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

Available Online at www.jptcp.com www.cjcp.ca

ABSTRACTS / RÉSUMÉS

Multidisciplinary Approaches to Modern Therapeutics: Joining Forces for a Healthier Tomorrow

May 24-27, 2011
Montreal, QC

Canadian Society of Pharmacology and Therapeutics
La Société canadienne de Pharmacologie et de Therapeutique

Contents

(Presenters are underlined)

ORAL PRESENTATIONS
THURSDAY - MAY 26, 2011

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Effect of human equilibrative nucleoside transporter 1 (hENT1) expression on adenosine production from neurons

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Conflict of Interest: None declared

Background: Adenosine is produced in brain under ischemic conditions. Intracellular and extracellular pathways for adenosine formation have been described. Transgenic (Tg) mice with neuron-specific expression of hENT1 were developed (Parkinson et al., 2009 J. Neurochem. 109:562-572) to examine neuronal uptake and release of adenosine during ischemic conditions.
Objectives: The present study examined release of adenosine and inosine from primary cultures of cortical neurons from wild type (CD1) and hENT1 Tg mice under basal and excitotoxic conditions.
Methods: Primary neuronal cultures were incubated with ^{3}H-adenine to radiolabel intracellular ATP. Cells were then treated for 30 minutes (37 ºC) with buffer or N-methyl-D-aspartate (NMDA; 100 µM). The effects of dipyridamole (DPR; 30 µM), an inhibitor of ENT1 and ENT2, α,β-methylene ADP (AOPCP; 50 µM), an inhibitor of ecto 5'-nucleotidase, and S-(4-nitrobenzyl)-6-thioinosine (NBMPR; 100 nM), a selective ENT1 inhibitor, on adenosine and inosine production were assessed.
Results: NMDA significantly increased levels of both adenosine and inosine ($p < 0.05$); levels were significantly greater using hENT1 Tg neurons than using CD1 neurons ($p < 0.05$). DPR, but not AOPCP or NBMPR, significantly inhibited levels of both adenosine and inosine ($p < 0.05$).
Conclusions: Intracellular formation and transporter-mediated release of both adenosine and inosine occurs from neurons under ischemia-like conditions. This contrasts with findings from hippocampal slices treated with ischemic conditions, which exhibited transporter-mediated uptake of adenosine.

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Disposition of atorvastatin, rosuvastatin and simvastatin in Oatp1b2$^{-/-}$ mice and intraindividual variability in human subjects

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Conflict of Interest: None declared

Background: The HMG-CoA reductase inhibitors, or statins, are widely prescribed to reduce cardiovascular disease risk. There is considerable interindividual variation in statin exposure and response arising from variability in both transport and metabolism.
Objective: Our aim was to better understand the *in vivo* relevance of the organic anion-transporting polypeptide (OATP) 1B family to atorvastatin (ATV), rosuvastatin (RSV) and simvastatin (SVA) disposition in Oatp1b2$^{-/-}$ mice, and the role of metabolism *vs* transport by comparing ATV, RSV and SVA pharmacokinetics in healthy human subjects given all three statins.
Methods: Male Oatp1b2$^{-/-}$ and wild-type mice were dosed 1 mg/kg ATV, RSV or SVA IV, and liver and plasma concentrations measured 30 min later. Ten healthy subjects were administered a single oral dose of 20mg ATV, 20mg SVA and 10mg RSV in a cross-over study design. Statin acid concentration in plasma collected 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 10 h post-dose was measured by LC-MS/MS.
Results: Liver-to-plasma ratios were significantly lower in Oatp1b2$^{-/-}$ *vs.* wild-type mice for ATV ($p=0.002$) and RSV ($p=0.03$) but not SVA. In humans, plasma exposure of ATV and SVA acid were significantly related ($p<0.05$), while RSV profile was not predictive of ATV or SVA acid exposure.
Conclusions: In mice, Oatp1b2 appears important for the hepatic uptake of ATV and RSV. In humans, ATV and SVA, which are subject to CYP3A metabolism and transport, appear to share common mechanisms of elimination, in contrast to RSV, which is not significantly metabolised but a substrate of multiple hepatic uptake and efflux transporters.

3
Validation of the novel *in vitro* platelet toxicity assay (*i*PTA) for the diagnosis of drug hypersensitivity reactions

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Background: Drug hypersensitivity reactions (DHRs) are rare but potentially fatal adverse events which occur in susceptible patients. The diagnosis and prediction of DHRs is difficult due to their variable clinical presentation and the overlap of symptoms with other clinical conditions. Systematic rechallenge, the gold standard for DHRs diagnosis, is not always ethical to perform due to possible serious reactions. Current *in vitro* tests including the lymphocyte toxicity assay (LTA) are cumbersome. We have recently developed a novel *in vitro* diagnostic test, the *in vitro* platelet toxicity assay (*i*PTA) for DHRs.
Objective: To validate the iPTA as a diagnostic test for DHRs.
Methods: Twenty-eight individuals (14 DHS-sulfa patients and 14 healthy controls) were recruited. Blood samples were obtained and both LTA and iPTA were performed independently. Results were then compared to determine the degree of agreement between the two diagnostic approaches.
Results: There was concentration-dependent toxicity in the cells of patients when incubated with the reactive hydroxylamine metabolite of sulfamethoxazole for both the LTA and iPTA ($p<0.05$) and toxicity was significantly greater for the cells of patients versus controls ($p<0.05$). The two tests had a high degree of agreement (correlation coefficient: $R^2 = 0.97$). It was very clear that the iPTA was more sensitive than the conventional LTA test in detecting the susceptibility of patient cells to *in vitro* toxicity.
Conclusion: The novel iPTA has considerable potential as an investigative tool for DHS as it is cheaper to perform and requires no special reagents that make it more suitable for clinical wider use.

4
Role of efflux transporter P-glycoprotein (MDR1) in rivaroxaban drug disposition

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Conflict of Interest: None declared

Background/Objective: Thromboembolic events resulting from blood clotting disorders are a significant source of mortality and morbidity. The novel direct factor 10a inhibitor, rivaroxaban, was recently approved for thromboembolism prophylaxis following total knee or hip replacement. However, rivaroxaban renal elimination is greater than glomerular filtration rate; thus, we hypothesized that the efflux drug transporter, P-glycoprotein (MDR1), is involved in rivaroxaban excretion and disposition.
Methods: The ability of MDR1 to mediate rivaroxaban transport *in vitro* was assessed in LL-CPK cells overexpressing MDR1 (LMDR1). To determine the *in vivo* relevance of MDR1 to rivaroxaban disposition, plasma and tissue concentrations were determined in *Mdr1a* deficient mice (*Mdr1a*^{def}) following oral administration.
Results: A markedly higher vectorial transport of rivaroxaban was observed in the basolateral to apical direction (B-A) compared to A-B in LMDR1 cells (efflux ratio 5.6, n=5). Additionally, a selective inhibitor of MDR1 (LY335979) abolished the B-A transport of rivaroxaban (efflux ratio 1.2, n=3). Following oral administration of rivaroxaban *in vivo*, plasma concentrations did not significantly differ between wild-type and *Mdr1a*^{def} mice (n=6). Liver to plasma ratio of rivaroxaban concentration was significantly lower in *Mdr1a*^{def} mice ($P<0.01$), while kidney to plasma ratio was marginally higher compared to wild-type mice. Importantly, rivaroxaban brain concentrations did not differ, suggesting that other efflux transporters at the level of blood-brain barrier may be compensating for the absence of MDR1.
Conclusions: Overall, rivaroxaban appears to be a substrate MDR1 *in vitro*. However, further studies are required to elucidate additional efflux transporters involved in rivaroxaban disposition *in vivo*.

5
Limited placental transfer of 6-mercaptopurine is mediated by tissue binding and not active transport

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Conflict of Interest: None for all authors

Introduction: The immunosuppressant azathioprine is increasingly used in pregnancy for the treatment of autoimmune diseases or for organ transplant patients. Several studies have demonstrated that azathioprine does not increase the risk for major fetal malformations. Azathioprine is rapidly metabolized to 6-mercaptopurine (6-MP) and the placenta is considered a relative barrier to 6-MP. Because 6-MP

interferes with DNA synthesis, it is important to determine how the placenta restricts transfer in order to identify factors that could increase fetal exposure.
Objective: To determine if active drug transporters, tissue binding, or placental metabolism restrict 6-MP transfer.
Methods: Dual perfusion of a single human placental lobule *ex vivo* was utilized and 6-MP was added to the maternal circulation to determine transplacental kinetics. 6-MP was also introduced under equilibrative conditions to determine if 6-MP is actively effluxed into the maternal circulation. Metabolite formation was also measured during all perfusions.
Results: At a clinically relevant concentration (50ng/ml), 6-MP appeared in the fetal circulation after 30 minutes. After 180 minutes, the fetal:maternal 6-MP concentration ratio was 0.45±0.09 (n=3). After adding 6-MP to both the maternal and fetal circulations, the fetal:maternal concentration ratio was 1.205±0.177 (n=4) after 180 minutes. 6-methylmercaptopurine was the only metabolite detected and only in perfusions with 10-fold higher 6-MP concentrations.
Conclusions: Tissue binding to the placenta, together with a short half-life, limits placental transfer of 6-MP. Active transport does not play a major role, thus polymorphisms or drug interactions involving drug transport proteins are unlikely to leave a fetus more vulnerable to 6-MP exposure.

