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ABSTRACTS

ORAL PRESENTATIONS
THURSDAY JUNE 3, 2010

1
Effect of rho kinase inhibitor Y-27632 on the cardiac proteome in ischemia/reperfusion injury

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Conflict of interest: No conflicts to declare.
Funding Source: CIHR, Heart and Stroke Foundation

Background: The development of cardiac injury following ischemia/reperfusion (I/R) results in contractile dysfunction. Thus pharmacological interventions that protect mechanical function of the heart are desired. Y-27632 is an inhibitor of Rho kinase and has been shown to improve left ventricular function in models of myocardial I/R. However, the precise molecular mechanism of this protection has not yet been established.
Objectives: Because Y-27632 may have pluripotent actions, we studied the effect of Y-27632 on proteomes of hearts subjected to I/R.
Methods: Rat hearts perfused using the Langendorff model were subjected to 20 min of global no-flow ischemia followed by 30 min of reperfusion or aerobic (control) perfusion. Y-27632 was infused into hearts 10 min prior to the onset of ischemia and for the first 10 min of reperfusion. Hearts were subsequently frozen for biochemical studies.
Results: Y-27632 abolished the contractile dysfunction observed after ischemia. Proteomics analysis of heart homogenates by 2-dimensional electrophoresis revealed changes in the levels of 4 proteins affected by Y-27632, according to an arbitrarily set threshold for protein analysis. Y-27632 treatment normalized the levels of creatine kinase, increased during I/R, and decreased the levels of ATP synthase, in comparison to I/R. Also, Y-27632 further increased the levels of two proteins associated with energy metabolism. These proteins were identified as L-lactate dehydrogenase B and glyceraldehyde 3-phosphate-dehydrogenase.
Conclusions: Y-27632 treatment abolished the ischemia-induced decrease in mechanical function. The changes in the levels of metabolic enzymes suggest that carbohydrate metabolism and oxidative phosphorylation may contribute to the protective mechanisms of action of Y-27632.
Keywords: *Ischemia/reperfusion injury; cardioprotection; phosphorylation*

2
The incidence of central nervous system depression in breastfed infants of mothers taking codeine for postpartum pain relief

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Conflict of interest: No conflicts of interest to declare.
Funding Source: Canadian Institutes of Health Research (CIHR)-Canadian Pharmacogenomics Network for Drug Safety (CPNDS)

Background/Objectives: Case reports and case control studies have recently suggested CNS depression in breastfed neonates whose mothers have been using codeine for postpartum pain. The incidence of this serious adverse effect is not known. The objective of the present study was to define the incidence and relative risk of CNS depression in neonates breastfed by codeine-medicated women as well as genetic and non-genetic characteristics associated with increased susceptibility to codeine-induced ADR.
Methods: In this cohort study, 394 breastfeeding women (184 taking acetaminophen and 210 taking acetaminophen with codeine), who called the Motherisk Program between January 2004-2008 were interviewed to assess rates of maternal and infant CNS depression. Codeine-consuming mothers were genotyped to identify functional CYP2D6 duplication and UM status. T-test and Mann-Whitney U statistical analyses were performed within the codeine cohort as appropriate.
Results: Maternal exposure to codeine during breastfeeding was associated with 16.7% rate of CNS depression (35/210), as compared to 0.5% in the acetaminophen-only group (1/184) [OR 36.6 (95% CI

5-270)]. There was a dose-response relationship with mothers detecting neonatal CNS depression having used on average 50% more codeine per kg per day (median 1.4 (0.75-10.5) mg/kg/day ($p<0.001$)). Four mothers of babies with CNS depression carried *CYP2D6* gene duplications consistent with ultrarapid codeine metabolism. There was significant concordance between neonatal and maternal CNS depression [OR 21.1 (95% CI 7.4-60.6)].

Conclusion: Codeine during breastfeeding causes neonatal CNS depression in every sixth baby and cannot be considered safe in all cases.

Keywords: *Acetaminophen, codeine, CYP2D6 ultrarapid metabolism*

3

The role of uptake and efflux transporter polymorphisms to steady-state statin plasma concentrations in patients on long-term statin therapy

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Conflict of interest: No conflict of interest declared

Funding Source**:** Canadian Institutes of Health Research (MOP-89753 and Vanier CGS to MKD), and the Canadian Foundation for Innovation

Background: Systemic exposure to the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), is influenced by transporter polymorphisms in healthy volunteers, including common variants in organic anion-transporting polypeptide 1B1 (OATP1B1/*SLCO1B1*), breast cancer resistance protein (BCRP/*ABCG2*), and p-glycoprotein (MDR1/*ABCB1*). Elevated statin exposure is associated with increased risk for statin-induced muscle injury. Close to 3 million Canadians take statins to reduce plasma LDL-cholesterol and cardiovascular disease risk, however the role of genetic variation in mediating statin exposure in these patients is not well understood. **Methods**: In our patients at London Health Sciences Centre on long-term atorvastatin and rosuvastatin therapy, we examined the influence of *SLCO1B1* c.388A>G and c.521T>C, *ABCG2* c.34G>A and c.421C>A, and *ABCB1* c.3435C>T polymorphisms on steady-state trough plasma concentrations of acid and lactone forms of statin determined by LC-MS/MS. **Results**: In patients taking 10-80mg atorvastatin daily (n=46), we noted marked (30-fold) interpatient variability in plasma levels, even among patients on the same dose. Individuals homozygous for the *SLCO1B1* *1b haplotype (c.388G/c.521T) had lower trough levels than *1a (c.388A/c.521T). Carriers of the *5 allele (c.388A/c.521C) had high acid:lactone ratios. Individuals with the reduced function *ABCG2* c.421A allele had moderately elevated atorvastatin acid concentrations. We did not detect a significant effect of *ABCB1* polymorphisms on trough atorvastatin concentrations. In patients on rosuvastatin therapy (n=34), no statistically significant effect of *SLCO1B1*, *ABCG2* or *ABCB1* polymorphism was observed. **Conclusion**: *SLCO1B1* and *ABCG2* variants appear to influence steady-state atorvastatin concentrations in our patients. To our knowledge, this is the first characterization of transporter polymorphisms and statin concentration in a clinical setting.

Keywords: *Drug transport, statin, pharmacogenetics*

4

Age and CYP3A5 genotype on tacrolimus dosing requirements directly after transplant in pediatric heart recipients

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Conflict of interest: No conflict of interest is declared.

Funding Source: Restracomp, SickKids, Toronto and ter Meulen Fund, KNAW, the Netherlands

Background: Tacrolimus is the drug of choice for organ transplants. In the first weeks after transplantation large variation in pharmacokinetics exist resulting in an increased risk of transplant loss. Age and genotype may contribute to this large variation. Limited data exist on the effect of CYP3A5 and MDR1 activity on this large variation.

Objectives: To determine the influence of age, recipient CYP3A5 and MDR1 genotype on tacrolimus dosing requirements in the first 14 days after transplant in pediatric heart transplant recipients.

Method: We studied the relationship between tacrolimus dose/kg and trough levels up to 14 days post-transplant, age, CYP3A5 and MDR1 genotype in pediatric heart recipients receiving tacrolimus. Tacrolimus dosing was adjusted based on therapeutic drug monitoring (TDM).

Results: Thirty-nine heart transplant recipients received tacrolimus post-transplant (median age: 6.0 yrs, range: 45 days-21.0 yrs). Younger children (<6yrs)

needed higher doses (0.08 (0.02-0.22) vs. 0.05 (0.02-0.13) mg/kg, P=0.038). In addition expressors of the CYP3A5 genotype required higher doses of tacrolimus compared to non-expressors (0.14 (0.09-0.21) vs. 0.06 (0.02-0.27) mg/kg/12h, P=0.001). Also, CYP3A5 expressors had lower tacrolimus trough levels than non-expressors (7.7 (2.3-10.3) vs. 9.8 (6.7-16.5) ng/ml, P=0.032). This relationship was not seen with MDR1 genotype.

Conclusion: Our study shows that in the first 14 days after heart transplantation, younger age and CYP3A5 was associated with lower tacrolimus levels despite higher tacrolimus doses. Knowledge of CYP3A5 genotype may help optimize dosing strategy early after heart transplantation. Further research is needed to determine if individualizing of tacrolimus dosing will result in improved outcome.

Keywords: *Pharmacogenetics, tacrolimus, cardiac-transplantation*

5
Molecular determinants of warfarin pharmacokinetics and pharmacological response

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Conflict of interest: No conflict of interest to declare.

Funding Source: Drug Innovation Fund from the Ontario Ministry of Health and Long-Term Care and Canada Graduate Scholarships from Canadian Health Research Institute

Background/Objectives: Warfarin is a widely prescribed anticoagulant known for marked interindividual variation in drug responsiveness. Genetic polymorphisms in genes affecting warfarin metabolism (CYP2C9) and activity (VKORC1) together with patient clinical parameters explain only 50% of dose variation. Therefore, we hypothesize that the interplay between warfarin (agonist) drug levels and vitamin K (antagonist) status is a key predictor of pharmacokinetic (PK) and pharmacodynamic (PD) responses.

Methods: We characterized warfarin PK-PD responses in patients initiating therapy. On treatment days 3, 5 and 8, plasma was obtained to measure response (INR), warfarin levels (LC-MS/MS), and vitamin K status (ELISA).

Results: We note that clearance of *S*-warfarin differed significantly between females and males, suggesting gender-dependent differences in the metabolic capacity for warfarin. *S*-warfarin clearance and vitamin K status were negatively associated with age indicating that both PK and PD differences are responsible for the increased warfarin sensitivity observed in the elderly. In addition, warfarin dose requirement related to genetic variation in the vitamin K metabolizing enzyme CYP4F2 (V433M). Importantly, therapeutic *S*-warfarin plasma levels segregated with VKORC1 haplotype. Furthermore, VKORC1 haplotype-dependent warfarin sensitivity was significantly related to increase in maximum inhibitory response (I_{max}). Furthermore, western blot analysis of our liver bank microsomes demonstrated that VKORC1 haplotype correlates to altered VKOR protein levels.

Conclusions: Our data reveal important new mechanistic insight regarding warfarin dose variability. Better understanding of warfarin PK-PD interaction will be essential to creating a more predictive algorithm for individualized warfarin therapy.

Keywords: *Warfarin, pharmacogenetics, pharmacokinetics-pharmacodynamics*

6
Assessment of the contribution of *CYP4F2* genetic polymorphisms on warfarin dose requirements

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Conflict of interest: No conflict of interest to declare.

Funding Source: CIHR, Xanthus

Background/Objectives: CYP4F2 appears involved in the vitamin K1 metabolism. CYP4F2 variants may contribute to variance in warfarin dose requirements. Our aim was to study the association between CYP4F2 genotypes and warfarin dose requirements, before and after adjusting for CYP2C9 or VKORC1 genotypes.

Patients (n=143) initiating warfarin treatment were enrolled.
Methods: Demographics, doses and INR were recorded during a 14-day period. CYP4F2 genotyping for the rs2108622 variant was performed by a PCR allelic discrimination assay (Applied Biosystem). CYP2C9 and VKORC1 genotyping were performed by gene chip analysis using Autogenomic Infinity system.
Results: The allelic frequency of the CYP4F2 variant allele (n=126) was 0.32. Patients homozygous for the CYP4F2 wild-type allele required lower doses of warfarin than patients carrying one or two variant alleles (dose/INR: 1.3±0.6 mg vs. 1.8±1.2). We observed a significant cumulative effect of CYP4F2 variant alleles after adjusting for CYP2C9 and VKORC1 genotypes. Lowest doses of warfarin were required in patients carrying CYP4F2 wild-type alleles and variant alleles of VKORC1 (SNPs 3673, 6484 and 6853) (dose/INR; 0.7±0.46 mg). In contrast, patients carrying one or two CYP4F2 variant alleles and homozygous wild-type for VKORC1 (SNPs 3673, 6484 and 6853) required higher doses (dose/INR; 2.8±1.6mg). Finally, the wild-type patients for CYP4F2 and VKORC1 9041 needed lower doses compared to patients carrying at least one variant allele for these genes (dose/INR; 1.1±0.6 vs. 2.2±1.6mg).
Conclusions: We demonstrate an association between CYP4F2 genotypes and warfarin dose requirements. The modulatory role of CYP4F2 genotype is further revealed after adjusting for CYP2C9 and VKORC1 genotypes.
Keywords: *CYP4F2, warfarin, genetic polymorphism*

7
Peroxisome proliferator associated receptor-γ (PPARγ) regulates chemerin expression through direct activation of chemerin promoter

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Conflict of interest: No conflicts of interest to declare.
Funding Source**:** Canadian Institutes of Health Research

Background: Chemerin is a novel adipokine that promotes adipogenesis of precursor cells by activating the cognate receptor, CMKLR1. The expression of chemerin increases dramatically with adipogenesis, however, the mechanisms underlying this induction are unknown. PPARγ, the master adipogenic transcription factor, promotes the expression of various adipogenic genes and therefore may contribute to the induction of chemerin expression with adipogenesis.
Objective: To determine the role of PPARγ in regulating chemerin expression.
Methods: We used primary cultures of bone marrow stem cells and the murine colon carcinoma cell line, MCA38 cells to investigate the regulatory role of PPARγ on chemerin gene expression. We conducted chemerin promoter reporter activity studies to examine the direct activation of chemerin promoter by PPARγ. We evaluated the chemerin expression in different mouse tissues following the administration of rosiglitazone, a PPARγ agonist for 7 days @ 3 mg/kg, i.p.
Results: We first identified potential PPARγ binding sites in the proximal chemerin promoter that is activated by the PPARγ agonist, rosiglitazone. Knockdown of PPARγ expression abolished the transactivation of chemerin promoter by rosiglitazone. QPCR analysis demonstrated that rosiglitazone induced chemerin gene expression and the knockdown of PPARγ significantly reduced the magnitude of the rosiglitazone mediated induction of chemerin. Forced expression of PPARγ rescued the loss of adipogenesis induced by chemerin knockdown. Furthermore, rosiglitazone induced the chemerin expression and/or secretion levels *in vivo* in mice.
Conclusions: These results provide clear evidence that PPARγ regulates chemerin expression by activating the chemerin promoter.
Keywords: *Chemerin, PPARγ, gene regulation*

8
Extracellular proteases regulate adipose derived bioactive chemerin

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Conflict of interest: None to disclose.
Funding Source: CFI, CIHR, NSHRF, DMRF, DPEF

Background: Chemerin, a hormone-like protein, affects cell differentiation, metabolism and inflammation. It is released from cells as inactive prochemerin and is converted to active chemerin by extracellular proteases. Obese humans have elevated serum chemerin, which may contribute to inflammatory or metabolic pathologies in obesity. Treatment of adipocytes with the obesity-related inflammatory mediator TNFα increased the ratio of active chemerin to total chemerin (active chemerin plus prochemerin) in adipocyte media. We hypothesize that this effect is mediated by adipocyte proteases that differentially activate prochemerin under basal and inflammatory conditions.

