Journal of Population Therapeutics and Clinical Pharmacology
INCORPORATING FETAL ALCOHOL RESEARCH
Journal de la thérapeutique des populations et de la pharmacologie clinique

Published online at: www.jptcp.com www.cjcp.ca

MODERN THERAPEUTICS 2012: ADVANCES IN PHYSIOLOGY, PHARMACOLOGY AND PHARMACEUTICAL SCIENCES

June 12 -15, 2012
University of Toronto
Toronto, Ontario, Canada

Joint Conference of:

Canadian Physiological Society
CSPT SCPT
CSPS

MODERN THERAPEUTICS 2012: ADVANCES IN PHYSIOLOGY, PHARMACOLOGY AND PHARMACEUTICAL SCIENCES

June 12-15, 2012

THURSDAY JUNE 14, 2012

CSPT TRAINEE ORAL PRESENTATION ABSTRACTS

1

HLA-B*1502 and HLA-A*3101 as genetic markers for carbamazepine-induced hypersensitivity reactions in children

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Conflict of Interest: None declared
Funding: Canadian Institutes of Health Research, Canada Foundation for Innovation, Child & Family Research Institute, Canadian Dermatology Foundation, and Genome Canada

Background: The use of the anticonvulsant carbamazepine (CBZ) is limited by the occurrence of hypersensitivity reactions that include drug-induced hypersensitivity syndrome (HSS) and Stevens-Johnson syndrome (SJS). Although rare, HSS and SJS are life-threatening adverse drug reactions with a very high morbidity and mortality. Two genetic variants in the HLA region, HLA-B*1502 and HLA-A*3101, have been associated with CBZ hypersensitivity. However, no study so far has investigated these associations specifically in children. Here, we assessed the association of HLA-A*3101 and HLA-B*1502 with CBZ hypersensitivity in 42 children with CBZ hypersensitivity, and 91 CBZ-tolerant controls. DNA and comprehensive clinical data on the adverse events from all patients were obtained through the Canadian Pharmacogenomics Network for Drug Safety. Genotyping was performed using real-time PCR. A significant association of HLA-A*3101 was observed with CBZ-HSS (OR 26.0, p=0.0008) and maculopapular eruptions (MPE; OR 7.3, p=0.0007), but not for SJS. Conversely, HLA-B*1502 was associated with CBZ-SJS (OR 38.6, p=0.002), but not HSS and MPE. Combined, the two risk variants were strong predictors of all CBZ hypersensitivity reactions (OR 7.6, $p=2.3x10^{-5}$). This study is the first to replicate the association of HLA risk variants with CBZ hypersensitivity in pediatric patients. Our results also provide new insights on the importance of these predictive biomarkers in a multi-ethnic North American population.

2

Effect of human equlibrative nucleoside transporter 1 and ecto-5'nucleotidase (eN) in adenosine formation by astrocytes under ischemic conditions

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Conflict of Interest: None declared
Funding: Canadian Institutes of Health Research

Background: Under ischemic conditions, levels of adenosine (ADO) increase up to 100-fold in brain. Intracellular or extracellular pathways of ADO formation from neurons or astrocytes could contribute to these rising levels of ADO.
Objectives: The present study examined release of ADO from primary cultures of cortical astrocytes from wild type C57bl6 (wt) or CD73 knock out (KO) mice, under basal or ischemia-like conditions.
Methods: Astrocytes cultured from wt or CD73 KO mice were incubated with 3H-adenine to radiolabel intracellular ATP. Astrocytes were then subjected to glucose deprivation (GD) or oxygen-glucose deprivation (OGD) conditions by treatment with 2-deoxyglucose (10mM) in glucose-free buffer for 30 min (37oC) in a humidified chamber or for 1 hour (37oC) in 95% N2 and 5% CO2. The effects of dipyridamole (DPR; 30 µM), an inhibitor of ENT1 and ENT2, or alpha, beta-methylene ADP (AOPCP; 50 µM),

an inhibitor of CD73 on [3H] purine release from astrocytes was tested.
Results: CD73 KO astrocytes produced less ADO under GD and OGD conditions ($p< 0.001$) than wt astrocytes; INO levels did not differ between wt and CD73 KO cells. Under GD and OGD conditions, ADO levels were significantly higher in wt cultures ($P < 0.001$).
Conclusions: Astrocytes produce ADO, but not INO, via an extracellular pathway that requires CD73. These data confirm the role of CD73 in the extracellular pathway contributing to rising levels of ADO formation under ischemia like conditions.

3

The effect of N-acetylcysteine on the antitumour efficacy of ifosfamide in a mouse xenograft model

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Conflict of Interest: No conflict of interest
Funding: CHRI, Canadian Institutes of Health Research, and the Pediatric Oncology Group of Ontario

Background: Nephrotoxicity is a serious side effect, affecting 30% of children treated with the drug ifosfamide. N-acetylcysteine, currently used in children for acetaminophen overdose, has mitigated this renal toxicity in cell and rodent models. Before this treatment can be realized clinically, we must show it does not affect the antitumour efficacy of ifosfamide.
Methods: In a Ewing's sarcoma xenograft mouse model we compared the efficacy of ifosfamide with and without n-acetylcysteine. Ewing's sarcoma tumours were implanted into mice, which were then randomized to receive one of the following treatments: 1) Saline 2) ifosfamide 3) ifosfamide + concurrent n-acetylcysteine 4) pretreatment with n-acetylcysteine + ifosfamide 5) n-acetylcysteine. Tumour volumes were assessed 3 times/week by caliper.
Results: Median tumour volumes of control mice (n=6) were significantly different from median tumour volumes in mice treated with ifosfamide alone (n=8), concurrent NAC, and pretreatment NAC ($p<0.05$). Moreover, when compared to median tumour volumes of mice treated with ifosfamide alone, those treated concurrent and pretreatment NAC, showed no significant difference in tumour growth, and tended to have lower tumour volumes.
Conclusion: These data show no evidence that NAC might interfere with the antitumour efficacy of ifosfamide. They further support to need for a clinical trial to assess the effectiveness of NAC to protect against ifosfamide-induced nephrotoxicity in children.

4

Investigation of the cytotoxic effects of novel jadomycins in drug-sensitive and drug-resistant breast cancer cells

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Funding: NSERC-CIHR Collaborative Health Research Grant

Background: Multidrug resistance (MDR) remains a major obstacle in the treatment of metastatic breast cancer. Novel anticancer agents that are efficacious in resistant tumours are needed. Jadomycins are polyketide-derived natural products produced by the soil bacteria *Streptomyces Venezuelae* (ISP 5230). We hypothesise that some jadomycins analogues evade efflux by ABC transporters, and as a result those analogues will exhibit higher cellular accumulation and improved efficacy over existing anticancer agents in drug-resistant tumour cells.
Methods: The cytotoxicity of jadomycin analogues was determined using MTT assays in drug-sensitive (control) and drug-resistant (ABCB1, ABCC1 or ABCG2-overexpressing) MCF7 breast cancer cells. The cellular efflux of the ABCB1, ABCC1 and ABCG2 substrates, respectfully, rhodamine 123, doxorubicin and Hoechst 33432 in stably transfected HEK cells with or without pharmacologically active concentrations of jadomycins was used to determine which jadomycins inhibit these drug efflux transporters.
Results: In comparison to control MCF7 cells, jadomycins G, DNV, B and N were equally toxic to ABCB1-overexpressing MCF7 cells;

jadomycins DNV, SPhG and N were equally toxic ABCC1-overexpressing MCF7 cells; and only jadomycin N was equally toxic to ABCG2-overexpressing MCF7 cells. None of the jadomycin analogues inhibited the efflux of ABCB1, ABCC1 or ABCG2 probe substrates in transport assays.
Conclusion: The ability of jadomycins to retain cytotoxic activity in the corresponding drug resistant MCF7 cells stems from their ability to circumvent interactions with the ABCB1, ABCC1 and ABCG2 drug efflux transporters. Based on the favorable pharmacokinetic properties we are further exploring the mechanisms of action and chemotherapeutic potential of these jadomycins.

5

Ethyl glucuronide crosses the human placenta and represents maternal and fetal exposure to alcohol

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Conflict of Interest: None declared
Funding: Ontario Graduate Scholarship

Background: Alcohol consumption during pregnancy can lead to Fetal Alcohol Spectrum Disorder, and because maternal self-reports are often unreliable, a biomarker of alcohol use during pregnancy is needed to accurately determine fetal exposure. Ethyl glucuronide (EtG) is a direct metabolite of ethanol that has been detected in the meconium of infants born to mothers who consumed alcohol during pregnancy.
Objective: To determine to what extent EtG crosses the human placenta.
Methods: Placentae (n=4) from consenting women undergoing elective Caesarian section at St. Michael's Hospital in Toronto were taken to the on-site perfusion laboratory. After cannulation and establishment of dual circulation, 1 mcg/mL EtG was added to the maternal reservoir and samples were taken throughout the 3h experiment. Measurements of placental viability were oxygen transfer, pH, glucose consumption, hCG production, fetal reservoir volume, and fetal arterial inflow pressure. EtG was analyzed in perfusate samples and placental tissue by GC-MS after solid phase extraction.
Results: After 3h, the fetal-to-maternal ratio was 0.30 ± 0.02 and net maternal-to-fetal transfer was still occurring. Triplicate averages of EtG concentrations in perfused placental lobules ranged from 140-414 ng/g. Placental validation markers were within normal ranges for all perfusions.
Conclusions: EtG appears to cross the human placenta and, hence, to represent both maternal and fetal exposure to alcohol.

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Embryonic catalase protection against ethanol embryopathies in acatalasemic and human catalase-expressing mice in embryo culture

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Funding: Canadian Institutes of Health Research

Background: Alcohol (ethanol, **EtOH**) consumption during pregnancy can cause a spectrum of structural, cognitive and behavioural anomalies termed the Fetal Alcohol Spectrum Disorder (**FASD**). Reactive oxygen species (**ROS**) have been implicated in the teratogenic mechanism, but the protective role of the embryonic antioxidative enzyme catalase is unclear, as embryonic activity is less than 5% of maternal levels. We addressed this question in a whole embryo culture model. C57BL/6 (**C57**) mouse embryos expressing human catalase (**hCat**) or their wild-type (**C57 WT**) controls, and C3Ga.Cg-*Cat*^{b}/J catalase-deficient, acatalasemic (**aCat**) mouse embryos or their wild-type C3HeB/FeJ (**C3H WT**) controls, were explanted on gestational day (**GD**) 9 (plug = GD 1), exposed for 24 hr to 2 or 4 mg/mL EtOH or vehicle, and evaluated for functional and morphological changes. hCat and C57 WT vehicle-exposed embryos developed normally, while EtOH was embryopathic in C57 WT embryos, evidenced by decreases in anterior neuropore closure, somites developed, turning and head length, whereas hCat embryos were protected ($p < 0.001$). Maternal pretreatment of C57 WT dams with 50 kU/kg PEG-catalase (**PEG-Cat**) 8 hr prior to embryo culture, which increases embryonic catalase

activity, blocked all EtOH embryopathies ($p < 0.001$). Vehicle-exposed aCat mouse embryos had lower yolk sac diameters compared to C3H WT controls, suggesting endogenous ROS are embryopathic. EtOH was more embryopathic in aCat embryos than WT controls, evidenced by reduced head length and somite development ($p < 0.01$), and trends for reduced anterior neuropore closure, turning and crown-rump length in aCat embryos. Maternal pretreatment of aCat dams with PEG-Cat blocked all EtOH embryopathies ($p < 0.05$). These data suggest that embryonic catalase is a determinant of FASD risk, and that ROS contribute to the embryopathic mechanism.

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Lysophosphatidicacid (LPA)-induced enhancement of blood-brain barrier (BBB) permeability as a potential method for enhancing drug delivery to the brain

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Funding: NSERC and Manitoba Health Research Council

Background: Delivery of drugs to the CNS is limited due to the restrictive nature of the BBB.Transient modulation of BBB permeability is one method for enhancing drug delivery to the brain and may have potential CNS drug delivery applications.
Objectives: Characterize the extent of LPA-induced modulation of BBB permeability and provide initial proof-of-concept for use of LPA to enhance drug delivery to the brain.
Methods: Alterations in BBB permeability were characterized in Balb/C mice using a small (gadolinium contrast-enhanced agent (Gad)), and large (IRdye800cw PEG) vascular permeability imaging agent. In addition, Rhodamine 800 (R800) imaging agent was used to monitor changes in P-glycoprotein-mediated BBB permeability. Mice were also administered 3H-methotrexate, either alone or in the presence of LPA to determine improvements in brain delivery of chemotherapeutic agent.
Results: The magnitude of BBB disruption was greatest for Gad with increases of 20-fold. Macromolecule marker, IRdye 800cw PEG, showed approximately 3-fold enhancement in brain accumulation following LPA. Increased brain penetration of R800 was observed following LPA exposure. The brain accumulation of methotrexate was increased 17-fold in LPA treated mice compared to vehicle.
Conclusions: LPA produced a rapid and reversible increase in BBB permeability to a wide variety of agents. Use of LPA in combination with therapeutic agents may be an effective strategy to increase drug delivery to the brain.

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Ligand-dependent and receptor-selective effect of non-nucleoside reverese transcriptase inhibitors on the activity of human pregnane x receptor and constitutive androstane receptor

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Funding: Canadian Institutes of Health Research

Background: Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are used routinely along with other anti-HIV drugs for treating HIV-1 infection. However, when used in combination, certain NNRTIs are known to cause drug interactions. *In vitro* studies have shown that NNRTIs increase mRNA levels of drug-metabolizing enzymes and transporters, the expression of which is regulated by nuclear receptors. However, the mechanism involved in NNRTI-mediated induction of drug-metabolizing enzymes and transporters is not understood. This study was aimed to investigate the interaction between selected NNRTIs and nuclear receptors, such as pregnane x receptor (PXR) and constitutive androstane receptor (CAR). Cytotoxicity of NNRTIs was determined by the lactate dehydrogenase (LDH) assay. The effect of NNRTIs on the activity of human PXR (hPXR) and human CAR (hCAR) (and its spliced variants) was assessed in transiently transfected HepG2 cells. Reporter gene expression was quantified using the Dual-Luciferase Reporter Assay System. Each of the selected NNRTI at concentrations up to 5 µM did not increase LDH release. Efavirenz

(7.3-fold), etravirine (17.1-fold), and rilpivirine (11.4-fold), but not nevirapine or delavirdine, activated hPXR. None of the NNRTIs activated hCAR or its spliced variants, regardless of whether or not cells were co-transfected with retinoid x receptor α. Our results indicate a ligand-dependent and receptor-selective effect of NNRTIs on the activity of hPXR and hCAR.