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Incidence of central nervous system (CNS) depression of neonates breastfed by mothers receiving oxycodone for postpartum analgesia

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Conflict of Interest: None declared

Background: Oxycodone has recently replaced codeine for postpartum pain relief in some institutions. However, the neonatal safety of oxycodone during breastfeeding is unknown.
Objective: To quantify the incidence of neonatal CNS depression in oxycodone-medicated mothers compared to codeine, and acetaminophen-only group.
Methods: A retrospective study consisting of 3 cohorts in 533 breastfeeding mother-infant pairs exposed to oxycodone (n=139), codeine (n=210) or acetaminophen-only (n=184) was conducted. A standardized telephone questionnaire was administered to elucidate adverse maternal and neonatal events temporally related to either drug according to maternal self-reports.
Results: The incidence of neonatal CNS depression for oxycodone was 20.1% (28/139) compared to 16.7% (35/210) for codeine [p>0.05, OR 0.79 95% CI 0.46-1.38] and 0.5% for acetaminophen (1/184) [p<0.0001, OR 46.16 95% CI 6.2-344.2]. Mothers of symptomatic neonates in the oxycodone and codeine cohorts took significantly higher doses of medication compared to mothers of asymptomatic infants in the same cohorts [oxycodone p=0.0005 (median 0.4 (0.03-4.06) vs. median 0.15 (0.02-2.25) mg/kg/day and codeine p<0.001 median 1.4 (0.7-10.5) vs. 0.9 (0.18-5.8) mg/kg/day]. There was significant concordance between neonatal and maternal CNS depression in both oxyodone and codeine groups [p=0.0006: OR 8.86, 95% CI 2.00-39.24, p<0.001: OR 21.1 95% CI 7.4-60.6 respectively].
Conclusion: Oxycodone is not a safer alternative than codeine in breastfed infants.

7
Effects of chronic renal failure on brain drug transporters in rats

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Conflict of Interest: None declared

Background: Many studies demonstrated that chronic renal failure (CRF) significantly affects the expression and activity of intestinal, hepatic and renal drug transporters. These drug transporters are also expressed in brain cells and at the blood-brain-barrier (BBB) where they limit the entry and distribution of drugs in the brain. Perturbations in brain drug transporters equilibrium caused by CRF could lead to central toxicity of drugs.

Objective: To evaluate how CRF affects the expression and activity of drug transporters at the BBB and in the brain using nephrectomised rats.
Method: Protein and mRNA expression of influx transporters (organic-anion-transporting-polypeptide [Oatp], organic-anion-transporter [Oat]), and efflux transporters (p-glycoprotein [P-gp], multidrug-resistance-related-protein [MRP]) was measured in CRF and control rat brain biopsies. Intra-cerebral accumulation of radio-labelled benzylpenicillin (substrate of Oats and MRPs) and digoxine (Oatps, P-gp) was used to evaluate BBB permeability to drugs. Protein expression of transporters was evaluated in rat brain endothelial cells (RBEC) and astrocytes incubated with control and CRF rat serum.
Results: We demonstrated significant 30-50% decreases in protein and mRNA of MRP2-4, Oat3, Oatp2-3 and P-gp in CRF rat brain biopsies, astrocytes and RBEC. MRP5 was unchanged. We found a 30% decrease in BBB permeability of benzylpenicillin and no change in digoxine permeability. We hypothesize that similar reductions in the expression and activity of influx and efflux transporters prevented drug accumulation in the brain and that competition with accumulating endogenous organic anions explains the reduced permeability of benzylpenicillin.
Conclusion: Even with decreased drug transporters, BBB integrity seems to be conserved in CRF.

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Repeated ethanol exposure of fetal sheep in late gestation and fatty acid ethyl esters in meconium

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Conflict of Interest: None for all authors

Background: Meconium fatty acid ethyl esters (FAEEs) are established biomarkers of prenatal ethanol exposure. We hypothesized that meconium FAEEs content correlates with ethanol-induced fetal pathology, thus validating their use in identifying newborns at-risk for ethanol-induced disabilities.
Objective: To determine the relationship between various markers of fetal neuropathology and organ-system injury and FAEEs content in meconium of fetal sheep exposed to ethanol in late gestation.
Methods: From 95-133 days of gestational age (DGA; term~147 days), chronically catheterized pregnant ewes received daily 1-hr infusions of either 0.75 g ethanol/kg (*n*=13) or saline (*n*=9). On 134 DGA, ewes and fetuses were euthanized and fetal tissues, as well as meconium, were collected for analysis. Meconium FAEEs (palmitic, linoleic, oleic, and stearic) were quantified using headspace solid-phase microextraction and gas chromatography-mass spectrometry.
Results: Total FAEEs content was significantly higher in ethanol-exposed fetuses as compared with controls ($P<0.05$), with mean content in meconium of 0.174 nmol/g (range 0-0.788) for ethanol and 0.013 nmol/g (range 0-0.068) for controls. Ethyl stearate and ethyl palmitate were measurable only in ethanol-exposed fetuses; ethyl oleate was measurable in both ethanol and controls, but in higher amounts in ethanol-exposed fetuses; while ethyl linoleate was undetectable in both groups.
Conclusions: Ethanol exposure in late gestation resulted in elevated FAEEs in meconium of fetal sheep. Correlational analysis of meconium FAEEs content with post-mortem measures of neuropathology in immersion-fixed fetal brain slices and with fetal organ-system pathology will be conducted to determine whether increased FAEEs content is predictive of particular manifestations of ethanol teratogenicity.

POSTER PRESENTATIONS WEDNESDAY - MAY 25, 2011

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Thiopurine methyltransferase and inosine triphosphate pyrophosphohydrolase genotypes among patients with inflammatory bowel disease treated with azathioprine

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Conflict of Interest: None declared

Background: Azathioprine (AZA) is a well established treatment option for patients with

inflammatory bowel disease (IBD, ulcerative colitis or Crohn's disease). However, in clinical practice AZA is not always effective, and nearly 20% of patients discontinue AZA due to adverse events. Functional polymorphisms of thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphohydrolase (ITPA), two enzymes involved in thiopurine metabolism, have been previously associated with toxicity. It has been proposed that known TPMT and ITPA variant carrier status may predict clinical response and toxicity in IBD patients treated with AZA.

Aims: To determine if TPMT and ITPA genotype is a predictor of clinical response and adverse effects (myelotoxicity, hepatitis, pancreatitis, diarrhea and myalgia) among patients with inflammatory bowel disease treated with azathioprine.

Methods: Patients diagnosed with IBD undergoing AZA therapy or those previously treated with AZA were enrolled. Adverse effects and clinical response were evaluated and correlated with TPMT and ITPA genotypes. Genotyping of previously described polymorphisms including TPMT 238G>C (*2 allele), TPMT 460G>A and 719A>G (*3A allele), as well as ITPA 94C>A, was performed by TaqMan Real-time PCR and polymerase-chain reaction-restriction fragment length polymorphism (PCR-RFLP). The TPMT wild-type allele was designated TPMT*1.

Results: A total of 56 patients were enrolled in the study, with 38 patients (67.9%) on therapy and 18 patients (32.1 %) currently off treatment. Among those, 16 patients (28.6%) responded to AZA therapy, 12 patients (21.4%) partially responded and 15 patients (26.8%) did not respond to therapy. Among 17 patients who experienced adverse events, 10 were unable to tolerate AZA therapy. Three patients (5.4%) experienced severe myelosupression (WBC< 2.0 or neutrophils <1.0). 4 out of 5 heterozygous carriers for TPMT*3A developed adverse events compared to 13 out of 48 wild-type carriers (80% vs. 27%, P = 0.032). 2/4 heterozygous carriers for TPMT*3A responded well to therapy compared to 14/48 of wild-type (50% vs. 29%, P= 0.58). No TPMT*2 was detected. 3/7 heterozygous patients for ITPA 94C>A developed adverse events compared to 14/46 of wild-type patients (43% vs. 30%, P = 0.67), and 1/7 heterozygous patients for ITPA 94C>A were responders to AZA therapy compared to 15/46 of wild-type patients (14% vs. 33%, P = 0.66).

Conclusion: Our result suggests that genotyping for TPMT may help predict clinical response and adverse events among patients initiated on AZA therapy. However, there was no correlation between clinical effectiveness and adverse events among patients carrying the ITPA polymorphism.

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The effect of patient education on the incidence of CNS depression in neonates of breast feeding mothers taking codeine: preliminary analysis:

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Conflict of Interest: None declared

Background: Previously, we reported CNS depression and one fatality in breastfed neonates of codeine-prescribed mothers. This has led us to develop guidelines which are now routinely given to breast feeding mothers taking codeine containing analgesia.

Objectives: We undertook this study to evaluate the effect of patient education on the incidence of CNS depression in neonates of breastfeeding mothers taking codeine as compared to our previous studies.

Methods: This prospective cohort study was conducted between December 2009 and January 2011 at St. Michael's Hospital, in Toronto Canada. The breastfeeding mothers taking codeine for postpartum pain relief following Caesarian section were educated by the study coordinator on the mechanism of action of codeine, its possible side effects in mothers, and what signs and symptoms should be monitored in neonate, and recommended duration of codeine use (four days). Mothers also had 24 hour access to the study physician in case of concern. Telephone follow up was conducted after 7 days.

Results: Out of 220 participants recruited and educated only 2 (0.9%) reported adverse drug reactions in their infants, as compare to 17 (23.6%) out of 72 (P = 0.00005). It is twenty fold lower than the previous report. Demographic characteristics like maternal age, parity, gestational age and fetal weight were not different between the two groups.

Conclusions: Patient education regarding the maximum duration of codeine use and symptoms of possible adverse reactions, including what actions to take, significantly decreases the CNS depression in infants of breast feeding women taking codeine containing medications.

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Hypertonicity-induced expression of human CYP3A in a humanized transgenic mouse model

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Conflict of Interest: None declared

Background: The Nuclear Factor of Activated T-cells 5 (NFAT5) mediates hypertonicity-induced human CYP3A expression *in vitro* through an enhancer sequence located within intron 2 of CYP3A7. In order to characterize this phenomenon *in vivo*, we used a humanized transgenic mouse model that contained the entire CYP3A4 and CYP3A7 as transgenes, including introns, 5' and 3' regulatory elements. First, we observed that neither human CYP3A4-3A7, nor known Nfat5 target genes were induced by acute intestinal hypertonicity (12h) or acute systemic intra-vascular hypertonicity (6h), suggesting time-dependency of Nfat5 activation *in vivo*. We then employed prolonged hypertonic conditions in the mouse model: a) intestinal hypertonicity (one week of 8% high-salt diet); and b) prolonged dehydration (one week of dehydration by cycling 24h water deprivation and 24h water ad-lib recovery phases). Stronger Nfat5 protein expression was observed in the duodenum after intestinal hypertonicity, and in the liver and kidney after prolonged dehydration. Consequently, Nfat5 target gene expression was increased, which is consistent with hypertonicity-induced Nfat5 activation. Human CYP3A4 transgene expression also increased in the duodenum after intestinal hypertonicity (4.7±0.9 fold compared to low salt-diet [0% NaCl]: M±SEM; n=6-12, $p<0.05$), and in the liver (10.8±4.0 fold; n=4-14, $p<0.05$) and kidney (2.5±0.5 fold; n=4-10, $p<0.05$) after prolonged dehydration. Furthermore, human CYP3A total protein and activity were increased in these tissues. Our findings indicate ambient hypertonicity-induced human CYP3A4 expression *in vivo*, suggesting a novel mechanism of human CYP3A4 expression control.