Objective: To identify the proteases that control bioactive chemerin production by adipocytes under basal and inflammatory conditions.
Methods and Results: Treatment of 3T3-L1 adipocytes for 24 hours with a general protease inhibitor cocktail increased media concentration of active chemerin determined by a cell-based bioassay. Analysis of adipose tissue and adipocyte mRNA identified tryptase, elastase, plasminogen and angiotensin converting enzyme (ACE) as possible proteases involved in chemerin processing. Given its clinical relevance, the effect of inhibiting ACE with captopril was investigated. Treatment of adipocytes and mice with captopril increased bioactive chemerin levels in media and serum, respectively, implicating ACE as a regulator of chemerin degradation *in vitro* and *in vivo*.
Conclusion: The results support the hypothesis that extracellular proteases regulate chemerin activity. Understanding how adipocytes process chemerin may serve as the basis for reducing the inflammatory or insulin resistance effects of chemerin in obesity, which in Canada afflicts 25% of the population and is a major cause of disability and premature death.
Keywords: *Adipocyte, chemerin, metabolism*

9
The role of OATP18 transporters in irinotecan disposition

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Funding Source: Lawson Innovation Prize (LHSC, London, ON)

Background: Metastatic colorectal cancer is commonly treated with Irinotecan. Irinotecan is hydrolyzed to form the active metabolite SN-38, which is subsequently deactivated by UGT1A enzymes. Human hepatic drug uptake transporters OATP1B1 and OATP1B3 have been shown to affect the transport of SN-38. Therefore, understanding the role of OATP1B molecules may aid in optimizing a patient's response to irinotecan therapy. To investigate the role of hepatic OATP1B transporters to irinotecan disposition we utilized newly created Oatp1b2 (murine homolog of human OATP1B1 and 1B3) knockout (KO) mice to test the hypothesis that absence of this transporter would result in a lower liver-to-plasma ratio of irinotecan and SN38. Male wildtype (WT) and Oatp1b2 null mice (8-12 wks, n=8/group) were injected with irinotecan (10mg/kg, i.v.) and a pharmacokinetic study was performed by sampling blood via saphenous vein puncture at 15, 30, 60 and 90 min post-injection (p.i.). At 120 min p.i. mice were euthanized and blood and liver was collected. Drug levels were determined in plasma and liver homogentates by LC-MS/MS. The plasma and liver levels of irinotecan and SN-38 in the WT and KO groups were not significantly different, with a trend of lower liver-to-plasma ratio for SN-38 in the KO group. This suggests that Oatp1b2 does not play a major role in the hepatic uptake of irinotecan in mice. Currently, we are assessing plasma levels of irinotecan and SN-38 in patients treated with irinotecan to determine the effect of SNPs in OATP1B1 and 1B3 to irinotecan disposition.
Keywords: *OATP1B transporters, irinotecan, colon cancer*

10
Oseltamivir pharmacokinetics in morbid obesity (OPTIMO Trial) – preliminary results

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Conflict of interest: The study investigators and authors received no remuneration for their work on the OPTIMO trial.
Funding Source: Canadian Centre for Vaccinology and an unrestricted grant from Hoffman La-Roche Pharmaceuticals

Background: Morbid obesity exceeds 2.7 % of the adult Canadian population. Some drugs require dose adjustment in obesity but for many drugs, detailed pharmacokinetics to guide drug dosing for the morbidly obese patient is lacking. Oseltamivir, which is widely used for influenza treatment and prophylaxis has not previously been investigated in this way.
Objectives: 1) To characterize the single dose and steady state pharmacokinetics of oseltamivir and its active carboxylate metabolite in healthy morbidly obese subjects (BMI > 40) and non-obese controls (BMI < 30) and 2) to develop a dosing strategy for morbidly obese patients if the disposition of oseltamivir is significantly different from non-obese patients.
Methods: OPTIMO was a single center, non-randomized, open label study. Oseltamivir and oseltamivir carboxylate were measured in plasma obtained from ten morbidly obese patients and ten healthy non-obese patients that received: 1) a single dose of 75 mg oseltamivir and 2) 75 mg of oseltamivir twice daily for 5 days (steady-state). Pharmacokinetic

parameters determined by non-compartmental analysis were compared using a two-way repeated measure ANOVA.
Results: For oseltamivir, the mean C_{max} was 50% lower and t_{max} increased 1.5-fold in the obese versus control group ($P < 0.05$). For oseltamivir carboxylate, CL/F and V_d/F were 2-fold higher in the obese versus non-obese group at steady-state ($P < 0.001$). The $t_{1/2}$ of elimination of the parent drug and metabolite were not altered in obese individuals.
Conclusions: The results indicate that morbid obesity significantly affects the pharmacokinetics of oseltamivir and oseltamivir carboxylate.
Keywords: *Oseltamivir, pharmacokinetics, obesity*

POSTER PRESENTATIONS WEDNESDAY JUNE 2, 2010

11
Potential regulation of micro-RNA by the oxidative stress-responsive transcription factor NRF2

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Conflict of interest: No conflict of interest declared.
Funding Source: CIHR

Background: Nrf2 is a chief transcription factor responding to cellular oxidative stress by up-regulating >150 antioxidant, cytoprotective genes. Recent studies have shown that constitutive hyperactivity of Nrf2 in cancer cells leads to their multi-drug resistance, increased cell proliferation, and hence poor prognosis. MicroRNAs (miRNAs) are small hairpin-like RNAs having roles in carcinogenesis, cell growth and proliferation, and drug resistance. Their regulation, however, remains largely unknown. We hypothesized that Nrf2 may additionally regulate a subset of miRNA in cancer cells.
Methods: We utilized small-interfering RNAs to knockdown Nrf2 expression in A549 cells which is a well-established lung cancer cell-line with constitutively active Nrf2. Analysis of 1146 miRNA candidates was carried out on the Illumina® miRNA profiling array. Panel miRNAs found to be significantly altered by Nrf2 knockdown on this array were individually verified by real-time PCR.
Results: Upon 24-hour treatment with siRNA targeting Nrf2, more than 80% reduction of Nrf2 mRNA occurred to A549 cells with significant suppression of Nrf2 protein and important Nrf2-regulated genes. The miRNA profiling assay revealed that Nrf2 knockdown in A549 cells resulted in significant suppression of hsa-miR-25 and hsa-miR-23a, but increase of hsa-miR-125b-2. These changes were successfully verified by real-time PCR.
Conclusion: The miRNAs found to be potentially regulated by Nrf2 are involved in cell growth and proliferation. This is the first report demonstrating a potential regulatory role of Nrf2 in miRNAs of lung cancer cells. Whether these Nrf2-regulated miRNAs are implicated in cancer cell proliferation and may serve a potential drug target merit further studies.
Keywords: *Nrf2, MicroRNA, drug resistance*

12
Frequency of three transported gene polymorphisms associated with statin transport in the Canadian Caucasian population

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Conflict of interest: No conflict of interest declared.
Funding Source: Heart and Stroke Foundation of Quebec

Background and Objectives OATP1B1 (SLCO1B1) and BCRP (ABCG2) are known transporters of statins such as rosuvastatin. A mutation in these membrane transporters can modify plasma and intracellular concentrations of statins which can lead to myopathy. The aim of the study was to identify the frequency of important polymorphisms in OATP1B1 and BCRP genes in the French Canadian Caucasian population.
Methods Blood samples were obtained from Canadian Caucasian young men without cardiovascular disease (n=242). DNA was extracted from the leucocytes and stored at -20°C. Genotyping of three single nucleotide polymorphisms (SNPs) at ABCG2-Q141K, SCLO1B1-D130N and V174A was performed by Taqman SNP Genotyping Assays (Applied Biosystems (ABS)). In brief 20 ng of genomic DNA was amplified using Taqman Universal PCR Master Mix (ABS) and a specific probe. PCR and genotype analysis were performed using the real-time rotary PCR analyser Rotor-Gene 6000 by software (Corbett research).

Statistical analyses were performed using the chi-square test.
Results The frequencies obtained for SCLO1B1-V174A, SCLO1B1-D130N and ABCG2-Q141K were the following: (69.8; 27.7; 2.5), (20.2; 49.4; 30.4) and (87.2; 12.0; 0.8) for homozygous wild type, heterozygous and homozygous mutant, respectively. The results for each polymorphism were in equilibrium with the Hardy-Weinberg principle.
Conclusions Percentages observed in our French Caucasian population were similar to those reported for other Caucasian populations. These results should help us better established the risk of statin-induced muscular toxicity.
Keywords: *Polymorphisms, transporters, statin*

13
A randomized controlled trial of analgesia during vaccination in adults

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Conflict of interest: No conflict of interest to declare.
Funding Source**:** Non-restricted research grant from the Gebauer Company. The liposomal lidocaine and vapocoolant spray were provided by the manufacturers, Ferndale Labs., and The Gebauer Company, respectively.

Background: Although immunization injections are the most common painful medical procedures performed worldwide, pain relieving interventions are not routinely used. Topical anesthetics are considered the gold standard for analgesia, however, identified barriers include cost and inconvenience.
Objective: To compare the analgesic effectiveness of topical anesthetics to: 1) vapocoolant spray, 2) tactile stimulation, and, 3) distraction, in adults undergoing immunization.
Methods: Open randomized controlled trial in a convenience sample of 352 adult hospital employees and volunteers. Prior to immunization, each subject received: 1) topical anesthesia using liposomal lidocaine; 2) vapocoolant spray using a proprietary blend of 1,1,1,3,3-Pentafluoropropane and 1,1,1,2-Tetrafluoroethane; 3) nurse-administered tactile stimulation; or 4) self-directed distraction by means of reading a magazine article. Study outcomes included self-reported pain using a 100 mm visual analog scale (VAS) and global assessment of analgesic effectiveness.
Results: The percentage of VAS scores $\leq$ 20 mm was higher for liposomal lidocaine (79.3%) compared to distraction (63.6%) p=0.02; but did not differ between liposomal lidocaine and vapocoolant spray (79.5%) or tactile stimulation (79.1%); p=0.97 for both analyses. Median VAS scores and the percentage of subjects reporting effectiveness was higher for liposomal lidocaine compared to distraction (p=0.05 and p=0.03, respectively).
Conclusions: When used to reduce pain during immunization, liposomal lidocaine was more effective than distraction but not different from vapocoolant spray or tactile stimulation. This information can be incorporated in current routine mass immunization programs to improve pain management practices and consumer satisfaction with the immunization experience.
Keywords: *Adult, immunization, pain*

14
Endocytosis-dependent and –independent functions of the bradykinin B_2 receptor

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Conflict of interest: No conflict of interest to declare.
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Background: The bradykinin (BK) B_2 receptor (B_2R) is a heptahelical receptor that is phosphorylated and internalized following agonist binding and upon endosomal degradation of BK, is recycled at the cell surface. Our group has shown that B-9972, a degradation-resistant agonist caused persistent internalisation of the B_2R in β-arrestin$_{1/2}$-labelled endosomes and prolonged ERK1/2 activation.
Objective: This study sought to evaluate what functions of B_2R are dependent on the endocytosis of the agonist-receptor complex.
Methods: HEK 293a cells transiently transfected with myc-tagged rabbit B_2R (myc-B_2R) constituted the exploited cellular model. Endocytosis was inhibited by a dominant negative form of dynamin (dynK44N). The activation of myc-B_2R by the agonists was measured by calcium mobilization and ERK1/2 phosphorylation (immunoblot, also applied to quantify myc-B_2R). A novel fluorescent version of BK, [5(6) carboxyfluorescein]- ε-aminocaproyl-BK (CF-εACA-BK, characterized by radioligand binding assay and

human umbilical vein contractility assay), allowed visual assessment of dynK44N's efficacy.
Results: Despite a large loss of affinity, B_2R-dependent CF-εACA-BK uptake in β-arrestin$_1$-positive endosomes was observed. DynK44N inhibited the uptake of the radiolabelled agonist ([^{3}H]BK (4.11 fmol compared to 30.10 in control myc-B_2R cells) as well as that of CF-εACA-BK (microscopy). Its co-expression with myc-B_2R did not affect agonist-induced calcium mobilization, nor did it alter ERK1/2 phosphorylation. Co-transfection with dynK44N abolished down-regulation of myc-B_2R following 12 h-treatment with B-9972 and inhibited β-arrestin$_1$ recruitment to the receptor.
Conclusions: Inhibition of receptor endocytosis had no effects on the measured signalling events, but prevented cellular ligand uptake, β-arrestin recruitment and receptor downregulation upon prolonged stimulation.
Keywords: *Bradykinin, endocytosis, receptor*

15
Factors influencing pain management during routine school-age and adolescent immunizations: findings from a focus-group discussion with public health nurses

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Conflict of interest: No conflict of interest to declare.
Funding Source: CIHR

Background: Despite the availability of a variety of evidence-based interventions, it has previously been reported that the majority of infants and children undergo vaccinations without the benefit of an analgesic. Public health nurses administer a substantial number of injections.
Objectives: To identify the attitudes and practices of public health nurses with regards to acute pain during vaccine injection.
Methods: A focus group including 10 public health nurses that immunize children in the city of Toronto was conducted whereby participants reported their attitudes and practices with regards to vaccine injection pain and pain management.
Results: Three key themes emerged: environmental and process factors, perceptions about the effectiveness of analgesic interventions, and perceptions about the importance of acute pain. Participants reported they felt that there was a lack of control over their environment, and that this resulted in fear and discomfort for children. They recommended increased support from external partners, such as school teachers and administrators. Participants reported that pharmacological interventions such as topical local anaesthetics were ineffective, but that psychological interventions such as distraction were effective analgesic strategies. Overall, fear was believed to be more important than vaccine injection pain.
Conclusions: Public health nurses reported vaccination setting, analgesic effectiveness, and relative unimportance of pain as important factors for pain management during vaccine injections. Additional studies involving public health nurses in other geographical regions are needed to corroborate these findings. Educational tools and programs aimed at addressing the identified themes and knowledge gaps are recommended.
Keywords: *Focus group, public health nurse, vaccination, pain*

16
Systematic review and meta-analysis of the effect of warming local anaesthetics on subcutaneous injection pain

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Funding Source: Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarships - Master's Award.

Background: Subcutaneous injection of local anaesthetics (LAs) is the mainstay for pain management during procedures such as laceration repair and other minor surgeries; however, injecting LAs is painful. Warming LAs has been proposed as a cost-free intervention that reduces injection pain. A systematic evaluation of the effectiveness of this technique has never been undertaken.
Objective: To determine the effectiveness of warming LAs to reduce pain during subcutaneous LA injections.
Methods: Data Sources: MEDLINE (1950-June 2009), EMBASE (1980-June 2009), CINAHL (1982-June 2009), Cochrane Library (issue 2, 2009), International Pharmaceutical Abstracts (1970-June 2009). We included studies with randomized or pseudo-randomized designs and healthy subjects or patients receiving subcutaneous injections of LAs that were warmed (body temperature) or not (room temperature).

