3.92) when compared to neonates with no heavy intrauterine ethanol exposure. Furthermore, a 2.97-fold increase in amphetamine use, a 2.41-fold increase in benzodiazepine exposure and a 2.08-fold increase in methadone exposure was detected; however, these results did not reach statistical significance, possibly due to small sample size. Discussion: More cases are required in order to increase power and validate the relationship between intrauterine ethanol exposure and amphetamines, benzodiazepines, and methadone. Conclusion: Neonates of mothers who used opiates in pregnancy should be tested for intrauterine ethanol exposure. Early detection of ethanol exposure can facilitate early intervention to both the neonate and the mother, thus decreasing the risk of secondary disabilities associated with FASD.

701

Pharmacologic Treatment of Preeclampsia and the New Possibilities in the Management of Hypertension in Pregnancy

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Introduction: Arterial hypertension during pregnancy is the most common medical complication of gestation. It is important to consider additional entities in the differential diagnosis, because of the two pregnancy-specific disorders: preeclampsia, a complication associated with substantial maternal and fetal morbidity, and gestational (transient) hypertension of pregnancy. Arterial hypertension occurs in 7-10% of pregnancies. There is no medication with ideal efficacy and safety which could be routinely recommended as the antihypertensive drug of choice during pregnancy. Nebivolol is a highly selective beta1 – blocker, combining beta 1 – adrenergic blocking activity with a vasodilating effect mediated by the endothelial L-arginine/NO pathway. Material and Methods: We followed up a cohort of 45 pregnant women during two years to assess the new drug nebivolol in the treatment of arterial hypertension in pregnancy. 17 patients sufferred from preeclampsia, 3 of them had the severe form. The effect of nebivolol was compared with other beta – blockers and also with other antihypertensive agents. Results: Nebivolol had ideal efficacy and safety. During the 2 years´ period we didn´t observe any adverse event regarding to the nebivolol treatment both by pregnant women ant the newborns. Conclusion: Nebivolol may have an important role in the treatment of hypertension in pregnancy and is safe for the pregnant woman and her fetus.

702

Significantly Altered Bioavailability of Atorvastatin Acid Following Laparoscopic Gastric Bypass in Morbidly Obese Patients

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Morbid obesity is a growing global health issue. Bariatric surgery, using the laparoscopic gastric bypass procedure, is an effective treatment alternative. From a pharmacokinetic perspective it is likely that this combined restrictive and malabsorptive procedure affects bioavailability of drugs. In the present study the impact of laparoscopic gastric bypass on the pharmacokinetics of the HMG-CoA reductase inhibitor atorvastatin was investigated. Twelve consenting patients on atorvastatin treatment (20-80 mg/day), listed for bariatric surgery, were included in the study. The average (range) age of the 8 female and 4 male patients was 52 (29-63) years with an average BMI of 43 (38-47) kg/m2. Eight hour pharmacokinetic investigations at steady state were performed the day before and median (range) 4.6 (3.3-6.3) weeks after surgery. Patients treated with 40-80 mg/day of atorvastatin, who had high systemic exposure of atorvastatin acid before surgery, showed a 48% reduction (95% CI: 0.44-0.53) in the area under the curve (AUC0-8) of atorvastatin acid following surgery ($P < 0.05$). Patients with low systemic exposure prior to surgery rather showed increased AUC0-8 of 18% (95% CI: 0.96-1.46) after surgery. Patients on 20 mg/day atorvastatin also increased (44%, 95% CI: 1.03-2.02). No adverse events were reported during the study period. These data show that both dose and patient characteristics influence the effect of gastric bypass on atorvastatin bioavailability. The results should be considered when optimizing dosing of atorvastatin, and possibly other drugs subjected to the same metabolizing enzymes, after surgery.

703

Maturation of CYP2D6 and Postnatal Changes in Tramadol Urinary Excretion

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Tramadol is an orally-active and centrally-acting opioid analgesic with an efficacy and potency similar to codeine that makes it useful for the treatment of moderate to severe pain. The drug is widely used in adults and the prescribing in children, infants and neonates increased in recent years considerably. Conversion of tramadol to its main active metabolite is catalyzed by polymorphic CYP2D6. The activity of this enzymatic pathway is reported to develop in early postnatal age from 5-10% of adult values. The aim of our study was to evaluate the maturational changes in tramadol and O-demethyltramadol urinary excretion. Totally 11 subjects (3 neonates aged 0-1 month, 5 infants aged 1-12 months, and 3 older than 1 year) were given 2mg/kg dose of tramadol. Tramadol was indicated as a pain treatment in all subjects. Samples of urine collection at 3hours were analyzed by GC-MS. The

log tramadol/O-demethyltramadol ratios decreased with increasing age with the mean values 1.38, 1.06, and 0.43 in age groups 0-1, 1-12, and older, respectively. Only borderline statistical significance was observed in the regression model, probably due to low number of subjects in relatively wide age groups(r=-0.57; p=0.065). Metabolism of tramadol is affected by maturational development of cytochrome P450 during postnatal age.

