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CSPT 2009 CONFERENCE

INNOVATIONS IN PHARMACOLOGY

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ABSTRACTS

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1

Comparing hair mercury content of women reproductive age with a lowest-observable-adverse-effect-level for neurodevelopmental effects of prenatal mercury exposure through maternal fish consumption

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Background: Methylmercury is an environmental pollutant that can cause irreversible effects on neurodevelopment. While there is no doubt that high exposure can cause neurodevelopmental deficits, the threshold that adversely affects the developing fetus is not well defined.
Objectives: To: 1) define a Lowest Observable Adverse Effect Level (LOAEL) for neurodevelopmental effects; 2) examine fish consumption habits and hair mercury content in women of reproductive age; and 3) compare hair mercury content with the LOAEL.
Methods: Mercury in hair samples was measured using Inductively Coupled Plasma Mass Spectrometry (ICPMS). Seafood consumption habits were recorded. Hair mercury concentrations and seafood consumption were correlated and compared between three groups; 1) Women who had called the Motherisk Program, 2) A Japanese population and 3) A cohort of mainstream Canadian women. A Kruskal-Wallis 1way ANOVA statistic was used to analyze variance.
Results: The LOAEL was defined at 0.3 µg mercury/g hair. The median hair mercury concentrations of the groups differed significantly ($p<0.0001$): Japanese population (1.7µg/g), Motherisk callers (0.4µg/g), and 0.2µg/g for the mainstream Canadian women. The median estimated ingested dose of mercury per month of each group also differed significantly ($p<0.0001$): 130.0, 54.4, and 24.5, respectively. Both number of fish servings and estimated ingested dose of mercury were significantly correlated with hair mercury ($p< 0.0001$).
Conclusion: Many of the Motherisk callers (68%) and all of the Japanese women exceeded the LOAEL, compared with 20% of the third group. Analysis of hair mercury content prior to pregnancy may be a novel method to protect the fetus.

2

The hypotensive and neuroprotective effects of the non-pyschotropic, atypical cannabinoid, abnormal cannabidiol, in the rat retina

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Background/Objectives: Cannabinoids and endocannabinoids have hypotensive (lower intraocular pressure), vasodilatory and neuroprotective effects in the eye. The pharmacological actions of many cannabinoids in the eye are mediated by CB1 and CB2 receptors. However, recent findings have also identified the involvement of a non-CB_1R/CB_2R cannabinoid receptor (CBx) that is activated by the behaviorally inactive atypical cannabinoid, abnormal cannabidiol (Abn-CBD). This research examined the effects of Abn-CBD on: 1) intraocular pressure (IOP). 2) retinal ganglion cell (RGC) survival in a rat model of optic nerve injury.
Methods: IOP was measured in Brown Norway rats using a hand-held rebound tonometer (tonolab). IOP measurements were made every15 minutes for two hours after i.p. drug administration. In experiments examining neuroprotection, RGCs were retrogradely labeled using fluorogold (FG) 7 days prior to axotomy. Abn-CBD was administered via intraocular injection 3 days prior to axotomy and again at 7 days after axotomy. Animals were sacrificed at 14 days post-axotomy and eyes enucleated and fixed. FG+ cells were counted in fixed retinal whole-mounts across 4 retinal quadrants.
Results: Abn-CBD produced a dose-dependent reduction of IOP in rat eyes ($p<0.01$). The IOP-lowering effect of Abn-CBD was blocked by O-1918

($p<0.01$), a selective antagonist of CB_x receptor, but was unaffected by the CB_1R antagonist AM251 or the CB_2R antagonist, AM630. Abn-CBD administration resulted in reduced retinal ganglion cell loss at 1 and 2 weeks after axotomy ($p<0.01$).

Conclusions: The ocular hypotensive and neuroprotective actions of Abn-CBD were independent of CB1R and CB2R and may be mediated by a novel CBx receptor. These findings suggest that the atypical cannabinoids that lack pyschotropic side-effects may be useful in reducing IOP and increasing neuronal survival in optic neuropathies such as glaucoma, in which increased IOP is a major risk factor.

3

Comparative study of the ocular efficacy and safety of diclofenac sodium 0.1% ophthalmic solution with that of ketorolac tromethamine 0.5% ophthalmic solution in patients with acute seasonal allergic conjunctivitis

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Objective: Only one of the several available non steroidal anti-inflammatory drugs (NSAIDs), ketorolac tomethamine is currently FDA approved for use in acute seasonal allergic conjunctivitis (SAC). The present study evaluates the efficacy and safety of diclofenac sodium in acute cases of SAC, which is already approved for relief of ocular signs and symptoms of chronic allergic conjunctivitis.

Material and methods: Sixty patients with signs and symptoms of SAC were enrolled in an open randomized parallel group study comparing diclofenac sodium (0.1%) with ketorolac tromethamine (0.5%) ophthalmic solutions. Patients instilled 1 drop 4 times daily for 14 days. Ocular signs and symptoms were evaluated at day 0, 3, 7, and 14. The principle symptoms (ocular itching, burning, discharge, photophobia) and signs (ocular inflammation, lid edema, chemosis, conjunctival mucous, keratitis) were evaluated. Significant clinical and statistical (student't' test) reductions in signs and symptoms from baseline were observed.

Results: Diclofenac sodium 0.1% was superior to ketorolac tromethamine 0.5% in reducing ocular itching ($p<0.05$) and ocular inflammation ($p<0.05$), at the final examination. At the study end (day 14), overall therapeutic response evaluated, rated diclofenac superior to ketorolac ($p<0.05$).

Conclusion: The results of this study demonstrate that diclofenac sodium 0.1% and ketorolac tromethamine 0.5% acted similarly to reduce the ocular signs and symptoms associated with acute seasonal allergic conjunctivitis. However, there was a statistically significant advantage for the diclofenac group to be free from symptoms at day 7 visit as compared to ketorolac.

4

Pharmacogenetics of tacrolimus-associated nephrotoxicity: A systematic review

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Background: Tacrolimus is presently the drug of choice for most organ transplants. Tacrolimus-induced nephrotoxicity is a serious adverse event with high rates of morbidity. Despite rigorous therapeutic drug monitoring, a substantial number of patients develop nephrotoxicity, bringing to attention the potential role of pharmacogenetic variability in adverse renal response to tacrolimus. We aimed at determining what genes have been associated with tacrolimus-induced nephrotoxicity through a systematic review.

Methods: A literature search was conducted using Pubmed/Medline, Embase and Google with the search terms 'tacrolimus", "genetics", and "nephrotoxicity" from their inception until November 2008. Additionally, reference lists of articles were searched.

Results: Of the initial 40 articles we found, 9 were relevant for our study aim (humans, genes in relation to nephrotoxicity). These studies showed positive associations between tacrolimus-related nephrotoxicity and genetic polymorphisms in CYP3A5 (kidney transplant), MDR1, CYP2C8 and ACE (liver transplant), and TGF-β (heart, kidney transplant). CYP3A5*1 and MDR1 11/22 haplotype were associated with a higher incidence of nephrotoxicity (range: 40-50% vs. 11.2%). An odds ratio of 16.67 ($CI_{95\%}$: 2.8-99.6) was found for CYP2C8*3. An increased risk of 2.6 and 4.3 was reported for Pro carriers in two codons of the TGF-β pro gene and ACE D/D gene, respectively.

Conclusion: Promising results are shown in these studies to identify patients at a higher risk of developing tacrolimus-induced nephrotoxicity after transplantation. However, further studies are needed to verify these reported associations in the same and other

organs. Presently, these results have only been obtained in adults.

5

Elevated hair cortisol levels in patients with acute myocardial infarction

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Introduction: Physical and emotional stress have been recognized as precipitants of myocardial infarctions (MI). Previous studies have found elevated salivary concentrations of the stress hormone cortisol in acute MI patients compared to control subjects. However, it is unclear if these high levels are caused by the MI, or if they reflect stress that contributed to the development of the MI. Hair analysis is a unique tool that allows longitudinal assessment of cortisol levels *prior* to an acute event. The objective of this study was to measure hair cortisol levels of acute MI patients compared to controls.

Hypothesis: Hair cortisol levels in acute MI patients reflect stress before the onset of the MI, and are increased compared to a less stressed control group.

Methods: Hair samples were collected from 2 groups: A) 60 patients admitted to hospital with acute MI within 48 hours of admission, and B) 60 control patients, admitted for other indications excluding MI. Cortisol was measured from the most proximal 3cm of hair, which represents the most recent 3 months of exposure. A modified salivary cortisol enzyme immunoassay kit was used for analysis.

Results: Hair cortisol levels are presented as median (range) and were 295.3 (105.4-809.3)ng/g in MI patients and 224.9 (76.58-949.9)ng/g in controls (P=0.006, Mann-Whitney U).

Conclusions: This study demonstrates that hair cortisol levels are elevated in acute MI patients compared to controls. Therefore chronic stress may be a contributing factor for MI, as indicated by increased hair cortisol in the 3 months prior to the event.

6

Impact of drug transport on the tissue distribution of rosuvastatin: Studies in Mrp1 knockout mice

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Background: The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, or statins, are important drugs used in the treatment and prevention of cardiovascular disease. Recently, we demonstrated a role for Organic Anion Transporting Polypeptide (OATP) 2B1 and Multidrug Resistance Associated Proteins (MRP) 1, 4 and 5 in the uptake and efflux transport as well as toxicity of statins in skeletal muscle cells *in vitro*. Here, we examined the *in vivo* role the transporters in regulating the tissue distribution of rosuvastatin in a mouse model lacking Mrp1.