WEDNESDAY JUNE 13, 2012

CSPT TRAINEE POSTER PRESENTATION ABSTRACTS

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Stability of brominated flame-retardants (PBDEs) measured in hair

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Conflict of Interest: None declared
Funding: Institute for Human Development, Child and Youth Health, Canadian Institutes of Health Research

Background: Polybrominated diphenyl ethers (PBDEs) are chemicals added various consumer products as flame-retardants, and have been labeled as endocrine disruptors. They persist in the environment and studies suggest hair as a suitable matrix for examining human exposure. Since more than 80% of exposure is due to contaminated dust in our environment, we hypothesize that hair PBDE levels will be stable over time, as one's environment remains unchanged.
Objective: To measure the intra and inter-individual stability of PBDE levels detected in hair over a period of one year.
Methods: Hair from 22 females was collected at The Hospital for Sick Children as part of another study. To assess stability, hair samples were separated into four 3 cm segments, which represents a one-year period. Segments were analyzed for eight PBDE congeners, BDE-28,-47,-99,-100,-153,-154,-183 and -209 by GC-MS.
Results: The total ΣPBDEs (pg/mg) varied among individuals. Smaller amounts were observed in more recent segments; first (48.7±5.3), second (67.9±8.5), third (87.2±10.7), and fourth (138.3±15.6). BDE-47 (113±10.5) and BDE-99 (81.6±8.1) comprised of 59% of the total ΣPBDEs in hair.
Conclusion: As expected BDE-47 and 99 were the primary congeners. Results indicate that PBDEs are relatively stable over time. However, further data is needed to determine how PBDEs accumulate in hair over time as a result of multiple sources and pathways of exposure.

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Lactobacillus reuteri DSM 17938 versus placebo in the treatment of infantile colic: A randomized double-blind controlled trial

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Conflict of Interest: None declared
Funding: Hospital for Sick Children

Background: Infantile colic is the most commonly reported medical problem in the first 3 months of life and although it is not a life-threatening condition, it causes appreciable distress for parents and pediatricians. The pathogenesis of colic remains unclear; however, multiple origins are suggested to be involved. Consequently, a variety of treatment options have been proposed, but there is currently no preferred approach to alleviate this condition.
Objectives: To investigate the effectiveness of the probiotic, *Lactobacillus reuteri* DSM 17938, in reducing infantile colic symptoms compared to a placebo treatment.
Methods: 100 infants diagnosed with either colic symptoms (>3 hrs of crying on >3 d/wk), fussy-gassy or gastroesophageal reflux (with or without esophagitis) will be enrolled in this randomized double-blind placebo controlled study. Colicky infants will be randomly assigned to receive *L. reuteri* or placebo at a standard dose of 10^8 colony-forming units 30 minutes following breast-feeding once daily for 21 days. The primary outcome is the reduction of mean crying time

from baseline to the end of treatment with *L. reuteri* compared to a placebo treatment.
Discussion: A lack of characteristic manifestation of colic and the day-to-day variability of crying time, it is necessary to adopt a safe therapeutic approach to alleviate colic symptoms. As such, treatment with *L. reuteri* is believed to be a safe and effective treatment to treat infantile colic.

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Functional activity of CYP450 2J2, 3A5 and 2E1 in human heart ventricles

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Conflict of Interest: No conflict of interest
Funding: Canadian Institutes of Health Research

Background: Cardiac CYP450s functional activity may contribute significantly to the local metabolism of drugs. Using a cocktail of probe drugs we characterized CYP2J2, CYP3A5, CYP2E1 and CYP2B6 functional activities in human heart microsomes (HHM).
Methods: HHM were isolated by differential centrifugation from the human heart right ventricle. Ebastine (CYP2J2; 0 to 25 µM), chlorzoxazone (CZX) (CYP2E1; 0 to 630 µM), midazolam (MDZ) (CYP3A5: 0 to 5 µM) and bupropion (CYP2B6; 0 to 775 µM) constituted the substrate cocktail. Incubation mixture contained 100 mM Phosphate buffer, substrates and NADPH-generating system. Reaction was initiated by adding microsomal proteins. Samples were incubated at 37°C for 60 min. Metabolites were quantified by LC-MSMS.
Results: Investigation of in vitro Km and Vmax shows significant metabolism of CZX in 6-OH-CZX, of ebastine in OHebastine (Km; 0,52 µM, Vmax; 0,49 pmoles /min/ mg prot) and of MDZ in 1-OHMDZ (Km; 4,8 µM, Vmax; 0,01 pmoles /min/ mg prot). CZX did not achieve enzymatic saturation. CZX is also a substrate of CYP1B1 with similar affinity and mRNA relative expression shows higher level of CYP1B1 than 2E1. Activities measured in HHM for ebastine and MDZ correlated well with mRNA levels measured for CYP2J2 and CYP3A5, respectively. No activity was observed for CYP2B6.
Conclusion: HHM shows functional activities of CYP2J2, 3A5 and 2E1 which may contribute to the cardiac metabolism of drugs.

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Oxycodone-induced central nervous system (CNS) depression in breastfed neonates: Pharmacological analysis

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Conflict of Interest: None declared
Funding: Canadian Institutes of Health Research catalyst grant

Introduction: Oxycodone is associated with an increased risk of CNS depression in both mothers and infants. The contribution of genetics in predicting toxicity in breastfeeding mother-infant pairs exposed to oxycodone is not known. The objective of this study was to assess the contribution of 18 polymorphisms in 4 genes involved in oxycodone metabolism and response in predicting both maternal and neonatal CNS depression.
Methods: A case-control study in 67 breastfeeding mother-infant pairs exposed to oxycodone was conducted. Cases were defined as parental reports of sleepiness in the infant temporally related to oxycodone exposure via breast milk. Maternal saliva samples were analyzed for 18 polymorphisms in 4 genes, *CYP2D6*, *CYP3A5*, *OPRM1*, *ABCB1* involved in oxycodone metabolism and response.
Results: Mothers of symptomatic infants were using oxycodone for a longer period of time during breastfeeding compared to asymptomatic infants ($p<0.0001$). None of the maternal genetic variants in the 4 genes were associated with oxycodone-induced depression in neonates. However, mothers carrying at least one copy of the *ABCB1 2677* T variant had an increased risk of experiencing sedation themselves [OR 2.35; 95% CI 1.06-5.28; p= 0.03].
Conclusions: Our study suggests that prolonged maternal use of oxycodone for greater than 4 days

increases the risk of CNS depression in the breastfed newborn. Maternal *ABCB1 2677T* was identified to be a risk allele for experiencing maternal CNS depression.

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Cocaethylene as a biomarker in human hair of concomitant alcohol and cocaine use in a high-risk population

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Conflict of Interest: No conflict of interest
Funding: Dr. Gideon Koren internal funding

Background: Cocaethlyene (CE) is a metabolite of cocaine formed only during cocaine and alcohol co-consumption. It is pharmacologically active, prolonging cocaine-related effects.
Objective: To determine whether CE can be used as a biomarker in hair testing to indicate chronic excessive alcohol consumption.
Methods: We used liquid-liquid extraction and solid-phase microextraction to isolate cocaine and its metabolites from hair, as well as fatty acid ethyl esters, a direct biomarker of alcohol consumption. The compounds concentrations were analyzed and determined using GC-MS.
Results: Of 588 individuals tested for cocaine and chronic alcohol abuse, 235 were positive for FAEE, indicating chronic excessive drinking. Of these, 99 individuals were also positive for cocaine use, representing 42.1% of FAEE positive results. Positive hair cocaine predicted chronic alcohol consumption (OR 1.767 P<0.05). For logistic regression, FAEE and positive cocaine use had odds ratios of 2.44 and 15.56 respectively, for positive CE, indicating that positive FAEE and cocaine use predict positive CE results. Critically, positive CE results identified 90.2% of individuals who are considered chronic alcohol abusers (FAEE>=0.5 ng/mg).
Conclusions: In our cohort, CE had a 90.2% positive predictive value for chronic alcohol abuse and it can be used clinically for that end.

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Characterization of rytvela: an allosteric modulator of IL-1β-induced inflammatory processes

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Conflict of Interest: No conflict of interest
Funding: McGill CIHR Drug Development Training Program - Vision Research Network

Background: Following an injury, Interleukin-1β (IL-1β) is secreted to regulate proinflammatory responses. It exerts its biological function by interacting with IL-1 receptor (IL-1R1) complex thus activating various downstream mediators. Of the current approved therapeutics, recombinant IL-1 receptor antagonist competes with IL-1β for receptor binding; however, numerous drawbacks limit its usage suggesting the need for small inhibitors offering preferable drug distribution. A short non-competitive IL-1R1 antagonist of the sequence rytvela, developed by our laboratory, was shown to be efficacious *in vivo.* Interestingly, it exhibits allosteric properties but its mechanism is unknown.
Objective: To study the effect of rytvela on IL-1β-regulatory actions.
Methods: The effect of rytvela on IL-1β-induced cytotoxicity in RGC-5 cells was measured using MTT assay. Furthermore, IL-1β-induced gene expression in rytvela-treated RGC-5 cells was analyzed using PCR. The suppression of IL-1β canonical NFκB and AP-1 pathways via rytvela was examined using NFκB/AP-1 reporter gene assay.
Results: rytvela restored viability in IL-1β-treated RGC-5 cells, supported by the reduction of caspase-3 expression. Moreover, it significantly decreased the gene expression of TNF-α, IL-6, and caspase-1; however, NFκB and AP-1 were unaffected.
Conclusion: rytvela can be an allosteric modulator of IL-1R1 function by selectively inhibiting downstream mediators without abolishing all signal transduction.

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Comparative performance of sprague-dawley rat hearts using DMSO and DMF as cryoprotectants

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Conflict of Interest: None declared

Funding: None declared

Background: Heart transplantation is one of the most effective treatment options for congestive heart failure. Current organ storage methods can preserve the human heart for only about four to six hours. The organ donor pool could be dramatically increased if the preservation time could be lengthened and hearts stored for weeks or even month's prior to transplantation. This study describes the performance characteristics of explanted Sprague-Dawley rat hearts before and after cryopreservation using 10 % dimethylsulphoxide (DMSO) and 30 % dimethylformamide (DMF) in Tyrode solution.

Methods: A modified Morgan perfusion model was used for this study. Male Sprague- Dawley (ethical approval AREC/2009/09/002) hearts were harvested and arrested in a cold (< 10 °C) Tyrode solution (pH 7.4) for 5 minutes. The hearts were mounted on the aorta and vena cava to allow reperfusion in a doubled walled water jacket at 37 °C for baseline (Control) performance studies. The hearts (n=3) were cooled to 4 °C, -20 °C, -80 °C and -196 °C (liquid nitrogen), and stored for 6 hours. This study was extended to 48 hours and 7 days at -196 °C (n=6). Cardiac output (aortic and coronary) and an electrocardiogram were obtained during baseline studies, followed by cryopreservation and after thawing at times T0, 10, 20, 40, 60, 120 min, 6, 8, 12 and 24 hours. Reperfused hearts were monitored for as long as possible. Ethical approval (AREC/2009/09/002) for the use of laboratory animals was obtained from the Tshwane University of Technology, Ethics Committee and the Animal Ethics committee before experimental work commenced.

Results: The average heart rate of the Sprague-Dawley rats reduced from 396 beats / minutes to 184 beats / minutes after anaesthesia. The average survival time of the hearts under the experimental conditions were 7 hours 32 minutes with an average aortic output at 8 hours of 0.62 ml and 0.52 ml at 12 hours for DMF and 0.61 ml for 8 hours and 0.35 ml for DMSO at average survival time of 9 hours 44 minutes. A 100 % recovery after cryopreservation with DMSO and DMF was achieved after storage for 6 hours, 48 hours and 7 days in liquid nitrogen. DMSO and DMF were equally effective cryoprotectants in this study. It was possible to preserve the hearts outside the body longer than 8 hrs as previously studied to 168 hour (7days) at – 196 °C with 100 % recovery using both DMSO and DMF as cryoprotectant.

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Quetiapine in human breast milk –population PK analysis of milk levels and simulated infant exposure

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Conflict of Interest: No conflict of interest

Funding: AstraZeneca

Background: Quetiapine is one of the widely used atypical antipsychotic drugs in women of childbearing age. However, the information on quetiapine excretion into human milk is limited, and no population-based prediction exists.

Methods: A pharmacokinetic study was conducted in lactating women who were taking quetiapine. Multiple milk and single blood quetiapine concentrations were measured. Population PK analyses of milk quetiapine profiles were performed using non-linear mixed-effects method by directly modeling concentration profiles in milk without plasma level data. Using the final PK model, quetiapine milk concentrations at steady state of 1000 individuals were simulated. All modeling and simulations were conducted in NONMEM® VI and R.

Results: Nine subjects receiving fast-release quetiapine (mean dose: 40 mg/day) were analyzed

at steady state. Mean milk/plasma ratio was 0.44 (range 0.098 -1.67). A two-compartment model with first order absorption was selected as the best model. Simulations based on the model parameters showed that 99% of the breastfed infants would ingest quetiapine at levels below 0.42% of the maternal weight-adjusted dose.
Conclusion: Infant exposure levels to quetiapine through breastfeeding are estimated to be very small. Based on the population simulation, we predict that the probability of significant exposure of the infant to quetiapine in milk is negligible.