704

Comparison of the Pharmacokinetics and Pharmacodynamics of Prasugrel in Subjects with End-stage Renal Disease and Healthy Subjects

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Introduction: Prasugrel, thienopyridine prodrug, is metabolized to an ADP-receptor antagonist, which potently inhibits ADP-induced platelet aggregation. This study compared the pharmacokinetics (PK) and pharmacodynamic (PD) response across a range of prasugrel doses in healthy subjects and subjects with end-stage renal disease (ESRD). Methods: In a single-dose escalation (5, 10, 30 and 60-mg prasugrel), open-label study in 32 subjects (16 ESRD, 16 healthy), each dose was given to 4 ESRD and 4 healthy subjects matched by weight, age, sex and race, when possible. Healthy subjects had creatinine clearance >80 mL/min; subjects with ESRD were on hemodialysis. The PK of prasugrel's active metabolite was measured through 24 hours. Maximum platelet aggregation (MPA) was measured by light transmittance aggregometry through return to near-baseline. Adverse events, vital signs and 12-lead electrocardiograms were collected. Results: Mean AUC(0-t_{last}) of prasugrel active metabolite was 40%, 47%, 31%, and 34% lower in ESRD subjects than in healthy subjects after 5, 10, 30 and 60-mg prasugrel, respectively, but profiles of MPA to 20 μM ADP were similar between ESRD and healthy subjects at each dose. MPA after the 60-mg dose was within 7 percentage points in ESRD and healthy subjects at all timepoints through the 6 to 8 day return-to-baseline. Recovery of platelet aggregation response was similar for subjects with ESRD and healthy subjects at each dose. Prasugrel was well-tolerated; no bleeding or drug-related adverse events were reported. Conclusions: Prasugrel's active metabolite exposure was lower in ESRD subjects but MPA response did not differ between ESRD and healthy subjects.

705

Influence of Repeated Painful Procedures and Sucrose Analgesia on the Development of Hypersensitivity to Subsequent Pain in Newborn Infants

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Introduction: Repeated exposure to painful procedures in full-term infants can lead to hyperalgesia during subsequent procedures. Our objectives were to determine the influence of cumulative exposure to painful medical procedures and sucrose analgesia on future pain responses. Methods: We studied 214 newborn infants enrolled in a clinical trial of oral sucrose prior to all painful medical procedures from birth until the newborn screening test. Infants were divided into two exposure groups according to number of painful procedures they had undergone [high (≥5) or low (≤4)] using the median cut-off technique. Pain was assessed using three validated measures [Premature Infant Pain Profile (PIPP), Visual Analog Scale (VAS) and cry duration] and compared between exposure (high vs. low) and treatment (sucrose vs. placebo) groups for the first (vitamin K injection) and last (venipuncture for newborn screening test) procedure. Results: MANOVA revealed effects of exposure (p=0.039) and treatment (p<0.001) on pain during venipuncture but not vitamin K injection. Follow-up univariate ANOVAs for venipuncture showed significantly more pain in the high versus low exposure group on the PIPP (p=0.012) and VAS (p=0.047) but not cry duration (p=0.382). Pre-treatment with sucrose resulted in less pain for all outcomes (p<0.001) with no interaction between exposure and treatment groups (p>0.05). Discussion: Infants exposed to ≥5 painful medical procedures demonstrated more pain during a subsequent venipuncture than infants exposed to ≤4 procedures. Sucrose reduced pain during venipuncture but did not prevent development of

hyperalgesia. Conclusion: Sucrose does not prevent hyperalgesia in newborn infants.