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Chronic hypertension in pregnancy: perinatal outcome in women exposed or unexposed to antihypertensive medications

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Conflict of Interest: None declared

Objective: Despite high rates of hypertension disorder during pregnancy, the effects of hypertension on perinatal outcome have not been appropriately separated from those of the medications used. We evaluated the safety of exposure to antihypertensive medications during pregnancy, when accounting for untreated hypertension.
Study Design: A population- based retrospective cohort study was performed, comparing all pregnancies of women exposed and not exposed to antihypertensive medications during pregnancy. A computerized database of medications dispensed from 1998 to 2008 was linked with computerized databases containing maternal and infant hospitalization records from the district hospital during the same period. Multiple logistic regression models were performed to control for confounders.
Results: During the study period 100,029 deliveries occurred; of those, 620 (0.6%) were exposed to at least one antihypertensive medication (methyldopa or atenolol) during pregnancy. A higher rate of low birth weight newborns (LBW<2500 grams, 24.4% vs. 10.3%; $p<0.001$), intrauterine growth restriction (IUGR, 5.2% vs. 2.2%; $p<0.001$) and preterm delivery (PTD<37 weeks, 24.4% vs. 8.1%; $p<0.001$) were noted among pregnancies of women who were exposed to antihypertensive medications during the third trimester of pregnancy, as compared to women without hypertension and not exposed to antihypertensive medications. The association between antihypertensive medications (in general), methyldopa and atenolol, and LBW, IUGR and PTD remained significant after adjusting for maternal age, ethnicity, smoking, diabetes mellitus, lack of prenatal care, multiple pregnancy and parity (OR=3.7 95%CI:2.9-4.8; OR=4.3 95% CI:3.0-6.3; OR=3.7, 95%CI: 2.9-4.8 respectively). Nevertheless, a similar association was noted while comparing untreated woman with chronic hypertension during pregnancy (n=1074) to woman without chronic hypertension and not exposed to antihypertensive medications (n=97,820) (OR=1.7 95%CI: 1.4-2.0, OR=2.1, 95%CI: 1.5-2.9, OR=1.9; 95%CI:1.6-2.3 for LBW, IUGR and PTD, respectively)
Conclusion: Chronic hypertension with or without treatment during pregnancy is an independent and

significant risk factor for adverse perinatal outcomes such as LBW, IUGR and PTD as compared to births of women without chronic hypertension and without exposure to antihypertensive medications.

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Intracellular mechanism involved in downregulation of hepatic cytochrome P450 by chronic renal failure and parathyroid hormone

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Background: Chronic renal failure (CRF) is associated with a decrease in drug metabolism, due to a down-regulation of hepatic cytochrome P450. Previous studies indicated that CRF modifies activity, protein and mRNA expression of different P450 isoforms *in vivo* and *in vitro* via circulating mediators. Parathyroid hormone (PTH) was identified as one of them. CRF and PTH cause an inhibition of P450. The mechanism remains to be defined.
Objective: The aim of this study was to evaluate the contribution of different signaling factors like; PXR, CAR, NF-κB, PKA and PKC.
Methods: Four groups of rats were studied CTL, CRF, CRF with parathyroidectomy (CRF-PTX) and CTL-PTX. Liver and hepatocyte CAR and PXR mRNA expression was measured by qPCR and their protein expression was measured by Western blots. Cultured hepatocytes were incubated with CTL, CRF, CRF-PTX and CTL-PTX sera, or with or without PTH, and nuclear extracts were obtained. Nuclear extracts were used for NF-κB flux cytometry and Western blots (p50 and p65).
Results: We observed down-regulations of PXR and CAR protein (43%, 44%, respectively) and mRNA (40%, 42%, respectively) CRF rat's liver. PTX prevents the mRNA down-regulation of CAR and PXR in CRF rats. We observed a NF-kB accumulation in liver's nuclei of CRF rats. Furthermore, blocking NF-κB with inhibitors counteracts the effect of CRF and PTH on P450 expression.
Conclusion: In conclusion, CAR, PXR and NF-κB could be implicated in the down-regulation of hepatic P450 by CRF and PTH.

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Quantitative in vitro to in vivo prediction of P-glycoprotein-mediated drug transport

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Introduction: It is well appreciated that membrane transporters play important roles in drug disposition, a critical determinant of the pharmacological and toxicological profile of all drugs. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is a clinically important and well-characterized efflux transporter affecting drug absorption, distribution and elimination. Despite that a number of in vitro assays for P-gp activity are commonly used, the in vivo relevance of data derived from such assays remains unclear due to both incomplete knowledge of transporter intrinsic clearance and a lack of predictive extrapolation strategies. Here we provide the theoretical and experimental strategy as well as preliminary data for in vitro to in vivo prediction of P-gp mediated transport.
Methods: We have selected sitagliptin, an unmetabolized drug used in Type 2 diabetes as a P-gp probe drug. P-gp transport of sitagliptin was examined in a model of cultured, polarized epithelial cells heterologously expressing varying amounts of transporter.
Results: By monitoring sitagliptin transcellular flux using liquid chromatography – tandem mass spectrometry in combination with mathematical modeling, we obtain a value for P-gp intrinsic clearance. This intrinsic transport clearance is normalized to P-gp protein content of cells as determined by quantitative proteomic analysis.
Conclusion: These data, which would be the first of their kind, are expected to form the foundation for quantitative methods for in vitro to in vivo prediction of drug pharmacokinetics and ultimately therapeutic efficacy.

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A comparison of folic acid pharmacokinetics in obese and non-obese women of childbearing age

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Conflict of Interest: None declared

Background: While maternal folate deficiency during the periconceptional period represents a major risk factor for neural tube defects (NTD), obesity has been recognized as an additional risk factor. Studies have identified an increased risk for NTD-affected births among obese mothers even after adjusting for folic acid supplementation. However, while folic acid intake may have been at the recommended dose in these samples, blood folate concentrations were not monitored to ensure protective levels were reached.
Objectives: To compare folic acid pharmacokinetics in obese and non-obese women of childbearing age.
Methods: Healthy obese (n = 12) and non-obese (n = 12) women of childbearing age volunteered to participate. Each obese participant was matched to a non-obese participant, and assigned an equivalent dose per kilogram body weight of folic acid. Folic acid was orally administered after a 6-hour fast, and blood samples were taken over a 10-hour period to evaluate pharmacokinetic parameters.
Results: Area under the curve (AUC) was found to be significantly higher in the obese group (P = 0.008). Defining AUC as a function of dose per lean body weight (LBW) was found to be a stronger predictor than dose per total body weight (r^2 = 0.90 and 0.76, respectively).
Conclusions: This indicates that the body tightly controls systemic exposure to folic acid, with 90% of variability in AUC controlled by the dose per LBW. Periconceptional supplementation recommendations may need to be adjusted to account for LBW differences in the obese population.

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Examination of superparamagnetic iron oxide nanoparticle accumulation and toxicity in various brain related cell culture models.

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Conflict of Interest: None declared

Background: Superparamagnetic iron oxide nanoparticles (IONPs) have shown great promise in biomedical imaging and drug delivery. However, issues pertaining to the toxicity of IONPs are a concern especially for those applications involving IONPs and the brain.
Objectives: To determine whether cell toxicity was correlated with cell accumulation of various IONP formulations and to examine the impact of magnetic field on both these parameters.
Methods: Bare, oleic acid and bovine serum albumin (BSA) coated IONPs were examined in a mouse brain microvessel endothelial cell line (bEnd.3) and mouse primary cultured neurons and astrocyte preparations in the presence and absence of a magnetic field. Accumulation of IONPs was examined over a 2 hour period using Prussian blue staining. Cytotoxicity was assessed by addition of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) after 24-hour exposure to various concentrations of formulated IONPs.
Results: In neurons, oleic acid coated IONPs had the greatest cell association compared to BSA coated IONPs. In contrast, rank order of cell association for IONPs in astrocytes was uncoated > oleic acid > BSA coated. The presence of magnetic field resulted in more IONPs within the cells regardless of formulation. None of the formulations produced significant (i.e. greater than 10%) toxicity at concentration up to 100ug/mL. Neurons appeared more sensitive to oleic acid coated IONPs at concentrations above100 ug/mL.
Conclusions: The various formulations of IONPs were safe in all cells examined at concentrations less than 100 ug/mL. Accumulation and cytotoxicity of IONPs was increased in the presence of a magnetic field.

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Down-regulation of human cytochrome P450 2C8 by 3-methylcholanthrene

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Conflict of Interest: None to declare

Background: 3-Methylcholanthrene (MC) is a model polycyclic aromatic hydrocarbon that induces cytochrome P450 1A1 (*CYP1A1*) expression via aryl hydrocarbon receptor activation. MC also down-regulates certain constitutive hepatic P450s in rodents, but the mechanism and human relevance of this response remain poorly understood. Recent reports suggest that MC down-regulates the expression of *CYP2C8* in primary human hepatocytes. This has potential clinical importance because human liver CYP2C8 metabolizes therapeutic agents, such as the antineoplastic paclitaxel, and endogenous signalling molecules, such as all-*trans*-retinoic acid.
Objectives: To determine if MC alters the expression of *CYP2C8* at the mRNA level in two human hepatocellular carcinoma cell lines, HepG2 and HepaRG.
Methods: Cells were treated with vehicle or MC (1 or 5 μM) for 24 or 48 h. Cytotoxicity was assessed by

trypan blue dye exclusion. CYP1A1 and CYP2C8 mRNA levels were measured by real-time RT-PCR.
Results: Under conditions that resulted in minimal cytotoxicity, MC induced CYP1A1 mRNA levels in HepG2 cells by approximately 245- to 425-fold. MC at 5 µM suppressed CYP2C8 mRNA levels in HepG2 cells by approximately 78% at 24 h and this response did not persist at 48 h. Similar studies are in progress using HepaRG cells as a more differentiated model that maintains higher basal levels of several constitutive P450s.
Conclusions: The demonstration of CYP2C8 mRNA suppression by MC in human liver-derived cell lines will facilitate our efforts to define the molecular mechanisms and functional impacts of the modulation of this important human P450 by environmental toxicants.