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Loss of equilibrative nucleoside transporter 1 (ENT1) in mice leads to progressive ectopic mineralization of spinal tissues resembling diffuse idiopathic skeletal hyperostosis (DISH) in humans

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Conflict of Interest: None declared
Funding: Canadian Arthritis Network, Canadian Institutes of Health Research, Schulich School of Medicine and Dentistry

Background: DISH is a non-inflammatory spondyloarthropathy, characterized by ectopic calcification of spinal tissues that occurs in 6-12% of North Americans, most over the age of 50. Its etiology is unknown and there are no specific treatments. *ENT1* mediates the Na^+-independent transport of hydrophilic nucleosides, such as adenosine, across plasma membranes. In mice lacking ENT1 (*ENT1*$^{-/-}$), we observed development of calcified lesions with remarkable resemblance to DISH in humans. MicroCT analyses revealed that *ENT1*$^{-/-}$ mice developed ectopic mineralization, starting in the cervical-thoracic spine and extending to the lumbar region with advancing age. Histological examination of decalcified samples revealed large, irregular accumulations of eosinophilic, amorphous material encapsulated by fibrocartilaginous cells, with no apparent inflammation. Plasma adenosine levels, determined by HPLC, were 2.8-fold greater in *ENT1*$^{-/-}$ compared to wild-type mice. In addition, quantitative RT-PCR analyses of spinal tissue from the cervical-thoracic region of 6-month-old mice revealed lower levels of adenosine A_1 receptor in *ENT1*$^{-/-}$ compared to wild-type mice. Lesions in the *ENT1*$^{-/-}$ mouse resemble DISH in humans and point to a role for purine metabolism in the regulation of biomineralization. *ENT1*$^{-/-}$ mice may provide a useful model to investigate mechanisms and to evaluate therapeutics for preventing pathological calcification in DISH and related disorders.

WEDNESDAY JUNE 13, 2012

CIHR-DSEN TRAINEE POSTER PRESENTATION ABSTRACTS

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Human hair cortisol analysis using an elisa: A comparison of the different reported methods

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Funding: Saudi Cultural bureau in Canada

Background: Recently, hair cortisol analysis has been a topic of global interest among researchers. Therefore, the need for critical examination of the analytical methods has to be done in order to standardize the method and allow uniform interpretation.
Objective: To assess the similarities and differences among methods published to date.
Methods: This study compares among four published laboratories procedures: Drs.Van Rossum, Kirschbaum, Laudenslager, and

Dr.Koren - Van Uum. We examined several common dimensions in their procedures.
Results: A major difference was the ELISA kit used. Alpco diagnostics (Salem, NH, USA) is used by Koren's group, Van Rossum uses DRG International, (USA) or DRG Instruments GmbH, (Marburg, Germany), Kirschbaum uses IBL (Hamburg-Germany), and Laudenslager uses Salimetrics, (LC). Koren and Van Rossum appear to have nearly the same mass of hair (10-15mg), do not wash the hair samples, have the same pulverization method which is mincing with surgical scissors, and the same amounts of the extraction and reconstituting solvents. In contrast, the other two groups use 50 mg of powdered hair and wash hair samples 2-3 times/3 minutes each with 2.5 ml isopropanol. Considerable other similarities were found.
Conclusions: Consensus toward developing one method that is comprehensive, convenient, and appropriate should be aimed.

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Long-term neurodevelopment of children exposed to above manufacturer recommended doses of Diclectin in utero

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Conflict of Interest: Gideon Koren has served as a paid consultant for Duchesnay
Funding: Duchesnay and Canadian Institues of Health Research

Background: Nausea and vomiting of pregnancy affects up to 90% of pregnancies. Doxylamine/pyridoxine is the only anti-emetic approved in Canada for NVP. The maximum dose is 4 tablets/day, at which lack of fetal toxicity, including longterm neurodevelopment has been established. However, some women receive higher doses, to 12 tablets/day. Although this dose has not been associated with major malformations, possible neurodevelopmental effects have not been investigated.
Methods: This is a prospective observational study. Four groups of mother-child pairs have been recruited: (1) NVP and>4 tablets (n=42), (2) NVP and <4 tablets (n=84), (3) NVP and no treatment (n=63) and (4) no NVP (n=49). At 6-9 months after birth, women were contacted for follow up. At ages 3-7, children received a full psychological assessment.
Results: All groups scored in the normal range for IQ and cognition tests. The NVP-exposed group scored significantly higher on the McCarthy numerical memory forward test (P<0.001), the NEPSY comprehension of instruction test (P=0.030), and the NEPSY visuomotor precision scale (P=0.010). Neither worst PUQE score or average dose were predictors of IQ.
Conclusion: Above manufacturer recommended doses of doxylamine/pyridoxine do not appear to harm neurodevelopment and should be considered safe for the treatment of NVP. NVP itself confers superior achievements in some tests among offspring of women with this condition.

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Safety of Levetiracetam in pregnancy

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Conflict of Interest: None declared
Funding: None declared

Background: Use of medications during pregnancy is often a challenge resulting in a lower compliance rate. For the medications where evidence is available and confirm the safety, women are more comfortable in continuing their use during pregnancy. There have been concerns regarding levetiracetam use during pregnancy and adverse pregnancy outcome.
Objective: To systematically review the available published evidence on the safety of Levetiracetam use during pregnancy with focus on birth defects.
Method: Pubmed, Embase, and Cochrane library data base were searched for studies, including pregnancy registries, observational studies, and abstracts regarding the use of levetiracetam during pregnancy.
Results: The study population included pregnant women exposed to levetiracetam as a monotherapy or polytheray in the first trimester. Ten studies met the inclusion criteria, 5 were observational studies in the form of pregnancy registries and 5 were published in abstract form. There were no well controlled studies on the use of Levetiracetam in pregnancy as ethically hard to

do. The available published data including from North American anti epileptic drug pregnancy registry, UK epilepsy and pregnancy registry, EURAP, the Dutch European registry of antiepileptic drugs, and Australian pregnancy registry showed 897 pregnancies exposed to levetiracetam. There were 20 major congenital malformation reported in these studies giving an overall risk of 2.2% (20/897). Although the exposure to monotherapy was associated with less risk 1.3% (8/604) as compare to poly therapy 4 % (12/296). Two studies have shown the development and language skills of exposed children at 3 to 24 months and 3 to 4 years were same like normal control.

Conclusion: The current evidence from all the registries suggests the overall risk of malformation after first trimester exposure are well within the baseline risk of 1-3 %. Long term developmental effects don't seem to be effected although the numbers are small.

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Malaria infection alters drug-disposition mechanisms in pregnancy

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Conflict of Interest: None declared

Funding: Canadian Institutes of Health Research operating grant

Background: Placental malaria has the potential to impact over 20 million pregnancies per year. Despite its high prevalence, little is known regarding the impact of malaria infection on drug-disposition mechanisms in pregnancy. As such, our objective was to characterize expression of key drug-disposition mechanisms in maternal, placental, and fetal tissues in an animal model of malaria.

Methods: 10^6 *P. berghei* ANKA-infected RBCs were injected i.v. into pregnant Balb/c mice at gestational day (GD) 13. Mice were monitored from GD13-19, sacrificed, checked for fetal viability, and maternal, placental, and fetal tissues were extracted and snap frozen from viable fetuses. RNA was isolated using TriZol reagent, assessed for purity using a NanoDrop, DNase I treated, and reverse transcribed to cDNA. Quantitative RT-PCR was used to assess changes in expression of drug transporters and drug-metabolizing enzymes from control and malaria-infected animals on GD19.

Results: Changes in expression of numerous drug-disposition mechanisms was observed in maternal liver, placenta, and fetal liver. Expression of placental transporters (Abcb1a, Abcb1b, Abcc1, Abcc2, Abcc3, Abcg2) was significantly down-regulated ($p < 0.05$). Expression of transporters in maternal liver illustrated significant and differential changes in the canalicular and basolateral hepatocyte domains. Expression of transporters in fetal liver revealed significant changes ($p < 0.05$), mirroring those observed in maternal liver. Both maternal and fetal Cyp3a11 were significantly down-regulated ($p < 0.01$).

Conclusions: Malaria-induced alterations in drug transporters and drug-metabolizing enzymes may significantly alter the pharmacokinetics of clinically-important therapeutics and other xenobiotics in pregnancy. Further studies are required to quantify the impact of these changes on maternofetal drug disposition.

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Clinical and transporter pharmacogenetic determinants of plasma atorvastatin and rosuvastatin concentrations in patients

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Conflict of Interest: No conflict of interest

Funding: Canadian Institutes of Health Research and Academic Medical Organization of Southwestern Ontario AFP Innovation Fund

Background: A significant barrier to statin therapy is muscle toxicity associated with elevated systemic exposure. Despite numerous clinical trials to assess statin safety and effectiveness,

there is little data describing plasma levels in patients, or contribution of clinical and pharmacogenetic variables to the exposure required for optimal therapy.
Objective: To assess interindividual variability and determinants of statin levels in patients.
Methods: We measured atorvastatin and rosuvastatin in 299 patients by LCMS, and evaluated the contribution of variants in transporter genes *SLCO1B1*, *SLCO1B3*, *SLCO2B1*, *ABCG2*, *ABCC2* and *ABCB1* by multiple linear regression.
Results: There was 45-fold variation in statin concentrations among patients on the same dose. After adjusting for gender, age, BMI, ethnicity, dose and time from last dose, *SLCO1B1* c521T>C ($p<0.001$) and *ABCG2* c421C>A ($p<0.01$) were important to rosuvastatin concentration whereas *SLCO1B1* c388A>G ($p<0.01$) and c521T>C ($p<0.05$) were significant for atorvastatin. The p-hydroxyatorvastatin to atorvastatin ratio was significantly correlated with *SLCO1B1* c521T>C ($p<0.001$) and prescription of fibrates ($p<0.05$), known *SLCO* transport inhibitors. This finding was confirmed in *Slco1b2*$^{-/-}$ mice. **Conclusions:** There is significant variability in statin exposure associated with uptake and efflux transporter polymorphisms. There appears to be heterogeneity of the genotype-drug interaction across members of the statin class.

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Fetal safety of cetirizine; a prospective cohort study and meta-analysis

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Funding: Motherisk Program

Background: Cetirizine, a second-generation antihistamine, is a major active metabolite of hydroxyzine and is broadly used in the treatment of allergies, hay fever, angioedema, and urticaria, but the data on fetal safety are incomplete.
Methods: Pregnant women who were counselled by the Motherisk Program about cetirizine exposure in the first trimester were enrolled in a controlled, prospective cohort study and compared to pregnant women counseled about non-teratogenic exposures. A systematic review was conducted to identify and synthesize all cohort studies that examined pregnancy outcome of women exposed to hydroxyzine or cetirizine while pregnant. All the studies including the current study were combined in one meta-analysis using a random effects model.
Results: There were no significant differences in the rates of major malformations found between the cetirizine exposed and comparison group in both the cohort study (P=1.00) and the meta-analysis study (OR= 1.31, 95% CI: 0.99-1.73). In the meta-analysis there were also no differences in the rates of other pregnancy outcomes.
Conclusion: Hydroxyzine and cetirizine are not associated with an increased risk of major malformations or other adverse fetal outcomes.

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CYP2C19, PON1, and the role of PPIs in clopidogrel bioactivation and *in vivo* antiplatelet response

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Conflict of Interest: None declared
Funding: The University of Western Ontario and the Canadian Foundation for Innovation.

Background: Clopidogrel bioactivation and response has been associated with cytochrome P450 2C19 (CYP2C19). However, a recent study identified paraoxonase-1 (PON1) as the main driver of its bioactivation and efficacy. This study aimed to elucidate the contribution of PON1 and CYP2C19 to clopidogrel metabolism and response. Additionally, the interaction potential between clopidogrel and proton pump inhibitors (PPIs) was assessed.
Method: The influence of CYP2C19, PON1 and PPI coadministration, on clopidogrel active metabolite (H4) AUC and antiplatelet response was assessed in healthy subjects (n=21). The in vitro metabolic profiling of clopidogrel metabolism was conducted in microsomes.

Result: There was a remarkable correlation between H4 AUC and antiplatelet response (r2=0.78). Furthermore, CYP2C19 but not PON1 genotype was predictive of H4 AUC and response. There was no correlation between paraoxonase activity and H4 AUC. Coadministration of PPIs did not significantly alter H4 AUC or response. Metabolic profiling of clopidogrel in vitro confirmed the role of CYP2C19 in bioactivating clopidogrel to H4. Conversely, PON1 cannot generate H4, but mediates the formation of another thiol metabolite, Endo. Importantly, Endo plasma levels are nearly 20-fold lower than H4 and was not associated with response. Conclusion PON1 does not mediate clopidogrel active metabolite formation or antiplatelet action, while CYP2C19 remains a predictor of clopidogrel pharmacokinetics and response.

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Hair cortisol concentrations in patients with obstructive sleep apnea

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Funding: None declared

Background: Obstructive sleep apnea (OSA) is a common sleep disorder with serious cardiovascular and metabolic co-morbidities that may be mediated by increased cortisol secretion. Recent studies have focused on the ability of continuous positive airway pressure (CPAP) to reduce cortisol secretion in OSA patients, but the results have been mixed and only point measures of cortisol measurement have been used. Hair cortisol analysis presents a means of non-invasively and retrospectively examining cortisol production in these patients.
Hypothesis: Hair cortisol concentrations are increased in OSA patients, and may be decreased with successful intervention with CPAP.
Methods: Patients were recruited after undergoing a polysomnogram. Physical exam information and medical history were recorded. Polysomnogram data including the apnea-hypopnea index (AHI), total hypoxemic time, and arousals per hour were recorded before and after CPAP. Additionally, a hair sample and Perceived Stress Scale (PSS) were collected before and after CPAP. Hair cortisol concentrations were determined using our modified salivary cortisol ELISA protocol.
Results: Ninety-two patients were enrolled in the study, of which 31 returned after 3 months of CPAP therapy. A trend towards increased hair cortisol concentrations was noted in moderate and severe OSA patients (P=0.056). Hair cortisol concentrations were weakly negatively associated with total hypoxemic time (r2=0.06, P<0.05). Hair cortisol concentrations were not significantly changed after placement on CPAP, but perceived stress was significantly reduced (P<0.001).
Discussion: Cortisol secretion may be up-regulated in severe cases of OSA. The psychological stress of OSA may be reduced with CPAP, however physiological stress may remain.

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CSPT POSTER PRESENTATION ABSTRACTS

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Development of biomarkers for pharmacogenetic testing

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Conflict of Interest: No conflict of interest
Funding: Canadian Pharmacogenomics Network for Drug Safety Consortium

Rationale: Cure rates for many diseases have improved significantly. Despite this, adverse drug reactions (ADRs) are a significant cause of morbidity and mortality. Genetic factors play a key role in the incidence and severity of ADRs. Pharmacogenomics aims to identify these factors.
Active ADR Surveillance: The Canadian Pharmacogenomic Network for Drug Safety (CPNDS) is a Canada-wide network using active surveillance to identify patients with severe ADRs with the goal of performing pharmacogenomic

studies. The CPNDS consortium includes 20 sites across Canada.