706

Regulation of alpha-Tocopherol Levels in Breast Milk during Lactation

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Human milk is a complex fluid capable of sustaining the total nutrition of a human infant. The composition of milk changes during lactation showing a rapid decline in fat –soluble vitamins and an increase in total lipids. Despite the observation that colostrum is a rich source of alpha-tocopherol, very little is known concerning the mechanism involved in the transfer of the component from the blood into milk. Vitamin E is well known for its antioxidant action. Alpha –tocopherol transfer protein (alpha-TTP) is purified in hepatocytes and plays an important role of determining the plasma alpha-tocopherol levels. In the current study, we have revealed alpha-TTP gene expression in mammary gland and the alpha-tocopherol secretion mechanism during lactation. We have showed the human alpha-TTP gene is a direct target of the oxysterol liver X receptor (LXR). LXR is one of nuclear receptor super families, ligand-dependent transcriptional factors and regulate cholesterol metabolism and lipogenesis. Analysis of the human alpha-TTP gene revealed the presence of a LXR response element. Moreover, LXR ligands have caused the increased alpha-tocopherol levels of liver and plasma in rats. However, LXR ligands cause the decreased alpha-tocopherol levels in rat milk during lactation. Alpha-tocopherol levels in milk and is reversely correlated with alpha-TTP gene expression in mammary gland during lactation. The change of alpha-tocopherol levels in milk throughout lactation may be regulated by LXR and alpha-TTP.

707

The Incidence of Poor Neonatal Adaptation Syndrome Following Exposure to Venlafaxine in Late Pregnancy

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Background: SSRIs are known to cause poor neonatal adaptation syndrome (PNAS) following late pregnancy exposure. However, data on SNRIs such as venlafaxine is lacking (4 case reports in the literature). Due to a shorter half-life and apparent higher incidence of abrupt discontinuation syndrome in adults, we hypothesized that the incidence of PNAS associated with the use of venlafaxine in late pregnancy would be higher than in SSRI induced PNAS, including paroxetine, which is considered to have the highest incidence. Methods: Women who called Motherisk regarding the safety of venlafaxine during pregnancy were contacted following delivery of their infant. Those who were exposed in late pregnancy were compared to two other groups of women; 1) exposed to paroxetine in late pregnancy and 2) unexposed to antidepressants. A standardized questionnaire was administered to each group, in an attempt to establish a diagnosis of PNAS. The three groups were compared to ascertain the incidence of PNAS. Results: To date, we have completed 390 follow-ups; 97 in the venlafaxine group with four (4.1%) cases of PNAS, 57 in the paroxetine group with one (1.8%) case and 64 in the non-exposed group with one (1.6%) case. We did not find a statistically significant difference among the three groups (p=0.54). Conclusions: Venlafaxine does not appear to increase the risk for PNAS compared to SSRIs or to no antidepressant use. In addition, our findings suggest that overall, with both SSRIs and SNRIs, the incidence is less than previously documented, which was approximately 30%.

708

Pre-clinical Assessment for the Impact of Secondhand Smoke Influences Aquaporin 1 and Novel Cytokines Involvement in Periodontal Disease

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Smokers present an increased risk for periodontitis and this effect is out of control even though there's adequate periodontal treatment. Secondhand smoke (SHS) is the only source of air-borne nicotine and contains chemical compounds that are known to cause inflammation. Specialized water channel proteins termed aquaporin1; AQP1 have been suspected in some pathological conditions involving inflammation. Rats were exposed to smoke

produced from cigarettes for 30-day experimental period, and maxillary first molar was received a ligature to induce periodontiis. We utilized immunocytochemistry to determine AQP1 expression. The species-specificity of various cytokines was investigated by cytokine antibody array. Here we report that periodontitis model rats with smoke inhalation developed rapidly progress of periodontal pocket formation in a time-dependent fashion. In parallel, the amount of inhaled toxic compounds correlated with periodontal destruction. The lesions were accompanied by up-regulation of AQP1 in the gingival epithelial cells, endothelial cells and inflammatory cells. In contrast, no periodontitis lesions were found in sham smoked that were submitted to identical procedures. Flactalkine, CINC-2, CINC-3, IL-1alpha, IL-1beta, MIP-3alpha, TNF-alpha, TIMP-1 and VEGF are abundantly expressed in the gingival tissues triggered by involuntary sidestream smoke. This study demonstrates that a sidestream smoke exposure during periodontitis directly affects periodontal status and induces AQP1 as well as novel cytokines expression. The outcome of our pre-clinical study may lead to the development of novel dental product approach for periodontal disease in both SHS and smokers.