Data were extracted onto pre-designed forms and verified by two reviewers. Quality was assessed using the Jadad quality score. The primary outcome was self-reported pain as assessed by a 100 mm visual analogue scale. Data were combined using mean differences with 95% confidence intervals (CI) using a random-effects model.
Results: A total of 14 studies with 695 adult patients were identified and meta-analyzed. A mean difference of -12.82 mm [95% CI -17.73, -7.92] on a 100 mm scale was found in favour of warmed LAs. Subgroup analysis of 5 studies investigating the effect of warming on buffered LAs showed consistent results: -12.26mm [95% CI -17.96, -6.56].
Conclusion: Warming LAs leads to less pain during subcutaneous injection. LAs should be warmed prior to administration in order to reduce injection pain.
Keywords: *Local anesthetics, pain, subcutaneous injection*

17
Placental formic acid and folic acid after chronic alcohol exposure: two sides to the story

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Funding Source: CFFAR grant no: 724 180 969 and CIHR NET grant # ELA-80227

Background: Formic acid, the toxic metabolite of methanol, has recently been detected in cord blood of infants born to alcohol-abusing mothers. Formic acid detoxification requires folic acid. However, in a rodent model, placental folate-receptors have decreased expression after alcohol exposure. We hypothesize that folate transport to the fetus will be decreased after chronic alcohol exposure and that these decreased levels will limit the ability to detoxify formic acid. These factors may, in part, contribute to deficits in the fetal alcohol spectrum disorders.
Objectives: To determine if alcohol consumption during pregnancy results in lower folate transport to the fetus and whether lower folate levels decrease the ability to detoxify formic acid.
Methods: Serum folate was measured in maternal and cord blood at delivery from alcohol-abusing mothers and controls. As an in vitro model, a choriocarcinoma cell line (BeWo) was characterized after culturing in folate-free conditions.
Results: The fetal:maternal serum folate ratio was ≤ 1.0 in half of the alcohol-exposed pairs (n=12), whereas all of the controls were >1.0 (n=8). Mean folate in cord samples was lower in alcohol-exposed cord than in the controls (25.83 ± 13.58 vs. 44.04 ± 10.86, $p<0.01$). BeWo cells were found to proliferate and maintain normal morphology when introduced into folate-free DMEM.
Conclusions: To our knowledge, this is the first study to show that folic acid transport to the human fetus is compromised in pregnancies affected by alcohol-abuse. BeWo cells can proliferate under folate-free conditions and will be utilized to investigate formate kinetics and toxicity.
Keywords: *Alcohol, folic acid, pregnancy*

18
Ethanol and nicotine induce CYP2B and CYP2E1 in unique regional and inducer-specific patterns in monkey brain

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Funding Source: CIHR MOP97751, Scholar Program for ICE-related research in Tobacco Control (CSF), Canada Research Chair (RFT)

Background: Human smokers and alcoholics have elevated levels of CYP2B and CYP2E1 in certain brain regions, which may contribute to effects such as altered neurotoxicity, increased metabolic tolerance and changes in drug efficacy.
Objective: To determine the effects of ethanol and nicotine on brain levels of CYP2B and CYP2E1 in a primate model.
Methods: Forty monkeys were randomized into four groups: an ethanol group, a nicotine group, an ethanol + nicotine group and a control (no drug) group. Monkeys in the ethanol and ethanol + nicotine groups were allowed to self-administer 10% alcohol in sucrose solution for 4 hours a day while the other groups consumed sucrose solution on the same schedule. Monkeys in the nicotine and ethanol + nicotine groups were injected with 0.5 mg/kg nicotine twice daily whereas the other groups were injected with saline on the same schedule. After 21 days on this schedule, the animals were sacrificed, tissue was collected from 8

brain regions and CYP2B and CYP2E1 protein levels were measured.
Results: Monkeys allowed to self-administer ethanol had induced levels of CYP2B protein in the caudate and putamen and CYP2E1 in the cerebellum. Monkeys treated with nicotine had induction of CYP2B in the caudate and putamen and CYP2E1 in the putamen. An interaction between nicotine and ethanol was demonstrated by induction of CYP2B in the thalamus of monkeys receiving ethanol + nicotine.
Conclusion: Ethanol and nicotine can induce CYP2B and CYP2E1 in unique regional and inducer-specific patterns as part of a neuroadaptation to drug exposure.
Keywords: *Ethanol, metabolism, nicotine*

19
Neurodevelopment of children exposed in utero to venlafaxine: preliminary results

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Funding Source: Wyeth Pharmaceuticals

Background/Objectives: When left untreated, maternal depression associated with stress and dysregulation of hypothalamic-pituitary-adrenal and placental axes leading to a potential risk for adverse fetal central nervous system (CNS) development. Venlafaxine (VLF) is an antidepressant widely used in pregnancy. Its effect on fetal CNS development has not been studied. The objective was to identify the long-term neurodevelopment of children exposed to VLF during pregnancy using standardized psychological tests, and to compare the outcome to those of children exposed to SSRIs and to healthy children exposed to non-teratogens.
Methods: Cohort study using a prospectively collected database. Three groups of children (Group 1: exposed to VLF, Group 2: exposed to SSRIs, Group 3: exposed to non-teratogens; n=62 in each group) matched for age and gender. The main outcome measure was children's Full Scale IQ. Statistical analysis included descriptive statistics, ANOVA, and regression.
Results: Children exposed to VLF and SSRIs did not differ in Full Scale IQ (p = 1.00). Children exposed to non teratogens achieved significantly higher scores than VLF and SSRI exposed children (112.3±11 vs. 104.7±14, p=.006; 113±11 vs. 105.1±13, p = .011 respectively). Maternal IQ and the number of depressive episodes following delivery were significant predictors for children's neurocognitive performance.
Conclusions: Maternal depression should be balanced against the potential teratogenic effects of antidepressant medication on the child's long-term neurodevelopment. Separating the effect of maternal psychiatric disorder from the effect of pharmacotherapy is the main challenge in drug safety studies in behavioral teratology.
Keywords: *Venlafaxine, pregnancy, child neurodevelopment*

20
Role of CYP2D6 in the pharmacokinetics and pharmacodynamics of oxycodone in healthy volunteers during co-treatment with placebo or quinidine

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Funding Source: CRCHUM

Background: A wide inter-subject variability is observed in the response to oxycodone. Drug metabolism studies have demonstrated that oxycodone is partially transformed by CYP2D6 into oxymorphone. Oxycodone affinity for opioid receptors is 10 to 40 times lower than that of morphine while oxymorphone has a 10-fold greater analgesic potency and a 3 to 5 times stronger affinity than morphine for the μ receptor. Hence, we hypothesized that variability in analgesic effects of oxycodone could be explained by inter-subject variability in the metabolic clearance of the drug.
Methods: Twelve healthy volunteers received a single oral dose of oxycodone (15mg) on two occasions: once alone and once during co-treatment with quinidine 60mg two hours prior to and 10 hours after the administration of oxycodone. Serial measurements of pain threshold were taken using a thermode placed on their triceps. Urine and plasma samples were collected between 0-24 hours.
Results: Five out of 8 volunteers had a decrease in the AUC of the relationship between pain level and time post-oxycodone dosing when oxycodone was administered with quinidine compared to placebo. A decrease in pain sensation was associated with a decrease in oxymorphone urinary excretion.
Conclusions: A decrease in CYP2D6 activity is likely to cause minimal changes in the pharmacokinetics of

oxycodone in plasma but appears relevant for the formation of the active metabolite oxymorphone. This may translate into significant changes in the overall analgesic efficacy of the drug. PK-PD models are being developed while considering plasma levels of oxycodone, noroxycodone and oxymorphone.
Keywords: *CYP2D6, oxycodone, drug interaction*

21
Ligand-based approaches to investigate the expression of angiotensin converting enzyme in intact human umbilical vein endothelial cells

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Background: Angiotensin converting enzyme (ACE) is a drug target and an effective bradykinin (BK)-inactivating ectopeptidase. Signaling pathways involved in physiopathological variation of this enzyme remain to be clarified.
Objective: We characterize the signaling of known (phorbol 12-myristate (PMA), cytokines) and novel stimuli that may influence ACE expression in human umbilical vein endothelial cells (HUVECs) using 2 types of ligands to quantify ACE.
Methods: A [3H]enalaprilat binding assay and 5(6)-carboxyfluorescein-BK (CF-BK) binding were used to evaluate the density of ACE at the surface of HUVECs treated with cytokines and/or pharmacological agents for 24 h. The translocation of NF-κB p65 subunit from cytosol to the nucleus in cells was studied by immunofluorescence. HUVECs lysates were immunoblotted to detect ACE and signaling molecules.
Results: TNF-α represses the expression of ACE while PMA upregulated it in 24 h (≈ 12-fold dynamic range, corroborated by ACE immunoblotting). In contrast, high glucose, kinins, insulin or EGF failed to affect ACE expression. The effect of TNF-α was abated by etanercept, the IKK2 inhibitor TPCA-1, or p38 inhibitor while that of PMA was reduced by inhibitors of PKC isoforms sensitive to phorbol esters and calcium. The short-term PKC- and MEK1-dependent increase of c-Fos expression was best correlated to PMA-induced ACE upregulation. CF-BK binding to HUVECs translated into punctate staining in PMA-stimulated cells and was displaced by enalaprilat.
Conclusion: Repression of endothelial ACE expression by TNF-α involves the NF-κB and p38 pathways, while PKC-mediated ACE upregulation may recruit MEK-1-ERK1/2 and c-Fos signaling.
Keywords: *5(6)-carboxyfluorescein-BK, [^{3}H]enalaprilat, cytokines*

22
Proteinase-activated receptor (PAR)-4 plays an important role in the regulating of eotaxin-1-induced eosinophil recruitment in the pleural cavity of mice

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Background/Objectives: Although proteinase-activated receptor (PAR)-4 has been implicated in inflammation, their role in regulating the eosinophil recruitment in response to chemoattractants has not yet been demonstrated. To investigate the contribution of proteinases and PAR-4 activation to eosinophil migration in response to eotaxin-1/CCL11 or leukotriene (LTB_4), we examined the effects of aprotinin or PAR-4 antagonist YPGKF-NH_2 (tcY-NH_2) on eosinophil migration induced by these chemoattractants.
Methods: BALB/c mice were immunized twice with ovalbumin (OVA) in aluminum hydroxide, and fifteen days after the first immunization challenged through the intra pleural (i.pl.) injection of OVA (1.0 µg/0.1 ml/ cavity) 1 h after subcutaneous (s.c.) administration of aprotinin (0.3 to 3.0 mg.kg^{-1}). Naïve BALB/c mice were pretreated s.c. with aprotinin (1.0 mg kg^{-1}) or tcY-NH_2 (30 µg) 1 h prior to the i.pl. injection of LTB_4 (50-500 ng) or eotaxin-1 (100 ng), and the number of infiltrating eosinophils in response to OVA or chemoatrractants were evaluated 48 h after. Statistical analyses were performed using One-Way ANOVA test, and differences between groups were assessed using Tukey post-test. $P<0.05$ was considered significant.
Results: Pretreatment with aprotinin (1mg.kg^{-1}) inhibited the eosinophil recruitment induce. In contrast, tcY-NH_2 treatment inhibited eosinophil recruitment in response to eotaxin-1 administration.
Conclusions: The results suggest that aprotinin-inhibited proteinases participate on d by OVA or eotaxin-1, but did not inhibit the eosinophil migration induced by LTB_4 eosinophil migration induced by allergen or eotaxin-1, and PAR-4 activation play an important role in regulating eosinophil recruitment in

eotaxin-1-induced pleurisy in mice.
Keywords: *PAR-4, Eosinophil-recruitment, Eotaxin-1*

23
Adverse events of benznidazole in children with Chagas Disease - a cohort study

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Introduction: Chronic Chagas disease (caused by infection with Trypanosome Cruzi) is associated with severe long term complications such as cardiac disease. Treatment of children with benznidazole can cure the disease and prevent chronic infection. Benznidazole is associated to a high incidence of adverse drug reactions (ADRs) in adults, including neuropathy and gastrointestinal symptoms. However, treatment in children seems to be associated with lower incidence and severity of ADRs. We aimed to describe ADRs in a cohort of children treated with benznidazole. **Materials/Patients:** A prospective study of children with Chagas disease treated with benznidazole in a tertiary pediatric centre in Buenos Aires, Argentina. **Results:** 107 children with Chagas were enrolled between 2004 and 2007. Mean age of the patients was 6.9 years (CI 95%: 10 days - 19 years). 62 ADRs (in 44 children) were observed, most (77.3%) in children over 7 years of age. ADRs could be classified as mild (50; 80.6%), moderate (10; 16%) and severe (2; 3.2%). 75% (32) of the ADRs occurred in the first 10 days of treatment. Skin was the most commonly affected organ (50%), followed by the central nervous system (22%), and gastrointestinal tract (21%). 7 patients did not complete treatment with benznidazole due to ADRs. **Conclusion:** Treatment of Chagas disease with benznidazole is well tolerated in children. Incidence of ADRs is strongly correlated with age (most ADRs occurred in children over 7 years). Severe ADRs frequently reported in adults were not observed in our cohort.
Keywords: *Chagas disease, Adverse Drug Reactions, pediatric clinical pharmacology*

24
Tissue expression and function of organic anion transporting polypeptide 2B1 splice variants

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Funding Source: The Canadian Institutes of Health Research

Background: The human Organic Anion Transporting Polypeptide 2B1 (OATP2B1) is a membrane transporter that facilitates the cellular uptake of a number of endogenous compounds and drugs. OATP2B1 is expressed in several tissues including the small intestine, liver, kidney and skeletal muscle, and is considered to play a role in the pharmacokinetics of substrate 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 but share common subsequent exons. These splice variations are expected to encode either a full length or truncated protein missing 22 amino acids from the N-terminus. Since little is known about OATP2B1 splice variants we investigated the relative expression of the splice variants in key tissues responsible for drug absorption and elimination, as well as the transport function of the truncated variant.
Methods and Results: Using variant-specific polymerase chain reaction, both the predicted full length and truncated forms of OATP2B1 were detected in liver, kidney and small intestine, albeit in differing proportions. With heterologous expression in cultured cells, we compared the transport kinetics (Vmax and Km) of the two forms of OATP2B1. Importantly, we demonstrate that the truncated variant was capable of transporting the known OATP2B1 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.
Keywords: *OATP2B1, transporters, alternate splicing*

25
Failure to maintain blood pressure at target following switch in nifedipine delivery technology in three patients

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Conflict of interest: No conflict of interest declared.
Funding Source: Unfunded clinical observation

Background: Hypertension is the commonest etiology of atrial fibrillation. Many atrial fibrillation patients require multiple antihypertensives, including dihydropyridines pushed to higher doses to reach target BP. Over the course of 6 months, 5 patients in our atrial fibrillation clinic who had been receiving nifedipine 60 mg/d in a zero-order osmotic pump had increases in their systolic pressure > 10 mmHg. Inspection of their medications revealed that the nifedipine formulation had been switched to an alternate pump not designed for zero-order delivery. **Objectives**: Indications were sought for a possible relation of BP to formulation to nifedipine formulation switch. Query of Health Canada identified 12 other reports of failure of therapy with the new formulation. A publication showing differences in delivery in vitro and in vivo were identified (Anschutz M, et al. Differences in bioavailability between 60 mg of nifedipine osmotic push-pull systems after fasted and fed administration. *Int J Clin Pharmacol Therap 2010; 48: 158-170).*
Methods: Three patients agreed to collect home BP in N-of-1 trials encompassing multiple back and forth switches between formulations at weekly intervals. **Results and Conclusions**: Of the 14 switches, 13 demonstrated lower BP on the zero-order pump vs. the alternate pump ($p<0.001$) on consecutive days, suggesting that in these patients, BP was better maintained by a zero-order delivery of the same dose. The prices are matched for both products, providing no cost-benefit to the switch. What percentage of patients have deterioration in BP control when switched to a non-zero-order nifedipine pump requires further study.
Keywords: *Antihypertensive, clinical-effect and drug-delivery*

26
The safety of quinolones use during pregnancy

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Funding Source: None

Background: The use of quinolones antibiotics has increased in the last decade. Little data exist on the safety of quinolones during pregnancy in human, although data on dogs suggest arthropathy after gestational exposure.
Methods: A computerized database of medications dispensed from 1998 to 2007 to all women registered in the "Clalit" health maintenance organization, in the Southern District of Israel, was linked with computerized databases containing maternal and infant hospitalization records from the district hospital. The following confounders were controlled for: parity, maternal age, ethnic group, maternal diabetes, smoking, and peripartum fever. Also, therapeutic pregnancy termination data were analyzed.
Results: There were 113,612 singleton births during the study period. A total of 81,703 of the infants (71.9%) were born to women registered in Clalit; 440 of the latter were exposed to quinolones during the first trimester of pregnancy, 120 were exposed during the second trimester and 96 during the third trimester. Exposure to quinolones in the first trimester was not associated with an increased risk for congenital malformations (adjusted OR = 1.05, 95% CI: 0.69-1.60); also, no such association was found when therapeutic pregnancy terminations were included in the analysis (adjusted OR = 1.01, 95% CI: 0.67-1.53). Exposure to quinolones during the first, second, third trimesters was not associated with an increased risk for other adverse pregnancy outcomes (i.e. low birth weight, mortality, low Apgar score and preterm delivery).
Conclusion: Exposure to quinolones was not associated with major congenital malformations, perinatal mortality, low birth weight, or low Apgar scores.
Keywords: *Quinolones, congenital malformations, pregnancy*

27
Optimizing the *in vitro* drug activation system of the lymphocyte toxicity assay: effects of species of origin and induction mode