Pharmacogenomic Predictions of ADRs: Studies are designed, biomarkers panel is developed, patients are genotyped, raw data is processed and quality controls and association studies are performed. CPNDS has identified markers for anthracycline-induced cardiotoxicity (ACT), cisplatin-induced otoxicity (CIO), codeine-induced infant/child mortality, vincristine-induced peripheral neuropathy and carbamazepine-induced skin rash. GWAS and sequencing are ongoing to further validate these markers and identify new ones.

Functional Validation and Pharmacokinetic Evaluation: CPNDS has begun functional validation (in vitro and in vivo) and pharmacokinetic evaluation for identified genetic markers for CIO and ACT. Preliminary results show that the overexpression of SLC28A3 in human cardiomyocytes protects against ACT.

Discussion/Conclusion: Identifying/validating genetic markers for ADRs are imperative for improving drug safety.

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Exposure to statins during pregnancy: Outcomes over a 13 year period

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Conflict of Interest: None declared

Funding: None declared

Background: Statins decrease cholesterol synthesis by competitively inhibiting the rate limiting step in the cholesterol synthesis pathway. Currently, statins are contraindicated during pregnancy because postponement of hypercholesterolemia therapy during pregnancy is not believed to have long term negative health effects and early case reports of exposure described major fetal malformations. However, controlled cohort studies and further case reports have not validated their FDA category X pregnancy classification.

Objective: To evaluate the outcome of 13 years of pregnant Motherisk callers exposed to statins.

Method: A cohort study of pregnant women exposed to statins from 1998-2010. Pregnancy outcomes and birth defect information were gathered by phone interviews using a standard questionnaire. The primary outcome was the report of major birth defects.

Results: From 1998-2010, 112 exposed pregnant Motherisk callers inquired about statins. Statin use ranged from 4- 19 weeks gestation. The exposure distribution was: 66 atorvastatin, 13 simvastatin, 25 rosuvastatin and 8 pravastatin. Outcomes collected to date are: 49 live births, 18 miscarriages, 3 abortions, and 1 neonatal death. No major malformations were reported, but 2 minor congenital anomalies were described: 1 soft tissue lump (surgically removed) and 1 case of ankyloglossia.

Conclusion: Statins do not appear to be major teratogens. However, further follow up is necessary as our sample size is small.

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Riboflavin treatment reduces the levels of BCRP-transported drug cimetidine into the milk

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Conflict of Interest: No conflict of interest

Funding: Canadian Institutes of Health Research, NSERC, Restracomp and U of T Fellowship

Background: Mother's milk provides a multitude of benefits to the offspring. However, drugs and toxins that are transferred into breast milk may pose a risk to the nursing infant. The Breast Cancer Resistance Protein (BCRP) is known to actively transport drugs (e.g. cimetidine) and toxins (e.g. PhIP) into breast milk. BCRP also transports nutrients such as riboflavin into breast milk, and together with recently identified riboflavin transporters (RFTs), may provide a mechanism for riboflavin secretion into breast milk. It is currently unknown if RFTs are expressed in the mammary gland. Our objective was to characterize BCRP and RFTs expression in

the mammary gland of FVB/N mice, and to investigate a potential strategy to decrease BCRP-transported xenobiotics excretion into the milk using a high-dose riboflavin intervention.
Methods: The expression of mRNA was analyzed using real-time quantitative PCR. Milk and plasma levels of riboflavin were quantified by high performance liquid chromatography. Levels of radioactive 3H-cimetidine in plasma and milk samples were determined by liquid scintillation counting.
Results: RFTs and BCRP expression was upregulated in the mammary gland of lactating mice. An intravenous injection of 5 μg/g body weight of riboflavin enhanced the levels of riboflavin in milk and plasma by 3.1- and 8.9–fold, respectively. Significant reduction in the levels of BCRP-transported 3H-cimetidine in milk was observed in the high-dose riboflavin treatment group.
Conclusion: RFTs upregulation in the lactating mammary gland suggests a potential role for these proteins in mammary riboflavin transport. Using riboflavin to exploit the function of mammary BCRP, significant reduction in milk levels of cimetidine was observed.

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Hepatoprotective activity of berberis aristata root extract against CCl4 induced acute hepatotoxicity in rats

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Funding: M M University

Background: CCL_4 is commonly used hepatotoxin in the experimental studies of liver diseases. Liver damage induced by carbontetrachloride (CCL_4) involves biotransformation of free radicals derivatives, increased lipid peroxidation and excessive cell death. *Berberis aristata* root extract, "Berberine chloride" is known to possess multiple pharmacological activities including anti-microbial, antiviral, anti-inflammatory, cholesterol lowering, anti cancer and anti-oxidant effects. The present study was conducted to evaluate the hepatoprotective activity of berberine in CCL_4 induced hepatotoxicity in rats.
Material &_Methods: The experimental protocol was approved be the IAEC. Adult wistar rats aged 7-9 weeks were injected intraperitoneally with 50% CCl_4 as 1:1 mixture in liquid paraffin. Berberine was administered i/p before or after CCl_4 treatment in various groups. Twenty-four hours after CCl_4 injection, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase(ALP) activities, total serum bilirubun levels and liver weight were measured. Histological changes of liver were examined with microscopy.
Results: The serum levels of AST, ALT, ALP and T. bilirubin were significantly increased ($p<0.01$) in CCl_4 treated group 2 rats. Group 3-5 rats treated with CCl_4 followed by Berberine chloride at doses of 5, 10 and 20 mg/kg, i/p respectively showed significant decrease ($p<0.05$) in AST, ALT, ALP and T. bilirubin levels when compared to group 2 in a dose-dependent manner. The percentage reduction of biochemical parameters after treatment with Berberine at 5, 10, 20 mg/kg in group 3-5 showed significant results ($p<0.05$) in AST (35%, 86%, 93%), ALT (50%, 87%, 91%), ALP (32%, 72%, 83), T. bilirubin (90%, 53%, 72%) and liver weight reduced to 6%, 12%, 20%. Histological examination showed lowered liver damage in berberine-treated groups.
Conclusion: The present study demonstrates that berberine possesses hepatoprotective effects against CCl_4-induced hepatotoxicity and that the effects are both preventive and curative. Berberine should have potential for developing a new drug to treat liver toxicity.

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Metabolic ratio of opioids in hair: a novel method to study population genetic polymorphisms]

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Conflict of Interest: No conflict of interest
Funding: Dr. Koren internal funding

Background: Codeine, still widely prescribed for

its analgesic effects, is subject to CYP2D6 polymorphisms affecting its metabolism. Metabolic ratio (MR) of morphine to codeine represents the extent of codeine metabolism to its active metabolite. These compounds can be found in hair, which has not been used as a matrix to study MR. Studying MR in hair can provide a simple method to evaluate population variability obviating the need for blood.

Methods: Hair samples were collected from the Motherisk Laboratory for testing as per request by social workers and other agencies. From July 2010 to December 2011, 1966 samples were tested for codeine and morphine through GC-MS analysis. All codeine positive samples and respective morphine results were used to calculate MR.

Results: Of the 239 codeine positive samples, 192 had an MR of 0, indicating no morphine detection. Nineteen samples had an MR <1 while 15 samples with an MR between 1 and 2 were found. In addition, 5 samples with an MR between 2 and 3 were detected and 8 samples were found to have an MR >3. Contrasting these data to blood metabolic ratios will allow for validation of this method.

Conclusion: From these results, wide differential incorporation of codeine and morphine into human hair has been found. With better understanding of codeine and morphine incorporation into human hair, polymorphisms can be studied in hair using MR. This is the first study to assess the use of human hair as a matrix to study polymorphisms.

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Acute hepatotoxic and inflammatory responses to liver carcinogens in the developing mouse

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Funding: None declared

Background: 4-Aminobiphenyl (ABP) is an aromatic amine procarcinogen found industrially and in cigarette smoke. ABP bioactivation may involve initial *N*-oxidation by CYP1A2 followed by *N*-acetyltransferase- (NAT) mediated conjugation to an intermediate that decomposes to a DNA-binding nitrenium ion. Neonatal exposure to ABP causes liver tumours in male but not female C57BL/6 mice, but NAT-deficient *Nat1/2(-/-)* mice are protected. However, biomarkers of DNA damage do not differ between sexes or strains. Diethylnitrosamine (DEN) shows the same sex difference as ABP. Adult mice exposed to DEN show an estrogen-dependent sex difference in acute hepatotoxic and inflammatory responses, suggesting a role for these processes in its carcinogenesis. We hypothesize that neonatal exposure to either ABP or DEN will cause sex and strain differences in acute hepatotoxicity. Mice were exposed to ABP and DEN using a tumor-generating dose. Mice were also exposed to DEN (25 mg/kg) using an established tumor-inducing protocol. Markers of liver damage (alanine aminotransferase (ALT) activity) and inflammation (interleukin-6 (IL-6) expression) were assessed. ABP or DEN treated mice had no treatment, sex or strain differences in ALT activity or IL-6 expression. Mice treated with 25 mg/kg DEN had increased IL-6 expression but no sex difference. Our results suggest that low doses of ABP and DEN are poor hepatotoxins, and that increased IL-6 in DEN treated mice may be due to dose and chemical specific factors.

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Chemokine-like receptor 1 regulates myogenic differentiation *in vitro* and *in vivo*

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Funding: Canada Foundation for Innovation, Canadian Institutes of Health Research, NSHRF, Pharmacy Endowment, Medical Research Foundation and Faculty of Health Professions, Dalhousie University

Background: The chemokine-like receptor-1 (CMKLR1) is a G-protein coupled receptor that is activated by the adipokine chemerin. Previous studies have shown that CMKLR1 is highly expressed in white adipose tissue and liver, and plays an important role in the regulation of adipogenesis and osteoblastogenesis. Based on the established function of CMKLR1 in cell

differentiation we invetigated the hypothesis that CMKLR1 regulates the differentiation of myoblasts into myotubes both *in vitro* and *in vivo*.
Methods and Results: In C2C12 mouse myoblasts, *CMKLR1* expression increased 3-fold with differentiation into multi-nucleated myotubes. Abolishing CMKLR1 expression, using an adenoviral-delivered shRNA, impaired the differentiation of C2C12 myoblasts into mature myotubes and reduced the expression of myogenic regulatory factors *myogenin* and *MyoD* as measured by quantitative real time PCR Embryonic (E12.5) CMKLR1$^{-/-}$ mice displayed significantly lower wet weights and a considerably diminished myotomal component of somites as revealed by immunolocalization of myosin heavy chain protein. These changes were associated with increased Myf5 expression in both C2C12 myoblasts and in mouse E12.5 embryos. Adult male CMKLR1 knockout mice exhibited significantly reduced bone-free lean mass and weighed less than the CMKLR1-expressing mice.
Conclusion: We conclude that CMKLR1 plays an essential role in myogenic differentiation of C2C12 cells *in vitro* and the CMKLR1 null mice exhibit a subtle skeletal muscle deficit beginning from embryonic life which persists during postnatal life.

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Neonatal adverse effects following exposure to benzodiazepines during breastfeeding

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Funding: Canadaian Pharmacogenomic Network for Drug Safety and The Ivey Chair in Molecular Toxicology

Background: Breast milk provides newborns with the ideal form of nutrition. The choice to initiate breastfeeding while taking medications chronically is a decision faced by many new mothers. Benzodiazepines are commonly prescribed anxiolytic agents and they have been detected in breast milk. Some studies suggest possible harmful effects in the suckling infant.
Objective: of this study was to assess the central nervous system depression and other adverse effects in infants exposed to benzodiazepines through breast milk.
Methods: Mothers who contacted the Motherisk program regarding the safety of benzodiazepines were invited to participate in a follow-up program regarding the effects of benzodiazepines on their infants during lactation.
Results: A total of 124 consenting women participated. Adverse outcomes, specifically sedation was identified in only 1.6% (2/124) of babies and was not associated with benzodiazepine dose, number of hours breastfed, or any demographic trait. Mothers reporting adverse outcomes in themselves [26% (32/124)] were more likely to be taking concomitantly a greater number of CNS depressants.
Conclusion: This study supports the continued recommendation to initiate breastfeeding while taking benzodiazepines postpartum.

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Techniques to answering epidemiological related health questions about pregnancy

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Funding: None declared

Background: Policy makers, researchers, clinicians and the public look for answers to population health related questions, yet reading a report of one randomized clinical trial (RCT) would often not provide a comprehensive picture to base critical decisions. Often, only observational studies are available. In pregnancy, RCTs are very rarely conducted.
Objectives: Describe several methods currently available to obtain answers to health related questions where RCTs do not exist or may provide insufficient information.
Methods: Experience drawn from our recently completed projects, supplemented by other reference materials will be used to describe techniques to answer population health related questions.
Results: Registries, administrative and population databases, such as the World Health Organization's (WHO) databases can be

important sources of data. For example, the WHO's databases can be used to compare trends across countries over time; comparing outcomes where practices/environment may differ. Systematic review and meta-analysis techniques can be used to bring together outcomes from different studies; providing more robust answers. The strengths and weakness of the various sources and techniques are discussed along with key aspects to pay attention to when conducting the analysis.
Conclusions: RCTs are not usually available to answer population related questions in pregnancy; however, alternative sources and techniques are available to answer these types of questions.

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Genotype specific approaches to preventing drug-induced liver injury in multiple sclerosis: Work in progress
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Conflict of Interest: No conflict of interest
Funding: Canadian Institutes of Health Research, British Columbia Clinical Genomics Network and Genomic Canada

Background: Multiple sclerosis (MS) is a neurodegenerative disease and the most common cause of neurological disability in young adults in the Western world. Interferon-beta (IFNβ) is widely prescribed for treating MS, yet its effectiveness is modest. A common adverse reaction associated with its use is the development of abnormal biochemical liver test results. Despite regular monitoring, cases of liver failure, necessitating a transplant are reported. We aim to identify genetic variants associated with IFNβ-induced liver injury, with the ultimate goal of predicting an individual's risk of developing this serious adverse reaction.
Hypothesis: There are genotypic differences between those who develop liver injury, compared to those who do not.
Protocol: Cases are defined as MS patients reaching the clinical threshold for drug-induced liver injury. Controls are matched to cases based on age, sex, area of residence and having taken IFNβ for >2 years with no evidence of drug-induced liver injury. Each patient's DNA will be genotyped to identify genetic variants associated with susceptibility to IFNβ-induced liver injury. Genotyping will be done using a panel of adverse drug reaction (ADR) □related genes consisting of HapMap tag SNPs and functional gene variants and CNV□specific assays for genes with known deletion and duplication variants. To date, 15 cases and 59 controls have been enrolled from BC, with recruitment beginning in Manitoba and London, Ontario by mid-2012.