709

Thiopurine Metabolite Levels in Relation to Age and Gender in Children with Inflammatory Bowel Disease

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Objectives: Thiopurines, 6-mercaptopurine (6-MP) and its pro-drug, azathioprine (AZA), are widely used in the management of inflammatory bowel disease (IBD). These drugs are extensively metabolized and previous pediatric studies have shown that their efficacy and toxicity are associated with specific 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) nucleotide levels in red blood cells (RBC). The polymorphic enzyme thiopurine methyltransferase (TPMT) is an important regulator of these levels; however, the effects of a child's age and gender on thiopurine metabolite levels have not been fully evaluated. Methods: RBC 6-TGN and 6-MMP levels were quantified by high performance liquid chromatography in 606 IBD patients (265 females and 3341 males) receiving 6-MP or AZA. The pubertal age was set at 12 and 14 years old for females and males, respectively. TPMT polymorphisms G462A and A719G were detected by PCR/ASO. Results: As expected, 6-TGN and 6-MMP levels in patients with TPMT variant alleles (heterozygous) were respectively higher and lower than those seen in patients without TMPT variants (wild-type). Regardless of age, there were no differences in 6-TGN levels between wild-type females and males or between heterozygous females and males. However, 6-MMP levels and the 6-MMP/6-TGN ratio in wild-type post-pubescent patients were lower in males than in females. A similar trend was also observed in heterozygous patients. Conclusions: These data suggest the presence of a more important thiopurine methylation process in post-pubescent females than males, and either the absorption of a higher fraction of an oral thiopurine dose or the administration of a larger thiopurine dose.

710

Inpatient versus Outpatient Treatment Compliance Assessment in Rwanda in Infants with Mild Malaria

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Introduction: Poor compliance to illness treatment is an important contributor to treatment failure. Evaluation of compliance is difficult, especially in developing countries where literacy is a major obstacle. Objectives: Three methods of patient compliance evaluation were compared: manual pill count, interrogation and electronic pill-box count using Medication Event Monitoring System (MEMS®, Aardex Corporation, Geneva, Switzerland). This was done in a hospitalized (inpatient) and an ambulatory (outpatient) setting.Methods: Fifty-six children (<5 years) with malaria were recruited at the University Hospital of

Butare, Rwanda. Patients were treated with quinine sulfate taste masked pellets during seven days: four days of hospitalization (inpatient), followed by three days in ambulatory (outpatient) setting. At the hospital, the drug was given at three fixed times per day. Upon discharge, parents were given the appropriate number of doses to complete the treatment and were instructed on dosing frequency and time. Medication was stored in pillboxes equipped with a microchip system (MEMS®) registering date and time of every opening.Results: Compliance data from 54 out of 56 patients were recovered. Manual pill count and interrogation method both yielded a compliance of 100% for in- and outpatient treatment periods. MEMS-compliance was 90.5+/-8.3%. Inpatient compliance (99.3+/-2.7%) was markedly higher than outpatient compliance (82.7+/-14.7%).Conclusion: During this short treatment period (seven days), overall compliance rate was good. However a significantly lower compliance in the outpatient setting was observed. This was only detected using MEMS®. This study shows that measuring patient compliance with MEMS® monitors is an asset also in Africa countries.

711

Routine Drug Level Monitoring of First Line ARV Regimen in a South African Paediatric HIV Roll-out Clinic

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Antiretroviral (ARV) therapy is increasingly complicated due to limited available paediatric dosage formulations, varying dosing regimens, toxicity, viral resistance, genetic variance, and drug-drug and food-drug interactions. This complexity contributes to patient nonadherence and is a major impediment to clinical success. Very few successful and acceptable tools are available to monitor adherence especially in a South African paediatric population. As part of a 24 month on-going pharmacokinetic, pharmacodynamic and pharmacogenetic study of efavirenz in 64 HIV-1 infected children (3-16 years), a sensitive and rapid LC-MS-MS method has been modified to quantify the first line ARV regimens in South Africa. An Agilent 1200 series liquid chromatographic system interfaced to a 6410 triple quadrupole mass spectrometer is used with a Zorbax Eclipse XDB-C18 column (4.6 x 150 mm). The novelty and advantages of this modified method is: 1) the MS is operated in positive multiple reaction monitoring mode only, 2) small quantities of plasma (100 ul) is used for the simple protein precipitation step and 3) a single injection is required to quantify efavirenz; stavudine; lamivudine or lopinavir; ritonavir; stavudine and lamivudine within the linear concentration ranges of 100-12000 ng/ml. The following tools are implemented and evaluated in this clinical trial to strengthen adherence and to accurately predict efavirenz through levels: counselling sessions prior to ARV therapy; ARV's are issued at a dedicated pharmacy; drug level monitoring; specific diaries and night time recordings of efavirenz dosing prior to a study visit; tablet counts and adherence checks are done at every study visit.