Methods: The expression of transporters in mouse tissues was examined by qPCR. Wild-type and Mrp1 knockout (KO) mice were administered [^{3}H] rosuvastatin (1 mg/kg) by tail vein injection. Mice were sacrificed after 6 hrs and the tissue content of [^{3}H] rosuvastatin was examined by liquid scintillation counting.

Results: The distribution of rosuvastatin in kidney, spleen, brain, heart, and testis was similar in Mrp1 KO mice in comparison to wild-type animals. However, Mrp1 KO animals had a significantly higher liver to plasma ratio of rosuvastatin compared to wild-type animals. Importantly, we observed a lack of difference in skeletal muscle rosuvastatin distribution between Mrp1 KO and wild-type mice. Gene expression analysis demonstrated elevated levels of Mrp 2, 4 and 5 in KO mouse tissues which normally express high levels of Mrp1.

Conclusion: Tissue distribution of rosuvastatin was not different in tissues of KO animals despite that this drug is an Mrp1 substrate. This unexpected finding may be the result of differences in expression of statin transporters other than Mrp1.

7

Communicating pharmacogenetic research results: An evaluation from the participants' perspectives

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Background: Whether and how to communicate individual pharmacogenetic results to research participants is a complex and unstudied challenge. We felt it was important to provide research participants who had taken codeine while breastfeeding their individual cytochrome P4502D6 (CYP2D6) genotype results if desired. We evaluated their perceptions in regards to the communication of pharmacogenetic research results, psychosocial implications of receiving this information, and the perceived benefits.
Methods: A questionnaire was designed with help from experts in pertinent fields. Overall study results and individual genetic results were communicated. 62 codeine-prescribed individuals previously recruited in a research study answered the questionnaire; 44 "controls" (asymptomatic breastfed infants), and 18 "cases" (symptomatic breastfed infants.)
Results: 57% of those interviewed originally participated in the study for access to genetic information. 33% were negatively affected by media coverage of codeine use during breastfeeding. All participants wished to receive their genetic results; 99% felt positive/reassured after receiving their results. Pharmacogenetic information was the most important study information received for 57% of participants. 33% did not want their doctors to receive these results and 7% did not have a family doctor. Cases were significantly more likely to avoid taking codeine and/or breastfeeding in future scenarios [p=0.03; OR 4.3; 95%CI 1.3-14.5]. 68% would take codeine again while breastfeeding after receiving their genetic results.
Conclusions: Research participants want to receive their pharmacogenetic results; this does not appear to be associated with negative psychosocial impact. Communication of individual pharmacogenetic information may benefit participants by empowering them to make more informed medical decisions.

8

Preliminary analysis of cysteine mutants of human equilibrative nucleoside transporter 1

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Background: Human equilibrative nucleoside transporter 1 (hENT1) mediates the movement of endogenous nucleosides and cytotoxic nucleoside analogues across cell membranes. Studies using N-ethylmaleimide have shown cysteine residues are important in ENT1 function. To elucidate which of the 10 cysteines in hENT1 are of importance in this regard, we have embarked on a study involving the mutation of each of these cysteines to serine and the subsequent creation of stable cell lines expressing each mutant. hENT1 function was assessed by binding of the ENT1-selective probe, [^{3}H]nitrobenzylthioinosine (NBMPR), and the uptake of [^{3}H]2-chloroadenosine (2-CADO), in the presence and absence of the membrane permeable methanethiosulphonate reagent MMTS (1 mM). We have established that the C87S, C193S, C333S, and C378S mutants are comparable to wild-type hENT1 in their NBMPR binding and 2-CADO uptake characteristics. Treatment with MMTS resulted in an increase in the Bmax of NBMPR binding by ~30% for wild-type hENT1 as well as the C87S, C193S, C333S, and C378S hENT1 mutants. This suggests that the effect of sulfhydryl reagents on the binding of NBMPR involves one or more of the remaining cysteines in hENT1. In contrast to the effects on NBMPR binding, MMTS treatment resulted in a significant reduction in 2-CADO influx (Vmax) by wild-type hENT1 as well as by the aforementioned cysteine mutants. The mechanism underlying this differential effect on ligand binding and substrate uptake is currently undefined, but may reflect MMTS-induced changes in hENT1 oligomeric state.

9

Inhibition MLC1 phosphorylation decreases its degradation by MMP-2 and protects contractile function of the heart from ischemia reperfusion injury

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Background: Degradation of contractile proteins is known to occur, however, the precise mechanism for myocardial stunning remains unexplained. Matrix metalloproteinase-2 (MMP-2) contributes to cardiac dysfunction due to ischemia-reperfusion (I/R) injury by proteolysis of contractile proteins including myosin light chain 1 (MLC1). We hypothesized that MLC1 is

phosphorylated in I/R injury which increases MLC1 susceptibility to degradation by MMP-2.
Methods: We analyzed MLC1 from Langendorff-perfused rat hearts subjected to 20min of global, no-flow ischemia followed by 30min of reperfusion. Control hearts were aerobically perfused for 75min. Cardiac mechanical function was assessed. To protect from contractile dysfunction during I/R, perfusion with ML7 (inhibitor of MLC1-kinase) or Y-27632 (MLC1 phosphatase activator) was performed. MLC1 was isolated by 2-D PAGE from cardiac tissue homogenates and phosphorylation of MLC1 was analyzed by mass spectrometry (MS). The role of MLC1 phoshorylation in its degradation by MMP-2 was verified by *in vitro* studies.
Results: I/R significantly decreased mechanical function down to 33.8±7.5% of preischemic values. Both drugs (ML7, Y-27632) have shown protective effect (80.7±4.8% and 95.2±3.5% recovery respectively, in both groups as compared to the I/R group). An *in vitro* study showed increased degradation of phosphorylated MLC1 which was prevented by ML7. MS analysis of MLC1 from I/R hearts showed phosphorylations of tyrosines77, 78 and 189, as well as of threonine170.
Conclusions: Phosphorylated tyrosine189 and threonine170 occur in direct proximity to the MMP-2 cleavage site. Pharmacological treatment resulting in decreased MLC1 phosphorylation could be a novel therapeutic target to reduce cardiac systolic dysfunction resulting from I/R injury.

10

Characteristics of pregnant women using methamphetamine

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Background: Methamphetamine (MA) is a CNS stimulant that has gained popularity due to low cost, relative easy of access and high potential for abuse. This study aims to determine the characteristics of pregnant women who use MA and help identify potential reproductive risk factors associated with this group of women.
Methods: This prospective, comparative study enrolled pregnant women from The Motherisk Program who reported using MA, and a comparison group of pregnant women not exposed to any amphetamines. Maternal characteristics, patterns of alcohol, MA and other illicit drug exposures, psychiatric condition were documented. The MA exposed and non-exposed groups were matched for gestational age at initial time of call.
Results: The 218 pregnant women who used MA were significantly younger (mean 26.6 vs. 30.3 years, P<0.001), with greater risk for unplanned pregnancies compared to pregnant nonusers (100% vs. 47.3%, RR=2.1, CI(1.8-2.4)). MA users were predominantly single (71.1% vs. 20.6%; RR=3.4, CI (2.6-4.5)), and recognized their pregnancies much later (8.9 wks vs. 4.5 weeks, P<0.001). Next to alcohol and cigarette, THC (32.1%) and cocaine (25.7%) were the most concurrent illicit drugs 168 women used in addition to MA. Other agents used included were: opiods, ecstasy, psilocybin, ketamine and GHB.
Conclusions: Smoking, heavy alcohol intake, and polydrug use, combined with a higher than expected rate of unplanned pregnancies, increases the risk of fetal exposure to potentially harmful substances. By understanding the associated reproductive risk factors such as unprotected sex and polydrug use, education and preventative counseling can be targeted towards this vulnerable group of young women.

11

Hypoxia decreases equilibrative nucleobase transporter 1 (ENBT1) function in microvascular endothelial cells

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Background: In response to hypoxic and ischemic insult, extracellular adenosine levels increase in tissues leading to protective cellular responses. The vascular endothelium rapidly accumulates and metabolizes the excess adenosine resulting in increased inosine and, more importantly, hypoxanthine (HX) production. Upon the return of oxygen, HX metabolism by xanthine oxidase releases superoxide anion, contributing to oxidative damage to the endothelial cells. ENBT1 is a recently characterized, high affinity HX transporter. Since regulation of intracellular HX levels may affect later oxidative damage, we investigated the impact of hypoxia on the HX transport function of ENBT1 in microvascular endothelial cells (MVECs). In human cardiac and mouse skeletal muscle MVECs, as well as PK15 nucleoside transporter deficient (PK15NTD) cells, induction of hypoxia by layering the cells under a mineral oil barrier for 2 hours caused dramatic decreases (>50%) in

ENBT1 mediated [^{3}H]HX uptake. In contrast, this treatment had no effect on substrate uptake by ENT1 (equilibrative nucleoside transporter) in the MVECs. To test if this effect was mediated via stabilization of hypoxia inducible factor α (Hif), mMVECs and PK15NTD cells were incubated with 100μM $CoCl_2$ for 2 hours. $CoCl_2$ treatment resulted in a change in V_{max} of [^{3}H]HX uptake in mMVECs to 21 ± 10% of control ($P<0.05$) but had no effect in PK15NTD cells. These data suggest that ENBT1 is down-regulated under hypoxic/ischemic conditions by both Hif- dependent and -independent mechanisms, with potentially significant consequences for purine metabolism and oxygen free radical production by endothelial cells.