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Drug-drug interactions between rosuvastatin and β-blockers through the OATP1A2 transporter
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Funding: Canadian Institutes of Health Research, CHUM Foundation

Background: OATP1A2 is a membrane transporter potentially involved in the absorption of various drugs, such as rosuvastatin and β-blockers. The concomitant use of more than one substrate of OATP1A2 can result in a drug-drug interaction and this could modify the pharmacokinetic profile of the medications. The goal of this study was to determine whether there is interaction in vitro between rosuvastatin and several β-blockers through OATP1A2.
Methods: A HEK293 cell line overexpressing OATP1A2 was used as model for the study. First, the cells were grown to confluence on 12-well plates. Then, they were co-incubated in the presence of rosuvastatin and increasing concentrations of the β-blockers: carvedilol, alprenolol, metoprolol, propranolol, timolol, acebutolol, celiprolol, nadolol, atenolol, and sotalol. The amount of rosuvastatin transported in the cells was measured by UV-HPLC.
Results: The β-blockers carvedilol, alprenolol, metoprolol, propranolol, and timolol were capable of inhibiting the uptake of rosuvastatin through

OATP1A2 with IC_{50} of 7.7, 40.0, 51.0, 51.6, and 53.8μM, respectively.
Conclusions: This study shows that a drug-drug interaction exists in vitro between rosuvastatin and several β-blockers, where carvedilol is the most potent inhibitor. Such an interaction may potentially occur under regular administration of those drugs. Consequently, some β-blockers may modulate absorption, distribution and metabolism of OATP1A2 substrates.

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The role of monolysocardiolipin acyl transferase - 1 (MLCL AT-1) in Barth syndrome

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Conflict of Interest: None declared
Funding: Canadian Institute of Health Research and Barth Syndrome Foundation of USA and Canada

Background: Barth Syndrome (BTHS) is a rare X-linked genetic disorder caused by mutations in the *TAZ* gene. The *TAZ* gene product, tafazzin, is responsible for remodeling Cardiolipin (CL) with the necessary acyl species. *TAZ* mutations can result in cardiomyopathy. Our laboratory has discovered a new enzyme involved in the remodeling of human CL, monolysocardiolipin acyltransferase-1 (MLCL AT-1).
Objective: We will examine if MLCL AT-1 complements tafazzin in the remodeling of CL.
Methods: Epstein-Barr virus transformed lymphoblasts from normal or BTHS patients were transfected with either *TAZ* RNAi or *MLCL AT-1* RNAi. Other groups were transfected with both *TAZ* RNAi and *MLCL AT-1* RNAi while the remaining groups were transfected with *TAZ* RNAi or *MLCL AT-1* RNAi plus an *MLCL AT-1* gene containing plasmid. *TAZ* and *MLCL AT-1* gene expression were analyzed using RT PCR. CL mass, MLCL AT-1 enzyme activity and incorporation of [1-^{14}C] Linoleic acid into CL were also analysed.
Results: *MLCL AT-1* gene expression increased when *TAZ* was knocked down. Expression of *MLCL AT-1* restored CL levels, increased [1-^{14}C] Linoleic acid incorporation into CL, and raised MLCL AT-1 enzyme activity in normal and BTHS cells in which *TAZ* was knocked down. Knockdown of *MLCL AT-1* and *TAZ* in normal or BTHS cells did not reduce MLCL AT-1 activity greater than knockdown of *TAZ* alone.
Conclusion: *MLCL AT-1* expression in BTHS cells may serve as a potential therapeutic approach to treat BTHS.

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Absolute quantification of P-glycoprotein drug transport activity for in vitro to in vivo pharmacokinetic prediction

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Funding: Canadian Institute of Health Research

It is well appreciated that membrane transporters play important roles in drug disposition, a critical determinant of the pharmacological and toxicological profile of all drugs. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is a clinically important and well-characterized efflux transporter affecting drug absorption, distribution and elimination. Despite that a number of in vitro assays for P-gp activity are commonly used, the in vivo relevance of data derived from such assays remains unclear due to both incomplete knowledge of transporter intrinsic clearance and a lack of predictive extrapolation strategies. Here we provide the theoretical and experimental strategy as well as preliminary data for in vitro to in vivo prediction of P-gp mediated transport. P-gp transport of probe drug sitagliptin was examined in a model of cultured, polarized epithelial cells heterologously expressing varying amounts of transporter using adenoviral gene delivery. By monitoring sitagliptin transcellular flux using liquid chromatography-tandem mass spectrometry in combination with mathematical modeling, we obtain a value for P-gp intrinsic clearance. This intrinsic transport clearance is normalized to P-gp protein content of cells as determined by quantitative proteomic analysis. These data, which would be the first of their kind, are expected to form the foundation for quantitative methods for in vitro to in vivo prediction of drug pharmacokinetics and ultimately therapeutic efficacy.

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Roles of cysteine 378 and cysteine 416 in human equilibrative nucleoside transporter 1 function and ligand interactions

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Funding: Schulich School of Medicine and Dentistry, NSERC and Canadian Institutes of Health Research

Background: Nucleosides and their analogues require specific transporters to enable their movement in and out of cells. Human equilibrative nucleoside transporter 1 (hENT1), is a bi-directional facilitative transporter with an 11 transmembrane topology. Previously, with heterologous expression in mammalian cells we showed hENT1 sensitivity to charged methanethiosulfonate (MTS) modifications. Positively charged MTSET inhibited binding of the prototypical ENT1 inhibitor [^{3}H]NBMPR while negatively charged MTSES affected its binding exclusively in cell membranes. In this study, we use cysteine-directed mutagenesis to identify residues responsible for charged MTS sensitivity. Mutation of the predicted extracellular C378 abolished MTSET effects seen in wild-type while mutation of C414 located in intracellular loop 5 (IL5) resulted in an enhancement of these effects. A double mutation of both C378 and C414 removed the inhibition of MTSET to [^{3}H]NBMPR binding indicating a structural link between the two residues. Loss of the cytoplasmic C416 (IL5) lead to a complete loss in [^{3}H]2-chloroadenosine transport, a classical substrate for ENT1. Additionally, C416S mutant was insensitive to MTSES treatment in broken cells identifying it as the residue reacting with MTSES to decrease [^{3}H]NBMPR binding. Taken together, we have identified two areas of importance in hENT1, the extracellular C378 and cytoplasmic C416 as residues contributing to inhibitor binding and substrate translocation sites.

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Pharmacogenetics and pain management: A review

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Funding: None declared

Objectives: To review the literature surrounding the role for pharmacogenetic (PG) testing in the medical management of patients with chronic pain.
Methods: A systematic literature review was performed.
Results: Current treatment strategies for chronic pain follow the WHO pain ladder and opioids remain the mainstay of medical treatment for chronic pain, both cancer and non-cancer. Several genes have been shown to be associated with altered PK and PD of certain pain management drugs. The majority of studies of PG testing in pain management patients focus on one gene and its affect on drug metabolism in isolation. There is a paucity of evidence that PG testing in chronic pain patients results in better analgesia with less adverse drug effects. There are data to suggest that monitoring of serum drug and/or metabolite levels may lead to better patient outcomes.
Conclusions: PG only partially explains the altered drug metabolism between individuals. Furthermore, the metabolism of most drugs involves more than one enzyme and can therefore be affected by multiple genes. The metabolism of pain medications can also be affected by many other factors, such as drug-drug interactions, dosing, disease co-morbidity, nutritional status, gender and age. Measurement of serum drug and metabolite concentrations likely provides more individualized information about the drug metabolism in a patient, since serum concentrations account for all of the various factors affecting drug metabolism.

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Safety of inhaled corticosteroids during pregnancy

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Funding: Canadian Institutes of Health Research

Background: Asthma is the most frequent chronic condition occurring during pregnancy but few prospective studies have been done to assess the safety of inhaled corticosteroids (ICSs) during this time. Pregnant women refrain from taking their ICS for fear of harming their baby, but poorly controlled asthma is also linked with adverse perinatal outcomes, such as prematurity or low birth weight. Oral corticosteroid therapy is associated with adverse drug reactions (ADRs) such as adrenal suppression, gastrointestinal complications, or decreased immunity. While inhaled corticosteroids are felt to be safer, some of the same ADRs as oral therapy have been found with their use.
Objectives: To assess maternal and fetal safety by employing active surveillance (AS), carried out by trained individuals, to identify maternal ADRs potentially linked with ICS use during pregnancy. Additionally, to collect detailed clinical and drug history, and prenatal outcome information from the pregnant women.
Methods: Using AS, a total of 150 asthmatic pregnant women on ICS therapy, and 75 negative controls (those not on ICS therapy), will be recruited through local asthma clinics and the Motherisk Program at SickKids Hospital. Assessment of ADRs will be by questionnaire. Where possible, chart reviews will be conducted, and hair samples collected to test cortisol levels as a sign of adrenal suppression.
Outcome: It is hoped evidence will show ADRs do not occur with ICS use during pregnancy.

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Dehydroepiandrosterone alters tocopherol levels and expression of tocopherol transfer protein

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Funding: None declared

Background: Dehydroandrosterone (DHEA) and its sulfate DHEA-sulfate ester (DHEA-S) are the most abundant adrenal steroids in humans. However, the physiologic roles of DHEA and DHEAS have not been clearly defined. High levels of DHEA have been reported to be associated with decreased risk of cardiovascular disease and there has been speculation about their possible role in the aging process. Vitamin E, which has a critical role as a lipid-soluble antioxidant and prevents lipid peroxidation in a variety of tissues in several pathological conditions.
Objectives: In this study, we examined vitamin E status in rats administered DHEA and investigated the expression of vitamin E related proteins including alpha-tocopherol protein (alpha-TTP), which binds selectively alpha-tocopherol, and regulates the distribution of tocopherol in the plasma and various peripheral tissues.
Methods: Wistar rats (four weeks, male) were assigned to two groups: a control group and a DHEA group fed the standard rat chow containing 0.4 % (wt/wt) DHEA, and fed for two weeks. Results: Plasma alpha-tocopherol level in DHEA administered rats are increased compared with controls. Hepatic alpha-TTP gene expression is significantly increased in DHEA administered rats. Expression of alpha-TTP may affect circulatory vitamin E status.
Conclusions: DHEA and DHEA-S are widespread as supplements for anti-aging. DEHA may have a synergetic effect as anti-oxidants with vitamin E.

THURSDAY JUNE 14, 2012

CSPT POSTER PRESENTATION ABSTRACTS

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Sulphamethoxazole-hydroxylamine reduces levels of Peroxiredoxin 1 in Jurkat T cells expressing the HIV-1 Tat protein

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Background: Treatment of HIV infection requires antiretroviral agents as well as drugs such as the antimicrobial Sulphamethoxazole (SMX), which is used as prophylaxis and first line therapy for Pneumocystis pneumonia, a common AIDS-defining disease. Hypersensitivity adverse drug reactions (ADRs) to a variety of drugs are common in HIV-infected individuals and cause significant morbidity with SMX remaining a major culprit. While the pathophysiology of drug hypersensitivity remains incompletely understood, ADRs to SMX have been linked to a reactive metabolite SMX-HA (SMX-hydroxylamine) which acts as a hapten following covalent conjugation with cellular proteins. We have also shown that the HIV-1 Tat protein contributes to ADRs in the HIV population. As the formation of hapten-protein conjugates is exacerbated by oxidative stress, we sought to elucidate the effects of Tat on the cellular redox proteome. We performed redox 2D gel electrophoresis, which enabled us to distinguish between thiol protein targets, using Tat-expressing Jurkat T cells in the absence and presence of SMX-HA. Exposure of the Tat-expressing cells to 200μM SMX-HA, led to a 2- to 3-fold increase in thiol protein oxidation as well as a significant decrease in the protein level of peroxiredoxin 1 compared to both the parent and HIV infected cell lines. This decline of peroxiredoxin 1 protein is indicative of significant oxidative stress that in turn lead to increased apoptosis.

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Pharmacogenomic diversity within African populations and between the African and European ancestries

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Funding: Canadian Pharmacogenomics Network for Drug Safety Consortium

Background: Genetic diversity influences drug response and risk of adverse drug reactions (ADR). Therefore, it is important to characterize genomic variations at drug response loci by population.
Aims: 1) Characterize key pharmacogenomic variations in drug absorption, distribution, metabolism and excretion (ADME) genes in ethnically diverse African populations; 2) explore differences between the African and European ancestries and their relevance to drug metabolism/toxicity.
Study Population/ Methods*:* 281 Africans (AFR) and 907 Europeans (EUR) genotyped for 4536 SNPs in over 300 key ADME genes. Genetic ancestry ascertained by principal component analysis.
Results: Frequencies of risk ADR-associated SNPs were dramatically increased in AFR vs EUR, consistent with the known increased in the incidence of these ADRs in AFR. Cancer pharmacogenomics: *UGT1A6* rs17863783 for anthracycline-induced cardiotoxicity - P = 5.98E-019, 12.68% AFR vs. 2.48% EUR and *TPMT* rs1800462 (*TPMT* *2) for thiopurine toxicity - P = 1.34E-53, 15.20% AFR vs 0.33% EUR. Antiretroviral drug toxicity associated SNPs: *CYP2B6* rs34097093 for Efavirenz – P = 1.24E-300, 34.19% AFR vs 0.17% EUR; *CYP3A* rs2740574 for Indinavir - P = 6.65E-229, 37.99% AFR vs 2.98% EUR and *ABCC2* rs8187710 for Lopinavir – P = 1.50E-025, 21.25% AFR vs 5.47% EUR. Variations were also observed within AFR populations.
Discussion/Conclusion*:* Increased frequency of risk ADR-associated SNPs in Africans could explain the increased incidence of ADRs.