712

The Safety of Proctofoam-HC® (Pramoxine + Hydrocortisone) in the Third Trimester of Pregnancy

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Background: Upto 25% of women suffer from hemorrhoids during the third trimester of pregnancy. However, none of the currently used topical anti-hemorrhoidal agents have been assessed for safety in pregnancy. This study will evaluate the fetal safety of the topical application of a commonly used anti-hemorrhoidal preparation; Proctofoam-HC®; during the last trimester of pregnancy. We will assess its safety by comparing birth weights of babies born in the treatment to those in the control group. Methods: In this prospective observational, open labelled study; 200 women will be recruited each in the treatment and control groups. Subjects will then complete two telephone assessments, the first on enrolment and the second post-partum. During these interviews, subjects will be asked a series of questions; and variables such as birth weight, gestational age at birth and delivery methods will be

recorded and analysed. Results: Thus far, 28 women have completed the study. There were 28 live births, with no major malformations. The mean birth weight was 3486.62 +/- 423.9 grams and 3630.84 +/- 355.47 in the treatment and control groups respectively. The mean gestational age was 39.43 weeks (range: 34 – 41.14) in the treatment group and 39.6 (range: 38 – 41.71) in the control group. No significant difference in birth weights between the treatment and control group was found (p=0.16). Conclusions: With the current data, no increased risk for major malformations and adverse fetal events were noted following the use of Proctofoam-HC®. Further, no difference in birth weights or birth outcomes was observed**.**

713

Concurrent use of Antimalarial Drugs with Traditional Medicinal Preparations might Affect Malaria Treatment Outcomes

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Background: Traditional antimalarial medicinal preparations are widely used concurrently with antimalarial drugs in malaria endemic areas. The effect of this practice on malaria treatment outcomes is not well documented. This study investigated the interactions between artemisinin and *Aspilia africana* (Pers.) C.D. Adams, a plant commonly used for traditional treatment of malaria in Eastern Africa. Methods: Ethyl acetate extracts of *A. africana* were investigated for *in vitro* interactions against artemisinin. The NitroBlue Tetrazolium based parasite lactate dehydrogenase was used to determine the antiplasmodial activity of extract-drug combinations against the D10 and K1 strains of *P. falciparum.* Radiouptake studies were carried out with dihydroartemisinin to study the effect of the extract on drug accumulation. Results: Interactions between the extract from this plant with artemisinin against two strains of *P.falciparum* showed an antagonist relationship against both the chloroquine-sensitive D10 and the chloroquine- and sulphonamide-resistant K1 strains of *P. falciparum.* The extract reduced accumulation of radiolabelled dihydroartemisinin by erythrocytes infected with the chloroquine-and sulphonamide-resistant K1 strain of *P. falciparum* while it increased its accumulation by erythrocytes infected with the chloroquine-sensitive D10 strain. Discussion: There is little information on antimalarial drug-herbal interactions as there is no simple approach to such studies. This study reports herbal-drug interactions between artemisinin derivatives and *A. africana* a plant which belongs to the same family as Artimisia annua. L. Conclusion: Complex interactions occur between some antimalarial medicinal plants and artemisinin and might affect treatment outcomes. An *in vitro* approach to investigating interactions between antimalarial drugs and traditional medicines is also illustrated.