12

Effect of organic anion transporting polypeptide 1B1 (OATP1B1/*SLCO1B1*) polymorphisms on plasma concentrations of atorvastatin and its metabolites in patients

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Background/Objective: The statin class of HMG Co-A reductase inhibitors is widely prescribed to manage hypercholesterolemia by inhibiting cholesterol synthesis in the liver. High systemic exposure may increase the risk of developing serious side effects including the rare but life-threatening form of muscle injury known as rhabdomyolysis. However, plasma levels of statins in patients are rarely measured or published, due to technical and practical challenges associated with quantification. There is growing appreciation for the importance of transport processes in mediating statin disposition. In particular, a relatively common polymorphism in organic anion transporting polypeptide 1B1 (OATP1B1/*SLCO1B1*) has recently been shown to be an important risk factor for the development of statin-induced muscle toxicity. We hypothesize that *SLCO1B1* genotype affects plasma concentration of atorvastatin and its metabolites in our patient population.

Methods: A highly sensitive method for measuring plasma concentrations of atorvastatin and its metabolites in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using lovastatin as an internal standard was developed. We obtained Research Ethics Board approval to collect blood samples from consented patients receiving statin therapy in order to measure plasma statin levels as well as genotype for *SLCO1B1* polymorphisms.

Results: Preliminary analysis indicates extensive interindividual variability in plasma atorvastatin acid and lactone concentrations, even among patients taking the same dose.

Conclusion: Characterization of atorvastatin levels and transporter polymorphisms will provide a better understanding of the range and variability of systemic exposure to atorvastatin in our patients, and further insight into the role of *SLCO1B1* polymorphisms in mediating this exposure.

13

Reducing toxicity in patients receiving warfarin and amiodarone for atrial fibrillation through extended anticoagulation clinic services

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Funding Source: None

Background: Anticoagulation clinics already see patients regularly to monitor warfarin in patients with atrial fibrillation, many of whom will also receive amiodarone during their association with the clinic. However, a knowledge gap exists regarding the monitoring of amiodarone in the community. Therefore, we explored ways that anticoagulation pharmacists could extend monitoring to include amiodarone with minimal extra effort. A knowledge synthesis of recommendations for amiodarone monitoring, tailored by local stakeholders, suggested simplified monitoring using only 3 parameters (freeT4, ALT, serum amiodarone) be added to INR at 1, 3 and 6 months following drug initiation. A decision tree enables screening for early abnormalities or undesirable amiodarone exposures that should be referred to a physician for further assessment. Our objective was to qualitatively describe acceptance of this tool to promote knowledge of amiodarone safety monitoring. This project showed that anticoagulation pharmacists and patients accepted the small inconvenience of extra tests and physicians became more aware of amiodarone-warfarin interactions as well as the importance of ongoing amiodarone monitoring. During the pilot, 1 patient was referred with thyroid abnormalities, 1 with elevated ALT, and 4 with undesirable amiodarone concentrations. A pathway outlining systematic amiodarone monitoring appears to be an acceptable tool for translating

knowledge to the community. Early indications suggest it is practical to apply this as a simple extension of existing anticoagulation monitoring and has the potential to identify patients at greatest risk of amiodarone toxicity for closer attention and dose adjustment by a physician.

14

Attenuated arterial and venous constriction in a rat model of type 2 diabetes

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Background: This study investigated if mean arterial pressure (MAP) and mean circulatory filling pressure (MCFP, index of body venous tone) responses are impaired in a new model of type 2 diabetes, induced through high fructose feeding (60% of caloric intake) and subsequent injection of streptozotocin (STZ, 60 mg/kg *i.v.*). Wistar rats (5 weeks old) were fed a normal or high fructose diet starting day 0 until the end of the study. After 2 weeks, half of the rats in each diet regimen were given STZ. Following 6 weeks of feeding, the effects of noradrenaline on MAP and MCFP were determined in the four groups of conscious rats. Both the STZ and fructose-STZ groups had significantly higher blood glucose and reduced MCFP responses to noradrenaline, relative to the groups not given STZ. The fructose-STZ rats also had increased ED_{50} (reduced potency) of MAP response to noradrenaline compared to the other three groups; however, maximum MAP responses were similar among the four groups. These results show that arterial and venous constrictions are attenuated in the fructose-fed, STZ-induced model of type 2 diabetes.

15

Optimizing periconceptional folic acid supplementation: is there a role for therapeutic drug monitoring?

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Conflict of Interest: Dr. G. Koren is a medical consultant for Duchesnay Inc. and a primary investigator for several studies investigating prenatal multivitamin supplementation. All other authors declare no conflict of interest.

Introduction: Folic acid fortification and guidelines for supplementation have helped reduce the incidence of neural tube defects (NTDs). However, current data indicate that many women could further reduce their risk for NTDs by improving their folate status.

Objective: To review and synthesize the evidence for TDM of folic acid for the prevention of NTDs.

Methods: PubMed and MEDLINE searches were conducted from their inception to January 2009. Search terms included "folic acid", "neural tube defects", "folate concentrations", and "preconception care".

Results: There is an inverse relationship between maternal folate concentrations and risk for NTDs. Erythrocyte folate concentrations above 906 nmol/L are associated with a very low risk for NTDs. The upper limit for folate concentrations is not known, however, potential risks of chronic exposure to high levels of folate have been proposed. Many factors affect a woman's folate status, but only few are considered in current guidelines for supplementation. As a result, achieving highly protective folate concentrations remains a challenge for many women. There are no outward manifestations of folate concentrations below 906 nmol/L but above the cut-off for clinical folate deficiency. However, reliable methods for quantifying blood folate concentrations exist and are utilized in many clinical laboratories.

Conclusions: Periconceptional folic acid supplementation for the prevention of NTDs satisfies the criteria for TDM. Monitoring of folate concentrations could be an effective tool for healthcare providers to identify and counsel individual women who possess a higher-than-baseline risk for NTDs due to sub-optimal folate status.

16

Does carbon monoxide play a role in dependence on cigarette smoke?

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Funding Source: Heart & Stroke Foundation of Ontario

Background/Objectives: Nicotine is generally credited for dependence on cigarette smoke. But cigarette smoke comprises over 3,000 chemicals each of which has the potential for biological activity. In the last decade, the gasotransmitters- nitric oxide, carbon monoxide (CO) and hydrogen sulfide- have made their mark as biologically important regulatory molecules. We addressed the hypothesis that dependence on cigarette smoke requires nicotine and CO.

Methods: A total of 131 healthy smokers (62 females and 69 males with informed consent) were recruited. Subjects were randomly assigned to receive either CO (4% in air) or air, and either nicotine nasal spray or nasal spray placebo (the nasal sprays were a gift from Pharmacia Upjohn Sweden). Each subject was studied on three consecutive days. Craving for cigarette smoke was evaluated by means of a questionnaire (Schiffman-Jarvik). Analysis: Means and standard deviations were calculated for time by group for each study day. Repeated measures general linear modelling (GLM) was used to examine differences in change scores from baseline to follow-up across study group.

Results: All treatments, including air/nasal spray control, resulted in significant decreases in craving. There was substantial variability in decreases in craving within each day and between days. The combination of CO/nicotine usually induced the greatest decrease in craving for cigarette smoke. There was no difference in the volume of gas inhaled among the four groups.

Conclusions: The placebo effect on craving for cigarette smoke was very strong. CO may play a role in craving for cigarette smoking. The hypothesis deserves further investigation.

17

Determination of quetiapine in human breast milk

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Funding Source: None

Background: The advantages of breastfeeding are well documented. Risk assessment becomes complicated if the mother needs medications. Psychiatric disorders are not uncommon in women of childbearing age. Drug therapy is often necessary. Data on quetiapine excretion in to breast milk is lacking.

Methods: Nine breast feeding women who were taking quetiapine or planed to stop breast feeding while using quetiapine were included. We collected 7 milk samples (before medication, 1, 2, 4, 7, 12, 24 hr after) of each individual and also 1 blood sample 2 hrs after taking medication. Quetiapine in milk and plasma was quantified by high-performance liquid chromatography. For each individual area under the milk concentration time curve (AUC), milk/plasma drug concentration ratio (M/P ratio) at maximum milk concentration, and maximum milk concentration/dose (C/D) ratio were calculated.

Results: One patient received an extended formulation 300 mg/day. We summarized the remaining 8 patients who received immediate-release formulation (mean daily dose: 36.7 mg; range 6.25-100 mg). Mean M/P ratio was 0.41 (range: 0.14-1.67). Mean C/D ratio was 1.51(range: 0.78-2.22). No medical event was experienced in the group.

Discussion & Conclusion: Given the maximum C/D is 2.22, estimated maximum milk concentration of quetiapine is 222 nmol/L(0.085 mg/l) at 100 mg/day maternal dose. Estimated maximum infant dose of the drug through milk is 13 microgram/kg/day (roughly 10% of the lower maternal dose on a weight basis). Since the drug is mainly metabolized by the most common cytochrome (P450 3A4), maternal quetiapine therapy does not pose significant exposure risk to the breastfed infants.