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Hepatic drug metabolism in varying degrees of renal function using rat models of renal failure

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Background: Chronic renal failure (CRF) is the result of decreasing renal function over time. Expression and activity of drug metabolizing enzymes such as CYP3A are decreased in end stage renal disease. However, only a small percentage of patients with CRF are at the final stage of the disease.
Objectives: This study aimed to determine the changes in drug metabolizing enzyme function and expression in rats with varying degrees of renal failure.
Methods: Sprague-Dawley rats underwent either 2/3 or 5/6 nephrectomy by partial left kidney resection followed by complete right nephrectomy. Control rats underwent sham laparotomies. Rats were sacrificed on day 42 and CYP3A activity was determined in liver microsomes by evaluating midazolam metabolism using ultra-performance liquid chromatography with photodiode array detection.
Results: On day 42, serum creatinine levels were 23.0 ± 1.4, 37.4 ± 1.0 and 75.4 ± 14.7 µM in control, 2/3 and 5/6 nephrectomized rats, respectively. V_{max} values for 4-OH and 1-OH midazolam formation were lower in 2/3 nephrectomized rats (29% and 45%, respectively) and 5/6 nephrectomized rats (36% and 48%, respectively) compared to controls ($P < 0.05$). CYP3A2 protein expression was significantly decreased in both experimental groups compared to controls ($P<0.05$). Vmax values for midazolam metabolism were weakly correlated with day 42 serum creatinine levels ($P < 0.05$).
Conclusions: Our results demonstrate that CYP3A activity and expression is decreased in mild renal failure, which suggests that drug therapy for patients in early stages of kidney failure may be compromised for drugs that are substrate for CYP3A.

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Mechanism of convolvulus arvensis induced relaxation of thoracic aorta in rabbit

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Conflict of Interest: None declared

Background: *Convolvulus arvensis* L plant (CAL) L is shown to produce relaxation in some smooth muscle. It is used in traditional herbal medicine for many purposes, especially as anti-spasmodic in Middle East.
Objective: Experiments were undertaken to determine whether ethanolic extract obtained from CAL has vasorelaxant activity in the rabbit aorta rings and, if so, to elucidate the underlying mechanism.
Methods: Rabbit aorta rings were suspended in organ chambers for the measurement of changes in isometric tension in the presence of pheylephrine or glibenclamide.
Results: CAL decreased vessels contraction by phenylephrine in *both presence* and *absence* of an intact *endothelium* groups compared with the control group (IC_{50} = 358 mg/ L with endothelium and 399 mg/ L without endothelium, P< 0.05). In addition, pretreatment of aortic rings with glibenclamide inhibited partially CAL induced relaxation in Phenylephrine–contracted aortic tissues.
Conclusions: These data indicate that calcium might play an important role in the mechanism of CAL induced relaxation of aorta tissues. Also, it might be involved, at least in part, potassium channel in the relaxation activity of CAL. The present findings suggest that CAL could be a candidate of herbal medicine for cardiovascular diseases associated with aorta tissues dysfunction.

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In vitro metabolism study of specific cyp450 substrates in breast cancer cell lines

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Conflict of Interest: No conflict of interest to report

Background/Objectives: A treatment failure is commonly observed in breast cancer patients due to an innate or acute resistance to anti-cancer agents. Understanding, the local metabolism of anti-cancer agents by CYP450s in breast cancer cells could help in the development of a more targeted treatment approach. Our goal is to evaluate if measurable metabolism of specific CYP450 substrates is present in breast cancer cell lines, and to compare this metabolism to the expression of CYP450 mRNAs.
Method: Seven commonly used breast cancer cell lines (and one normal breast cell line) were cultured and then plated in 24 well plates. Once confluent, the cells were incubated in the presence of specific substrates, and the metabolism was determined by measuring the appearance of metabolites by LC-MS-MS. In order to correlate metabolism with mRNA expression, RNA was isolated from each cell line, and the mRNA relative expression was determined by RT-PCR (TaqMan Assay) for 19 CYP450 isoforms.
Results: RT-PCR results demonstrated that each cell line showed differential expression of CYP450 mRNAs. The expression of CYP2J2 is of interest because several cell lines showed elevated expression of this isoform, such as MCF-7 cells, whereas others, such as Hs578T cells, showed little expression. Metabolism was observable in cells lines for the specific CYP2J2 substrate, Ebastine, where MCF-7 cells demonstrated measurable metabolism in as little as 30 minutes.
Conclusions: Our results suggest that the local metabolism of various anti-cancer agents could be significant enough to greatly impact the resistance that is observed in breast cancer patients.

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A systematic review of the fetal safety of interferon alfa

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Conflict of Interest: None declared

Background: Interferon alpha (INF) is an effective treatment for a variety of conditions. Since these conditions could happen in pregnancy, Information regarding the safe use of this medication in pregnancy is essential. This systematic review attempts to summarize all published data on outcomes of INF alpha exposed pregnancies.
Methods: Using key words INF alpha, pregnancy, we searched Pub Med, EM BASE, and Google, since INF alpha was introduced in the market. We were able to locate only case reports of INF alpha exposure in pregnancy. All cases were collected and included in our review. We also collected 71 cases that were diagnosed with essential thrombocythemia, but did not receive any medication in pregnancy.
Results: Among 61 INF alpha exposures in pregnancy we located, mean maternal age was 31±4 years, median 33 years, and range 23-43 years. Mean full term babies' weight was 3010, median 2680 grams and the range 1350-3800. Mean gestational age at delivery was 37±3 weeks, median 38, range 30-41 weeks. No major malformation or stillbirth was reported. There was one spontaneous abortion, and 12 preterm deliveries (20 % of all the cases). In 71 cases with the same underlying diseases who did not receive any medication in pregnancy, 46 out of 71 had early or late abortion. There were 3 cases of stillbirth and 4 cases experienced preterm delivery. 18 cases had term normal babies.
Conclusion: This systematic review suggests that INF alpha does not increase the risk of abortion, major malformation, still birth, and premature deliveries, and probably it has a protective effect on prevention of early/late spontaneous abortion and still birth in this particular population.

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The Motherisk cancer in pregnancy forum: a unique way of counseling

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Background: Cancer occurs in approximately 0.07-0.1% of pregnancies. The diagnosis of cancer in pregnancy complicates optimal treatment due to potential risk to the fetus. The Motherisk program established The Consortium of Cancer in Pregnancy Evidence (CCoPE) in an effort to address the

deficiency in information regarding the care of cancer and associated therapies in pregnancy. Subsequently, a Cancer in Pregnancy Forum was created to provide unique, evidence-based counseling to women and their medical professionals.
Objectives: To describe the characteristics of the cases counseled by the Cancer in Pregnancy Forum.
Methods: Review of cases documented in the Cancer in Pregnancy Forum since its inauguration. Data were collected on the person who initiated the post, the type of cancer, the treatment plan, response time and completeness of information.
Results: There were a total of 129 inquiries on the Cancer Forum from 1999 to 2011. Healthcare providers and scientists initiated 41% of the postings, whilst women or their partners made up the remaining 59%. Inquiries regarding maternal chemotherapy were the most frequent (30%), followed by questions concerning specific cancers (20%), radiation (17%), paternal chemotherapy (13%), other therapies (12%), breastfeeding (5%), female fertility (1.5%) and male fertility (1.5%).
Conclusions: A diagnosis of cancer during pregnancy is a major stress for the expecting mother and her family. The Cancer in Pregnancy forum is the only forum of its kind worldwide, providing women and medical professionals evidence-based information regarding diagnosis, treatment, symptoms and other concerns with respect to cancer during pregnancy and lactation.

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Ototoxicity in Mexican children receiving cisplatin based chemotherapy

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Introduction: Cisplatin has been widely used as a chemotherapeutic agent for a variety of paediatric malignancies. One of the most incapacitating adverse drug reactions reported on patients under cisplatin therapy is ototoxicity, associated with permanent bilateral hearing loss. Even though extensive publications are available in the literature, information on Latin-American patients is scare.
Objective: To describe the frequency and severity of ototoxicity in paediatric patients treated with cisplatin based chemotherapy in a third level paediatric hospital in Mexico City.
Methods: Three audiology evaluations were prospectively conducted in paediatric patients (<18 years) with solid tumour cancers, at baseline and at the end of each of two chemotherapy cycles. Audiology data was used to classify hearing loss according to the Common Terminology Criteria for Adverse Events.
Results: Forty-two patients participated in this study, aged 4 to 17 years, 52% were female, being osteosarcoma (78.6%) the predominant cancer type. Bilateral hearing loss was observed in 31% of patients at the end of the first chemotherapy cycle, while 62% report hearing loss at the end of the second cycle, mainly in frequencies over 4,000hz ($p<0.001$). This represent an increased risk of hearing loss (OR= 2.00, [CI 95% 1.26-3.17], $p=0.005$) from the first to the second cycle of chemotherapy. Cumulative dose, age, treatment scheme and tumour type were not related to hearing lose.
Conclusion: Hearing loss in Mexican children under cisplatin therapy is similar to that reported previously in other populations. Although ototoxicity was related to chemotherapy cycle number, it cannot be positively correlated with cumulative dose of cisplatin, tumour type or age. Further research is required to correctly characterize ototoxicity in Mexican patients.