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Funding Source: This work was funded by the Ivey Chair in Molecular Toxicology, Dept of Physiology

and Pharmacology, the University of Western Ontario, London, Ontario, Canada

Background/Objectives: Adverse drug reactions (ADRs) represent a major clinical problem. One of the rare but potentially fatal types of ADRs is the drug hypersensitivity syndrome (DHS). Diagnosis of DHS is difficult because of lack of any safe and reliable test. The lymphocyte toxicity assay (LTA) is an *in vitro* test used to diagnose DHS. However, the clinical usability of LTA is hindered by its complicated procedure, poor reproducibility and undefined predictive value. One aspect of the test procedure is the generation of reactive metabolites (TMs) of the culprit drug *in vitro* using isolated liver microsomes (MICs), a process that has not been precisely characterized or standardized. The objective of this study was to explore the process of generation of drug TMs using different types MICs.
Method: Non-induced MICs form human origin and from five different animal species, as well as rat liver microsomes induced either by phenobarbital (PHB), 3-Methylcholanthrene (3-MC), dexamethasone, or clofibrate were tested for their ability to generate TMs using death of jurkat-E 6.1 cells as an end-point.
Results: MICs from murine origin generated more TMs than MICs from other species. In addition, PHB and 3-MC induced MICs were more efficient as *in vitro* drug activators than non-induced MICs or MICs induced by other chemicals.
Conclusion: The results indicate that MICs species of origin and their induction mode are important factors that may affect the LTA final results. This data can have important impact on the clinical use of LTA as a diagnostic tool for DHS.
Keywords: *Adverse Drug Reactions, the Lymphocyte Toxicity Assay, drug hypersensitivity, diagnosis*

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Hair cortisol levels in patients with adrenal insufficiency on glucocorticoid replacement therapy

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Funding Source: None

Background: Patients with adrenal insufficiency require life-long treatment with exogenous glucocorticoids to replace this deficiency. Several studies have shown impaired subjective health status in these patients, which may be caused by glucocorticoid over-replacement. Our laboratory has developed a technique to measure cortisol in hair to obtain a historical record of systemic cortisol exposure. The objective was to compare hair cortisol levels in patients receiving glucocorticoid (hydrocortisone) replacement therapy with controls.
Methods: Using a mail-out study, hair samples, demographics, medical history and perceived stress questionnaires were collected from 57 patients across North America, diagnosed with primary or secondary adrenal insufficiency. The patient data was compared to individuals residing in the same household (control group, N=57). Cortisol was measured from the proximal 2cm of hair, representing the most recent 2 months of exposure. A modified cortisol enzyme immunoassay was used for analysis.
Results: The median (range) hair cortisol concentrations were 222.0 (22.7-1438.0) ng/g and 182.0 (57.7-1479.0) ng/g in the patient and control groups, respectively ($p>0.05$). Although these findings were not significant, the patients and controls differed in their perceived stress scale scores: 16.2 ± 7.2 and 12.7 ± 6.2 (mean ± SD, N=56, $p<0.005$).
Conclusions: Hair cortisol concentrations of patients with adrenal insufficiency were not found to be significantly different than their controls. However, the trend toward higher levels in the patient group may suggest some are over-treated and, hence, may be at risk for the adverse effects of cortisol. A greater sample size may be needed to detect this important difference.
Keywords: *Adrenal insufficiency, glucocorticoid, hair*

29
Strategies for improving the use of artemisinin combination therapies (ACT) in the treatment of uncomplicated malaria in DRC

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Conflict of interest: No conflict of interest to declare.
Funding Source: None

Background: Sub Sahara Africa is the most affected area by malaria (90% of cases). Children under 5 years old and pregnant women bear the heavy burden of this disease. WHO recommend the use of Artemisinin combination therapies (ACT) in the treatment of uncomplicated malaria since the spread of resistance to Common antimalarials. However, 2 years after the adoption of Artesunate+Amodiaquine as the first

treatment for Uncomplicated Malaria, a prior study in Kinshasa proved the poor use of this combination. This study aims to determine strategies for improving the use of this combination in DRC.
Method: A cross sectional study has been conducted in 457 health centers, 524 pharmacies and 1781 households. Information was collected using a structured questionnaire and data capture that was done with the Software Excel and data analysis with the Software epi info and SPSS.
Results: More than 100 drugs including conventional antimalarials, Artemisinin combination therapies (ACT) and herbal remedies are used in the treatment of Uncomplicated Malaria at Kinshasa. Artésunate used alone (25, 6% and 35, 6%) and quinine (21, 2% and 25, 8%) are the most prescribed antimalarial in Health centers and Pharmacies. Artesunate + amodiaquine which are the recommended ACT by National Policy is the third Antimalarial prescribed. (18, 8% of prescriptions) and represented 4, 5% of antimalarial sale to the pharmacies. In households, quinine and artesunate used alone are the most used antimalarials.
Conclusion: Decisions must be taken by government concerning imports, quality control and distribution of ACT.
Keywords: *ACT, strategies, Artesunate+amodiaquine*

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Buprenorphine transdermal system (BTDS) for opioid therapy in patients with chronic low back pain

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Conflict of interest: Authors Eisenhoffer, Piraino, Quigley, Harsanyi and Darke are or were employees of the sponsor.
Funding Source: Purdue Pharma, Canada

Objective: This randomized, double-blind, crossover study compared the efficacy and safety of a 7-day BTDS and placebo, in patients with low back pain of moderate or greater severity for at least 6 weeks.
Methods: Pre-study analgesics were discontinued the evening prior to randomization to 5µg/hr BTDS or placebo, with acetaminophen 300mg/codeine 30mg, 1-2 tablets q4-6h prn for rescue analgesia. The dose was titrated to effect weekly, if tolerated, to 10 and 20µg/h BTDS. Each treatment phase was 4 weeks. Data were analyzed using three-way analysis of variance.
Results: Fifty-three patients were evaluable for efficacy (completed ≥ two weeks in each phase). Baseline pain was 62.1±15.5 mm (100 mm VAS) and 2.5±0.6 (5-point ordinal). BTDS resulted in lower mean daily pain scores than placebo (37.6±20.7 vs. 43.6±21.2 mm VAS, p=0.0487 and 1.7±0.6 vs. 2.0±0.7 ordinal, p=0.0358). Most patients titrated to the highest dose of BTDS (59% 20µg/h; 31% 10µg/h; 10% 5µg/h). There were improvements from baseline in Pain and Disability, Pain and Sleep, Quebec Back Pain Questionnaire, and SF-36 for both BTDS and placebo, without significant differences between treatments. While there were more opioid-related side effects with BTDS treatment than with placebo, there were no serious adverse events. 82% of patients chose to continue BTDS in a long-term open-label evaluation, where improvements in pain intensity, functionality and quality of life were sustained for up to 6 months without analgesic tolerance.
Conclusion: BTDS (5-20µg/hr) represents a new treatment option for initial opioid therapy in patients with chronic low back pain.
Keywords: *Buprenorphine, low back pain, transdermal system*

31
Prolonged neutropenia after irinotecan-based chemotherapy in a patient with Rhabdomyosarcoma: polymorphisms in UGT1A1 and SLCO1B1

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Funding Source**:** SS and FG-B are supported by the Canadian Pharmacogenomics Network for Drug Safety. FG-B is also supported by the Clinician-Scientist training programme at the Hospital for Sick Children.

Background: The topoisomerase inhibitor, irinotecan, has been used increasingly in the treatment of childhood malignancies. Genetic polymorphisms of uridine diphosphate glucuronosyl transferase 1A1 (*UGT1A1*), and *SLCO1B1* coding organic anion-transporter polypeptide 1B1 (OATP1B1) are independent risk factors known to increase irinotecan toxicity in adults. Although combined occurrence of polymorphisms in these 2 genes is likely to influence

susceptibility to irinotecan toxicity, data are scarce, especially in children.
Objectives: We report an 11 year old female with severe and prolonged neutropenia after irinotecan-based chemotherapy for rhabdomyosarcoma. The clinical course was complicated by grade 4 neutropenia after each cycle of irinotecan therapy. To explore the possible cause of her prolonged neutropenia, genotyping of *UGT1A1* and *SLCO1B1* were conducted.
Methods: A blood sample was obtained and genotyped for UGT1A1 and SLCO1B1 to investigate the cause of her prolonged neutropenia.
Results: Genotyping of *UGT1A1* revealed that the patient was heterozygous with one wild type copy and one copy of the *UGT1A1*28* haplotype, which is known to lead to decreased activity of the enzyme detoxifying SN-38, the active metabolite of irinotecan. Genotyping of *SLCO1B1* revealed that the patient was heterozygous at the 521 and 388 loci (521T>C, 388A>G), which is associated with decreased liver uptake of irinotecan, leading to larger systemic exposure to the drug.
Conclusion: To our knowledge, this is the first case report of combined genotyping of both *UGT1A1* and *SLCO1B1* in a child. A combination of variant alleles in both genes may have contributed to the severe irinotecan toxicity in this case.
Keywords: *Irinotecan, UGT1A1, OATP1B1*

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Safety and effectiveness of Proctofoam-HC® in the third trimester of pregnancy

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Conflict of interest: Dr. Koren is a medical consultant for Duchesnay Inc.Canada.
Funding Source: This study was funded by Duchesnay Inc. Canada.

Background: Hemorrhoids are highly prevalent in pregnancy. Although many products are available for the treatment of hemorrhoids, none have been studied for safety and effectiveness in pregnancy. Proctofoam-HC® is commonly used rectal medication in pregnancy.
Objective: The objective of this study is to assess the safety and effectiveness of Proctofoam-HC® in the third trimester of pregnancy. Birth weight is the primary outcome used for assessment of safety, while changes in the severity of each of the hemorrhoidal symptoms, as well as their impact on Well-Being Score, are used for the assessment of effectiveness.
Methods: 210 pregnant women suffering from hemorrhoids were recruited from six different clinics; all were prescribed Proctofoam-HC®. Prenatal and postnatal interviews were conducted to collect information on medical history, maternal characteristics, delivery complications, neonatal health and birth weight. Severity of hemorrhoids and their impact on women's well-being were assessed using a visual analogue scale before and after treatment. Statistical analysis was performed to detect any difference in the primary outcome between treatment and comparison groups, as well as improvements in the symptoms.
Results: No significant difference was found in primary and secondary outcomes between treatment and comparison group. Significant improvement in all symptoms was detected after treatment with Proctofoam-HC®.
Conclusion: Proctofoam-HC® is a safe and effective anti-hemorrhoidal medication for use in the third trimester of pregnancy.
Keywords: *Hemorrhoids, pregnancy, Proctofoam-HC*

33
N-acetylcysteine as a novel prophylactic treatment for Ifosfamide-induced nephrotoxicity in children; translational pharmacokinetics

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Background: Ifosfamide (IFO) is a highly effective chemotherapeutic agent, treating a wide variety of solid tumours. Its use is associated with 30% risk for

nephrotoxicity in children. Nephrotoxicity is believed to be due to oxidative stress; therefore use of an antioxidant for its attenuation is feasible. NAC is a synthetic thiol which is used clinically in children as an antidote for acetaminophen overdose and has been demonstrated to be effective in preventing IFO nephrotoxicity in a rodent model. Our objective is to compare the systemic exposure of NAC in children treated for acetaminophen overdose, to the systemic exposure associated with prevention of nephrotoxicity in rats. Blood samples were collected from male Wistar Albino rats treated with the dose schedule of NAC shown to prevent IFO-induced nephrotoxicity. In parallel, blood samples were collected from children (n=10) who had received NAC for acetaminophen overdose. For both the rat and pediatric patients NAC was measured by High Performance Liquid Chromatography and systemic exposure determined by calculating the area under the curve. The mean AUC of NAC in rats who were given therapeutically effective levels of NAC was 18.72 mM•Hr, similar to the AUC in children treated with NAC for acetaminophen overdose (14.56 mM•Hr). The range of AUC distribution was similar between the rats and pediatric patients. These findings support the use of NAC to prevent IFO-induced nephrotoxicity in a clinical setting. This study is significant in the advancement toward effective prevention of life threatening IFO-induced nephrotoxicity in children.

Keywords: *Ifosfamide, nephrotoxicity, N-acetylcysteine*

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Nutritional pharmacology of cholesterol "Interaction mechanisms between the cardioprotective amino acids and cholesterol feeding in rats"

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Conflict of interest: I am currently working on interdisciplinary course that including pharmacology & nutrition, Nutritional Pharmacology, which is considered as a new area. The new course, should be explore the impact of nutrition on health. This includes diet, lifestyle, and the pharmacological actions of nutritional supplements and other elements. There is much controversy in this area of pharmacology, and this course will cover many of these issues and their clinical applications. Therefore, this new discipline will not only cover the physiological effects of diet on the body but it also covers the pharmacological actions of the diet on the different tissues & organs and the therapeutic applications of nutritional pharmacology. Thus, Nutritional issues of concern to pharmacologists, including the popular use of nutritional supplements for physiological enhancement and disease prevention, drug-nutrient interactions, therapeutic implications for nutritional pharmacology, and product availability. Such products should be under control and be sure of their value in the prevention and cure of diseases and avoid many clinical complication, so we grantee these products will be dispense by expertise subjects.

Problem Statement: The effects of the cholesterol intake on free amino acids in the plasma and the heart after long-term administration of rats were investigated.

Approach: *In Vivo* treatment was carried out to study the influence of cholesterol intake on free amino acid patterns in the hearts of rats. Also, plasma cholesterol, triglycerides, lipoproteins and γGT were determined by colorimetric methods.

Results: Feeding cholesterol intake 100mg/kg/day for 20 weeks significantly elevates the contents of aspartate from 135 ± 014 to 187 ± 008 µmol /100 g wet tissues (P< 0.005), glutamine from 204 ± 017 to 293 ± 025 µmol /100 g wet tissues (P< 0.01), ornithine from 7 ± 001 to 114 ± 008 µmol /100 g wet tissues (P< 0.001), histidine from 44 ± 005 to 79 ± 008 µmol /100 g wet tissues (P< 0.005) and arginine from 74 ± 009 to 390 ± 010 µmol /100 g wet tissues (P< 0.001) in the heart. These increases are concomitant with slight increase in cardiac taurine from 1891± 156 to 2064 ± 136 µmol /100 g wet tissues. However, the increase in the level of taurine in the heart was insignificant (P > 0.05). Chronic oral treatment of rats with cholesterol 100mg/kg/day for 20 weeks significantly elevated the plasma level of taurine from 235.5 ± 23 (control) to 349 ± 35 µmol /L (P< 0.05), Glutamate from 94 ± 7.5 to 131.1 ± 15.3 µmol /L (P< 0.01), alanine from 526.5 ± 40.5 to 649 ± 34.2 µmol /L (P< 0.05),valine from 161 ± 12.5 to 200 ± 13.6 µmol /L (P< 0.05), phenylalanine from 59.6 ± 4.4 to 75.4 ± 05.5 µmol /L (P< 0.05). These increases were associated with a significant decrease in the plasma level of ornithine from 98 ± 14.1 to 62.6 ± 06.4 µmol /L (P< 0.05). Meanwhile proline was not detected in the plasma of treated animals. In these experimental conditions, the chronic intake of cholesterol had no significant effects on plasma cholesterol or other plasma lipids parameters tested except plasma triglycerides which was significantly increased. Results also indicated that feeding cholesterol significantly increased the plasma γGT activity.

Conclusion: On broad basis, the mobilization of the protective amino acids in the cardiac tissue indicates that there is a mechanism (or mechanisms) responsible for the interaction between cholesterol

intake and certain amino acids. These mechanisms are considered the significant factor for generating metabolic events which might be responsible for the physiological functions in the protection of myocardium muscle from the actions of the cholesterol intake. These mechanisms also promote high resistance of rat to atherosclerosis. Thus, under pathological conditions, these benefits changes may disturb and that leads to enhancement of the risk of atherosclerosis and coronary heart disease.