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Placental ABC efflux transporter expression in insulin-managed diabetes

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Conflict of Interest: None declared

Funding: Canadian Institute of Health Research operating grant; NSERC CGS-M (AMC); CIHR CGS-D (GJA)

Background: Drug efflux transporters in the placenta can significantly influence the materno-fetal transfer of a diverse array of drugs and other xenobiotics. To determine if clinically important placental drug efflux transporter expression is altered in pregnancies complicated by insulin-managed gestational diabetes mellitus (GDM-I) or insulin-managed type 1 diabetes mellitus (T1DM-I), we compared the expression of multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein 2 (MRP2) and the breast cancer resistance protein (BCRP) via western blotting and quantitative real-time polymerase chain reaction in samples obtained from insulin-managed pregnancies to healthy term-matched controls. At the level of mRNA, we found significantly increased expression of MDR1 in the GDM-I group compared to both the T1DM-I ($p < 0.01$) and control groups ($p < 0.05$); however significant changes in the placental protein expression of MDR1, MRP2 and BCRP were not detected ($p > 0.05$). Interestingly, there was a significant, positive correlation observed between plasma hemoglobin A1c levels (a retrospective marker of glycemic control), BCRP protein expression ($r = 0.45$, $p < 0.05$) and BCRP mRNA expression ($r = 0.58$, $p < 0.01$) in the insulin-managed DM groups. Collectively, the data suggest that the expression of placental efflux transporters is not altered in pregnancies complicated by diabetes when hyperglycemia is managed; however, given the relationship between BCRP expression and plasma hemoglobin A1c levels it is plausible that their expression could change in poorly managed diabetes. Further studies are required.

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Potential role of 4-hydroxy-2-nonenal in the development of nitrate tolerance

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Funding: Canadian Institutes of Health Research and OGS

Background: Tolerance to organic nitrates such as glyceryl trinitrate (GTN) is associated with a loss of vasodilator activity, oxidative stress, and inactivation of aldehyde dehydrogenase 2 (ALDH2). 4-hydroxy-2-nonenal (4HNE) is a by-product of oxidative stress-induced lipid peroxidation and this toxic aldehyde can form adducts with proteins, resulting in cell dysfunction. ALDH2 plays a major role in the detoxification of 4HNE. Taken together, these findings raise the possibility that tolerance development could be mediated by increased levels of 4HNE resulting from GTN-induced inactivation of ALDH2. In the present study we used an *in vivo* GTN tolerance model and a cell culture model of nitrate action to assess whether GTN exposure resulted in HNE adduct formation, and whether exogenous 4HNE affected GTN-induced relaxation and cGMP accumulation.
Results: In aortae from rats treated with GTN (0.4 mg/hr for 2 days) or in porcine kidney epithelial cells (PK1) incubated with 1μM GTN, a marked increase in HNE-protein adducts was observed. Preincubation of PK1 cells with HNE (10, 30 and 100μM) resulted in a dose-dependent decrease in GTN-induced cGMP accumulation, whereas the cGMP responses to the NO donor, DEA/NO,were unaffected. Preincubation of isolated rat aorta with HNE (0.1, 1 and 10μM) resulted in a dose-dependent decrease in vasodilator responses to GTN, thus mimicking GTN-tolerance.
Conclusions: Together, these results indicate an association between GTN-induced HNE adduct formation and decreased responses to GTN, suggesting a role for HNE in the development of GTN tolerance.

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Validation of the *in vitro* Platelet Toxicity Assay (*i*PTA) for the diagnosis of suspected hypersensitivity reactions to sulfonamides

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Funding: The Ivey Chair in Molecular Toxicology, CIHR-GSK Chair in Pediatric Clinical Pharmacology and Schulich School of Medicine and Dentistry

Background: Drug hypersensitivity syndrome (DHS) is a rare but potentially adverse drug reaction (ADR). A valid diagnostic test for DHS would be a major advance in patient care and evaluation of possible ADRs during drug development and clinical trials. We have recently developed a novel diagnostic test for DHS called the in vitro platelets toxicity assay (iPTA) using peripheral blood platelets as a surrogate cell model as oppose to the traditional lymphocyte Toxicity Assay (LTA) that uses peripheral lymphocytes. This work was to validate the use of the iPTA for the diagnosis of DHS. Forty-seven individuals (25 DHS patients and 22 healthy controls) were recruited to participate in this research. Blood samples were obtained and both LTA and iPTA were performed independently. Results were then compared to determine the degree of agreement between the two diagnostic approaches. There was concentration-dependent toxicity in the cells of patients in both the LTA (lymphocytes) and iPTA (platelets) and toxicity was greater in cells from patients thn from controls. The iPTA was significantly more sensitive than the conventional LTA test in detecting the susceptibility of patient cells to in vitro toxicity. The novel iPTA has considerable potential to be used as an investigative tool for DHS.

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Meta-analyses of small cohort studies in teratology – Do they predict later results of large cohorts?

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Funding: Libyan Government

Introduction: Meta-analyses have become increasingly useful in the area of clinical teratology. Observational studies provide an important and often solitary source of information to be included in such meta-analyses. However, the quality of published meta-analyses of small observational studies is variable.
Objective: The aim of the study was to examine the validity of the conclusions reached by meta-analyses of small cohort studies. These results were compared to more recent, much larger database-derived cohort studies.
Methods: All meta-analyses published in peer review journals by the Motherisk Program were identified. The results of these meta-analyses were compared to those of large cohort studies published at later dates.
Results: Out of about 60 meta-analyses published by Motherisk on medicinal drugs between 1985 and 2011, 9 different meta-analyses were successfully matched to large cohort studies published later on the same exposure. There were 7 "negative" meta-analyses (showing no teratological effects) and 2 "positive" ones. In all nine instances, the meta-analyses accurately predicted the results of the later, large cohort studies.
Conclusion: Meta-analysis of smaller studies generates the correct signal in estimating human teratogenicity years before a large and methodologically superior cohort studies are published.

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Relationship between meconium fatty acid ethyl esters and measures of structural and functional toxicity in the fetal guinea pig following chronic maternal ethanol administration

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Funding: Canadian Institutes of Health Research

Background: FAEE are ethanol metabolites present in high concentrations in the meconium of prenatally ethanol-exposed human neonates. This study examines associations between gestational age, hippocampal weight, CYP2E1 activity, HPA-axis function, and FAEE in the meconium of ethanol-exposed third-trimester equivalent fetal guinea pigs. Pregnant guinea pigs were randomized into two groups; ethanol-exposed (4g/kg/day, n = 12) and isocaloric-sucrose pair-fed controls (n = 12). On gestational day (GD) 45, four animals from each group were euthanized. Littermates were euthanized and hippocampi were dissected and weighed. Plasma was analyzed for

ACTH and cortisol. Fetal liver was excised; mitochondrial and microsomal fractions were assessed for CYP2E1 activity. Meconium was collected and analyzed by GC-MS; ethyl palmitate, stearate, oleate, and linoleate were quantified. This was repeated at GD 55 and GD 65. Negative correlations were found between FAEE and hippocampal weight (Spearman r = -0.549; p = 0.003) as well as microsomal CYP2E1 activity (Spearman r = -0.799, p = 0.002) at GD 65. A positive correlation was found between mean littermate FAEE and total maternal ethanol dose.These data indicate that meconium FAEE constitute a biomarker of hippocampal injury and cumulative ethanol exposure. The negative correlation between fetal hepatic CYP2E1 activity and meconium FAEE indicates that FAEE production in the fetus may be inversely dependent upon fetal oxidative metabolism capacity.

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Pharmacological blockade of the androgen signaling stimulates androgen glucuronidation in prostate cancer cells

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Conflict of Interest: None declared

Funding: Canadian Institutes oh Health Research, Canada Foundation for Innovation, Fonds de Recherche Quebéc-Santé

Background: Androgen receptor (AR) activation is a crucial event for both prostate cancer (PCa) initiation and progression. An efficient way for androgen inactivation in prostate cells consists in their conjugation with the highly hydrophilic glucuronide moiety. This reaction, catalyzed by the UDP-glucuronosyltransferase (UGT)2B15 and UGT2B17 enzymes, produces inactive and easily excretable glucuronide derivatives in the human prostate. AR was previously identified as a negative regulator of UGT2B15 and UGT2B17 genes expression. Based on these observations, ex vivo and in vivo experiments were performed to test the possibility that clinically used anti-androgens may affect this AR-dependent down-regulation. Using the PCa cell models LNCaP and LAPC-4, we show that AR antagonist Casodex causes a time- and dose-dependent induction of UGT2B15 and UGT2B17 genes expression, as well as an improved androgen glucuronidation. The contribution of AR in these regulatory events was confirmed using LNCaP cells knock-downed for AR. In addition, tissue microarray experiments demonstrated that PCa samples from patients exposed to neoadjuvant hormonal therapy exhibited increased UGT2B15 protein levels. UGT2B17 levels were transiently increased in patients treated for up to 5 months. Overall, these observations illustrate an unexpected anti-androgenic effect for the pharmacological blockade of the androgen signaling in prostate cancer cells.

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Azole-based heme oxygenase inhibitors and their effects on breast cancer growth and metastasis in vivo

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Conflict of Interest: None declared

Funding: Ontario Institute of Cancer Research

Background: Heme oxygenase (HO) is responsible for the breakdown of heme to biliverdin, free iron, and carbon monoxide (CO). The two major isoforms, HO-1 (inducible) and HO-2 (constitutive) are involved in a variety of physiological functions, including apoptosis, inflammation and angiogenesis - important to the progression of some cancers. Previous work using metalloporphyrin-based compounds has identified HO inhibition as a potential target for the prevention of tumour growth and progression. These compounds are limited by their lack of selectivity for HO. Novel azole-based HO inhibitors have demonstrated increased in vitro selectivity for HO and were tested for their effects on breast cancer growth & metastasis and cancer-related angiogenesis. *In vitro* experiments included observing the effects on AC2M2 cell viability and endothelial tube growth in an aortic ring model. *In vivo* experiments involved the

implantation of GFP-labelled AC2M2 cells into the mammary fat pad of female nude mice, which then received metronomic treatment with an azole-based compound or vehicle. Primary tumours were removed on day 21 and metastases were allowed to grow for another 10 days. We hypothesized that the azole-based HO inhibitors would decrease primary tumour volume, decrease the incidences of lung metastases and that resulting secondary tumours would be smaller in size compared to control. The findings will help determine whether HO is an appropriate target in the treatment of breast cancer.

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Preventing asthma exacerbations with a short course of oral steroids at the earliest signs of upper respiratory tract infection: Preliminary results of an ongoing policy trial

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Conflict of Interest: No conflict of interest

Funding: Canadian Institutes of Health Research, Michael Smith Foundation and Provincial Health Service Authority

Background: Up to 80% of asthma exacerbations are caused by upper respiratory tract infections (URTIs). Despite recommendations in guidelines, prescription of a short course of oral steroids at the earliest signs of URTI is not widely accepted. We are conducting a policy trial to determine barriers to this practice and patient outcomes of this intervention.

Methods: Patients with two or more URTI-related ED-visits in the past year were recruited into the trial upon diagnosis of asthma exacerbation. Patients receive a dispensed prescription of oral steroids with detailed instructions to use the medication at the earliest sign of URTI. A second, matched group of patients follow the standard of care. Patients' outcomes are collected for one year from follow-up interviews on quality of life, asthma exacerbation management, URTI signs, symptoms and frequency, ED use, as well as health provider's acceptance of this practice.

Results: We enrolled 97 patients; 27 in the intervention arm. Several factors have been identified as barriers for health providers to completely accept this preventative drug prescribing: the belief that families will not be able to correctly use the oral steroid at the time of URTI onset, that oral steroids will be administered but the child will not receive medical follow up, and that there is questionable value of giving a medication for future exacerbations.

Conclusions: We have been able to document the value of this intervention as well as some of the barriers healthcare providers have in order to completely accept this intervention. Next steps will be to conduct focus groups with healthcare providers and families of patients to further understand barriers of intake.

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Methadone pharmacokinetics in Opiate Dependent patients

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Conflict of Interest: No commercial or financial conflicts

Funding: None declared

Background: In Opioid Dependence programs treatment with Methadone has been successful. Although the patient is on a maintenance dose they often ask for re-adjustment of their dose. Almost all of these patients have co-morbidities and require additional medication so drug-drug interaction cannot be ruled out. Methadone half-life is the functional marker of drug-drug interactions and reflects the state of methadone's metabolism.

Method: Pre-dose and post dose blood samples, dose, weight and patient's medication list was obtained. The assays for methadone and metabolite were done by immunoassay that had previously been validated against both HPLC and

GC. We calculated t½, Cl and Vd for both methadone and its metabolite.
Result: From 2002 to Jan 2012, 239 patient samples were analysed. Since 2006 on 65 patients we also had the list of medications. Six of these had no other medication and the 59 remaining had received an average of 3.5 medications (range 1 to 13). These included substrates and inhibitors of methadone. Methadone: Half-life could be calculated on 214 patients t½ ranged from 6.6h to 167h. Removing extreme values <15h to >50h, mean 27.9h, n=177 was obtained. Half-life correlated significantly with dose in mg/kg, weight and pre-dose methadone blood levels at r=-1954, p<0.032; r= .3612, p=000 and r=.5939, p=0.000 respectively.
Conclusion In this large series changes in methadone half-life in many patients could be attributed to the medication the patient was prescribed.

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Regulation of expression and transport function of organic anion transporting polypeptide 2B1 transcriptional start site variants

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Conflict of Interest: None declared
Funding: Canadian Institutes of Health Research

Background: Organic Anion Transporting Polypeptide 2B1 (OATP2B1) is a membrane transporter that facilitates the cellular uptake of numerous endogenous compounds and drugs. Recently, it has been shown that differential promoter usage in tissues results in the expression of several OATP2B1 splice variants which utilize 5 distinct first exons and promoters but share common subsequent exons. These splice variations are expected to encode either a full length or truncated protein. Since little is known about OATP2B1 splice variants we investigated the transport function and relative expression in key tissues responsible for drug absorption and elimination.
Methods and Results: Both the predicted full length and truncated forms of OATP2B1 were detected using variant-specific PCR in liver and small intestine, albeit in differing proportions. The transcriptional activity and regulation of the truncated variant SLCO2B1 gene promoter was examined by dual luciferase reporter assay and ChIP. We determined that HNF4α was able to transactivate the truncated variant promoter but not the full length. Transport kinetics were determined after heterologous expression in cultured cells, demonstrating that the truncated variant was capable of transporting the known OATP2B1 full length variant substrates, estrone sulfate and rosuvastatin.
Conclusion: These findings indicate that differential regulation of OATP2B1 splice variant expression in tissues could contribute to variation in drug response.