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Statins and their category X pregnancy classification: pravastatin may merit reconsideration for a novel indication

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Background: Animal models of recurrent pregnancy loss (RPL) and preeclampsia have implicated elevated levels of tissue factor (TF), and resultant hypercoagulability and inflammation, as a key factor in placental and fetal damage and pre-eclampsia. Using a mouse model that shares features with human RPL and pre-eclampsia, treatment with pravastatin (HMG CoA reductase inhibitor) down-regulated TF and rescued pregnancies. However, the FDA has given a category X classification for all statins (i.e. contraindicated in pregnancy). Yet, unlike lipophilic statins that comprise the majority of this drug group, pravastatin is

hydrophilic and hepatospecific, and therefore less likely to enter fetal circulation.
Objective: To evaluate the FDA category X classification for pravastatin.
Methods: Literature review of teratogenic effects of pravastatin exposure.
Results: Contraindication of statins in pregnancy is based on animal testing and a small number of case reports involving two lipophilic statins, lovastatin and simvastatin. Increased risk of teratogenicity has not been established. Contrary to lipophilic statins, pravastatin did not exhibit teratogenicity in animal testing or human cases. Pravastatin has not been shown to be teratogenic in multiple cohort studies.
Conclusions: Despite the lack of established teratogenic risks, pravastatin remains contraindicated in pregnancy. Since suspension of hypercholesterolemia therapy during gestation is considered safe, this contraindication has not previously been questioned. However, promising new indications for prevention of adverse pregnancy outcomes and reassuring reports of its safety during gestation support both reevaluation of its contraindication and implementation of controlled clinical studies to establish the efficacy of pravastatin for treatment of RPL and preeclampsia.

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Switching from brand-name to generic psychotropic medications: a literature review

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Background: Current world economics encourage use of generic medications which are less expensive than brand name originals. There is however controversy as to their clinical equivalence. Clinical deterioration, adverse effects and toxicity have been described with generic substitution.
Objective: To explore issues about generic substitution of psychotropic medications reported in the literature.
Methods: Pubmed was searched from January 1, 1974 to March 1, 2010. The MeSH term "generic, drugs" was combined with "anticonvulsants", "mood stabilizers", "lithium", "antidepressants", "antipsychotics", "anxiolytics" and "benzodiazepines." Articles in English, French, or Spanish were considered if they discussed clinical equivalence of generic and original medications, generic substitution, or issues about effectiveness, tolerability, compliance, or economics encountered with generics. Additional articles were obtained by searching the bibliographies of relevant references.
Results: Formulation substitution of anticonvulsants/mood stabilizers (carbamazepine, valproate, lamotrigine, gabapentin, topiramate, lithium) antidepressants (amitriptyline, nortriptyline, desipramine, fluoxetine, paroxetine, citalopram, sertraline, mirtazapine, bupropion, venlafaxine) antipsychotics (clozapine, risperidone) and anxiolytics (clonazepam, alprazolam) has been linked to clinical deterioration, decreased tolerability/toxicity and pharmacokinetic changes. Decreased compliance may result from a change in formulation. Generic substitution is not always economically profitable when the consequences of relapses and decreased compliance are taken into account.
Conclusions: Publication bias and heterogeneity of the studies are limitations of this review. It is yet premature to determine the true equivalence between generic and original medications. Caution is still advised when a patient's medications are switched to another formulation. Health professionals should be sensitized to the possible consequences of generic substitution.

26 WITHDRAWN
The epidemiology of intentional self-poisoning: a population-based study

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27
CYP2C19 genotyping in two infants with gastroesophageal reflux

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Conflict of Interest: None declared

Background: Although the impact of CYP2C19 genotypes on PK of proton pump inhibitors (PPI) has been extensively investigated, clinical utility of CYP2C19 genotyping is still not clear. Also, the optimal PPI dosage for children under 2 years old has not been yet determined. Here we show 2 infant cases

with severe gastroesophageal reflux who failed to respond to high dose of pantropazole.
Case 1; 11 months old (5.6kg). Pantoprazole was administered via continuous infusion 8.5mg/kg/day (standard dose: 1mg/kg/day), but gastric pH remained low. CYP2C19 genotyping showed that the patient was a heterozygote of CYP2C19 wild type (*1) and high function genotype (*17). Apparent clearance was 0.37L/kg/h (reported clearance at 2-4 y.o; 0.20±0.23 L/kg/h). Pantoprazole was then switched to 15mg tid oral omeprazole with successful therapeutic response.
Case 2: 5 months old (5.4kg). She received 14.0mg/kg/day of pantoprazole without response. Her CYP2C19 genotype was *1/*1 (wild type). Pantoprazole was changed to oral omeprazole with remarkable therapeutic response.
Discussion: These 2 infants required 8.5 times and 14 times higher doses of pantoprazole than normal recommended dose for infants (1mg/kg/day). The high clearance was confirmed in Case 1, which is consistent with CYP2C19*17 genotype. On the other hand, Case 2 suggests a wide range of functionality within the CYP2C19 *1/*1 genotype and/or pharmacodynamics (PD) variations.
Conclusions: CYP2C19*17 genotype may be sufficient but not necessary as a cause of pantoprazole therapeutic failure in infants. Studies of CYP2C19 genotype - pantoprazole PK/PD correlation are needed to determine the optimal dose for infants.

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The incidence of tacrolimus-induced nephrotoxicty in children: do we really know?

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Background: Tacrolimus is a calcineurin-inhibitor and the drug of choice for pediatric solid organ transplants. Although the renal effects of calcineurin-inhibitors have widely been studied, the data on tacrolimus-induced nephrotoxicity in children is limited.
Objectives: To determine the incidence of tacrolimus-induced nephrotoxicity in children by systematically reviewing the literature.
Methods: Pubmed/Medline, Embase and Google were searched from their inception till February 2nd 2011 with the search terms "tacrolimus", "nephrotoxicity", "transplantation" and "children". References of relevant articles were screened as well.
Results: Fifteen of the 87 articles were considered relevant. Ten papers researched liver transplant recipients, 4 kidney transplant recipients and one abdominal and thoracic transplant recipients. The incidence of tacrolimus-induced nephrotoxicity ranged from 0%-76.5%. This range is a direct result of the differences between the studies. Follow-up times ranged from pre-transplant until 8 years post-transplantation. However, five studies did not report their follow-up times. Seven studies did not clarify their definition for tacrolimus nephrotoxicity. Two of these studies did not mention the type of tacrolimus toxicity reported. Those studies reporting a definition for tacrolimus-induced nephrotoxicity, mainly used grading systems based on GFR. One study used histological grading systems. However, cut-offs for nephrotoxicity were not reported in any of the grading systems.
Conclusions: Due to the many differences between the studies, a decisive incidence number cannot be given. Further studies need to address the implications of these different definitions on the incidence number to have a better understanding of the vastness of the problem.

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Assessing interactions between herbal medicines and drugs: a review

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Background: Herbal products are generally considered safe, but not necessarily when used concurrently with drugs. A reference tool for rapid identification of known herbal medicine-drug interactions was created to help clinicians.
Objective: To review the herb-drug literature and update the herb-drug interaction grid.
Methods: Searches were conducted in MEDLINE and EMBASE between 2007 and 2010, using the following search terms: 34 commonly used herbs combined with 'clinical trials', 'case studies', and 'case reports'.

Reference lists of relevant review articles were analyzed for additional papers, and a tertiary source was consulted to identify other herb-drug interactions. Data extraction included the amount of evidence regarding the severity and likelihood of the interactions between each herb and each category of pharmaceutical agents. The interactions were classified into four groups: 1) No reported or theoretical interactions, 2) Theoretical interactions based on animal of in vitro data, 3) Theoretical interactions extrapolated from clinical data, and 4) Interactions supported by clinical evidence.

Results: A total of 1553 references were identified by the searches. 1514 articles have been screened, and 120 have been included for extraction. Full results will be ready in October.

Conclusions: The herbal medicine-drug interaction grid will allow clinicians to have a guide on potential harms based on the most recent literature. This quick reference guide should facilitate clinicians' ability to answer questions about the concurrent use of herbal medicine products and prescription medicines, thus enhancing patient safety.

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Role of metabolite receptor GPR91 in post-stroke recovery

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Conflict of Interest: None declared

Background: Numerous events affecting normal cerebral blood flow can cause hypoxia-ischemia (H-I), which can lead to major neurological disabilities in infants and adults. There is currently a void in treatment modalities to counter cerebrovascular injury. We have recently demonstrated that the Krebs cycle product succinate, links capillary function to tissue metabolic needs via the G protein-coupled receptor (GPCR) GPR91. The functional benefits of this coupling and its effect on post-stroke recovery are still unknown.

Objectives: We assess the role of succinate in orchestrating brain angiogenesis and recovery during post-ischemic events in the newborn.

Methods: To test our hypothesis the Rice-Vanucci model was employed. Succinate levels were evaluated by mass spectrometry at different time-point following H-I. Expression of GPR91 was determined by immunohistochemistry and western blot. Expression of pro-angiogenic genes was evaluated by real-time pcr.

Results: We detected a rapid accumulation of succinate following the insult. GPR91 was primarily localized in neurons within the cerebral cortex. To determine the role of succinate in this injury paradigm, we injected succinate intraventricularly in newborn animals and detected a 30% increase in blood vessel formation which was corroborated *ex vivo* with a robust increase in vessel density in cortical explants. Stimulation of primary neuronal cultures with succinate lead to a 2.1-fold increase in VEGF mRNA.

Conclusion: Collectively our data demonstrate that succinate strongly affects the cerebral vascular response to ischemia. These results offer a new and potentially important target to optimize recovery following cerebral vascular injury.

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A drug-drug interactions study between rosuvastatin and pantoprazole after oral administration in healthy male subjects

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Conflict of Interest: On behalf of all authors, cannot identify any potential conflict of interest.

Purpose: Rosuvastatin (ROSU) disposition involved intestinal and hepatic membrane transporters such as OATP1B1, 1B3, 2B1, ABCG2 (BCRP) and NTCP. Pantoprazole (PANTO) is a potential substrate/inhibitor of ABCG2. Our objective was to select PANTO as a clinical probe to study the impact of ABCG2 inhibition on the pharmacokinetic of ROSU.