18

Nausea and vomiting of pregnancy; using the 24 hour pregnancy unique–quantification of emesis (PUQE-24) scale

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Conflict of Interest: Motherisk NVP line is supported by an unrestricted grant by Duschenay, Inc. Canada, although no specific financial support was given for this study. Dr. Gideon Koren is the holder of the Research Leadership for Better Pharmacotherapy during Pregnancy and Lactation (Toronto), and the Ivey chair in Molecular Toxicology (University of Western Ontario)

Background/Objective: Nausea and vomiting in pregnancy (NVP) is a clinical condition affecting between 50-80% of all pregnancies. In 2002 the Pregnancy Unique Quantification of Emesis (PUQE) was developed by Motherisk from the Rhodes scale and was the first scale of its kind to focus on nausea and vomiting specific to pregnancy. In 2006 we revised the scale to a 24 hour scale such that the time spent sleeping is accounted for. The objective of the present study was to validate this new 24 hour version of PUQE.

Method: Information regarding daily liquid intake, number of hours and quality of sleep, multivitamin usage, hospitalization, and well-being scores, was collected from 315 women calling Motherisk NVP line between 2005-08. The PUQE score for each patient was calculated using the three criteria for NVP assessment: hours of nausea, and number of episodes for retching and vomiting in 24 hours. Linear regression analysis was used between PUQE-24 scores and well-being, hours of sleep and liquid intake. Chi square analysis was performed to compare the severity

of PUQE-24 and quality of sleep, multivitamin use, and rates of hospitalization.
Results: PUQE-24 scores were highly predictive of women's propensity to take multivitamins; rate of hospitalization due to NVP, and well-being.
Conclusion: PUQE-24 is a reliable tool for the assessment of the severity of NVP symptoms. The simplicity of its use and its specificity to NVP symptoms make it a valuable assessment tool for clinicians and researchers.

19

Unexposed siblings as controls for genetic confounders in behavioral teratology studies. Novel methodology: preliminary results

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Funding Source: None
Background/Objectives: The neurocognitive development of children exposed to medications in pregnancy is an inseparable part of drug safety assessment in teratology. Neurodevelopmental studies are complicated by multiple confounders, and study design plays an integral role controlling for them. The authors present a novel approach to studying behavioural teratology, where genetic and environmental factors are controlled by testing unexposed siblings.
Methods: IQ scores of children exposed to Venlafaxine (VLF) (n=27) are compared to those of their unexposed siblings (n=27) and to healthy controls (n=27). Participants were matched for age, gender and order of delivery. The primary outcome measure was the children's Full Scale IQ measured by the WPPSI-III Scales of Intelligence. Statistical analysis accounted for the clustering effect, with correlation and regression also performed.
Results: Full Scale, Performance and Verbal IQs were not different between the VLF-exposed children and their unexposed siblings (102±13 vs.106±13; 100±14vs104±12; 105±12vs107±16), respectively. Healthy controls scored significantly higher than the VLF-exposed on Full Scale (113±12, P=0.02), Performance (108±12, P=0.004), and Verbal IQs (114±13, P=0.04). Maternal IQ and the number of depressive episodes after delivery were significant predictors of children's cognitive performance. There were no differences between the groups in maternal IQ, socioeconomic status or children's physical characteristics.
Conclusions: Preliminary results showed that VLF is not neurotoxic. Sibling assessment should be included into behavioural teratology study design. It helps to separate the effect of the drug from genetic and environmental factors, and to provide strong evidence in drug safety studies.

20

Increased degradation of MLC1 by MMP-2 in cardiomyocytes subjected to ischemia is associated with nitration and nitrosylation of MLC1 molecule

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Funding Source: Canadian Institutes of Health Research and the Saskatchewan Health Research Foundation
Background: Increased level of peroxynitrite ($ONOO^-$) may lead to the development of systolic dysfunction of heart following ischemia reperfusion injury (I/R). It has been shown that cardiac myosin light chain 1 (MLC1) becomes nitrated during biological aging. MLC1 is a substrate for matrix metalloproteinase-2 (MMP-2) during I/R injury in the heart. In this study we hypothesize that modification of MLC1 by $ONOO^-$ during ischemia plays an important role in the regulation of its susceptibility to degradation by MMP-2.
Methods: Isolated rat cardiac myocytes were subjected to 15 and 60 minutes of ischemia and MLC1 levels were measured by 2-dimensional electrophoresis. MMP-2 activity was assessed by zymography. In in vitro studies cardiac MLC1 was incubated with 10μM to 1mM ONOO- which was followed by incubation with MMP-2. Nitrations and nitrosylations of MLC1 were identified by mass spectrometry (MS). The nitration level of cardiomyocyte MLC1 was evaluated by immunobloting and HPLC.
Results: Analysis of nitrotyrosine levels in cardiomyocytes (marker of ONOO- formation) indicates that ischemia enhances nitration of MLC1 and the in vitro study showed that nitration/nitrosylation increases its degradation by MMP-2. Mass spectrometry analysis of MLC1 showed nitration of tyrosine 190, which is localized in the cleavage site for MMP-2, and nitration of tyrosine 78, as well as nitrosylation of cysteine 81. High correlations between duration of ischemia, MMP-2 activity and MLC1 level were observed.

Conclusion: Simultaneous inhibition of MLC1 nitration/nitrosylation and inhibition of MMP-2 activity may provide a novel pharmacological approach for heart protection in clinical settings of I/R injury.

21

In-utero exposure to ionizing radiation in a woman with Hodgkin's lymphoma – A case report and a review of the literature

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Background: Exposure to high doses of ionizing radiation in pregnancy correlates with an increased risk of malformations and other complications in the offspring. Few cases on radiotherapy use during pregnancy are reported, all with normal outcomes. However, short follow-ups prevent definitive conclusions.

Objective: Our objective was to present a child exposed in- utero to radiation in the first trimester, whom we followed until 18 months of age and to systematically review the literature on effects of radiation on pregnancy outcomes.

Methods: We conducted a literature search using Medline to identify publications regarding radiation exposure during pregnancy and postnatal outcomes.

Results: Congenital anomalies and growth impairment are reported among children exposed in-utero to radiation, but effects are only seen after high doses (50 cGy). In our case, estimated dose to the fetus was 12 cGy, given to the mother in the mediastinum for Hodgkin's disease. The child was born without malformations or growth retardation, but had delayed postnatal growth noted at age of 18 months, in particular his head circumference (3rd percentile).

Conclusion: Radiotherapy should not be contraindicated in pregnant patients with cancer, especially if remote from the pelvis. Fetal radiation doses should be estimated by qualified medical personnel (i.e. medical physicist or radiation oncologist) and discussed on an individual basis with the patient. In our case report, correlation between small head size of the infant and radiation exposure during pregnancy is still uncertain and requires more in depth investigations to rule out other etiologies.

22

Prediction of infant drug exposure through breastfeeding: A proof-of-principle study with population PK modeling and simulation of fluoxetine

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Background: Risks of significant infant drug exposure through breastmilk are poorly defined for many drugs. We used population pharmacokinetics (PopPK) modeling and simulation to obtain population estimate of infant drug exposure through breast milk.

Methods: Using plasma and milk fluoxetine (FX) and norfluoxetine (NFX) concentration profiles from 25 breastfeeding women, a PopPK model was developed with NONMEM. An integrated 1 compartment model with scaling factors was chosen as most appropriate. Individual variation was modeled as an exponential distribution. PopPK parameters including milk-to-plasma ratio (MPR) were estimated. Infant FX and NFX dose relative to maternal dose and infant plasma concentrations were estimated from 1000 simulated mother-infant pairs, using random assignment of feeding times and milk volume ingested. A conservative estimate of CYP2D6 activity of 20% of the weight-adjusted adult value was assumed.

Results: Derived model parameters, including MPR (median: 0.59 FX; median: 0.49 NFX) were consistent with those reported in the literature. Visual predictive check and other model diagnostics showed no model misspecifications. Estimated FX RID was below 10% in >99% of simulated infants. Predicted median infant-mother Css ratio was 0.093 (range 0.033 - 0.31), consistent with literature reported values (mean=0.07; range 0-0.59). Moreover, predicted incidence of infant-mother Css ratio of >0.2 was <1.3%.

Conclusion: Predictions from our model are consistent with clinical observations, suggesting that substantial exposure to FX through breastmilk is rare. Our approach may be valid for other drugs, and may allow in silico prediction of drug PK in extreme, rare, situations.

23

A multi-center analysis of severe adverse cutaneous reactions in pediatric patients

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Introduction: Severe cutaneous drug adverse reactions such as Stevens–Johnsons syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) are rare, but life-threatening complications of medications. Their characteristics, long term sequelae and response to therapy in particular, are not well described in children.

Methods: We analyzed all SJS/ TEN cases treated in two tertiary-care pediatric hospitals in North America between 2004 and 2007. Cases were identified using ICD-10 discharge codes; data was manually extracted from patients' charts.