714

Tolerability of Topical Cyclosporine Eye Drops is Improved by Pretreatment with Topical Loteprednol Induction Therapy

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Introduction: Cyclosporine (tCSA) eye drops are efficacious for mild to moderate chronic dry eye disease (CDED), but are poorly tolerated by 17% of patients. This study evaluated induction therapy with loteprednol etabonate suspension (LE) to improve tolerability. Methods: This prospective, randomized, placebo controlled, multi-center trial enrolled 118 CDED patients (27-80 years, 93 females, 25 males) with 61 in the LE treatment group and 57 in the artificial tears (AT) control group, Patients received either LE or AT for two weeks QID before initiation of tCSA therapy BID accompanied by AT or LE BID for an additional 6 weeks. Evaluation parameters included visual acuity (VA), Ocular Surface Disease Index (OSDI), global self assessment, fluorescein and lissamine green staining, slit lamp examination, Schirmer's tear test, and IOP. Results: LE pretreatment significantly reduced tCSA stinging ($p<0.05$) with concomitant improvement in artificial tear use, Schirmer's, fluorescein and lissamine staining. OSDI improvement was significantly greater in the LE group. Both pretreatment strategies improved global self assessment scores. IOP and VA did not change in either group. Conclusions: LE induction therapy 2 weeks before beginning tCSA improves subjective and objective clinical parameters compared to AT

alone, thereby accelerating clinical improvement. Because LE also reduces the stinging side effect of tCSA drops, the most common cause of tCSA therapy failure, LE induction therapy should be considered for patients with mild and moderate CDED, particularly when compliance may be an issue. LE induction could increase the overall number of patients who benefit from long term tCSA maintenance therapy.

715

Normal Distribution of Palpebral Fissure Lengths in Canadian School Age Children

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Fetal alcohol syndrome (FAS) is a syndrome that includes small palpebral fissures (PF) and accompanying brain dysfunction. The size of the eye and the PF relate to early growth and development of the brain and are potential physical markers for identifying children with underlying brain abnormalities. There is concern that gender and racial/ethnic populations may have different normal PF that would compromise medical interpretation of "borderline" size. Moreover, norms with standard deviations that accurately reflect the Canadian population are not available. Existing studies of racial variation have predominately focused on the American population. A large population based study was carried out to determine normative values accurately reflecting the Canadian population. A normative sample of school age children was identified in Vancouver, British Columbia and Winnipeg, Manitoba to reflect the diversity of racial and national groups in Canada. The sample included students in grades 2, 4, 6, 8, and 10 from 17 schools in Vancouver. Schools were selected based on racial diversity obtained from data from the 2001 Statistics Canada census. 1170 students in Vancouver and 900 students in Winnipeg were photographed in a standardized way, capturing only the eyes (response rate 50.5%; drop-out rate 4.36%). Photographs were analyzed using the "FAS Facial Photographic Analysis Software". Early analysis suggests that PFs do grow with age and there is little difference among boys and girls in each age group. Furthermore, results suggest that it is possible to define Canadian standards without reference to racial or regional origin.

716

Nebivolol Improves Vascular Dynamics and Endothelial Function in Healthy Subjects

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Introduction: Nebivolol is a novel selective beta1-blocker with nitric oxide (NO)-potentiating, vasodilatory effect. We evaluated the effect of single dose nebivolol on indices of vascular hemodynamic and endothelial function in healthy volunteers. Methodology: Twelve healthy volunteers (M/F: 8/4; Age- 38 ± 10 yrs) participated in the study. We measured "carotid femoral pulse wave velocity" (CF PWV- an index of large artery stiffness) and "reflection index" (RI-a marker of vascular tone), non-invasively after single oral dose of 5 mg nebivolol. We also determined digital artery endothelial dependent vasodilatory function (expressed as percentage drop in RI) to salbutamol challenge. Results: Acute administration of 5 mg nebivolol did produce significant decrease in blood pressure and heart rate. The maximal fall in systolic, diastolic blood pressures as well as heart rate (HR) were 10.3 ± 7.1 mmHg, 3.5 ± 5.0 mmHg, 18.3 ± 9.1 beats/min, respectively from baseline. *Per se*, there was no significant change either in small artery tone or large artery stiffness from baseline except for a slight increase of 1 ± 0.8 % in RI and 57.2 ± 58.9 cm/sec in CF PWV after nebivolol therapy. Inhalation of salbutamol did increase the heart rate 9.8 ± 4.3 beats/min and decreased the RI 6.0 ± 8.5 % from baseline. Treatment with nebivolol did not antagonize increase in heart rate (11.8 ± 3.8 beats/min) to salbutamol challenge. However, it potentiated the drop in RI (endothelial dependent vasodilator function) from 6.0 ± 8.5 % to 19.9 ± 7.7 %, $P<0.001$. Conclusions: Nebivolol not only has blood pressure lowering activity, but also has a favorable profile on vascular dynamics, such as preservation of vascular resistance and amplification of endothelial dependent vasodilator function.

AUTHOR NAMES BY ABSTRACT