Keywords: *Cholesterol intake, amino acid analyzer, cardio- protective amino acids*

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The fetal safety of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers

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Conflict of interest: No conflict of interest to declare.
Funding Source: None

Background: Angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) are known to cause fetal renal damage in pregnancy. Their safety after first trimester exposure has been debated, with both positive and negative studies.
Objectives: Our aim was to determine whether the use of ACE inhibitors or ARBs in the first trimester of pregnancy is associated with an increased risk for major malformations or other adverse pregnancy outcomes.
Methods: All subjects were prospectively enrolled from among women contacting the Motherisk Program in Toronto. At initial contact, details of maternal medical history and exposures were collected. Pregnancy outcome was recorded on follow up interview after the completion of pregnancy. To control for the potential effects of hypertension, a control group of hypertension treated with other antihypertensives, as well as healthy controls were also recruited.
Results: Maternal characteristics (age, pregnancy history, alcohol consumption and smoking habits) were not different among the three groups. There were no differences in rates of major malformations. Both the ACE-ARBs and disease matched groups exhibited significantly lower birth weight and gestational ages than the healthy controls ($p<0.001$ for both variables). There was a significantly higher rate of miscarriage noted in the ACE/ARB group ($p<0.001$).
Conclusions: These results suggest that ACE inhibitors/ARBs are not major human teratogens, however, they may increase the rate of miscarriage with first trimester exposure.

Keywords: *Angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, pregnancy teratogens, birth defect*

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Prenatal exposure to Mycophenolate Mofetil – report of three cases and review of the literature

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Funding Source: None

Introduction: Mycophenolate Mofetil (MMF) has become a major therapeutic option for the management of patients undergoing transplantation, as well as for the treatment of autoimmune conditions. Limited human studies and post marketing surveillance have suggested that MMF use during pregnancy increases the risk for congenital malformations. This is of utmost importance given that many patients receiving the drug are women of child-bearing age. As many pregnancies are unplanned, it is imperative to assess the teratogenic risk of MMF. We prospectively identified and followed pregnant women exposed to MMF during pregnancy and report the pregnancy outcomes.
Methods: We searched all records of women who contacted the Motherisk Program at the Hospital for Sick Children, and the Special Pregnancy Program-Maternal Unit, Mount Sinai Hospital in Toronto between 2000 and 2008.
Results: Three pregnancies exposed to MMF during the first trimester of pregnancy were identified. Two women gave birth to healthy babies and one had an early miscarriage. The two babies did not exhibit any malformations, as evaluated by obstetricians and pediatricians involved in their follow up. Postnatal follow-up showed normal growth and development.
Discussion: We did not observe malformations in our prospectively followed patients, which are in

agreement with another small prospective series that also failed to find any malformations, and in contrast with the information obtained from retrospective case series. We believe that it is possible that the rate of malformations associated to MMF use in pregnancy has been exaggerated by selection bias, and may be smaller than previously believed.

Keywords: *Mycophenolate mofetil, immunosuppressant, pregnancy, birth defects, SLE, inflammatory bowel disease*

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Inhibition of clopidogrel metabolism by proton-pump inhibitors *in vitro*

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Funding Source: Canadian Institutes of Health Research (MOP-89753 and CMS), and the Canadian Foundation for Innovation.

Background: Clopidogrel is a thienopyridine prodrug that is bioactivated by cytochrome P450 (CYP) enzymes to exert its antiplatelet effect. Clopidogrel is first converted to 2-oxo clopidogrel, then to its pharmacologically active metabolite (clopidogrel-AM). Recent *in vitro* studies have suggested that the proton-pump inhibitor (PPI), omeprazole, may substantially reduce the formation of both metabolites. This interaction may contribute to a loss of antiplatelet effect and an increased risk of myocardial infarction in patients taking clopidogrel and PPIs concomitantly.
Objectives: This study aims to evaluate the enzyme kinetics of clopidogrel metabolism and to determine whether the inhibition of clopidogrel bioactivation by PPIs is a class-specific effect.
Methods: Using microsomes prepared from baculovirus/insect expressing human CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and pooled human liver microsomes (HLM), the Michaelis-Menten kinetics were determined for the formation of 2-oxo clopidogrel and clopidogrel-AM. Inhibition kinetics of 2-oxo clopidogrel and clopidogrel-AM formation by were assessed by pre-incubating reactions with omeprazole, lansoprazole, rabeprazole and pantoprazole. Clopidogrel and its metabolites were quantified using liquid chromatography/tandem mass spectrometry (LC-MS/MS).
Results: We determined the Michaelis-Menten kinetics of the 2-oxo clopidogrel using the recombinant CYP microsomes and HLMs and we will have data on clopidogrel-AM production using this system. We will soon be able to demonstrate the extent of inhibition and rank order of potency of PPIs on clopidogrel metabolism.
Conclusions: Our ongoing study will provide new *in vitro* data that will serve as the mechanistic basis for the clinically important drug-drug interaction that is thought to occur between clopidogrel and PPIs.

Keywords: *Drug metabolism, clopidogrel, pharmacogenetics*

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Assessment of rational use of artemisinin based combination therapies (ACT) at Kinshasa (DRC)

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Funding Source: None

Background: The spread of resistance to Common anti malarial in sub Saharan Africa is a concern. Irrational use of these drugs enhances incorrect dosage, inappropriate treatment and drug interactions. More than fifteen African countries including DRC have adopted artesunate+amodiaquine as first line treatment Policy of Uncomplicated Malaria. Two years after the adoption of this combination in DRC, this study aims to assess the rational use of this combination.
Method: A cross sectional study has been conducted in 457 health centers and 524 pharmacies at Kinshasa, capital of the DR Congo where Malaria transmission is perennial. Information was collected by interview of health professionals using a structured questionnaire and in reviewing patient case file and sales. The data captures have been done with the Software Excel and data analysis with the Software epi info and SPSS.
Result: 20, 6% of Kinshasa's health workers claimed to give rationally the combination artésunate + amodiaquine that means in accordance to the directives of National program of fighting against Malaria (PNLP) in adults; 17, 1% of drugs sellers in pharmacies recommended a rational use of this combination to patients. 74% of Kinshasa's health workers that prescribed the combination Artesunate + amodiaquine in treatment of Uncomplicated Malaria claimed to use it for children under 6 month in contrast to the directives of National Malaria Control (PNLP).
Conclusion: The use of Artesunate+amodiaquine at Kinshasa is irrational and will contribute to the spread of resistance to this combination.

Keywords: *Rational use, perennial, Artesunate+amodiaquine*

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Accidental clonidine ingestion in an infant: case report

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Conflict of interest: No conflict of interest to declare.
Funding Source: None

Background: Clonidine exposure in children can be life-threatening. We present a case of accidental clonidine intoxication in a 2 year-old male infant and review the literature.
Case: A 2 year-old male infant presented with a history of lethargy. Vital signs were notable for hypotension; pupils were constricted and neurological examination revealed hypotonia. The Glasgow coma score progressively worsened, leading to intubation. Naloxone was given without response. Urine toxicology revealed high concentrations of clonidine, but no opiates. Intravenous atropine 0.02 mg/kg was administered as well as rapid boluses of isotonic crystalloids with good response. The patient was subsequently discharged home with pediatric and community services follow up. Review of the literature shows that all reported pediatric patients with accidental clonidine poisoning were symptomatic, and that 2 died. Bradycardia responded to atropine, and response to naloxone has been reported in some cases. Central nervous system and respiratory depression, and cardiovascular instability were the most commonly reported features.
Discussion: Clonidine intoxication should be considered in children presenting with lethargy, miosis and respiratory depression, who fail to respond to naloxone. Initial management consists of establishing a patent airway, IV fluids, atropine, if indicated, and naloxone to rule out opiate co-exposure. Unintentional poisoning is a real danger to children because small amounts of clonidine can be fatal.
Keywords: *Clonidine intoxication, pediatric clinical pharmacology, pediatric toxicology*

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The effect of Reiki on pain in women after elective caesarean section - a double blinded randomized controlled trial

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Funding Source: None

Background: Reiki is an ancient Japanese form of healing where the practitioners transfer healing energy through their hands to their patients. While Reiki has been reported to reduce pain in patients, a recent systematic review showed that all of the studies published to date are of poor methodological quality. Yet an estimated 1.2 million Americans are using this method. Our objective was to assess whether remote Reiki (i.e., the practitioner and patient are not in the same room) is effective in reducing pain following elective caesarean section.
Methods: A randomized, double blinded study in women scheduled to undergo non-emergency caesarean section allocated to either Reiki (n=40) or Control (n=40). The Reiki group received three sessions of remote Reiki on each of their three days post surgery in addition to usual care. The Control Group received only usual care. Neither patients nor treatment team were aware of women's allocation to treatment.
Results: Reiki did not have a measurable effect on the primary measure (i.e. Area Under the Curve of a Visual Analogue pain Scale). None of the other secondary pain measures showed a significant difference when compared (i.e. dose of pain medication consumed, proportion of women who needed opioids, rate of healing, or number of adverse events). There was a small but significant reduction in the heart rate 4 hours post surgery and in systolic blood pressure on day 3 in the Reiki group.
Conclusion: The remote Reiki treatment had no measureable effect on women's pain response following elective caesarean section.
Keywords: *Pain, Reiki, CAM*

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Effectiveness of probiotics in the treatment of infantile colic: a randomized, placebo-controlled trial

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Conflict of interest: No conflict of interest to declare.
Funding Source: None

Background: Infantile colic is one of the most commonly reported medical problems within the first three months of life causing appreciable distress for both parents and pediatricians. The reported incidence of infantile colic ranges from 3% to 28% in prospective studies and up to 40% in retrospective surveys. The pathogenesis of infantile colic remains elusive despite decades of research. Consequently, various treatment approaches have been tried to alleviate this condition. Recent studies have suggested that changes of intestinal microflora of a newborn may play an important role in pathogenesis of infantile colic. Therefore, dietary supplementation with probiotics has been proposed for the improvement of this condition.
Objective: To investigate the effectiveness and safety of probiotic supplementation with *Lactobacillus reuteri* in the treatment of infantile colic.
Methods: 100 exclusively breastfed healthy term infants between 20 and 90 days of age, with symptoms of infantile colic defined as crying more than 3 hours per day on more than 3 days per any week, will be randomly divided into two groups to receive once-daily supplementation with *Lactobacillus reuteri* (treatment group) or placebo (control group) for 21 days in a double blind fashion. *Outcome measurement*: The mean change in crying time from baseline to the end of the treatment period (primary outcome); number of colic episodes per day, parents' quality of life, use of additional interventions by parents (secondary outcomes).
Results: In progress.
Conclusion: The findings of this study will provide rigorous scientific evaluation of the possible alternative treatment for infantile colic.
Keywords: *Infantile colic, probiotics, lactobacillus reuteri*

POSTER PRESENTATIONS THURSDAY JUNE 3, 2010

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Examining the health and hair test results for drugs of abuse among a cohort of children found in drug producing homes

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Conflict of interest: None declared.
Funding source: Canadian Institute for Health Research

Background: Residential drug production often imposes a variety of safety and health issues, particularly pertaining to child inhabitants. Authorities estimate that thousands of children across the country may be residing in these environments and are concerned about the associated risks; therefore if drug production discovered (or in some provinces even suspected) these children are frequently apprehended and separated from their parents. In the Motherisk Clinic at the Hospital for Sick Children we assessed 75 children (mean age 6.5 years) that were residents of either marijuana grow operations (80%) or clandestine illicit substance laboratories (including methamphetamine, MDMA, and crack cocaine). A paediatric evaluation and hair analysis for drugs of abuse were used to determine if the environments of these children's homes were precipitating any remarkable effects on their health and wellbeing. Overall, occurrences of mild eczema, asthma, and attention/learning deficits among a few children of this cohort were evident (between 4-6%); however these fell well below values reported in the literature for Canadian reference populations. Moreover, only 30% of hair tests were positive for illicit substances suggesting that the majority of these children are likely separated from the drug producing activities of their parents. In summary, we found the majority of these children to be relatively healthy and drug-free. In light of this, we believe that automatic removal of children from their parents is unjustified and unethical, and may impose tremendous risks for these children's wellbeing. Comprehensive evaluations should be performed on a case by case basis in order to determine what is ultimately in the best interest for the child.
Keywords: *Marijuana grow operation, drug endangered children, hair analysis, child-welfare*

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Effects of strain and gender on 4-aminobiphenyl-induced hepatotoxicity in the mouse

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Conflict of interest: No conflict of interest to declare.
Funding Source: CIHR

Background: The aromatic amine liver carcinogen 4-aminobiphenyl (ABP) appears to require metabolic activation to exhibit its carcinogenic potential. Our recent studies have shown significant protection against the development of ABP-induced liver tumors in mice lacking the *N*-acetyltransferases Nat1 and Nat2

and marked protection in wild-type (C57BL/6) female mice compared to males, but little evidence for either strain or gender differences in levels of ABP-induced DNA damage. Recent studies suggest that the acute cytotoxicity of carcinogen reactive metabolites may result in a tumor-promoting inflammatory environment. Protection of female mice against liver tumor development has been observed with diethylnitrosamine, and may be related to gender differences in the inflammatory response to its acute cytotoxicity.
Objectives: To determine whether gender and/or strain differences exist in the acute cytotoxicity or inflammatory responses to ABP.
Methods: Hepatotoxic and inflammatory responses to ABP were compared between C57BL/6, Nat1/Nat2 null and Cyp1a2 null mice by measuring serum levels of the liver damage biomarker ALT and of the inflammatory cytokines TNFa and IL-6, 24 hr after an ABP dose of 20 mg/kg i.p.
Results: ABP produced significant hepatotoxicity in wild-type male mice but not in females, and had no effect in Nat1/2 or Cyp1a2 null mice of either gender. Levels of IL-6 and TNFa were not markedly elevated in any mice.
Conclusion: Our results suggest that acute hepatotoxicity of ABP may be related to the increased tumor incidence in wild-type male mice, but that inflammatory mechanisms are not involved or not evident at the time points investigated.
Keywords: *Aromatic Amines, cytotoxicity, hepatocarcinogenesis*

44
CMKLR1 regulates energy homeostasis in mice

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Conflict of interest: None to declare.
Funding Source**:** CIHR and NSHRF

Background: Obesity, characterized by an excess of adipose tissue, is an established risk factor for cardiovascular disease and type 2 diabetes. Different mechanisms linking obesity with these comorbidities have been postulated, but remain poorly understood. Adipose tissue secretes various hormone-like compounds, termed adipokines that regulate a number of biological functions including appetite and energy balance, glucose homeostasis, and inflammation. Alterations in the synthesis and secretion of adipokines with obesity are believed to contribute to the development of obesity and obesity-related diseases. Chemerin is a novel adipokine that has a role in adaptive and innate immunity, and regulates adipocyte differentiation and metabolism by binding to and activating the, G protein-coupled receptor chemokine like receptor-1 (CMKLR1).
Objective: To determine the *in vivo* role of CMKLR1 in energy homeostasis.
Methods: Wildtype and CMKLR1 null mice were weighed, and food intake was measured weekly from 6 to 30 weeks of age. Mice were subjected to regular glucose tolerance and insulin sensitivity tests, and dual energy X-ray absorptiometry to measure body composition. At 30 weeks of age, mice were euthanized, and tissues collected.
Results: CMKLR1 null mice had lower weight, food consumption, total fat mass, and percent body fat than wildtype controls. In addition, 12 week old CMKLR1 null mice were more glucose intolerant and had larger adipocytes than wildtype control mice. However, this difference was no longer observed at 30 weeks.
Conclusion: Collectively, these data indicate that CMKLR1 influences adipose tissue development and glucose homeostasis, and may contribute to the metabolic derangements characteristic of obesity and obesity-related diseases.
Keywords: *Adipokines, adiposity, diabetes*