Results: We identified 33 cases, 20 males and 13 females. The mean age at presentation was 9 ± 3 years (range 1–18). Twenty nine children had SJS and 4 had TEN. Eleven patients had confirmatory skin biopsy. Length of hospital stay ranged from 2 to 54 days. The most common confirmed etiologies were drugs (11 [33%]; antiepileptics [7], sulfonamide antibiotics [4]) and mycoplasma infection (10); specific drug was undetermined in 12 patients. Treatment included systemic corticosteroids (9) and intravenous immunoglobulins (7), which showed beneficial effects. Both treatments were used in 6 cases. There were no deaths. 7 patients (21%) had recurrent SJS/TEN episodes following different drug exposures, 5 had multiple recurrences. 14 patients (42%) had long-term sequelae, including skin hyper/ hypopigmentation (10), ophthalmologic complications (8) and bronchiolitis obliterans (1).

Discussion: Prognosis of SJS/ TEN in children appears more favourable compared to adults, but long-term sequelae are common. Certain individuals may be prone to recurrences. Long term follow up of these patients is important.

24

Antioxidant effect of a methanol extract of garcinia kola biflavonoid "Agbilu"

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Funding Source: None

Background: *Garcinia kola* is used in treatment of various diseases such as diarrhea, hepatitis, asthma, dysmenorrheal and dysentery. Spasmolytic interaction between *G. kola* and oxytoxin in the estrogen primed virgin rat uterus markedly diminished toxic spasms. Antioxidants such as vitamin C appear to limit the damage caused by free radicals. The effect of a methanolic extract of *G. kola* on oxidation was evaluated.

Methodology: A titration method using 2,6-dichlorophenol-indophenol was used to evaluate the antioxidant properties. The effect of $CuSO_4$, NaCl, cysteine and a methanolic *G. Kola* extract was evaluated in the presence of ascorbic acid.

Result: The titration values at 20 minutes were 6.12 ml for ascorbic acid alone, 15.00 ml for NaCl with ascorbic acid, 21.53 ml with cysteine, 2.63 ml with $CuSO_4$ and 34.92 ml with the *G. kola* extract in the presence of ascorbic acid.

Conclusion: The *G. kola* methanolic extract inhibited vitamin C oxidation. It might therefore be considered as exhibiting antioxidant or anti-oxidant synergist properties.

25

Characterization of the porcine constitutive androstane receptor (CAR) and its splice variants

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Background: The Constitutive Androstane Receptor (CAR; NR1I3) acts as a xenosensor, regulating the expression of enzymes involved in the metabolism of xenobiotic and endobiotic compounds. The goal of this study was to characterize CAR from the pig (pgCAR) compared to CAR from mouse (mCAR) and human (hCAR). The wild type pgCAR coding region was 86% and 75% homologous to hCAR at the nucleotide and protein levels, respectively. Five alternatively spliced forms of pgCAR were identified, all of which had frameshift mutations resulting in the premature stop codons and truncated protein products. Real-time PCR analyses showed that these splice variants were present in liver cDNA samples from 4.61% to 9.20% of the total pgCAR present. In a luciferase reporter assay, pgCAR and hCAR responded similarly for more

ligands than hCAR and mCAR. The hCAR agonist CITCO also activated pgCAR, while the mCAR agonist TCPOBOP had no effect on pgCAR. 5β-Dihydrotestosterone was identified as a novel inverse agonist for both pgCAR and hCAR, with no effect on mCAR. None of the pgCAR splice variants were active in the luciferase reporter assay on their own. When present with the wild type pgCAR, splice variant 2 (SV2) had a potent dominant negative effect on the activation of pgCAR by CITCO and all splice variants attenuated the repression of the wild type pgCAR by 5β-dihydrotestosterone. In summary, pgCAR activity is controlled by some ligands that also affect hCAR, and alternatively spliced variants of pgCAR can have dominant negative effects on the wild-type receptor.

26

Warfarin oral anticoagulation therapy: *R/S*-warfarin and vitamin K levels as determinants of maintenance dose

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Funding Source: The Drug Innovation Fund from the Ontario Ministry of Health and Long-Term Care
Background/Objectives: Warfarin is a widely prescribed anticoagulant known for marked interindividual variation in drug responsiveness. Polymorphisms in genes affecting warfarin metabolism (cytochrome P450 2C9) and activity (vitamin K epoxide reductase complex 1) only explain 50% of the dose variation in patients, suggesting that other patient-associated parameters are important determinants of the observed warfarin response. We hypothesized that in addition to genetic factors, the interplay between *R/S*-warfarin (antagonist) and vitamin K (agonist) plasma levels is a key predictor of warfarin response.
Methods: Consented patients starting warfarin therapy were enrolled in our study and blood samples were collected on treatment days 3, 5, and 8. Highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays were developed and validated for measuring plasma *R/S*-warfarin enantiomers as well as vitamin K levels. The calibration curves for warfarin enantiomers and vitamin K were linear over the range of 1-1000 ng/mL with a lower limit of quantification of 1 ng/mL.
Results: Warfarin levels were analyzed in 25 patients and it was evident that variable warfarin levels are at least in part responsible for the unpredictable anticoagulant response.
Conclusions: We describe robust, specific, and sensitive assays for warfarin enantiomers and vitamin K in plasma and their application in the assessment of anticoagulation response in patients. It is expected that warfarin drug levels coupled with plasma vitamin K level analysis will provide further insight and a basis for creating a more robust and predictive genetics-based dosing algorithm for individualized warfarin therapy.

27

Pharmacogenetics of fatal hydrocodone intoxication in a child

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Funding Source: The Canadian Pharmacogenetic Network for Drug Safety
Background: Hydrocodone is a semi-synthetic upload with analgesic and antitussive properties and is widely used for the treatment of cold-related cough in adults and children. Half of total hydrocodone clearance is determined by oxidative metabolism via cytochrome P4502D6 (CYP2D6) and P4503A4 (CYP3A4) into hydromorphone and norhydrocodone respectively. Fatal upload toxicity occurred in 5 year 9 month old child who received high doses of hydrocodone for a respiratory tract infection.
Methods: Postmortem blood was screened using gas chromatography mass spectrometry (GC-MS) for the presence of common drugs and/or poisons. Allele discrimination of CYP2D6 *4, *9, *10, and *41 was performed and CYP2D6 copy number was quantified.
Results: A full autopsy revealed no pathological etiologies for the death. Blood concentration of hydrocodone was determined at 0.14 μg/ml; this concentration is associated with fatality in adults. In contrast, hydromorphone concentration was below the limit of detection. Genetic analysis revealed the child had a reduced capability to metabolize the drug via the CYP2D6 pathway. The co-administration of clarithromycin further prevented the elimination of the drug from the body by inhibiting CYP3A4, and thus contributing to the lethal hydrocodone concentration observed.

Conclusions: Pharmacogenetic factors must be considered when children are prescribed hydrocodone, and the concomitant administration of hydrocodone and clarithromycin should be avoided.

28

Long-term neurodevelopment of children exposed in utero to venlafaxine

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Introduction: Venlafaxine (VLF) is widely used by women of childbearing age. Its effects on fetal central nervous system development have not been studied. Objectives were to determine neurocognitive development of children exposed *in-utero* to VLF using standardized psychological tests, and to compare outcomes to children exposed to SSRIs and to non-teratogens.

Methods: Cohort study using a prospectively collected database. Three groups of mother-child pairs (VLF-exposed, SSRI-exposed, and exposed to non-teratogens) were matched for maternal age at conception, and child's gender and age at testing. Psychometrists were masked to subjects' exposure. The main outcome measure was children's Full Scale IQ. Statistical analysis included ANOVA, t-test, GLM repeated measures, and linear regression.

Results: VLF and SSRI-exposed children did not differ in Full Scale IQ (104.1±13, 105.1±12, p=1.00). Non-teratogen-exposed children achieved significantly higher scores on Full Scale IQ than VLF-exposed (112.3±11vs.104.1±13, p=.002) and SSRI-exposed children (113±11vs.105.1±12, p=.007). Similar results were achieved in Performance and Verbal IQ scores. Regression analysis revealed that maternal IQ, duration of maternal depression, and number of depression episodes after delivery were significant predictors of children's neurocognitive performance.

Conclusion: Exposure to VLF or SSRIs was not associated with child's brain neurotoxicity. Genetic and environmental factors, such as maternal IQ, duration of maternal depression and number of depressive episodes following delivery, were strongly associated with children's intelligence. If indicated, VLF can be used to control maternal depression in pregnancy.

29

Functional impact of a novel single nucleotide polymorphism (SNP) in the proton coupled folate transporter

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Background/Objective: Folates are essential nutrients required for purine and thymidylate synthesis. Folate deficiency is associated with pathological conditions including neural tube defects and cardiovascular disease. Recently, the proton-coupled folate transporter (PCFT) has been characterized as a pH-dependent folate transporter. Loss-of-function mutations in this transporter have been identified as the molecular basis for hereditary folate malabsorption. Recently, a PCFT SNP with a T insertion in exon 4 (PCFTins) has been identified. The objective of this study was to determine the functional relevance and allele frequency of this polymorphism. As this polymorphism causes the formation of a truncated protein product we hypothesized that it would confer decreased transport activity.

Methods: Folate and methotrexate uptake studies were carried out *in vitro*, using HeLa cells transiently transfected with wild-type PCFT or PCFTIns. We also created MDCKII cell lines stably expressing wild-type GFP-PCFT or GFP-PCFTIns to assess localization using confocal microscopy. In addition, we developed an RFLP genotyping method to determine the allele frequency of PCFTIns.