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The free radical spin trap α-phenyl-N-t-butylnitrone (PBN) reduces postnatal cognitive deficits caused by in utero exposure to methylmercury

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Funding: Canadian Institutes of Health Research

Background: Methylmercury (**MeHg**) causes neurodevelopmental deficits in infants, possibly in part via the formation of reactive oxygen species (**ROS**). ROS-mediated oxidative damage to cellular macromolecules like DNA has been implicated in embryopathies caused by several xenobiotics. We previously found that fetuses exposed to a single maternal dose of 4-8 mg/kg MeHg chloride exhibited a dose- and time-dependent increase in oxidatively damaged DNA in fetal brain. Herein we investigated the role of ROS in postnatal cognitive deficits in CD-1 mice caused by in utero exposure to the lower dose of MeHg. Pregnant dams were injected i.p. on gestational day (**GD**) 17 with 4 mg/kg MeHg or its phosphate-buffered saline vehicle, with or without pretreatment with the free radical spin trap α-phenyl-N-t-butylnitrone (**PBN**). The offspring were evaluated in the object recognition test (**ORT**) at 6 weeks of age, or in the passive avoidance test (**PAT**) at 4 months, and using von Frey filaments at 6 weeks and 4 months of age.

MeHg caused cognitive deficits in both the ORT ($p < 0.05$) and PAT ($p < 0.05$). No change in mechanosensitivity was observed in the von Frey test, confirming that PAT results were not confounded by reduced sensory function. Pretreatment with PBN reduced MeHg-initiated deficits in both the ORT ($p<0.05$) and the PAT, with no effect in the von Frey test, implicating ROS in the mechanism of MeHg-initiated cognitive deficits, to which the developing fetal brain is highly sensitive.

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Drug-transporter interactions: inhibition of MCT1 and MCT4 by statins and other acidic drugs

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Funding: CHUM foundation

Background: The muscle injury, also called myopathy, is a well known side effect associated with statin medications. In addition to statins, many drugs have been implicated as causes of myopathies and in some more serious cases, rhabdomyolysis. We hypothesized that these side effects could be related to an intracellular accumulation of lactic acid. Our objective was to use cell models expressing monocarboxylate transporters, MCT1 and MCT4, to determine whether the use of statins and other acidic drugs can inhibit the transport of lactic acid.
Methods: Cell lines used as models for MCT1 and MCT4 were Hs578T and MDA-MB-231, respectively. The cells were incubated with [^{14}C]lactic acid at 37°C and the intracellular concentration of radioactive lactic acid was measured. Inhibition studies were conducted by co-incubating the cells with different concentrations of acidic drugs and lactic acid.
Results: 15 drugs have been tested for the inhibition of MCT1 and MCT4. Among these drugs, atorvastatin, irbesartan showed the highest inhibition with IC_{50} of 20-50 µM; losartan, valsartan showed an intermediate inhibition with IC_{50} of 200-500 µM; and salicylic acid showed a lower inhibition with IC_{50} >1000 µM.
Conclusions: These results imply that atorvastatin and other acidic drugs can lead to the accumulation of lactic acid due to the blockage of MCT1 and MCT4. Further studies are required to link the intracellular accumulation of lactic acid to the muscle pain.

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Tricyclic drugs inhibit the uptake of rosuvastatin through the OATP1A2 transporter

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Conflict of Interest: No conflict of interest
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Background: OATP1A2 is a membrane transporter involved in the absorption of various drugs. Previous results from our group demonstrated that the uptake of rosuvastatin through OATP1A2 can be inhibited by several β-blockers, where carvedilol is the most potent inhibitor. Carvedilol structurally differs from the other β-blockers tested by its tricyclic moiety. The goal of this study was to determine whether the tricyclic structure of carvedilol is responsible for its strong inhibitory effect on OATP1A2.
Methods: A HEK293 cell line overexpressing OATP1A2 was used as model for the study. First the cells were grown to confluence on 12-well plates. Then, they were co-incubated in the presence of rosuvastatin and increasing concentrations of different tricyclic drugs: amitriptyline, carazolol, carbamazepine, carbazole, chlorpromazine, imipramine, and phenothiazine. The amount of rosuvastatin transported in the cells was measured by UV-HPLC.
Results: The tricyclic drugs carazolol, amitriptyline, imipramine, and chlorpromazine inhibited rosuvastatin uptake through OATP1A2 with IC_{50} of 4.0, 5.0, 8.0, and 29.6 µM, respectively.
Conclusions: This study shows that the inhibitory component is made up of the tricyclic ring with a short carbon chain and that a tricyclic ring alone is not enough to inhibit OATP1A2 transport.

Consequently, drugs composed of a tricyclic ring with a short carbon chain may strongly modulate the transport of OATP1A2 substrates.

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Pregnancy outcome and child neurodevelopment following in utero exposure to maternal cancer

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Funding: Breast Cancer Foundation

Background: Limited data exists on cancer-complicated pregnancy outcomes and management guidelines are inconsistent.
Objectives: This report outlines existing knowledge of perinatal cancer and presents pediatric and neurodevelopmental outcomes of children exposed in utero to maternal malignancy.
Methods: 24 children (aged 3-12) prenatally exposed to maternal malignancy were assessed. Information on maternal malignancy and pediatric outcomes was documented. Children's neurodevelopment was assessed using standardized psychological tests.
Results: 15 children were exposed to chemotherapy and/or radiation. 9 children exposed to maternal cancer or surgery served as controls. Control children had shorter gestations (37.2 wks vs. 35.2 wks) and lower birth weights (3115 gm vs. 2600 gm). Children from both groups were similar in their developmental milestones; anthropometric measurements; Full-scale, Verbal, and Performance IQs; and CBCL scores at testing.
Conclusions: Child's physical and neurological development was within population norms for both groups. Shorter gestations and low birth weights among controls were due to planned deliveries in order to start treatment. Prematurity is associated with increased child morbidity and mortality and should be minimized. These results should be considered when weighing the benefits of timely versus postponed maternal treatment. More research is needed to support these results and ensure optimal maternal treatment and fetal safety.

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Testing knowledge acquisition in nurses and parents from educational tools about managing pain during childhood immunization

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Funding: None declared

Background: An evidence-based clinical practice guideline (CPG) for managing childhood vaccine injection pain was recently published (CMAJ 2010).
Objective: To evaluate parents' and nurses' knowledge about effective pain management (PM) methods before and after exposure to educational materials (pamphlet and video) developed from the CPG.
Methods: Participants included 29 nurses [mean age (SD) in years, 40(13)] and 37 parents [33(4)] on the postnatal ward of Mount Sinai Hospital in Toronto. Participants completed the same knowledge test containing 10 true/false questions regarding the effectiveness of various PM methods. Percent correct scores were compared within groups using RM ANOVA. Only answers whereby participants reported both the correct response and complete certainty in their level of confidence regarding their response were included as correct.
Results: The mean percent correct scores at baseline, post-pamphlet, and post-video were 20% (SD=19), 61% (21), and 72% (16) for parents and 34% (18), 64% (21), and 70% (15) for clinicians. Statistically significant ($P<0.01$) increases in knowledge were observed at each level of intervention for both groups.
Conclusions: This study provides evidence of knowledge acquisition from the educational pamphlet and video. Importantly, that knowledge was further increased by the video after reading the pamphlet suggests that both should be used

together for future vaccine pain management education.

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Normal subjects exposed to nifedipine via differing osmotic delivery systems have differing patterns of nocturnal dipping

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Funding: None declared

Background: Several patients with unexplained increases in BP >10 mmHg were observed following silent switching of nifedipine from a 2-compartment (Adalat XL=AdN) to a 1-compartment osmotic delivery system (Mylan=MyN). To explore potential differences, we obtained 24-h ABPM recordings in 3 healthy, normotensive subjects. Recordings were made for each formulation on Day 7 of 30 mg/d dosing. Delay in nocturnal dipping to 85 mmHg was observed for MyN in all 3 subjects. Table. Subj: Nocturnal Dip Delay, 24-h mean MAP's for MyN vs and A: 5 h, 99 ± 09 vs 94 ± 10, B: 1 h, 98 ± 07 vs 95 ± 10, C: 4 h, 99 ± 10 vs 93 ± 15 Clinical dosing of nifedipine produces concentrations on the steep portion of the dose-response curve and its T1/2 is only 2 h. Therefore, changes in delivery rate during a partial dosing interval (2-4 h) would be expected to change BP response, even though differences in hourly concentrations might not be detected on 24-h AUC's. Despite being deemed bioequivalent (24-h AUC/Cmax diff ≤ +/- 20%), differering release technologies have differences in time-release profiles. This is most clearly apparent in healthy subjects taking no other medications, as seen in these preliminary data. Clinically, some patients also appear sensitive to differences in nifedipine delivery formulations. These preliminary data in 3 subjects suggest that nocturnal dipping should be scrutinized on 24-h ABPM pressures if there is a question that patients have different responses to nifedipine formulations.

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Aryl hydrocarbon receptor-dependence of dioxin effects on constitutive mouse hepatic cytochromes P450 and growth hormone signaling components

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Conflict of Interest: No conflict of interest
Funding: Canadian Institutes of Health Research

Background: The aryl hydrocarbon receptor (AHR) mediates most responses to the pollutant, 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD). A readily metabolized AHR agonist, 3-methylcholanthrene, disrupts mouse hepatic growth hormone (GH) signaling components and suppresses cytochrome P450 2D9 (*Cyp2d9*), a male-specific gene controlled by pulsatile GH via signal transducer and activator of transcription 5b (STAT5b).
Objectives: Using *Ahr* $^{-/-}$ mice as a model to examine AHR-dependence of responses, we studied effects of TCDD, a poorly metabolized AHR agonist, on hepatic expression of selected constitutive P450s, GH signaling components and STAT5b target genes.
Method: Male *Ahr* $^{-/-}$ and wild-type C57BL/6J mice received a single dose of TCDD (1000 µg/kg) or vehicle by gavage and liver was harvested 19 h later. Levels of mRNAs were assayed by RT-PCR.
Results: Two STAT5b target genes, *Cyp2d9* and major urinary protein 2 (*Mup2*), were suppressed by TCDD with AHR-dependence. TCDD also decreased GH receptor, Janus kinase 2, and STAT5a/b mRNA levels with AHR-dependence. Without triggering acute inflammation, TCDD caused AHR-dependent induction of *Cyp1a1* and NADPH-cytochrome P450 oxidoreductase (*Por*) and suppression of *Cyp3a11*. Basal mRNA levels for CYP2D9, CYP3A11, POR, serum amyloid protein P, and MUP2 were influenced by *Ahr* genotype.
Conclusions: AHR activation *per se* leads to dysregulation of hepatic GH signaling components and suppression of some, but not all, STAT5b target genes.

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The detection of cortisol in human sweat: Implications for measurement of cortisol in hair

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Conflict of Interest: No conflict of interest
Funding: None declared

Background: Hair cortisol analysis is an effective measure of chronic stress. Cortisol is assumed to enter the hair via serum, sebum, and sweat, however the extent to which sweat contributes to hair cortisol content is unknown.
Hypothesis*:* It was hypothesized cortisol would be found in sweat. Further, exposures to a hydrocortisone solution with a sweat-like cortisol concentration were hypothesized to affect hair cortisol concentrations, but would be normalized with washing.
Methods*:* Sweat and saliva samples were collected from 17 subjects, and analyzed by salivary ELISA. Subsequently, an *in vitro* test on hydrocortisone exposure was conducted. Residual hair samples were immersed in a 50ng/ml hydrocortisone solution for periods lasting 15 minutes to 24 hours, followed by a wash or no-wash conditions. Hair cortisol content was determined using a modified salivary ELISA protocol.
Results*:* Sweat cortisol concentrations were 74.62±41.51ng/ml (mean±SD) and ranged from 8.16-141.7ng/ml. Hair exposure to a 50ng/ml hydrocortisone solution for 60 minutes or more resulted in significantly increased hair cortisol concentrations ($P<0.01$). Washing did not affect immersion-increased hair cortisol concentration.
Conclusions*:* Human sweat contains cortisol that likely contributes to hair cortisol content. Subjects with prolonged sweating at the time of hair collection may have increased hair cortisol concentrations that cannot be decreased with conventional washing procedures.

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Pharmacogenomics of adverse drug reactions: establishing priorities for research programs

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Background: The importance of genetic factors in the incidence and severity of many adverse drug reactions (ADRs) is being increasingly recognized. A tool was developed to facilitate the prioritization of drugs and their associated ADRs for pharmacogenomic studies.
Methods: Scores were based on 25 criteria that are relative for clinical and genetic research: pharmacoepidemiology of drug use and ADR prevalence, likelihood of genetic basis of the ADR, pharmacology of the drug and ADR mechanism, as well as study feasibility within a given research setting (required resources, patient recruitment, timelines). The tool was applied to five drug/ADR combinations by two researchers independently and scores were compared using the intraclass correlation coefficient (ICC).
Results: Total scores for target ADRs ranged from 33% (19.5/60) to 73% (44/60) of the maximum possible score. The tool performed as expected, with a frequently occurring and severe ADR previously studied receiving the highest score, while a rare ADR with difficult clinical characterization and a milder ADR scored lower. Good agreement was observed between the scientific, feasibility, and total scores from two reviewers (ICC values = 0.895, 0.980, and 0.983, respectively).
Conclusion: This tool allows the direct comparison of strengths and weaknesses of drug/ADR study targets and can be used by research teams to better understand which pharmacogenomic studies are best suited for their research environments.

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Optimizing periconceptional and prenatal folic acid supplementation: steady-state red blood cell and plasma folate levels achieved with 5mg vs. 1.1mg folic acid in prenatal multivitamin supplements among pregnant women

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Conflict of Interest: Dr. Koren, is a medical consultant for Duchesnay Inc.
Funding: Duchesnay Inc.

Background: Folic acid supplementation before and during pregnancy reduces the risk of neural tube defects (NTDs). Maximal protection against NTDs is achieved through maternal red blood cell (RBC) folate concentrations of 900nmol/L or greater.
Objectives: To compare the steady-state periconceptional and gestational RBC and plasma folate levels in women supplementing with prenatal multivitamins containing 1.1mg vs. 5mg folic acid.
Methods: 8 women, who were early in pregnancy or planning, and were not previously taking folic acid, were enrolled in the study after obtaining informed consent. Participants were randomized to either 1.1mg or 5mg of folic acid-containing multivitamins daily till 30 weeks gestational age. Plasma and RBC folate levels were measured at baseline, and at 6, 12 and 30 weeks of gestation using a competitive-binding receptor assay.
Results: No significant difference was observed between the baseline RBC concentrations of the 2 groups (baseline was 2849 ±143nmol/L and 2840 ±230nmol/L in the 1.1mg and 5mg groups respectively). However, differences were observed in RBC concentrations between the groups at 6, 12, and 30 weeks gestation: RBC folate concentrations by 30 weeks gestation were 4149 ±321nmol/L in the 1.1mg vs. 6175 ±394nmol/L in the 5mg group.
Conclusions: The use of 5mg folic acid produced and maintained higher blood folate concentrations compared with 1.1mg folic acid, thus rendering greater protection against NTDs.