45
Insulin sensitization in aging by voluntary exercise is mediated through the hepatic insulin sensitizing substance (HISS)

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Conflict of interest: No conflict of interest to declare.
Funding Source: The Canadian Institutes of Health Research (CIHR)

Background: A progressive decrement in postprandial glucose utilization is an early feature of age-associated insulin resistance. The post-meal reflex from gut regulates release of the hepatic insulin sensitizing substance (HISS), which defuses the postprandial glycemic spike and neutralizes the unwanted effects of hyperglycemia. The abnormalities in HISS release underlie and constitute the primary metabolic defects of insulin resistance. With physical exercise demonstrating therapeutic reversal of insulin resistance, there is a scope of examining its efficacy to intervene the HISS pathways of glucose utilization in aging.
Objectives: The aim of the study is to test the hypothesis that, improved insulin sensitivity by voluntary running-wheel exercise in aging rats is attained through preserving the HISS-function. We also investigate the possible association of exercise-mediated beneficial changes in metabolic conditions

and body compositions with the improved HISS-dynamics.
Methods: We tested insulin sensitivity in 9, 14 and 21-week old rats with/without exercise. The postprandial glucose, serum insulin, whole-body adiposity, and visceral fat masses were determined in age-matched control and exercise rats.
Results: Voluntary exercise caused improvements in HISS-dependent glucose uptakes in 9, 14 and 21-week age-groups by 29.1 ($p=0.052$), 27.6 ($p<0.001$), and 23.6 ($p<0.01$) mg kg^{-1} respectively. This contributed to the major benefits of insulin sensitization response to exercise. The older rats ran less, but received more benefits with per-km run. The body compositions and metabolic conditions were also beneficially changed with exercise-induced improvements in HISS-response.
Conclusion: The therapeutic efficacy of voluntary exercise against insulin-resistance in aging is achieved through restoration of the HISS-action.
Keywords: *Aging, exercise, Type 2 diabetes, insulin, HISS*

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Effect of folic acid supplementation on plasma concentrations of oxidized folic acid

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Conflict of interest: GK is a medical consultant for Duchesnay, Inc. and a primary investigator for several studies investigating prenatal multivitamin supplementation.
Funding Source: Duchesnay, Inc (Blainville, QC)

Background: Folic acid is a fully oxidized form of folate that is found only in supplements and fortified foods. The liver has limited capacity to convert folic acid to reduced, physiological forms of folate. Folic acid intake that exceeds this capacity results in the appearance of oxidized folic acid (FA) in plasma. Circulating FA may contribute to the proposed adverse effects of chronic exposure to high folate intakes and blood folate concentrations.
Objectives: To assess the effects of folic acid supplementation on plasma FA in healthy women who are also exposed to fortification of staple foods.
Methods: Fasting plasma samples were collected as part of a randomized trial evaluating blood folate concentrations among women consuming 1.1 mg or 5 mg of folic acid daily over a 30 week period. Oxidized folic acid was quantified by reverse-phase HPLC.
Results: There was a significant effect of time ($p = 0.01$), but not dose ($p = 0.31$), on FA levels. Fasting plasma FA tended to increase after 6 and 12 weeks of folic acid supplementation; however, the differences were not significant ($p = 0.11$, 0.06). Plasma FA decreased significantly between weeks 12 and 30 ($p = 0.01$); the difference between baseline and week 30 was not significant ($p = 0.12$).
Conclusions: Folic acid supplementation increases fasting plasma FA, however, concentrations appear to remain low with once daily dosing up to 5 mg. Our data suggest that there are mechanisms that regulate the absorption and metabolism of folic acid to maintain folate homeostasis.
Keywords: *Folic acid, dietary supplements, pharmacokinetics*

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The effect of acyclovir on the renal tubular secretion of creatinine in LLC-PK1 cell monolayers

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Funding Source: Canadian Institute of Health Research (CIHR)

Background: Acyclovir is a widely used antiviral agent in children. Acyclovir is generally well tolerated, however, in some cases, nephrotoxicity has been observed. A rapid increase in plasma creatinine levels occurs within 24 – 48 hours in some children. The rapid increase in plasma creatinine levels appears to precede other signs of nephrotoxicity. The human organic cation transporter (hOCT) system plays a role in the renal tubular transport of creatinine and acyclovir. Additionally, several drugs are known to compete with creatinine for renal tubular transport and subsequently, induce a rapid increase in plasma creatinine levels. Therefore, it is possible that the rapid increase in plasma creatinine levels observed in some children receiving acyclovir may be partially due to the inhibition of the renal tubular secretion of creatinine by acyclovir.
Objective: To determine if acyclovir inhibits the renal tubular secretion of creatinine.
Methods: Porcine renal proximal tubular (LLC-PK1) cell monolayers cultured on porous membrane filters were exposed to [^{14}C] creatinine (5 µM) and acyclovir (22 – 89 µM) in medium. The basolateral to apical

transport of [^{14}C] creatinine was assessed using a liquid scintillation counter. Statistical analyses were performed using unpaired student t-tests. Results were considered statistically significant if $p<0.05$.
Results: Acyclovir did not inhibit the renal tubular secretion of creatinine in LLC-PK1 cell monolayers.
Conclusion: The study is the first to address the effect of acyclovir on the renal tubular handling of creatinine. The results suggest that acyclovir does not affect the renal tubular secretion of creatinine, and hence, the increase in plasma creatinine levels is genuine.
Keywords: *Creatinine, acyclovir, LLC-PK1 cells*

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Is nifurtimox compatible with breastfeeding?

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Conflict of interest: No conflict of interest to declare.
Funding Source: FGB has received funding from the Clinician Scientist Training Program. This program is funded, fully or in part, by the Ontario Student Opportunity Trust Fund - Hospital for Sick Children Foundation Student Scholarship Program.

Background: Women with Chagas disease receiving treatment with nifurtimox are discouraged from breastfeeding. Many patients who would receive treatment with nifurtimox live in extreme poverty, and have limited access to resources such as clean water and baby formula and may not have safe alternatives to breast milk. We aimed to estimate, using limited available pharmacokinetics data, potential infant exposure to nifurtimox through breast milk.
Methods: Original nifurtimox plasma concentrations were obtained from published studies. Pharmacokinetic parameters were estimated using nonlinear mixed effect modeling with NONMEM version VI. One thousand nifurtimox plasma-concentration profiles were simulated and used to calculate the amount of drug that an infant would be exposed to, if breastfed 150 ml/kg/day.
Results: Estimation of Breast milk concentrations on the basis of peak plasma levels (1,361 ng/ml) and milk-plasma ratio were estimated. We calculated infant nifurtimox exposure of a breastfed infant of a mother treated with this drug to be below 10% of the maternal weight-adjusted dose, even if milk – plasma ratio were overestimated. Simulation led to similar estimates.
Conclusion: Risk for significant infant exposure to nifurtimox through breast milk seems small and below the level of exposure of infants with Chagas disease receiving nifurtimox treatment. This potential degree of exposure may not justify discontinuation of breastfeeding.
Keywords: *Pediatric clinical pharmacology, Chagas disease, population pharmacokinetics, modeling*

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Population pharmacokinetics study of benznidazole in children with Chagas disease

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Conflict of interest: No conflict of interest to declare.
Funding Source: FGB has received funding from the Clinician Scientist Training Program. This program is funded, fully or in part, by the Ontario Student Opportunity Trust Fund - Hospital for Sick Children Foundation Student Scholarship Program.

Background: Chagas disease is caused by *Trypanosoma cruzi*, and leads to long term cardiac and gastrointestinal complications. Treatment of children with benznidazole is effective, but no information on its pharmacokinetics and no pediatric formulation are available.
Patients and Methods: Population pharmacokinetics study in children with Chagas disease, aged 2-12 years (clinicaltrials.gov #NCT00699387). Enrolled children (target N=50) were treated with oral benznidazole 5–8 mg/kg/day BID for 60 days, as per current pediatric Chagas protocols. At least 3 blood samples were obtained after the first dose, at steady state or after the last dose. Benznidazole was measured in blood by HPLC.
Results: 25 children were enrolled to date. Mean age was 6 years (range 2.2–12 years). Median benznidazole dose was 6.3 mg/kg/day. Median steady-state (trough) benznidazole concentration was 0.99 mg/L, and highest observed concentration (Cmax) was 11.07 mg/L. All children treated had a positive response, with negativization of PCR for T. cruzi DNA, and marked decrease in anti T. cruzi antibody titers.
Discussion: Observed benznidazole concentrations in children were markedly lower than those reported in adults (treated with comparable mg/kg doses). In spite of these lower concentrations children treatment was effective and well tolerated, with few adverse drug

reactions (ADRs). Unlike adults, ADRs in children are uncommon, and severe ADR are rare. It is possible that the lower blood concentrations, while still providing therapeutic effect, may be responsible for this lower incidence of ADRs. If confirmed, our results would suggest that dosing modifications in adults may be beneficial.

Keywords: *Pediatric clinical pharmacology, Chagas disease, population pharmacokinetics*

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Placental transport and metabolism of 6-mercaptopurine

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Conflict of interest: No conflict of interest to declare.
Funding Source: CIHR

Introduction: The immunosuppressant azathioprine is increasingly used in pregnancy and is extensively metabolized to 6-mercaptopurine (6-MP) after oral administration. Animal studies suggest that high doses of 6-MP are teratogenic. However, a large study of women receiving azathioprine showed no increased risk for malformations. Only 1-2% of maternal 6-MP concentrations are found in cord blood indicating that the placenta acts as a barrier to fetal 6-MP exposure.
Objective: To determine how the placenta restricts transfer of 6-MP to the fetus in order to predict those at risk of higher exposure to 6-MP *in utero*.
Methods: Dual perfusion of a single human placental lobule under equilibrative conditions was utilized to determine whether 6-MP is actively effluxed into the maternal circulation. Metabolic capacity of the placenta was determined by incubating cytosolic fractions with 6-MP and monitoring metabolite production by HPLC.
Results: After adding 50 ng/ml or 500 ng/ml to both the maternal and fetal circulations, the fetal:maternal ratios of 6MP were 1.205 ± 0.177 (n=4) and 1.170 ± 0.093 (n=3) respectively after 180 minutes. After incubation of term placental cytosol with 6-MP, half was metabolized to 6-thioinosine-5'-monophosphate by 12 hours. Small amounts of the inactive metabolites 6-thioxanthosine and 6-methylmercaptopurine and none of the active 6-thioguanine nucleotide were produced.
Conclusions: Under equilibrative conditions, 6MP concentrates in the fetal circulation, likely as a result of ion trapping, and thus is not effluxed into the maternal circulation. The placenta has the ability to metabolize 6-MP and this may contribute to the limited transfer to the fetus.

Keywords: *Pregnancy, 6-mercaptopurine, placenta*

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Investigating differential signalling through chemerin receptors

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Conflict of interest: No conflict to declare.
Funding Source: Canadian Institutes of Health Research (CIHR)

Background: Adipokines, a group of signaling proteins secreted from adipocytes, contribute to the regulation of adipose function, inflammation, and energy homeostasis. Recently, our lab identified a novel adipokine, chemerin, which regulates adipocyte differentiation and metabolism, and possibly glucose and bone homeostasis through its action at the G protein-coupled receptor (GPCR) chemokine-like receptor-1 (CMKLR1). Because chemerin also binds and activates G protein-coupled receptor 1 (GPR1) with high affinity, we hypothesize that GPR1 also plays a fundamental role in mediating chemerin action.
Objective: To characterize chemerin activity and signaling at CMKLR1 and GPR1.
Methods: A Tango assay, which couples receptor activity to a luciferase reporter, allowed quantitative measurement of wild-type and mutant (generated by site-directed mutagenesis) receptor activation. GFP fusion proteins allowed visualization of receptor localization and internalization. Signaling was examined using cell lines which stably contain luciferase reporters linked to tandem repeats of transcription factor binding sites specific to signaling pathways of interest.
Results: Mouse GPR1 exhibits high basal activity and elevated intracellular localization compared to human and rat GPR1 independently of a conserved (D/E)R(Y/W) motif typically associated with constitutive activity. In contrast to CMKLR1, which signals through a MAPK/ERK pathway, MAPK/ERK is not a signaling pathway for chemerin action at GPR1.
Conclusion: Collectively, these data suggest that there is a species specific divergence in chemerin signaling through GPR1 and CMKLR1, which may contribute to cell type-specific variations in chemerin activity. These variations may be critical for regulation of both normal physiology and in various pathologies associated with aberrant chemerin activity.

Keywords: *Adipokines, G protein-coupled receptors, Chemerin*

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Systemic bioavailability of the doxylamine-pyridoxine delayed release combination (Diclectin®)

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Conflict of interest: Motherisk NVP helpline is supported by an unrestricted grant from Duchesnay, Inc. Canada. GK, is holder of the Research Leadership for Better Pharmacotherapy During Pregnancy and Lactation (Toronto), and The Ivey Chair in Molecular Toxicology (University of Western Ontario). He has been a paid consultant of Duchesnay, Inc.
Funding Source: Duchesnay Inc.

Background: Diclectin®, composed of 10 mg of doxylamine succinate and 10 mg of pyridoxine hydrochloride, is the drug of choice for the management of NVP; however, there is large variability in its onset and duration of action among women. In order to understand and improve its effectiveness, its systemic bioavailability must be studied.
Objectives: To determine the systemic bioavailability of the two components of Diclectin® after oral administration.
Methods: 18 non-pregnant, non-lactating, healthy females between 18 and 45 years of age were administered 2 tablets of Diclectin® with 240 mL of water under empty-stomach conditions. Blood samples were analyzed for doxylamine and PLP concentrations using tandem mass spectrometry. Pharmacokinetic values were adjusted for bodyweight, and their variability were calculated.
Results: The mean DOX-$AUC_{0\rightarrow\infty}$ was calculated to be 3137.22 ± 633.57 ng·hr/mL (range 2056.59 to 4376.06 ng·hr/mL). The mean pyridoxal-5'-phosphate(PLP)-$AUC_{0\rightarrow\infty}$ was calculated to be 5513.10 ± 2362.35 ng·hr/mL (range 1572.56 and 10 153.77 ng·hr/mL). Based on studies were pyridoxine was administered to volunteers intravenously, we calculated the bioavailability as 100%.
Conclusion: There is 2.1-fold variability in the DOX-$AUC_{0\rightarrow\infty}$ and 6.5-fold variability in the PLP-$AUC_{0\rightarrow\infty}$ after oral administration of 20 mg of Diclectin®. These interindividual differences may be important sources of variability in the effectiveness of the doxylamine succinate/pyridoxine hydrochloride combination for the management for NVP, and may need to be addressed in dosing guidelines.
Keywords: *Pharmacokinetics, doxylamine succinate, pyridoxine hydrochloride*

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Pregnant women and receiving the H1N1 vaccine: perception of risk and determinants of decision-making

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Funding Source: None

Background: Following availability of the H1N1 vaccine to the public in October 2009, Motherisk received an increased number of calls from pregnant women inquiring about the safety of the H1N1 vaccine.
Objectives: To determine 1) how many received the H1N1 vaccine following their call to Motherisk; 2) reasons for their decision; and 3) the sources of information regarding the vaccine and how this affected their decision-making.
Methods: Observational cohort study, using a questionnaire administered via telephone. All pregnant women who called Motherisk in October and November of 2009, requesting counseling regarding safety of the H1N1 vaccine, and who had not yet received the H1N1 vaccine, were contacted. A structured questionnaire consisting of 10 questions that included quantitative and semi-qualitative questions was used.
Results: To date, we have completed 35 follow-ups. Twenty seven (80%) pregnant woman received the vaccine and 7 (20%) declined. Of those who received vaccine, 61% cited concern about the risk of H1N1 infection for the baby as the reason for their decision, while 71% of those who declined, cited concern about the safety of the vaccine for the baby. Regarding information received, the women stated that the information from Motherisk (63%) and their direct health care providers (54%) was helpful, in contrast to the media of which only 11% found helpful.
Conclusions: In studying pregnant women's perceptions and determinants of their decision-making in receiving the H1N1 vaccine, we can gain important information for public education to be applied to future preventative health interventions.
Keywords: *H1N1 influenza, vaccine, pregnancy*