Results: We observed a near complete loss of folate and methotrexate uptake in PCFTIns compared to wild-type in transiently transfected HeLa cells ($p < 0.05$). Confocal microscopy showed proper cell surface expression of this transporter. Screening of 100 human DNA samples failed to detect the PCFTIns polymorphism suggesting very low allele frequency.

Conclusion: Our data clearly demonstrate that PCFTIns has impaired transport of folate and antifolate drugs, indicating a severe loss-of-function and that this SNP is likely to be rare among folate replete healthy subjects.

30

In vitro platelet toxicity assay (PTA): A novel diagnostic test for drug hypersensitivity syndrome

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Background: Drug hypersensitivity syndrome (DHS or DRESS) is a rare but potentially fatal adverse drug reaction (ADR) which develops in susceptible patients following exposure to certain drugs including aromatic anticonvulsants and sulphonamides. Diagnosis of this type of ADR has always been a difficult task due to its ill-defined clinical picture and lack of validated and safe diagnostic tests. The lymphocyte toxicity assay (LTA) is an *in vitro* diagnostic test that has been used with some success for the diagnosis of DHS; however, its true sensitivity and specificity have always been controversial. Furthermore, the procedure of the LTA involves isolation of peripheral blood monocytes (PBMCs), a step that complicates and prolongs the test.
Objectives: To develop and validate a simple *in vitro* diagnostic test for DHS based on platelets.
Method: Blood samples were withdrawn from healthy volunteers with no prior exposure to the tested drugs and from patients with medical history highly suggestive of developing DHS. Platelets were isolated by differential centrifugation and their susceptibility upon incubation with the culprit drugs was measured using MTT method.
Results: Upon incubation with the culprit drugs, platelets from hypersensitive patients were significantly (20%to 60%, $p< 0.05$) more susceptible to cell death than platelets from healthy volunteers.
Conclusion: Platelets can efficiently replace PBMCs as an easy surrogate cell model in detecting susceptibility of patient cells to death upon incubation with the drug. This procedure provides a potential simple and quick test to screen for patients susceptible to develop DHS prior to prescribing the drug.

31

Increased degradation of myosin light chain 1 by matrix metalloproteinase-2 results in cardiac dysfunction in an animal model of neonatal asphyxia

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Background: Asphyxia causes adverse cardiovascular effects in neonates. Increased myosin light chain1 (MLC1) degradation could lead to heart injury. Hypoxia-reoxygenation (H-R) is observed during asphyxia and resuscitation of newborns. This study aimed to determine if MLC1degradation by matrix metalloproteinase-2 (MMP-2) is associated with H-R and whether this results in decreased heart function. Impact of peroxynitrite ($ONOO^-$) on the degradation in experimental H-R was investigated.
Methods: 12 newborn piglets were instrumented to measure hemodynamic parameters. Animals were randomized to a normoxia group and a H-R group (exposed to normocapnic hypoxia (2h) followed by reoxygenation (4h)). Hearts were snap-frozen for subsequent biochemical analyses. MLC1 level was assessed by 2-dimensional electrophoresis and immunoblot. MMP-2 activity was measured using zymography. Modifications of MLC1 were investigated using MS/MS analysis. Nitrate levels were measured as a marker of $ONOO^-$ biosynthesis.
Results: In H-R, MLC1 was degraded due to increased activity of MMP-2 and $ONOO^-$production. MLC1 level was correlated with myocardial function during H-R. Increased $ONOO^-$ production activated MMP-2 and modified MLC1 (nitration and nitrosylation of amino acids located in the cleavage site for MMP-2) thus enhancing its susceptibility to enzymatic degradation. Strong correlations between MLC1 level and MMP-2 activity in normoxic hearts suggest that MMP-2 plays an important role in physiology by modulation of MLC1 level.
Conclusions: In newborn piglets with H-R, the impaired myocardial function was associated with MLC1 degradation and MMP-2 activation. Controlled reoxygenation and pharmacological treatment with MMP-2 inhibitors and/or inhibitors of MLC1 nitration/nitrosylation may help reduce heart injury in neonatal asphyxia.

32

Use of octreotide in children with sulfonylurea intoxication at the Hospital for Sick Children

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Introduction: Unintentional poisoning with sulfonylureas is a significant danger to infants and children as the ingestion of relatively small amounts can be fatal. Administration of octreotide is considered effective in patients hypoglycaemic despite glucose administration. However, experience in children is limited. We reviewed the clinical features of children presenting with sulfonylurea intoxication at The Hospital for Sick Children in Toronto.

Methods: A retrospective chart review of all patients with sulfonylurea intoxication presenting between April 2001 and November 2008 at The Hospital for Sick Children.

Results: Four patients were identified as sulfonylurea overdoses (mean age: 8.2 years; range 1.5 – 15). All patients were exposed to glyburide and developed severe hypoglycemia. Two patients were toddlers and two teenagers. Ingestion was accidental in the case of the toddlers, and suicidal attempts in the case of the adolescents. All patients were initially treated with glucose infusions. Both toddlers also received subcutaneous octreotide with good response and no rebound hypoglycemia; Blood sugar levels remained stable with no further symptoms of hypoglycemia. On the other hand, the two teenagers were treated only with prolonged glucose infusions; in both cases rebound hypoglycaemia and increased glucose requirements were observed.

Discussion: Glyburide-induced hypoglycemia was pronounced in all patients identified. Treatment with octreotide seemed effective in the 2 infants treated. This is in agreement with the limited experience reported to date in the literature, and suggests that octreotide should be considered the treatment of choice also in children.

33

Nicotine replacement therapy (NRT) in pregnancy: recommend or not recommend?

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Funding Source: None

Background: Smoking during pregnancy increases risks of maternal and fetal complications and adverse neonatal outcomes. Although pregnancy is often a strong motivator for smoking cessation, many pregnant women continue smoking. Thus, effective smoking cessation strategies during pregnancy are clearly needed. Behavioural support provided by prenatal smoking cessation programs is safe and effective during pregnancy but generates relatively modest reduction in smoking cessation rates. Hence, addition of Nicotine Replacement Therapy (NRT) may offer an effective alternative to help pregnant women to quit smoking. This suggestion is based on the convincing research evidence of effectiveness of NRT in general population. Currently there is no consensus among health care providers whether or not recommend NRT during pregnancy. The main concern is about its safety and effectiveness for a pregnant woman and her fetus.

Objective: To review up-to-date data on safety and effectiveness of NRT trials in pregnancy and provide recommendations for clinicians regarding NRT use in this population.

Methods: An extensive literature search was undertaken to identify original studies and critical reviews that assess the evidence of safety and effectiveness of NRT use in pregnancy.

Conclusion: Based on the research data available, it is prudent to advise light pregnant smokers to quit without NRT, using behavioural support. Moderately and highly addicted pregnant women, who cannot achieve abstinence with behavioural intervention, may use NRT after considering possible benefits and risks of this intervention with their physicians. Future clinical trials should take into consideration changes in nicotine metabolism during pregnancy especially when planning doses schedule of NRT.

34

Reversing prediabetic insulin resistance by a synergy of drugs mimicking a feeding signal.

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Background: 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 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 pharmacological provision of both feeding signals in a prediabetic animal model.

35

C-tail mediated modulation of human somatostatin receptor type-4 (hSSTR4) homo-and heterodimerization, receptor internalization and cell signaling

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Background/Objectives: Somatostatin receptors (SSTRs) have shown great diversity in response to agonist-mediated homo-and heterodimerization in receptor specific manner. SSTR subtypes functions as dimers and display homo-and heterodimerization within the same family as well as with the members of distinct family. In the present study, using morphological, biochemical and biophysical techniques, we studied the status of hSSTR4 and determined receptor-homodimerization, receptor-coupling to adenylyl cyclase and effect on downstream signaling molecules with/without C-tail.

Results: *wt*-hSSTR4 and R4-CR5 exists as homodimers in basal as well as in presence of Somatostatin (SST) whereas hSSTR4 mutants R4□CT and R4-CR1 displayed no homodimers. hSSTR4 and R4-CR1 exhibited significant inhibition of ERK1/2 phosphorylation in presence of SST whereas R4-CR5 completely blocks ERK1/2 phosphorylation. Changes in ERK5 were opposite to ERK1/2 in presence of SST. hSSTR4 and different chimera exhibited increased $p27^{kip1}$ as an index of inhibition of cell proliferation consistent with 10-40% inhibition accomplished by MTT assay. *wt*-hSSTR4 exhibited strong interactions with *wt*-hSSTR5 and displayed relatively higher FRET efficiency in basal and treated conditions but not with *wt*-hSSTR1. In cells cotransfected with *wt*-hSSTR4/hSSTR5, forskolin-stimulated cAMP was inhibited by 59.5% in presence of SST in comparison to monotransfected with *wt*-hSSTR4 (33%) or cotransfected with hSSTR4/hSSTR1 (30%).

Conclusions: Our data demonstrates the negative regulatory effect of C-tail on receptor dimerization but positive effect on ERK phosphoryaltion and might provide important role to hSSTR4 in absence of its C-tail. hSSTR4 heterodimerize with hSSTR5 which implicates the possible role of SSTR4/SSTR5 heterodimers in the process of neurodegeneration and pain biology.