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Educating parents about pain management during immunization

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Funding: None declared

Background: A fact sheet for parents regarding effective pain management (PM) strategies for infants was developed from a recently published clinical practice guideline (CMAJ 2010).
Objective: To evaluate parents' self-reported knowledge and utilization of effective PM methods from the fact sheet.
Methods: A convenience sample of new mothers was recruited from the postnatal wards of two perinatal centres: Mount Sinai Hospital (MSH), Toronto and IWK Health Centre (IWK), Halifax). IWK was randomly allocated to passively distribute the fact sheet in parent hospital discharge packages. Participants were contacted 2-3 months after discharge and asked about knowledge and utilization of PM interventions.
Results: 86 and 92 parents from MSH and IWK, respectively, participated. Overall mean age was 32 years (SD=5); 66.4% were university educated. Knowledge was significantly increased for parents from IWK vs. MSH for PM methods, including: 1) sugar water (22.8% vs 11.6%), 2) local anesthetics 42.4% vs. 30.2%, and 3) parent behaviour; 90.7% vs. 97.8%); all $P<0.05$. Reported utilization did not differ; 2.17% vs. 0%, 1.09% vs. 0% and 98.9% vs. 97.7%, respectively (all $P>0.05$).
Conclusions: Passive dissemination of the fact sheet in discharge packages increased parental knowledge about PM methods at little cost. Knowledge is first step in behaviour change supplementing the fact sheet with other KT strategies is needed to influence parental behaviour.

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Characterization of aminosilane coated iron oxide nanoparticles for brain targeted delivery

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Funding: Manitoba Medical Services Foundation and NSERC

Background: Aminosilane coated iron oxide nanoparticles (AmS-IONPs) have been widely used in constructing complex and multifunctional drug delivery systems. However, suitability of AmS-IONPs for brain related drug delivery applications is unknown.
Objectives: To determine AmS-IONPs toxicity and cell accumulation in brain related cell cultures and assess permeability across cell culture model of the blood-brain barrier (BBB).
Methods: AmS-IONPs were examined in a mouse brain microvessel endothelial cell line (bEnd.3) and mouse primary neurons and astrocytes. Cell accumulation of IONPs was examined using Ferrozine assay and cytotoxicity was assessed by MTT assay. Permeability of AmS-IONPs in bEnd.3 monolayer grown on PET membrane inserts (1 μm pore) was evaluated.
Results: Acute toxicity was observed in neurons and astrocytes above 70 ug/ml. Rank order of accumulation of AmS-IONPs was astrocytes> bEnd.3 cells> neurons. Presence of a magnetic field increased cell uptake but had minimal effect on AmS-IONP toxicity. Negatively charged AmS-IONPs showed 16% flux across bEnd.3 monolayers after 24 hrs with aid of magnetic field.
Conclusion: AmS-IONPs were well tolerated by all cells examined. Permeability of positively charged AmS-IONPs across confluent bEnd.3 monolayers was negligible. Modification of surface chemistry of the AmS-IONPs improved the permeability profile in cell culture model of the BBB. Therefore, AmS-IONP is a promising candidate for delivery of drugs into the brain.

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Implementation of a clinical practice guideline about immunization pain management in a public health setting

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Conflict of Interest: Dr. Anna Taddio has received research support from Pfizer and research study supplies from Natus and Ferndale, and has consulted to Archimedes.
Funding: British Columbia Immunization Committee

Objective: To evaluate the impact of a new evidence-based program guideline about pain management during childhood immunization injections on public health immunizers' attitudes, beliefs, and analgesic practices.
Method: In a controlled, before-and-after methodology, participating intervention group public health nurses (PHNs) were educated about pain-relieving strategies according to a new British Columbia Immunization Program Manual. Attitudes and beliefs of PHNs towards immunization injections and pain management and percentage of children receiving newly recommended pain-relieving strategies during immunization visits were compared before and after intervention.
Results: A total of 516 children were immunized by 31 PHNs pre- and post-implementation in the intervention sites. Overall usage of at least one newly recommended strategy increased from 22.4% pre-intervention to 77.6% post-implementation ($p<0.01$). Post-implementation, attitudes and beliefs toward analgesic interventions were significantly ($p<0.05$) more positive. PHNs also reported significantly higher levels of confidence and satisfaction in their abilities to reduce immunization injection pain post-implementation.
Conclusion: Implementation of the new guideline improved PHN immunizers' attitudes, beliefs, and

practices regarding paediatric immunization injection pain. It is anticipated that province-wide implementation of the guideline will result in a better immunization experience for children, caregivers and immunizers.

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Acute olanzapine overdose in a toddler: a case report with pharmacological insights

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Funding: No funding

Background: Olanzapine, a dopamine and serotonin antagonist, is widely used and therefore available for accidental exposure in children. An atypical antipsychotic, olanzapine is supposed to have minimal extrapyramidal and hyperprolactinemia-related side effects.
Objectives: To define the clinical signs and pharmacokinetic properties of olanzapine in toddlers after overdose.
Methods: This report describes a 17 month-old female toddler who accidentally ingested 28 mg of olanzapine. The estimated half-life of olanzapine and its effect on prolactin secretion in toddlers is reported.
Results: The patient experienced prolonged respiratory distress, leading to four days of intubation. She also experienced fever and extrapyramidal symptoms. Olanzapine levels were measured at two different time points. At day five, her prolactin level was above the upper limits of the normal range for this age group. With supportive care, she was discharged on day seven without complications.
Conclusions: The elimination half life of olanzapine in this toddler was estimated to be 13.7 hours. This supports previous findings that the elimination half life found in toddlers is significantly shorter than in adults. This is the first case to measure prolactin levels in an olanzapine-overdosed toddler. More research is needed to elucidate the importance of this finding.

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Transcriptional regulation of hepatic drug metabolizing enzymes in chronic renal failure rats

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Conflict of Interest: None declared
Funding: NSERC

Background: Drug metabolizing enzymes such as CYP3A are highly regulated by nuclear receptors. Expression and activity of these enzymes are decreased in chronic renal failure (CRF); however the mechanism by which this is occurs is largely unknown.
Objectives: This study aimed to determine the mechanism of hepatic drug metabolizing enzyme down-regulation in CRF.
Methods: CRF in Sprague-Dawley rats was surgically induced by 5/6 nephrectomy. Control rats underwent sham laparotomies. Rats were sacrificed on day 42 and hepatic CYP3A1, CYP3A2 and CYP2C11 expression and activity were determined. Chromatin Immunoprecipitation (ChIP) was performed to determine the transcriptional activation of these enzymes by nuclear receptors.
Results: On day 42, serum creatinine levels were 23.1 ± 0.9 and 60.7 ± 10.4 μM in control and CRF rats, respectively.
Conclusions: Our results demonstrate that decreased CYP3A1/2 and CYP2C11 function and protein expression are secondary to the decrease in transcriptional activation. This study provides further insight into the mechanisms of variability in drug therapy in patients with CRF.

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Heme oxygenase: Selective HO-2 inhibition

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Conflict of Interest: No conflict of interest
Funding: Canadian Institutes of Health Research

Background/Objective: A program in our laboratories is concerned with the design and synthesis of selective inhibitors of heme oxygenase (HO). Several azole-based compounds have been synthesized and evaluated as novel inhibitors. Recently, a new series of benzimidazole derivatives has been synthesized and screened for HO inhibitory activity.
Methods: The compounds were tested as inhibitors of HO-1 (rat spleen microsomal fraction) and HO-2 (rat brain microsomal fraction) using an in vitro assay for heme oxygenase based on the quantitation of CO formed from the degradation of methemalbumin.
Results: Most of the compounds in this series were found to be potent inhibitors of the constitutive isozyme HO-2, showing little inhibitory activity against the stress-induced isozyme HO-1. This selectivity for HO-2 is in contrast to our previous findings from our exploration of imidazole–dioxolane derivatives, which led to the discovery of numerous potent and selective HO-1 inhibitors. The synthesis of these novel analogs and structure–activity relationships with respect to the inhibition of HO and other enzymes will be presented.
Conclusions: Our selective HO-2 inhibitors are anticipated to become useful tools in elucidating the physiological/pathological roles of heme oxygenase/carbon monoxide in mammalian and other biological systems, and complement our suite of selective HO-1 inhibitors.

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Role of sxidative stress in 4-Aminobiphenyl-Induced liver tumorigenesis in mice

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Conflict of Interest: None declared
Funding: None declared

Background: 4-Aminobiphenyl (ABP) is a probable human environmental carcinogen that also produces liver tumors in mice. Neonatal exposure of mice to ABP leads to a dramatically lower liver tumor incidence in females than males. This sex difference parallels that found in human liver cancer, where women have a three- to five-fold lower incidence than men. Given that oxidative stress represents a major etiological factor in human liver cancer, we hypothesize that sex differences in ABP-induced oxidative stress correlate with liver tumor incidence in mice. We quantified the formation of reactive oxygen species (ROS) using the dichlorofluorescein assay, lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS), and oxidative DNA damage by immunochemical detection of the □H2AX protein. ABP exposure of cultured Hepa1c1c7 mouse hepatoma cells led to increased ROS formation which could be blocked by the antioxidant *N*-acetylcysteine, as well as a slight but non-significant increase in lipid peroxidation. The major oxidative ABP metabolite *N*-hydroxy-ABP also increased oxidative DNA damage in these cells. In preliminary *in vivo* studies, ABP-treated neonatal mice also showed an increase in oxidative DNA damage in liver. These results suggest that ABP is capable of producing liver oxidative stress. Ongoing studies are focused on comparing ABP-induced oxidative stress in male and female mice, and correlating these measures with liver tumor incidence. Funding for this project is received from the Canadian Institutes of Health Research and no conflict of interest is reported.

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Determinants of CYP3A4 expression and metabolic activity in the Huh7 human hepatoma cell model of non-alcoholic fatty liver disease

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Conflict of Interest: None declared
Funding: Canadian Institutes of Health Research

Background: With the increased prevalence of obesity, diabetes and metabolic syndrome, Non-Alcoholic Fatty Liver Disease (NAFLD) has become the most common liver disease. While medication use is common in NAFLD to treat comorbidities, little is known regarding the effect of hepatic steatosis on drug elimination and drug response. Studies in our laboratory have recently demonstrated that Cytochrome P450 (CYP) 3A4 metabolic activity and expression are decreased in

NAFLD. Here, we explored the potential molecular mechanisms that may be involved.
Objectives: To examine possible transcriptional mechanisms of CYP3A4 expression *in vitro* and *in vivo*.
Methods: Cultured Huh7 human hepatoma cells exposed with fatty acids to induce steatosis were transfected with a CYP3A4 promoter firefly luciferase reporter. C57BL/6 mice were fed a high fat diet for 5 weeks as a model of NAFLD. Hydrodynamic tail-vein injection was used to deliver the CYP3A4 luciferase reporter into the liver for functional analysis.
Results: Reporter gene analysis demonstrated that CYP3A4 transcriptional activity decreases in our *in vitro* and *in vivo* NAFLD models in comparison to control by 12% and 60% respectively.
Conclusions: We conclude that drug metabolism activity is reduced in NAFLD due to steatosis-induced reduction in CYP3A4 gene transcription. These findings are expected to provide the basis for further studies aimed at optimizing pharmacotherapy in NAFLD.

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Characterization of novel pathogenic roles of cytochrome P450s in endometrial cancer cells

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Funding: CHUM foundation

Background: Endometrial cancer (EC) cells show resistance to chemotherapy agents thereby resulting in resistance and failure in cancer treatment. This phenomenon could be due to the expression of membrane transporters in cancer cells as well as due to the expression of highly active drug metabolizing enzymes. In this study, we evaluated the implication of CYP450s in the local metabolism of drugs by EC cells by evaluating their expression levels in several cell lines. The metabolic activity of microsomes prepared from these cells was evaluated as well using probe drug substrates.
Methods: Extracted mRNA from 4 EC cell lines were analyzed by RT-PCR. Microsomes were prepared from an optimized procedure and activities measured for ebastatine (CYP2J2, 1-25 µM), chlorzoxazone (CYP2E1, 600 µM), midazolam (CYP3A5, 2 µM), bupropion (CYP2B6, 310-1550 µM), ethoxyresorufin (CYP1A1 and 1B1, 1-20 µM).
Results: The expression levels of CYP450 mRNAs showed great variability. CYP1A1 and 1B1 were highly expressed in HEC 1-B, KLE, and RL-95-2 cells while little expressed in AN3CA cells where CYP 2E1, 2A6 and 2D6 were major contributors. CYP2J2 and 2B6 are also major contributors in HEC1-B cells. Other types of CYP450s showed little to no expression. In HEC 1-B cells, CYP 2J2 had higher activity than CYP 2E1 and 3A5 while CYP1A1, 1B1 and 2B6 did not show any activity.
Conclusions: CYP450 are expressed and active at various degrees in various EC cell lines.

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Bioavailability of amoxicilin dissolved in human milk

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Conflict of Interest: No commercial or financial conflicts
Funding: WHO

Background: Since the safety of drinking water in some developing countries is a concern, breast milk could be an appropriate substitute of water to dissolve amoxicillin. No evidence is currently available to prove bioequivalence of drugs dissolved in breast milk compared to those dissolved in water.
Method: We conducted a randomized 2x2 cross-over single-dose PK study in healthy adult volunteers to characterize basic PK parameters of amoxicillin dissolved in human milk or water. Sixteen healthy adult volunteers (male or female) were enrolled. 500 mg amoxicillin was orally administered to each fasting volunteer (10 hrs). 8 blood samples were collected. Amoxicillin plasma concentrations were determined by HPLC-MS/MS. Amoxicillin PK parameters were estimated using a model-independent approach. Means of the bioequivalence parameters (C max and UAC) in 2 groups are compared using paired Student t-test.

Results: While the inter-individual variations of the concentration profiles were apparent, the intra (i.e., within)-individual differences between the water and milk arms were not remarkable. There was no statistically significant difference in C max and AUC between the 2 vehicle arms (i.e., water and human milk).