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African Americans have unique genetic variation in *CYP2B6*: implications for smoking cessation

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Conflict of interest: R.F.T. holds shares in Nicogen Research, a company that is focused on novel smoking cessation treatment approaches. None of the data contained in this manuscript alters or improves any commercial aspect of Nicogen; no Nicogen funds were used in this work. N.L.B. is a paid adviser to several pharmaceutical companies that market or are developing smoking cessation medications and also serves as a paid expert witness in litigation against tobacco companies. J.S.A. is a consultant to Pfizer.
Funding Source: NIH: CA091912

Background: Bupropion is a smoking cessation drug which is metabolized by the genetically polymorphic enzyme cytochrome P450 2B6 (CYP2B6). The smoking cessation outcomes of bupropion treatment, compared to placebo, are altered by genetic variation in *CYP2B6.*
Objectives: We investigated the frequencies of six non-synonymous *CYP2B6* variant alleles known to influence bupropion metabolism *in vitro* or acute bupropion pharmacokinetics *in vivo*.
Methods: African American light smokers were recruited and their *CYP2B6* genotypes were assessed by established *CYP2B6*4, *6 and *9* haplotyping and *CYP2B6*5* genotyping assays. Additionally, we developed a new *CYP2B6*16 (785A>G* and *983T>C*) and **18 (983T>C)* haplotyping assay to assess the frequencies and haplotype relationship of the novel *983T>C* variant.
Results: The newly developed assay reliably detects the alleles, which was confirmed by sequencing. Currently, 410 individuals have been genotyped for *CYP2B6*4, *5, *6, *9, *16* and **18* alleles. *CYP2B6*4, *9* and **16* alleles were rare (<1%) in this population, while *CYP2B6*5, CYP2B6*6,* and *CYP2B6*18* were prevalent with the allele frequencies of 2.9%, 33.8% and 5.2%, respectively. Each genotype was in Hardy-Weinberg equilibrium.
Conclusions: These data indicate that more than 70% of African American light smokers were *CYP2B6* slow metabolizers (SM, individuals with one or more copy of *CYP2B6*5, *6, *16,* or **18*) which is greater than observed in Caucasians (≈60% were *CYP2B6* SM) and much higher than in Asians (≈30% were *CYP2B6* SM). Next we will assess the impact of these variant genotypes on chronic bupropion pharmacokinetics and smoking cessation outcomes.
Keywords: *Bupropion, CYP2B6, smoking cessation*

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Biotransformation of triazolam human liver and heart microsomes

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Conflict of interest: No conflict of interest to declare.
Funding Source: Internal funding CRCHUM

Background: The CYP450-mediated metabolism of drugs in extrahepatic tissues is clinically relevant as many drugs present toxicity in other tissues. We studied the biotransformation of the probe drug triazolam to its hydroxylated metabolites α-hydroxytriazolam (α-OHTIA) and 4-hydroxytriazolam (4-OHTIA) in human liver and heart tissues.
Methods: Pooled human liver microsomes (HLM) and Supersomes® were supplied. Human heart microsomes were prepared from left ventricle samples of a patient and isolated by a series of ultracentrifugation according to our previously developed procedure. Incubations were performed (15 min: liver, 1 hour: heart) with 0 to 125 µM triazolam. Quantification of triazolam metabolites was carried out by GC-MS.
Results: Michaelis-Menten Kinetics of triazolam in HLM obtained for each metabolite were in agreement with data previously published. For α-OHTIA, a Km of 51.5 µM and Vmax of 2.7 nmoles of α-OHTIA/incubation/min/nmoles of P450 was obtained. For the 4-OHTIA, a Km of 166.5 µM and Vmax of 5.3 nmoles of 4-OHTIA/incubation/min/nmoles of P450 was obtained. Metabolism of triazolam by human heart microsomes was selective for the α-OHTIA; the 4-hydroxymetabolite could not be detected. Formation of α-OHTIA was 13 times less in heart microsomes compared to the formation observed in CYP2J2 over-expressed isozymes (Supersomes®) at 100 uM of triazolam.
Conclusion: Our study establishes and confirms CYP450-mediated kinetic parameters for triazolam in hepatic tissues. Moreover, our study confirms the presence of significant CYP450 activity in human heart tissues using the probe drug triazolam. Results obtained also suggest the involvement of CYP2J2 in the formation of α-hydroxytriazolam in cardiac tissue.
Keyword: *CYP-450, microsomes, triazolam*

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Investigating the impact of obesity on folate status in the prevention against neural tube defects; a systematic review

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

Background: Maternal obesity has previously been identified as a risk factor for neural tube defects (NTD) during pregnancy. As obesity becomes an increasingly prevalent public health concern, its association with teratogenic consequences may become more apparent. While adequate folic acid intake can aid in the reduction of NTD-affected births, it remains unknown if obese women achieve protective folate levels during the periconceptional period. The aim of this systematic review was to determine whether obesity and folate status are independent risk factors for NTDs, by examining the association between body weight and folate status.
Methods: Embase and Medline were searched for articles identifying an association between body weight and folate status. Additionally, reference lists of pulled papers were scanned to find any relevant studies. Both serum and erythrocyte folate were considered acceptable indicators of folate status. Located articles were classified according to primary or secondary outcome, in regards to analysis of folate status as a function of body weight.
Results: The search identified a total of 695 articles, of which 27 met the inclusion criteria. For serum folate, 6 of the primary outcome studies found a negative correlation, while only 1 found no apparent correlation. For erythrocyte folate, 1 primary outcome study found a negative correlation and 1 found no apparent correlation. No consistent trends were found among secondary outcome studies for either serum or erythrocyte folate.
Conclusion: The potential link between body weight and folate status remains unresolved. While there appears to be a trend in regards to serum folate, the lack of a congruent association among the investigated studies suggests that further work must be conducted to realize the impact of obesity on folate levels. If obesity and folate deficiency are not mutually exclusive risk factors of NTDs, optimization of folic acid dosing may aid in reducing birth defects among infants born to obese mothers.
Keywords: *Obesity, folic acid, neural tube defects*

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Fetal safety of cetirizine use in pregnancy – a prospective controlled cohort study

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Conflict of interest: No conflict to declare.
Funding Source: None

Background: Cetirizine, a non-sedating second–generation H1-receptor antagonist, is widely used to treat allergic rhinitis and urticaria, but the data on pregnancy outcome are limited.
Objective: To determine whether exposure to cetirizine during pregnancy increases the risk of major malformations or other adverse pregnancy outcomes.
Methods: Pregnant women who were counseled by the Motherisk Program in regard to cetirizine exposure (n=134) were enrolled in a controlled, cohort study and compared to an equal number of pregnant women counseled for non-teratogenic exposures. The primary outcome was the rate of major malformations and secondary endpoints were the rates of miscarriage, stillbirth, preterm delivery, gestational age at birth, and birth weight.
Results: There were no significant differences found between the cetirizine exposed and comparison group in the pregnancy outcome: rate of live births, miscarriages, stillbirths, and elective termination (p=0.345). There was also no difference in the rates of major malformations (2/130=1.5% versus 2/128=1.6%; p=0.625), gestational age at birth (38.96±2.3 versus 39.59±1.4 weeks; p=0.103), prematurity (p=0.047) and mean birth weight (3389±657 versus 3547±523 grams; p=0.081).
Conclusion: The use of cetirizine during pregnancy does not appear to be associated with an increased risk of major malformations or other adverse fetal outcomes.
Keywords: *Pregnancy, cetirizine, fetal outcome*

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A randomized, double-blind, crossover comparison of buprenorphine transdermal system (BTDS) and placebo in patients with chronic low back pain

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Conflict of interest: Authors Piraino, Eisenhoffer, Harsanyi, Darke and Michalko are or were employees of the sponsor.
Funding Source: Purdue Pharma, Canada

Objective: To compare the efficacy and safety of a 7-day BTDS and placebo in chronic low back pain.
Methods: Patients requiring at least 1 tab/day of an opioid preparation underwent opioid washout (2-7d) before randomization to 10µg/h BTDS or placebo and titrated weekly according to efficacy and tolerability to 20µg/h and 40µg/h. After 4 weeks, patients crossed over to the alternate treatment for another 4 weeks. Acetaminophen 650mg q4-6h prn was provided for rescue. Data were analyzed using three-way analysis of variance.
Results: Of 78 randomized patients, 52 (67%) met per-protocol criteria. BTDS produced significantly lower VAS (45.3±21.3 vs. 53.1±24.3mm, p=0.0219) and 5-point ordinal (1.9±0.7 vs. 2.2±0.8, p=0.0439) pain scores during the last week of treatment than placebo (confirmed by ITT analysis). The overall Pain and Sleep score was significantly better with BTDS than placebo (177.6±125.5 vs. 232.9±131.9, p=0.0268). There were no treatment differences on the Pain Disability Index or SF-36, but individual Quebec Back Pain items were significantly improved with BTDS (p≤0.0424). 66% of patients (p=0.0009) and 60% of investigators (p=0.0079) preferred BTDS, versus placebo (patients: 24%; investigators: 28%), while 10% of patients and 12% of investigators had no preference. 65% of patients (p=0.0139) and 63% of investigators (p=0.0139) assessed BTDS as moderately or highly effective. Several opioid-related AEs were reported more frequently with BTDS (p≤0.0201) but the frequency of constipation was not significantly different. 82% of patients chose to continue BTDS in a long-term open-label evaluation.
Conclusion: BTDS is effective for management of chronic low back pain.
Keywords: *Buprenorphine, transdermal system, chronic non-cancer pain*

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The safety of escitalopram in pregnancy: a prospective cohort study

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Conflict of interest: Authors declare no conflict of interest.
Funding Source: None

Background: Escitalopram (Lexapro®) is a 5-HT reuptake inhibitor prescribed for its antidepressant and anxiolytic activity. To date, no prospective studies documenting fetal outcome following exposure to escitalopram during pregnancy have been reported.
Objectives: To determine whether the use of escitalopram during pregnancy is associated with an increased risk of major malformations; secondly, to determine the rates of spontaneous abortions, therapeutic abortions, stillbirths, mean birth weight, low birth weight and prematurity.
Methods: A prospective, observational cohort study involving pregnant and planning women who contacted Motherisk Program in Canada and other TISs in Italy and Switzerland between January 2003 and August 2009 requesting information on the safety of escitalopram in pregnancy. Data was collected via standardized questionnaire. Two comparison groups of pregnant women exposed to other antidepressant medications (N=213) as well as non-teratogenic exposures (N=213) were matched for maternal age and gestational age at call. Statistical analysis was performed using Fishers exact test and Chi Square statistics.
Results: 639 patients have completed the study. Among the 213 escitalopram exposed, 38 (17.6%) reported spontaneous abortion, 3 (1.41%) reported still birth, 19 (8.9%) reported premature birth, and 6 (2.8%) described a major malformation. Mean birth weight of exposed infants was 3198 g while low birth weights were reported in 17 (8%). Comparison group results are pending.
Conclusion: Preliminary results reveal that escitalopram exposure during pregnancy does not increase the risk of major malformation above the baseline and does not appear to affect fetal survival rates, mean birth weights or duration of pregnancy.
Keywords: *Esitalopram, pregnancy, depression, teratogenicity*

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Comparing TWEAK and T-ACE to identify problem drinking in pregnancy

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Conflict of interest: No conflicts declared
Funding Source: None

Background: The TWEAK and T-ACE screening tools are validated methods of identifying problem drinking in pregnant women.
Objective: To compare effectiveness of both screening tools in women representative of problem drinkers using various cut-points (CPs).
Methods: Study participants consisted of women calling the Motherisk Alcohol Helpline for information regarding their alcohol use in pregnancy. In this cohort, concerns surrounding under-reporting are not likely as women self-report their alcohol consumption. Participant's self-identification, confirmed by her self-reported amount of prenatal alcohol use, determined whether or not the participant was classified as a problem drinker. This was the standard against which screener performance was assessed. The TWEAK and T-ACE tools were administered on both groups and subsequent analysis was done using student t-test, operational characteristic calculations and ROC analysis.
Results: The study consisted of 75 problem and 100 non-problem drinkers. Both TWEAK and T-ACE tools poorly identified potential at-risk women (PPV=0.54) using traditional CP of two and three, with high sensitivity (100%-99% and 100-93%, respectively) but low specificity (36 - 43% and 19 - 34%, respectively). However, performance of both tools improved using higher CP, where TWEAK was significantly better than T-ACE at CP of 4 or more.
Conclusion: Neither the TWEAK nor T-ACE tests were able to identify problem drinkers effectively in this cohort using the current recommended thresholds. These results provide further evidence that screening tools are population-dependent. If either tool is used, providers should follow-up with additional screening for antenatal alcohol use so that specificity can be improved.
Keywords: *Screening tools, alcohol, pregnant women*

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Adverse event related to the use of artemisinin based combination therapies (ACT) at Kinshasa

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Conflict of interest: All authors declare no conflicts.
Funding Source: None

Background: Malaria is an infectious disease that kills a lot of people in the world. About 300 to 500 million case of malaria are described each year including 1 millions of deaths. Children under 5-years old and pregnant women bear the heavy burden of this disease. More than 15 African countries including DRC have adopted artésunate+amodiaquine as first line treatment Policy of Uncomplicated Malaria. 2 years after the adoption of this combination in DRC, no pharmacovigilance study has been conducted in our country. This survey aimed to determine adverse event related to the use of ACT.
Method: A cross sectional study has been conducted in 457 health centers, 524 pharmacies and 1781 households. Information was collected by interview of health professionals and patients using a structured questionnaire. The data captures have been done with the Software Excel and data analysis with the Software epi info and SPSS.
Result: Asthenia represented the most reported adverse event in households, health centers and pharmacies for all ACT available in Congolese drug market (Artesunate+Amodiaquine; Artesunate+luméfantrine and Artesunate+Sp).
Conclusion: A prospective study is urgently needed to detect, evaluate, understand, prevent and treat true adverse drug reaction related to ACT in DRC.
Keywords: *Adverse event, ACT, adverse drug reaction*

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Effect of bicarbonate on neonatal serum ionized magnesium in vivo

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Conflict of interest: None to declare.
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Background: Sodium bicarbonate is used to treat metabolic acidosis, or to induce metabolic alkalosis in sick neonates. The aim of this study was to quantify the decrease in serum concentration of ionized magnesium ([Mg2+]) when sodium bicarbonate is administered in vivo.
Methods: We administered 1 mEq/Kg body weight sodium bicarbonate 4.2% for correction of metabolic acidosis (n= 11) for management of persistent pulmonary hypertension (n=3).
Results: After sodium bicarbonate treatment, serum pH increased by an average of 0.046, (p< 0.001), serum [Mg2+] decreased by an average of 0.07 mmol/L, (P< 0.01), and serum [Ca2+] decreased by an average of 0.06 mmol/L (P= 0.04). There was a significant correlation between baseline [Mg2+] and baseline [Ca2+] (R2 = 0.328, P= 0.032).
Conclusion: Sodium bicarbonate therapy in infants causes a significant decrease in [Mg2+] and serum [Ca2+]. We suggest that infusion of sodium bicarbonate be effected while monitoring serum [Mg2+] and serum [Ca2+].
Keywords: *Ionized magnesium, sodium bicarbonate, pH; preterm infants, term infants, metabolic acidosis*

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The influence of bromocriptine on the plasma and brain amino acid concentrations of rat 'taurine as a probable mediator and modulator'