36

Unique aspects of the metabolism and pharmacology of the antidepressant/antipanic drug phenelzine

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Background/Objectives: Phenelzine (PLZ) is a potent inhibitor of monoamine oxidase (MAO) -A and -B that has also been shown to be neuroprotective in an animal model of cerebral ischemia. PLZ also inhibits GABA-transaminase, resulting in marked and long-lasting elevations in brain GABA. PLZ is also a substrate for MAO, and MAO inhibition prior to PLZ administration abolishes its GABAergic effects, suggesting that a metabolite of PLZ formed by the action of MAO mediates that effect. It has been speculated that phenylethylidenehydrazine (PEH; a compound that we have shown to elevate brain GABA and exert neuroprotective effects) is that metabolite.

Methods: We have developed an assay for PEH using gas chromatography, and applied the assay to brains from rats treated with PLZ (and to *in vitro* experiments using human MAO).

Results: PEH was present in high concentrations in brains and livers of rats treated with PLZ, and inhibition of MAO with tranylcypromine prior to PLZ administration prevented the formation of PEH. *In vitro* studies using human MAO-A and -B extended our results to demonstrate that PLZ is a good substrate

for both human MAO isoforms (as measured by H_2O_2 formation).
Conclusions: PEH has now been demonstrated definitively in rats to be a metabolite of PLZ resulting from the action of MAO. These studies are now being extended using human MAO.

37

Heme oxygenase-mediated suppression of oxidative stress and extracellular matrix attenuates cardiac histopathological lesions in adult spontaneously hypertensive rats

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Funding Source: None
Background: Aldosterone and phospholipase C (PLC) stimulate nuclear factor-kappaB (NF-□B) and activating-protein (AP-1) causing fibrosis and hypertrophy. Besides harbouring binding sites for NF-□B and AP-1, heme oxygenase (HO-1) generates cytoprotective products including bilirubin and ferritin. The multifaceted interaction between HO-1 and aldosterone-PLC profibrotic axis in cardiac hypertrophy of spontaneously hypertensive rats (SHR) was studied.
Methods: HO-1 was induced with hemin or blocked with chromium mesoporphyrin (CrMP). The study groups included: (**A**) controls (SHR, WKY and SD), (**B**) SHR+hemin, (**C**) SHR+hemin+CrMP, (**D**) SHR+CrMP, and (**E**) SHR+vehicle. Histological and morphological/morphometrical, quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot, Enzyme Immunoassay (EIA) and spectrophotometric assays were used to assess the effect of the HO system on cardiac hypertrophy.
Results: Hemin therapy evoked a 3-month enduring cardioprotection in adult SHR by lowering blood pressure, and reducing left-to-right ventricular ratio, left-ventricular wall-thickness, left ventricle-to-body-weight ratio, whereas CrMP exacerbated cardiac fibrosis/hypertrophy. The cardioprotection was accompanied by reduced aldosterone, PLC, inositol-triphosphate, NF-□B, AP-1, heme and 8-isoprostane, a marker of oxidative stress, whereas HO-1, HO activity, cyclic guanosine monophosphate (cGMP), bilirubin, ferritin, superoxide dismutase and the total anti-oxidant capacity were increased. Correspondingly, extracellular matrix/remodelling proteins like fibronectin, collagen-1, collagen-IV, alongside cardiac histopathological lesions including fibrosis, scarring, muscular-hypertrophy, coronary-arteriolar thickening and interstitial/perivascular collagen deposition were attenuated.
Conclusion: Our study unveils sustained cardioprotection by hemin that may have clinical relevance.

38

Limited sampling strategies for tacrolimus monitoring in pediatric liver transplant recipients

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Funding Source: None
Background: Trough concentration (C_0) is currently used for tacrolimus (FK506) dose individualisation. AUC may be a better tool to predict tacrolimus efficacy and toxicity. However, such monitoring is often impractical in a clinical setting because it involves many blood samples. The aim of this study was to define a limited sampling strategy (LSS) that accurately predicts tacrolimus exposure in pediatric liver transplant recipients.
Methods: Thirty 12-hour pharmacokinetic profiles were performed in 23 stable pediatric liver transplant recipients (9.7 ± 6.5 years) after 55.7 ± 66.2 months post-transplantation. Tacrolimus concentrations were determined by MEIA II method. AUC_{0-12} was calculated by the trapezoidal method. Relationships between individual concentration points or abbreviated AUCs and AUC_{0-12} were determined using multiple regression analysis (R^2; % of absolute prediction error [APE]).
Results: Pharmacokinetic profiles showed high variability. Mean AUC_{0-12} was 134.0 ± 63.1 ng*h/ml. Calculated AUC using 3 concentration points (9.97 + 3.42 *C_0 + 1.51 *C_1 + 5.74 * C_4) accurately predicted AUC_{0-12} (R^2 = 0.97; APE = 6.0% ± 5.8%). Among single concentration point strategies, C_0 had the weakest correlation with AUC_{0-12} (R^2 = 0.62; APE = 17.7% ± 15.0%) while C_4 showed the highest one (R^2 = 0.91; APE = 11.4% ± 9.7%).
Conclusions: The abbreviated AUC using $C_0/C_1/C_4$ accurately predicts AUC_{0-12} and appears most suitable for clinical use as it requires three blood concentrations within the first 4 hours after tacrolimus administration. This LSS may now be employed for prospective validation studies in liver and other transplant populations.

39

Paternal exposure to drugs and chemicals: Review of six-year experience in the Motherisk Program

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Background: The Motherisk Program, established in Toronto in 1985, is a teratology information and clinical consultation service with a focus on drug safety during pregnancy and lactation. In addition to counselling on maternal issues, Motherisk has been receiving requests for counselling on paternal exposure (PEx), an area in which limited information is available. Here, we reviewed for the first time our experience with PEx.

Methods: This was an observational retrospective cohort study using a prospectively collected database. Telephone counselling records between Jan 1, 2002 and Dec 31, 2007 were screened to identify records concerning PEx. Data were extracted using a standardized form. Callers who requested counselling on PEx were contacted by telephone for their verbal consents to participate in a questionnaire for follow-up of pregnancy outcomes, if pregnancy occurred within 2 months following PEx.

Results: Of a total of 188,188 requests to Motherisk over these 6 years, 301 requests (0.16%) pertained to PEx. The available geographical distribution of these calls was as follows: 244 from Ontario, 49 from other provinces, and 5 from the U.S.A. Most requests concerned exposure to drugs, with the remainder being exposure to alcohol, chemicals, infection and radiation. The drugs on which counselling was most frequently requested were methotrexate (43), finasteride (28), prednisone (22) and azathioprine (22). For many drugs, there was no available information on PEx. Follow-up of pregnancy outcomes is in progress.

Conclusions: Our findings suggest that there is an ongoing need for safety data on PEx to selected drugs in the peri-conception period.

40

Outcome of infants exposed to poly antidepressant drug use during pregnancy: Results of a prospective, comparative study

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Funding Source: None

Background: There are no studies documenting possible adverse effects in infants exposed to poly antidepressant use during pregnancy.

Objective: examine whether poly antidepressant use increases the rates of major malformations, spontaneous abortions (SA) therapeutic abortions (TA), stillbirth, preterm birth and birth weight.

Methods: At The Motherisk Program, we analyzed pregnancy outcomes of 1243 women in our data base exposed to various antidepressants during pregnancy, and a comparison group of 1243 non exposed women. We identified 75/1243(6%) women who took more than one antidepressant during pregnancy. We compared this group to 2 other groups of pregnant women, 1) who took a single antidepressant and 2) unexposed.

Results: 8/75(10%) of the women took 3 antidepressants and 67(90%) took 2 antidepressants concomitantly and 12(16%) took more than one antidepressant during pregnancy, but not concomitantly. There were no statistically significant differences in any of the outcomes among the 3 groups that were analyzed. However, there were 2 cases of cerebral palsy in the infants exposed to 2 concomitant antidepressants (mirtazepine/sertraline) and (trazodone/sertraline) compared to none in either of the other groups.

Conclusions: Based on this small sample size, exposure to antidepressant poly therapy during pregnancy does not appear to increase the risk for infant adverse outcomes. The 2 cases of cerebral palsy are likely by chance, as there are 1) multiple causes of cerebral palsy and 2) antidepressant use during pregnancy is not been associated with an increase risk in studies that previously examined antidepressant use in pregnancy and adverse outcomes.

41

Protective effect of sphingosine 1-phosphate (S1P) in the cerebral microvasculature

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Background/Objectives: Sphingosine 1-phosphate (S1P), an endogenous phospholipid, is involved in pre-conditioning responses in the heart. The objective of this study is to evaluate whether exogenous S1P could have a similar protective role in the cerebral microvessel endothelial cells following hypoxic insult.

Methods: Primary human brain microvessel endothelial cells (HBMECs) were exposed to oxygen-

glucose deprivation (OGD) for 0-6 hours. Cell viability was assessed using MTT assay immediately after OGD. To determine the presence of active apoptosis, caspase-3 levels were measured using an enzyme-linked immunosorbent assay (ELISA). The effects of S1P on OGD-mediated cell toxicity was determined by pre-treating the cells with various concentrations of S1P (0-10 µM) for 0, 1, 12, or 24 hours.