Methods: Eight healthy White male subjects performed a single-center, 2-period, cross-over study. They received, on 2 occasions, 7 days apart, either an oral dose of PANTO (40 mg) or placebo in the morning followed by a single oral dose of ROSU (10 mg) 1 hour after. The next morning (24 hours after), subjects were given a 2nd 40 mg dose of PANTO. Genotyping for 3 single nucleotide polymorphism *ABCG2* C421A, *SLC01B1* T521C and A388G (OATP1B1) was performed. Blood samples were collected before and 0.25, 0.5, 0.45, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 48 and 72 hours after ROSU

administration. Urine was collected over 72 hours. Plasma and urine samples were analyzed by LC/MSMS. Data were analyzed by ANOVA with repeated measures adjusting for sequence and period for Cmax, AUC, CL/F and CLr. No effect boundary of 80-125% for the point estimates and the 90% confidence interval (CI) for the ratio of the geometric least-square means of all PK parameters were determined.

Results: Geometric means for Cmax, AUCinf and CL/F were respectively 4.3 ng/mL, 44.39 h·ng/mL and 3754.21 mL/min for ROSU alone, and 4.21 ng/mL, 45.89 h·ng/mL and 3631.30 mL/min for ROSU+PANTO. Ratio and 90% CI were 96.48% (83.67% - 114.24%), 100.20% (90.41% - 111.90%) and 99.58% (86.67% - 107.95%) for Cmax, AUCinf and CL/F, respectively. Geometric means for CLr were 245.82 mL/min (ROSU) and 232.24 mL/min (ROSU+PANTO). Ratio and 90% CI were 98.99% (87.73% - 101.73%). However, in one subject homozygous for variant alleles (521CC) associated with a loss of function in OATP1B1, an increase in plasma concentrations was observed following pantoprazole administration. Cmax and AUCinf were respectively 8.5 ng/mL and 83.83 h·ng/mL for ROSU alone, and 10.2 ng/mL and 90.33 h·ng/mL for ROSU+PANTO.

Conclusion: ROSU is an ABCG2 (BCRP) substrate for its elimination through the bile. However, this study showed that block of ABCG2 (BCRP) by pantoprazole does not alter rosuvastatin pharmacokinetics to a significant extent in healthy volunteers with functional activities of other membrane transporters involved in the disposition of the drug.

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Placental transfer of formic acid is rapid and decreases hCG secretion

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Conflict of Interest: None for all authors

Background: Formic acid has recently been detected in maternal blood and umbilical cord blood of infants born to alcohol abusing mothers. This toxic metabolite of methanol requires folate for detoxification. We hypothesize formic acid produced in the maternal circulation will transfer across the placenta and will be toxic to both the placenta and fetus.

Objectives: First, to determine whether formic acid transfers across the placenta and is toxic to the placenta. Second, to determine whether folate can decrease transplacental transfer of formic acid and mitigate toxicity.

Methods: Dual perfusion of a single placental lobule *ex vivo* was used to characterize the transfer of formic acid across the placenta. After a 1-hour control period, formic acid (2mM) was introduced into the maternal circulation with (n=4) or without folate (1uM) (n=4) and allowed to equilibrate for 3-hours.

Results: Formic acid transferred rapidly from the maternal to the fetal circulation and transfer was not altered with the addition of folate. Compared to the control period, there was a significant decrease in hCG secretion (p=0.03) after addition of formic acid. In contrast, there was no significant decrease when folate was present in the perfusate.

Conclusions: Formic acid rapidly transfers across the placenta and thus has the potential to be toxic to the developing fetus. Formic acid decreases hCG secretion in the placenta, which may alter steroidogenesis and differentiation of the cytotrophoblasts, and this can be mitigated by folate.

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Maternal fish consumption and mercury: risk perceptions and therapeutic monitoring

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Conflict of Interest: None declared

Background: While fish is rich in essential nutrients and women are encouraged to consume fish products, fish may contain methyl mercury which is an established neurotoxin to the fetus. Consequently, there are high levels of anxiety among women of reproductive age regarding fish consumption.

Objectives: 1) To investigate what motivates women of reproductive age to avoid eating fish during their pregnancy and to understand their perceptions towards consuming fish; and 2) to pilot an intervention program in women of reproductive age to ensure mercury levels are below the LOAEL.

Methods: We surveyed 100 women of reproductive age who consulted the Motherisk program about fish consumption on their perceptions regarding fish

consumption. Subsequently we implemented a therapeutic monitoring program for women who had hair mercury levels above 0.3 µg/g, the No Observable Effect Level (NOEL) for mercury for neurocognitive effects.
Results: The majority of women (90%) were aware of the potentially harmful effects of fish containing high levels of mercury. Most respondents were unable to describe specific toxic effects. When rating the level of anxiety from 0 (none) to maximal (10), the mean rate was 5, and 16 women were most worried. A pilot on 5 women testing above the LOAEL of 0.3 mcg/g (mean 0.78±0.46), after diet modifications reducing fish consumption, levels decreased significantly (to 0.33±0.22) ($P<0.01$).
Conclusion: Women are very concerned about fish consumption and potential fetal risk. Therapeutic monitoring appears to be effective.

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Transport of lactic acid by mct1 and mct4 in cancer cell lines

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Conflict of Interest: No conflict of interest to report

Background/Objectives: A decrease in oxygen supply to mitochondria is associated with a reduction in ATP production from pyruvate and an increased formation of lactate. Accumulation of lactate, and secondly of lactic acid, is associated with mild to severe pathophysiological conditions including severe muscle pain. Two monocarboxylate transporters namely, MCT1 (SLC16A1) and MCT4 (SLC16A3), are involved in the transport of lactic acid in several cell types. The objective of our studies was to develop cell models to dissect activity of these two transporters.
Methods: Seven different breast cancer cell lines were cultured and harvested for RNA. RT-PCR analyses were performed to determine the relative mRNA expression of the MCT1 and MCT4. For uptake transport assay, cells were plated and incubated with [^{14}C] lactic acid at 37°C. The level of lactic acid incorporated was determined by using a liquid scintillation analyzer.
Results: RT-PCR results obtained allowed us to identify, out of the seven breast cancer cell lines, two cell lines with interesting characteristics. First, MDA-MB-231 expressed MCT4 at a much higher level than MCT1 while, on the other hand, SKBR3 expressed MCT1 at a greater level than MCT4. Experiments conducted to assess the functionality of the transporters in these cell lines confirmed the lactic acid uptake in both of those cell lines.
Conclusions: Different cancer cell lines can be used as in vitro models for the transport study of lactic acid. Experiments are currently underway to link MCT1 and MCT4 transport activity to drug toxicity.

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Respiratory depression following therapeutic administration of opioids in the operating room: an opioid pathway pharmacogenetic analysis

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Conflict of Interest: None declared

Background: Systemic approaches are needed to understand how variations in the genes associated with opioid pharmacokinetics and pharmacodynamics can be used to predict clinical outcome. We present 2 cases of life threatening opioid-induced respiratory depression in the operating room. *Case One:* The patient had severe respiratory depression following 2 mg of subcutaneous morphine on top of intrathecal morphine administered for a Cesarean section. The patient had a history of near apnea with one dose of codeine/acetaminophen (30mg/500mg respectively), but tolerated hydromorphone. *Case Two:* Life threatening respiratory depression occurred following epidural morphine given at standard doses for surgical removal of tumor. Post-operatively, the patient needed only 0.6mg total of IV hydromorphone over 4 days for pain management.
Methods: Functional candidate polymorphisms in genes involved in opioid metabolism and action pathway (CYP2D6, UGT2B7, ABCB1, OPRM1, COMT) were genotyped by using SNaPshot® and TaqMan® Drug Metabolism Genotyping assays or by amplifying and re-sequencing the corresponding genomic regions.

Results: ***Case One:*** Genotype results revealed this patient had an increased propensity to generate active metabolites from both codeine (extensive CYP2D6 activity) and morphine (increased UGT2B7 activity) while having a functional μ-opioid receptor system. These active metabolites are not generated with hydromorphone.
Case Two: Collectively, this patient appeared to have increased exposure and overall sensitivity to morphine and hydromorphone. Decreased ABCB1 efflux transporter activity, in combination with low COMT activity associated with increased sensitivity of the μ-opioid receptor system may have predisposed the patient to this adverse outcome.
Conclusions: An opioid pathway pharmacogenetic approach along with clinical history may provide insight into severe respiratory depressive events in patients who received therapeutic doses of opioids and may be useful information to mitigate future adverse events.

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Rapid and reversible enhancement of blood-brain barrier (BBB) permeability using lysophosphatidic acid (LPA)

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Conflict of Interest: None declared

Background: The delivery of drugs to the CNS is limited due to the restrictive nature of the BBB. Transient modulation of BBB permeability is one method for enhancing drug delivery to the brain. One potential modulator of BBB permeability is LPA.
Objectives: Examine the use of the LPA, to transiently increase BBB permeability
Methods: LPA-induced alterations in brain microvessel permeability were examined in both cell culture and whole animal models. The permeability of fluorescein labeled dextran (FDX; MW3000) was examined using human brain microvessel endothelial cells, HBMEC, under control conditions and following exposure to LPA (0.1-10 μM). Effects of LPA on BBB permeability was examined in Balb/c mice using magnetic resonance imaging (MRI) and near infrared fluorescence imaging techniques.
Results: Exogenous LPA produced concentration-dependent increases in FDX permeability in HBMEC. Mice treated with LPA (1mg/kg) had significantly higher accumulation of Gadolinium contrast agent (Gd) compared to control mice in all regions of brain with the greatest increase observed in the posterior regions. The maximum enhancement of Gd occurred within 12 minutes following the administration of LPA. Re-establishment to normal barrier function was apparent within 20 minutes of LPA exposure. Examination of the permeability of a large near infrared fluorescent imaging agent, IRdye PEG, showed a qualitatively similar accumulation profile.
Conclusions: LPA produces a rapid and reversible increase in brain microvessel endothelia cell permeability. These studies indicate that administration of LPA in combination with therapeutic agents may be an effective strategy to increase drug delivery to the brain.