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Conflict of interest: The results may lead to new conceptualizations and some potential clinical approaches of hallucination and schizophrenia.
Funding Source: None

Problem Statement: In clinical trials in which Bromocriptine was administered with concomitant reduction in the dose of levodopa/carbidopa, the most common newly appearing adverse reactions were: nausea, abnormal involuntary movements, hallucinations, confusion, "on-off" phenomenon, dizziness, drowsiness, faintness/fainting, vomiting, asthenia, abdominal discomfort, visual disturbance, ataxia, insomnia, depression, hypotension, shortness of breath, constipation, and vertigo**.** It is well known that any interference with brain amino acid levels is likely to lead to brain disorder. Our previous research has shown that bromocriptine produced significantly changes in the heart and kidneys amino acid contents. It was confirmed that the brain amino acids concentrations are influenced by plasma amino acid levels. Therefore, it is therefore, thought of interest to investigate the effect of bromocriptine, sulpiride or their combination on the brain and plasma amino acid concentrations of rat.
Material and Methods: Approach: The influence of chronic treatment with bromocriptine 20 mg kg/ day I.P, sulpiride 20 mg kg/ day I.P. or their combination bromocriptine (20 mg Kg-I) + sulpiride (20 mg -1 (20 mg Kg); for 6 weeks on free amino acids in the brain and the plasma of rats were carried out. The amino acids were quantified using the LKB 4400 Amino Acid Analyzer and the Hami1ton's amino acid calibration standards.
Results: Bromocriptine significantly decreased the plasma content of glutamic acid, glycine and alanine. (P <0.05, n =7). Sulpiride did not affect basal or bromocriptine-induced changes in the plasma amino acid concentrations. Also, results indicate that bromocriptine decreased the brain content of taurine, glycine and aspartic acid. The mean % decreases were 40 ± 3, 55 ± 6 and 30 ± 4 respectively (P<0.0l, n = 7). Sulpiride prevented the decreases in glycine and aspartic acid only.
Conclusion: Since our previous studies reported that bromocriptine stimulated the isolated rabbit jejunum and the rat uterus and sulpiride or haloperidol failed to antagonize the induced contractions. Where the latter were abolished by pretreating the tissues with cyproheptadine or taurine. Therefore, activation of serotonergic receptors may underlie the appearance of some side effects such as hallucinations in Parkinson's disease patients ingesting large doses of bromocriptine. Further, the results direct the attention to the possible involvement of taurine and glycine in bromocriptine induced decrease in plasma prolactin and the improvement in sexual activity of sub-fertile males taking the compound.
Keywords: *Bromocriptine, sulpiride, amino acids, brain, plasma, taurine, glycine, hallucinations*

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Kinin B1 receptor (B1R) in diabetic complications under septic shock

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Conflict of interest: The authors have declared that no competing interests exist.
Funding Source: This work was supported by the Canadian Diabetes Association. G. Lemire received a Traineeship from Diabète Québec

Background: Septic shock induced by lipopolysaccharides (LPS) and diabetes induced by Streptozotocin (STZ) enhanced kinin B1R expression.
Objectives: To determine whether LPS combined with

STZ increases mortality, secondary to excessive blood clotting induced by B1R on platelets or following the release of pro-inflammatory mediators from other cells by B1R activation.
Methods: Rats treated with STZ 4 days earlier, received LPS (2 mg/kg, i.p.) or vehicle. B1R antagonist SSR240612 (10 mg/kg) was administered twice a day and fasted rats were sacrificed the following morning to measure its impact on body temperature, plasma insulin and glucose, edema and vascular permeability in various tissues. The mechanism underlying the mortality was further studied by using inhibitors of cox-1 (indomethacin), cox-2 (niflumic acid), nitric oxide synthase (NOS) (L-NAME) and iNOS (1400W) administered for 1-week. The presence of B1R on platelets was determined by Flow cytometry. Platelet aggregation using an agregometer was made on blood samples from rats treated with B1R agonist (des-Arg^9-BK) or antagonist (SSR240612).
Results: SSR 240612 prevented the mortality, fever, edema and increased vascular permeability induced by LPS alone or in combination with STZ. SSR240612 normalized hyperglycemia and improved insulin deficiency in STZ. No significant amount of B1R was found on platelets. Whereas SSR240612 prevented platelet aggregation, the B1R agonist increased it. Cox-2 and iNOS inhibitors prevented the mortality in LPS plus STZ.
Conclusion: SSR240612 produced anti-inflammatory, anti-pyretic, anti-diabetic and anti-thrombotic effects which improved rat survival. Hence, kinin B1R antagonists can be of therapeutic value in septic shock encountered in diabetic complications
Keywords: *Diabetes, peptides, septic shock*

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Comparative study of ligand receptor binding of several monoclonal antibodies to EGFR on different species

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Conflict of interest: All authors declare that there are not conflicts of interest.
Funding Source: Molecular Immunology Center, Havana, Cuba

Background: Drugs inhibitors of signal transduction are target specific for molecular alterations that are thought vital for cellular functions on cancer treatment. Safety evaluation program of biological derived products should be performed in relevant species, those in which the test material is pharmacologically active due to the expression of receptor. This work analyzes, comparatively, the affinity of binding constants (EGF, nimotuzumab and cetuximab) by the EGFR in placentas of 6 different species used in previous toxicological studies. One-way ANOVA and Duncan tests were used for Statistical analysis. Displacement curves of ligand-receptor for different MAb in several species showed to be dose dependent but with different affinity grade in the following order EGF < cetuximab < nimotuzumab. Human and monkeys species shown Kd$\leq 10^{-8}$M, while rabbit, mouse and rat shown a Kd $=10^{-7}$M. Values obtained in this study were between 10^{-9} M and 10^{-7}, which implies that those species with Kd > 10^{-8} M for nimotuzumab and cetuximab are not relevant species for toxicological studies. Nevertheless, species of monkeys are relevant for toxicological studies and the results of non toxic effects of nimotuzumab are valid.
Keywords: *Nimotuzumab, receptor binding*

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Reduced plasma all-trans retinoic acid levels in acute promyelocytic Leukemia patients with gastrointestinal complications

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Conflict of interest: No conflicts declared.
Funding Source: This work was supported by the Mother and Child Health Foundation (Japan), and Japan Leukemia Research Fund.

Background: All-trans retinoic acid (ATRA) is used as differentiation therapy for acute promyelocytic leukemia (APL). Resistance to ATRA has occurred recently, presumably because of low plasma ATRA levels, which may be due to increased oxidative metabolism of ATRA or expression of the multidrug-resisitance gene product. Several complications may affect the pharmacokinetics of ATRA in patients with APL, however there is rare investigation.
Objectives: To evaluate the pharmacokinetics of ATRA in gastrointestinal alternations, the pharmacokinetics of ATRA were determined in patients with APL.
Methods: Three patients with APL (two; administration via a feeding tube, one; Crohn's disease) were enrolled. All of the patients received oral ATRA (45 mg/m^2 per day) and chemotherapy, the pharmacokinetics of ATRA were determined.
Results: Cmax of ATRA level of all patients 10.0±4.0 ng/ml) were markedly decreased compared with that in APL patients with remission (352.0±173.6 ng/ml) described previously (Takitani, Am J Hematol, 2006). Two of them had incomplete remission and one had complete remission despite of lower ATRA levels.
Conclusions: Gastrointestinal complications may also have influenced absorption because our patients given an elemental diet via the feeding tube were cachectic.

The intestinal manifestations of Crohn's disease lead to impair absorption of protein and lipids such as fat-soluble vitamin. Therefore, we concluded that their low plasma ATRA levels might be due to impaired absorption. Further studies will be needed to develop appropriate administration schedules for patients with impaired absorption because of factors such as gastrointestinal complications.

Keywords: *Acute promyelocytic leukemia, all-trans retinoic acid, pharmacokinetics*

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Observational clinical study in patients with advanced stage epithelial tumors treated with nimotuzumab

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Funding Source: Molecular Immunology Center, Havana, Cuba

Background: The CIM has developed nimotuzumab, a recombinant MAb against EGF Receptor, effect that leads to the inhibition of cellular proliferation. This work evaluates the safety and clinical benefit of nimotuzumab in patients with advanced epithelial tumours, by an observational, prospective, multicentric, national, open study in cancer patients treated with nimotuzumab combined with standard therapies or monotherapy. All adverse events was collected and classified as per the CTCAE toxicity, NCI v3 scale. Results were processed and tabulated by descriptive statistics. Pediatric (19.1%) and adult (22.5%) population presented at least one adverse event. The safety analysis was based on the exposure to the antibody. From the 191 patients that received 1 to 6 doses, 40 (20.9%) had adverse events (grade I and II); causality relationship was attributed to nimotuzumab in only 7 (10.6%). 55 received >30 doses, 17 (30.9%) presented adverse events (grade I and II) where 14% were nimotuzumab attributed. Treatment with nimotuzumab provides clinical benefits on survival, as shown on previous clinical studies. The mean survival rate in patients with head-neck advanced cancer was 28.63 months. The median of this group had not been reached when the data was analyzed, whereas, the mean survival rate for patients with recurrent or methastasic tumours was 17.5 months with a median of 9.10. mean survival rate for patients with a new diagnosis of multiform glioblastoma were 15.73; median 13.97. Nimotuzumab probed be safe in all therapeutic and prolonged use treatments. As monotherapy or combined with standard therapies provide clinical benefits on patient survival.

Keywords: *Clinical study, epithelial tumours, nimotuzumab*

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Homocysteine as a biomarker for vascular disease in children and adolescents with epilepsy

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Conflict of interest: No conflict of interest.
Funding Source: Children's Hospital of Research Institute

Background: Epilepsy is a common neurological disorder in the paediatric population most often treated with antiepileptic drugs. Previous studies have demonstrated that chronic exposure of such drugs often resulted in elevated plasma homocysteine (Hcy) concentration. In turn, hyperhomocysteinemia is a risk factor for vascular disease and is often due to the interference of Hcy metabolism. While the mechanistic relationship between Hcy and vascular injuries remain unknown, studies have identified endothelial dysfunction to be an early indicator of vascular disease. The objective of this study is to examine the relationship between Hcy concentration in epileptic children being treated with valproic acid (VPA) and the effect on endothelial function. Validation studies will also be done *in vitro* to further strengthen the results obtained from patients. Plasma total Hcy, erythrocyte folic acid, and serum vitamin B12 concentrations along with endothelial function, will be assessed by high-performance liquid chromatography and peripheral arterial tomography in 40 epileptic children at baseline and six weeks into the study and compared to healthy controls. Statistical differences will be assessed by Student's t-test. Preliminary results show a trend towards increased plasma Hcy, erythrocyte folic acid, and serum vitamin B12 concentrations in epileptic children on VPA compared to controls. If elevated Hcy concentration is caused by VPA then it can serve a useful biomarker for vascular disease. These results could potentially yield new information on determinants of the development of vascular diseases, as well as planning interventions to prevent or diminish the negative effects of VPA induced hyperhomocysteinemia in epileptic children.

Keywords: *Antiepileptic drugs, homocysteine, vascular disease*

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Therapeutic use of opioids: a prospective, comparative study of pregnancy outcomes

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Conflict of interest: No conflict of interest to declare.
Funding Source: None

Background: Data on pregnancy outcomes of women taking medically prescribed opioid analgesics throughout pregnancy are incomplete. Although this class of medications does not appear to be teratogenic, a possible association between opioid analgesic use in early pregnancy and elevated risk of cardiac malformations has been reported recently. Adverse pregnancy outcomes other than malformations associated with maternal exposure to opioids are rarely reported. Little information exists on neonatal withdrawal syndrome due to opioids used for medical reasons.
Objectives: (1)To determine whether exposure to medically prescribed opioids is associated with increased risk of major malformations (2)To examine rates of spontaneous abortions, therapeutic abortions, stillbirths, birth weight and gestational age at delivery (3)To estimate the rate of neonatal withdrawal symptoms in infants exposed to prescribed opioids late in pregnancy.
Methods: From the Motherisk Program prospectively collected database, cohort of women who took opioid-containing medications for medical reasons at any stage of pregnancy will be identified and followed up. Pregnancy outcomes will be compared to those of two matched control groups: women with similar medical conditions who used non-opioid medications for pain relief (1) and women exposed to non-teratogens (2).
Results: To date, 35 follow ups on pregnant women exposed to opioid-containing medications have been completed. Among exposed women, 3/35 delivered prematurely. Among infants of exposed women, 1 died due to severe prematurity (to be confirmed by physician) and there were 4 malformations (2 hernias, 1 cleft lip and palate, 1 unspecified).
Conclusion: The study is ongoing and more data will be collected from the Motherisk database.
Keywords: *Drug safety, opioid analgesics, pregnancy*

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Effects of hypertonicity on CYP3A expression in vivo

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Conflict of interest: There is no conflict of interest.
Funding Source: Doctoral Research Award (CIHR) and Restracomp (The Hospital for Sick Children)

Background: The Tonicity Enhancer Binging Protein (TonEBP)/Nuclear factor of activated T-cells 5 (NFAT5) is responsible for the up-regulation of human CYP3A mRNA expression under hypertonicity in intestinal and hepatic cell lines. In this study, we first examine the effects of hypertonicity on CYP3A expression in human primary hepatocytes and observed a significant increase of CYP3A5 and CYP3A7 mRNA levels (2.1 and 3.6 folds respectively), but with no change in CYP3A4 mRNA, hepatic CYP3A total protein and activity levels. Second, the effect of hypertonicity is examined in humanized CYP3A mouse that encompasses human CYP3A4 and CYP3A7 and its 5' regulatory regions. Animals are examined by two approaches: a) one week of 8% high-salt diet and b) one week of twenty-four hour water deprivation cycles. Results show a stronger nuclear staining of Nfat5 at the a) villus tip of the proximal intestinal tract and b) kidney medulla respectively. The high-salt diet is capable of inducing human CYP3A transgene expression in the gut and kidney (95% confidence), and cyclic dehydration induced CYP3A transgene in the liver (90% confidence) and kidney (95% confidence). Taken together, tonicity may influence the level of CYP3A expression in extra-hepatic tissues, which may in turn influence the effectiveness of drug substrates via first-pass metabolism.
Keywords: *First-pass metabolism, NFAT5/TonEBP, P450 CYP3A*

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Impact of folic acid fortification/supplementation on antifolate chemotherapy: a systematic review

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Conflict of interest: The authors have no conflict of interest to declare.
Funding Source: Sickkids Foundation

Background/Objectives: Since 1998 in both Canada and the United States flour has been supplemented with folic acid at the rate of 140 mcg per 100 g to prevent

neural tube defects. The objective of this paper is to explore whether folic acid supplementation negatively impacts treatment outcomes with antifolate chemotherapy.

Methods: A systematic review was conducted searching Embase and Medline. Inclusion criteria consisted of articles related to the treatment of individuals with cancer using drugs that inhibit the enzyme dihydrofolate reductase alone or in combination with other chemotherapy.

Results: The pooled results yielded 750 articles. Forty-five articles were retrieved for full analysis resulting in zero studies meeting the inclusion criteria. Three articles discussed folic acid supplementation specifically, however, these did not meet the inclusion criteria. One article was a case series. The second explored erythrocyte folate levels in children supplemented with 75-200 mcg per day of folic acid while on maintenance therapy for acute lymphoblastic leukemia. The third study explored the impact of folic acid supplementation in host versus graft disease. Of note, since 1999 folic acid supplementation has been incorporated as part of the treatment protocol with pemetrexed.

Conclusions: The results of the systematic review document the paucity of data on potential interactions between folic acid supplementation and treatment outcomes in the use of antifolate chemotherapy. Because antifolates are used by millions of patients for cancer therapy and as antimicrobials, further investigations are urgently required to better assess the potential impact of folic acid fortification/supplementation in chemotherapy treatments.

Keywords: *Antifolate chemotherapy folic acid, oncology*

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