Results: Exposure of HBMECs to OGD resulted in a significant decrease (approximately 20%) in cell viability when assessed immediately after OGD. However, caspase-3 levels were not elevated at this time. Pre-treating HBMEC cells with S1P for 1 hr prior to OGD provided a dose dependent protection, with 1.0 and 10.0 µM S1P completely reversing the loss in cell viability associated with OGD at all time points examined.

Conclusion: Activation of S1P receptors within the brain microvasculature prior to hypoxic events may help prevent toxicity to the cerebral microvasculature. The receptors involved and the involvement of the Akt signaling pathway in the response to S1P are currently under investigation.

42

Ecosystem health in the Walpole Island First Nation (WIFN) community: An area of concern in the Great Lakes.

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Funding Source: Assembly of First Nations-Health Canada Regional First Nations Environmental Contaminants Program

Background: Walpole Island is located downstream from Sarnia on the St. Clair River. Members of the WIFN have been exposed to persistent organic pollutants (POPs) and heavy metals for decades. The purpose of this study was to determine baseline concentrations of POPs and heavy metals in volunteers from the WIFN community to assess the current level of exposure. We hypothesized that the WIFN volunteers would have higher concentrations of heavy metals and POPs when compared to literature values and 3 urban cohorts.

Methods: Plasma samples from 20 volunteers were analyzed using gas liquid chromatography with electron capture detection for POPs, and a panel of toxic metals were quantitated in hair and whole blood samples from 55 volunteers using inductively coupled plasma mass spectrometry. Statistical techniques to analyze data included one way ANOVA and the Mann-Whitney t test.

Results: The single POP with the highest concentration was the DDT metabolite, DDE which occurred at a median concentration of 236.5ng/g blood lipid. The median concentration of arsenic in hair was 0.2nmol/g.

Conclusion: Compared to the NHANES values reported for representative citizens in the USA, the DDE concentration in blood of WIFN volunteers was within the 25-50th percentile. The median concentration of arsenic was significantly lower than in an urban population of Japanese families. As a result of this survey, we were able to establish baseline concentrations for more than 100 environmental pollutants in Walpole Island volunteers from samples collected in 2008.

43

The mechanisms of acyclovir–induced nephrotoxicity in children

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Funding Source: Canadian Institute of Health Research (CIHR)

Background: Acyclovir is a widely used agent in the treatment of herpes simplex and varicella zoster in children. While generally well tolerated, nephrotoxicity, and in some cases, serious acute renal failure have been observed. Currently, it is believed that acyclovir induces nephrotoxicity by crystalluria leading to obstructive nephropathy. The avoidance of a rapid bolus intravenous dose and adequate hydration has been the main preventative strategies for acyclovir – induced nephrotoxicity. However, we have recently illustrated that proper hydration does not prevent nephrotoxicity in some children. Furthermore, evidence from several cases of renal biopsies illustrated that; acyclovir induces nephrotoxicity with no evidence of crystal formation and with tubular necrosis.

Hypothesis: We hypothesized that acyclovir induces nephrotoxicity via direct damage to renal tubular cells.

Methods: Human renal proximal tubular cells (HK-2) were exposed to acyclovir (Zovirax®; 500 – 2000 µg/mL) in complete growth media for 24 hours. Cytotoxicity was measured using the AlamarBlue™

assay. Statistical analyses were performed using unpaired student t-tests. Results were considered statistically significant if $p<0.05$.

Results: Cytotoxicity studies illustrated that acyclovir decreased HK-2 cell viability in a concentration – dependent manner. Compared to untreated control, acyclovir concentrations of 500, 1000, 1500 and 2000 µg/mL induced statistically significant ($p<0.05$) decreases (17, 32, 44, and 55 %) in cell viability, respectively.

Conclusion: This is the first experimental evidence which suggest that, in addition to crystalluria; acyclovir induces direct tubular damage by a yet unknown mechanism. Therefore, it is imperative to elucidate the mechanism of direct renal tubular damage.

44

Pharmacogenetics of fatal codeine intoxication in a toddler after adenotonsillectomy

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Funding Source: Canadian Institutes of Health Research (CIHR)-Canadian Pharmacogenomic Network for Drug Safety (CPNDS)

Background: Fatal upload toxicity occurred in a 2-year-old child following age-appropriate codeine dose after adenotonsillectomy. The second morning after surgery the child was found with no vital signs and resuscitation efforts failed.

Methods: Full postmortem examination including GC/MS for blood drug concentrations was performed. CYP2D6 allelic duplications or deletions were detected by restriction fragment polymorphism assay.

Results: Post-mortem examination showed evidence of tracheitis, aspiration of food particles, and bilateral bronchopneumonia of 1-2 days. Examination of drug that remained revealed that no excessive or extra doses were given. Femoral blood showed morphine concentrations of 32 ng/mL. No other poisons, drugs or metabolites were detected. CYP2D6 genotyping revealed functional duplications of CYP2D6 allele resulting in ultrarapid metabolizer (UM) phenotype.

Conclusions: Increased conversion of codeine to morphine due to CYP2D6 UM phenotype resulted in toxic accumulation of morphine in the toddler. Although codeine is a popular analgesic, its polymorphic metabolism precludes accurate prediction of its toxic effects. As many as a third of children remain symptomatic after adenotonsillectomy and thus, unresolved apnea and related breathing problems may contribute to upload susceptibility, respiratory depression and death. Due to the polymorphic nature of codeine metabolism and the fact that adenotonsillectomy does not reverse all cases of obstructive sleep apnea, codeine cannot be considered a safe outpatient analgesic in toddlers post-adenotonsillectomy.

45

Managing heartburn and acid reflux in pregnancy is associated with a reduction in the severity of nausea and vomiting of pregnancy

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Funding Source: None

Conflict of interest: Motherisk NVP helpline is supported by an unrestricted grant from Duchesnay, Inc. Canada. GK is holder of the Research Leadership for Better Pharmacotherapy during Pregnancy and Lactation (Hospital for Sick Children) and the Ivey Chair in Molecular Toxicology (Department of Medicine, University of Western Ontario).

Background/Objectives: Heartburn and acid reflux (HB/RF) in the non-pregnant population can cause nausea and vomiting; we wished to examine, in women with nausea and vomiting of pregnancy (NVP), whether treatment of HB/RF may decrease the severity of symptoms.

Methods: We collected a cohort of women experiencing NVP and heartburn, acid reflux or both (n=194), and compared it to a group of women experiencing NVP but no HB/RF (n=188) using the PUQE score. Multiple linear regression was used to control for the effects of confounding factors. We studied a second cohort of women experiencing NVP and HB/RF (n=60) who were used acid-reducing pharmacotherapy. The effectiveness of the acid-reducing medication in decreasing symptoms of both HB/RF, and NVP was measured.

Results: Women with HB/RF reported higher PUQE scores (9.6 ± 2.6 vs. 8.9 ± 2.6, p=0.02) and lower Well-being scores (4.3 ± 2.1 vs. 4.9 ± 2.0, p=0.01) compared to controls. Multiple linear regression documented that increased PUQE scores (P=0.003) and decreased Well-being scores (P=0.005) were due to the presence of HB/RF as opposed to confounding factors. Acid-reducing drugs resulted in significant decreases in PUQE (9.6 ± 3.0 to 6.5 ± 2.5) ($p<0.0001$), and Well-being scores (4.0 ± 2.0 to 6.8 ± 1.6) ($p<0.0001$) from the initial to the follow-up interview. After intervention with acid-reducing pharmacotherapy, a reduction in

acid symptoms correlated significantly with reduction in NVP (R^2=0.72, p<0.001).
Conclusion: HB/RF are associated with increased severity of NVP; management of HB/RF can reduce the severity of NVP. This new knowledge may allow development of better therapy for NVP.

46

A comparative study to evaluate the efficacy and tolerability of combination therapy of pantoprazole and itopride vs. pantoprazole monotherapy in gastroesophageal reflux disease patients

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Funding Source: None

Objective: Although a proton pump inhibitor (PPI) and a prokinetic drug are often combined for the medical treatment of gastroesophageal reflux disease (GERD), there are only a few clinical studies on the efficacy and tolerability of this therapy. This study investigates whether pantoprazole plus itopride leads to an additional benefit in comparison to pantoprazole alone in treatment of GERD patients.
Methods: It was a randomized, comparative, parallel group, open trial involving 50 patients suffering from GERD and had endoscopic evidence of esophagitis. Total patients divided into 2 groups A and B, group A patients received Pantoprazole and Itopride, whereas group B patients received Pantoprazole alone for 8 weeks. The treatment outcome was assessed after 4 and 8 weeks. The primary criterion was endoscopic healing after 8 weeks. Additionally relief of symptoms like heart burn and regurgitation was also studied at 4 & 8 weeks. All values were expressed as means±SD for continuous variables and as frequencies for categorical variables. The results were analyzed statistically using unpaired 't' test & chi square test with Yate's correction.
Results: After 8 weeks of treatment endoscopic healing rates were 68% and 56% in groups A and B respectively and the difference in healing rate was not significant statistically.
Conclusion: The drug itopride when combined with Pantoprazole leads to no additional benefit in comparison to pantoprazole alone in healing of esophagitis in GERD patients.

AUTHORS INDEX BY ABSTACT