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Ocular toxicity in children exposed in utero to antimalarial drugs: a systematic review of the literature

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Conflict of Interest: The authors state no conflict of interest to declare.

Background: There have been concerns regarding retinal toxicity in the offspring of women exposed to antimalarial drugs chloroquine (CQ) and hydroxycholoquine (HCQ) during pregnancy.
Objective: To systematically review the published evidence on safety of antimalarials during pregnancy with focus on ocular toxicity in the offspring.
Methods: Ovid MEDLINE(R), EMBASE and Cochrane Library databases were searched for randomized controlled trials (RCTs) and observational studies assessing visual function in the offspring of women exposed to antimalarials during pregnancy.
Results: 12 studies with a total of 588 exposed offspring met the inclusion criteria. Of 12 studies, 2 were RCTs and 10 were cohort studies 5 of which were lacking comparison group. Methods and time of visual assessment varied among studies. 5 studies reported no clinical visual abnormalities in all cases (n= 251). In a RCT on malaria prophylaxis, visual acuity in 251 infants exposed to CQ in utero did not differ from placebo group. Detailed ophthalmological examination was performed in 4 studies and normal results were reported in all children (n=59). Electrophysiological testing using electroretinogram was performed in 3 small cohorts of infants exposed to HCQ prenatally (n= 31) and were normal in all but six infants.
Conclusions: The current evidence from small and relatively low quality studies suggests no fetal ocular toxicity of antimalarials during pregnancy. The clinical

significance of early electroretinogram anomalies reported in a small subset of infants remains to be established. Larger follow up studies are warranted to confirm low risk of ocular toxicity in children following antenatal exposure to antimalarial medications.

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The Delirium Risk Evaluation and Assessment of Midazolam, EEG Recording and Sleep (DREAMERS) Study

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Background: Critically ill patients require adequate sedation to tolerate the invasive interventions necessary for their care. Benzodiazepines are commonly used to achieve sedation in the intensive care unit (ICU), however, they have been linked to the development of delirium. Midazolam, a benzodiazepine commonly used in ICU demonstrates significant interindividual pharmacokinetic (PK) variability in these patients.
Objectives: 1) To define the relationship between midazolam PK and electroencephalogram (EEG) in critically ill patients. 2) To clarify the effect of critical illness on midazolam pharmacokinetics. 3) To determine if impaired midazolam clearance is a risk factor for delirium.
Methods: Patients admitted to the ICU with sepsis and on a continuous infusion of midazolam were screened for study enrolment. Upon enrolment, continuous subhairline EEG was applied and daily blood samples were collected for plasma midazolam quantification. Clinical and laboratory parameters were followed and delirium onset was monitored using the Intensive Care Delirium Screening Checklist (ICDSC).
Results: Data is currently available for five patients. Patients on continuous midazolam infusions had maximal plasma midazolam concentrations ranging from 176 to 472 ng/ml (mean 336 ± 118). Corresponding EEG tracings demonstrated predominance of a delta wave pattern suggestive of deep sedation. Four out of 5 patients had ICDSC scores suggestive of delirium.
Comment: The midazolam concentrations observed suggest impaired clearance, and are higher than reported in other studies. Elevated midazolam levels, correlating with EEG recordings suggestive of oversedation may be a risk factor for the development of delirium. Further data is being analyzed and will be available for presentation.

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Systematic monitoring of amiodarone therapy produces high efficacy and low toxicity rates

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Background: Amiodarone, although highly effective, is renowned for potential adverse effects limiting its use. In clinical trials of patients receiving arbitrary doses and minimal monitoring, 15-20% discontinued amiodarone in the first year.
Objectives: To report outcomes in patients taking amiodarone who are managed in a specialized Atrial Fibrillation (AF) Clinic with regular monitoring, individual serum drug concentration guided amiodarone dose titration, and focused education.
Methods: 60 patients, who were naïve to amiodarone when started in outpatient clinic, were followed for ≥ 12 months. Thyroid, liver and lung function were recorded at baseline, then, monitored along with annual pulmonary metrics according to symptoms. Amiodarone dosing was adjusted using guidance from serum amiodarone concentrations.
Results: As a mixture of persistent and intermittent AF patients, 25% were in sinus rhythm at baseline (B). After 12 mo (E) of therapy, 0% discontinued amiodarone and 90% were in sinus without limiting adverse effects or clinically important changes in liver or thyroid function (ALT B=33 vs. E=39 and FT4 B=14.9 vs. E=18.3). Thyroid supplementation was required in 15 pts (13 already on thyroxin at baseline) and later 4 pts developed transient hyperthyroidism that resolved while still on amiodarone. Some had transient neurologic symptoms that resolved after loading. No pulmonary toxicity was observed.
Conclusion: Systematic monitoring and dose adjustment based on serum amiodarone concentrations provides a high rate of therapeutic success. Thyroid issues were common, but no patient required discontinuation of amiodarone for toxicity.

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Hair cortisol concentrations in patients with obstructive sleep apnea

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Background: Obstructive sleep apnea (OSA) is a common sleep disorder with serious cardiovascular and metabolic co-morbidities. OSA patients experience a repetitive collapse of the upper airway, resulting in intermittent episodes of hypoxemia. The adverse effects of OSA may be mediated by increased cortisol secretion. In this model, the frequent sudden arousals during sleep activate the hypothalamic-pituitary-adrenal axis resulting in increased nighttime cortisol secretion, a time when cortisol secretion is normally very low. Hair analysis is a non-invasive tool that can provide a retrospective, integral measure of cortisol production over several months.
Objectives: This study will assess if hair cortisol concentrations can be used as a biomarker of OSA severity. It is hypothesized that hair cortisol content correlates with the severity of OSA, and successful intervention with either CPAP or surgery will result in decreased cortisol concentrations.
Methods: Patients are recruited after undergoing a sleep study. Their hair cortisol concentrations are determined with an immunosorbent assay and compared with their apnea-hypopnea index (AHI), a score of the severity of a patient's OSA. A second, post-intervention sample will be collected from positively diagnosed patients undergoing an intervention.
Results: To date, 65 pre-intervention patients have been recruited, and many post-intervention hair collections will occur in upcoming months. A preliminary analysis of 39 patients using a linear regression did not detect a correlation between cortisol concentration and AHI.
Conclusion and Plan: In March, 22, post-intervention samples will be collected, allowing for a preliminary assessment of if cortisol concentrations decrease with effective interventions for OSA.

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Pharmacogenetics of warfarin in children

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Conflict of Interest: None declared

Background: Warfarin is a highly effective anticoagulant, but the large variation in dose requirements between patients makes dosing challenging. Inadequate warfarin dosing can cause serious adverse drug reactions (ADRs), such as blood clots or excessive bleeding. Polymorphisms in three genes (CYP2C9, VKORC1 and CYP4F2) have a significant effect on the required warfarin dose in adults. However, the validity of these findings have not been comprehensively assessed in children. Further paediatric studies are required to understand the predictive factors contributing to dose variation in children and prevent warfarin-induced ADRs.
Objectives: To determine the effect of genetic variation in CYP2C9, VKORC1 and CYP4F2 on warfarin response in children, as well as the importance of additional variation in genes involved in drug biotransformation and coagulation pathways that may be implicated in paediatric warfarin dosing.
Methods: We will collect a cohort of paediatric patients receiving warfarin therapy and test for associations of genetic variation in VKORC1, CYP2C9 and CYP4F2 with therapeutic dose, time to stable international normalized ration (INR), and warfarin-induced ADRs. We will also use univariate analysis to test for associations between therapeutic dose and additional genes studied.
Significance: The anticipated results of this study will provide insight into the genetic basis of warfarin dose requirements in children and the potential benefits of genetic testing in children prior to initiation of warfarin therapy. A paediatric-specific genetic dosing algorithm would allow early prediction of patients at risk for over- or underanticoagulation, and minimize the danger associated with warfarin therapy in this under-studied population.

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Creation and assessment of adenovirus-mediated drug transporter model expression system for the prediction of pharmacokinetic profiles in humans

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Introduction: Predicting the in vivo role and effects of functional genetic polymorphisms to observed PK profile of drugs in humans is important for optimal drug therapy. Currently, IVIVE (in vitro to in vivo extraporation) algorithms are widely utilized for the prediction of drugs which are metabolized by CYP enzymes, but not transporters. Therefore, our goal is to create an in vitro transporter expression system capable of expressing multiple drug transporters simultaneously so that the cell-based system better reflects human organs such as the liver. Accordingly, we have cloned a number of hepatic bile acid and drug uptake transporters into an adenovirus-based expression construct and tested the efficiency of transporter expression in a number of cell lines.
Methods: Adenovirus constructs containing uptake transporters such as NTCP and members of the human OATPs were constructed and transport activity, as well as, cell viability assessed in HeLa, MDCKII, LLC-PK1 and Caco-2 cell lines.
Results: In terms of cell viability, LLC-PK1, Caco-2 and Hela were able to tolerate MOI (Mutiplicity of Infection) of 1000, but lower for MDCKII at MOI 300. Transport function at the same MOI was the greatest in HeLa cells. Interestingly, MDCKII cells exhibited higher transport activity compared to LLC-PK1 cells when expressing NTCP, but LLC-PK1 was better when expressing OATP2B1.
Conclusions: Our findings suggest a number of cell lines can be transduced to express hepatic transporters using the adenoviral expression system. Therefore, our in vitro system has the potential serve as a physiologically relevant model for predicting in vivo PK.

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Choroidal antiangiogenic effects of lymphocyte-derived microparticles are mediated through PEDF and neurotrophin receptor p75NTR signalling pathways

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Purpose: The importance of identifying VEGF-independent pathways in pathological angiogenesis is increasingly recognized as a result of emerging drug resistance to anti-VEGF therapies. Human T-Lymphocyte-Derived Microparticles (LMPs) significantly inhibit angiogenesis in several ocular neovascularization (NV). Both pigment epithelium-derived factor (PEDF) and the neurotrophins (NT) low-affinity p75NTR receptor have shown antiangiogenic effects. Our study is designed to determine how LMPs modulate the pro and antiangiogenic microenvironments in choroidal angiogenesis.
Methods: Antiangiogenic effects of LMPs were determined by using rat model of choroidal explants. LMPs were produced by treatment of human T-lymphocytes with actinomycin D. Cell viability (MTT assay), proliferation ([3H]-thymidine DNA incorporation), migration assays and apoptosis, were tested in cell lines. Choroidal expression of VEGF, PEDF, nerve growth factor (NGF) and p75NTR were demonstrated by Western blots and RT- PCR.
Results: Choroidal NV was suppressed by more than 50% after 72h of LMPs treatments. LMPs targeting acted on multiple cell types important for choroidal angiogenesis, such as vascular endothelial cells (inhibit HREC cell proliferation by 55%), and RPE cells (ARPE19). At a molecular level, LMPs regulated neurotrophins and their receptors expression both in vitro and in vivo. Inhibition of p75NTR abolished the antiangiogenic effect of LMPs.
Conclusions: LMPs are important candidate for antiangiogenic therapy. PEDF and neurotrophin induction by LMPs may be of therapeutic value in treating ocular neovascular diseases. Our data demonstrate that choroidal tissues have the capacity to synthesize neurotrophins, and that various stimulations can up-regulate gene and protein expression of neurotrophins.

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Dehydroepiandrosterone alters retinol levels and expression of retinol related proteins

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Background: Dehydroandrosterone (DHEA) and its sulfate DHEA-sulfate ester (DHEA-S) are the most abundant adrenal steroids in humans. However, the

physiologic roles of DHEA and DHEAS have not been clearly defined. High levels of DHEA have been reported to be associated with decreased risk of cardiovascular disease and there has been speculation about their possible role in the aging process. In animal experiments, DHEA and DHEA-S have beneficial effects to obesity, diabetes, oncogenesis, atherosclerosis, and memory. Vitamin A is essential for vision, embryonic development reproduction, immunity, and growth. The affect of DHEA to retinol status has not been reported previously.
Objectives: In this study, we examined the retinol status in rats administered DHEA and investigated the expression of retinol related proteins including lecithin retinol acyltransferase (LRAT) and beta-carotene 15,15´ monooxygenase (BCM) genes, which are metabolic enzymes of retinol and beta-carotene respectively.
Methods: Wistar rats (four weeks, male) were assigned to two groups: a control group and a DHEA group fed the standard rat chow containing 0.4 % (wt/wt) DHEA, and fed for two weeks.
Results: Retinol levels of both plasma and liver in DHEA administered rats are decreased compared with controls. Hepatic BCM and LAT gene expression is significantly decreased in DHEA administered rats. Expression of both enzymes may affect circulatory retinol status. Conclusions: DHEA and DHEA-S are widespread as supplements for anti-aging. However, we should be aware that excess of DHEA intake might affect fat-soluble retinol status.

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Behavioural effects of enhanced expression of equilibrative nucleoside transporter 1 in mice

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Background: Adenosine is a neuromodulator that permeates cell membranes via nucleoside transporters. Mice with neuronal expression of human equilibrative transporter 1 (hENT1) have been generated (Parkinson et al., 2009 J. Neurochem. 109:562-572). Expression of hENT1 was associated with increased ataxic effects of ethanol and reduced stimulatory effects of caffeine, two drugs that act at least in part through adenosine signalling mechanisms.
Objectives: The present study examined the hypothesis that mice homozygous for the hENT1 transgene have significantly different behavioural responses to ethanol and caffeine compared with heterozygous mice.
Methods: To examine ethanol sensitivity, we tested loss of righting response (LORR) duration after injection (i.p.) of ethanol (3.6g/kg; 20% v/v in saline). To examine caffeine sensitivity, alterations in locomotor activity were monitored after injection of caffeine (25 mg/kg, i.p.).
Results: In behavioural assays, transgenic mice showed a greater response to ethanol and a reduced response to caffeine than wild type littermates, but no significant differences between heterozygous and homozygous transgenic mice were detected.
Conclusion: These data indicate that the increase in ENT1 function between wild type and heterozygous mice is greater than the increase in ENT1 function between heterozygous and homozygous transgenic mice. Therefore, homozygous mice do not offer a significant advantage over heterozygous mice for studies of ENT1 regulation of adenosine levels and adenosine dependent behaviours.

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Cardiac specific over-expression of membrane-associated human stem cell factor promotes epicardial activation post myocardial infarction

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Background: Myocardial infarction (MI) is one of the leading causes of death worldwide. We recently demonstrated that the cardiac specific over-expression of membrane-associated human stem cell factor (MA-hSCF) improves cardiac function and survival post-MI. Epicardium derived cells (EPDCs), a population of cardiac stem cells which are essential for embryonic heart development, has recently been shown to be able to be activated and involved in cardiac repair post-MI.
Objective: The aim of the present study was to investigate the effects of cardiomyocyte-specific over-expression of MA-hSCF on epicardial activation post-MI. We hypothesized that cardiac specific over-expression of MA–hSCF promotes epicardial activation in mice post MI.
Methods/ Results: Wild-type (WT) and the inducible cardiac-specific MA-hSCF transgenic (hSCF/ tTA) mice were subjected to MI. Activated EPDCs were increased in hSCF/tTA epicardium compared to WT mice (P<0.05) 3 days post MI as determined by Wt1 staining. E13.5 WT EPDCs were cultured and infected

with Ad-hSCF or Ad-EGFP. Proliferation was significantly enhanced in Ad-hSCF infected EPDCs as determined by cell counting ($P<0.05$). Moreover, a trans-well system was employed to evaluate the migration of E13.5 $EGFP^+$ EPDCs. Neonatal WT cardiomyocytes were cultured and infected with Ad-hSCF or Ad-LacZ in the lower compartment of the trans-well. $EGFP^+$ EPDCs were seeded in the upper compartment. Twenty-four hours later, EGFP signal in the bottom well was significantly increased in the Ad-hSCF infected group compared to Ad-LacZ ($P<0.05$).
Conclusions: Cardiomyocyte-specific over-expression of MA-hSCF promotes the activation of EPDCs post-MI. Over-expression of MA-hSCF enhances the proliferation and migration of EPDCs.

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Calcium involved in the vasorelaxant effect of convolvulus arvensis L extract on rabbit aorta rings

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Conflict of Interest: None declared

Background: Our previous finding suggests that calcium plays an important role in the relaxant effect of *Convolvulus arvensis* L (CAL) extract in rabbit's aorta.
Objective: As a result, further investigation is needed to determine calcium role in the CAL induce relaxation.
Methods: CAL extract was added cumulatively to the rabbit aorta pre-contracted with high K^+ Krebs solution. Also another procedure was performed using Ca^{2+}-free Krebs where aorta ring exposed to Phenylephrine (PE) as a control group. When the phasic contractions reached a plateau, $CaCl_2$ was added to the bath, causing a tonic contraction. This procedure effectively explain whether the relaxant action of CAL was due to intracellular Ca release from the endoplasmic reticulum (ER)or extracellular Ca^{2+}-release from cell surface calcium channels. The procedure was repeated in the same tissue after pretreatment of extract or Diltiazem.
Results: CAL significantly decreased vessels contraction induced by high concentration of K^+. Also, the CAL extract inhibited both calcium release from the ER and the entry of calcium through Ca^{2+} channels.
Conclusions: Our finding gives evidence that clearly CAL induced relaxation in rabbit Aorta rings via involvement in the mobilization of Ca^{2+} from ER as well as from Ca^{+2} channels. Our results might led to new therapeutic application of CAL in cardiovascular diseases.

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Synthesis and anticonvulsant properties of 1-(amino-*N*-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea derivatives

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Introduction: There is continuing demand for new anticonvulsant agents as several of the currently available antiepileptic drugs (AEDs) have been associated with severe side effects and fail to control seizures in about 30% of epileptic patients. Study revealed that isatin is a privileged lead molecule for designing potential bioactive agents.
Objectives: Considering extensive applications of isatin moiety in medicinal chemistry, an attempt has been made to synthesize amino-*N*-arylmethanethio urea derivatives containing isatin moiety that are comparatively more efficacious and safer than the currently used anticonvulsant agents.
Methods: A series of new 1-(amino-*N*-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (5a-p) were prepared. The pharmacological testing is done by National Institute of Neurological Disorders and Stroke (NINDS), USA under Anticonvulsant screening program (ASP). The Phase I pharmacological screening comprised MES, scPTZ and neurotoxicity. Some of the selected compounds were tested for their activity at 6 Hz model.
Results: The most potent anticonvulsant compounds found to be were 5f, 5h, 5i, 5k and 5l. In MES test, compound 5h and 5i showed marked protection at 100 and 300 mg/kg dose. In sc.PTZ screening compounds 5h and 5i were active at dose of 300 mg//kg. In 6 Hz screening compounds 5h and 5i showed significant protection and emerged as lead compounds.
Conclusions: Compounds 5h and 5i emerged as a lead compound compared to the standard drugs when they were subjected to preliminary anticonvulsant screenings. They showed marked lower neurotoxicity

and therefore a higher protective index. These can be regarded as strong candidates for future investigations.

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Bone marrow cells migrate to the retina and effect angiogenesis in a mouse model of oxygen induced retinopathy

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Background: The retina is a metabolically active tissue requiring a large amount of oxygen. Under hypoxic conditions, neovascularization occurs and can have severe negative repercussions on the retina. Retinopathy of prematurity (ROP), the leading cause of infant blindness, exemplifies the detrimental effects neovascularization can cause. These premature newborns acquire vaso-obliteration of micro vessels in the retina, followed by a pathological neovascularization once the retinal metabolic demand increases. Oxygen induced retinopathy (OIR) is an animal model that replicates both phases of ROP and is achieved by exposing newborn mouse pups to 80% oxygen from post-natal day 7 to 12. There have been many advances in stem cell research regarding coronary and renal revascularization demonstrating a possible role for stem cells in angiogenesis.
Objective: Utilizing bone marrow derived stem cells, we aimed to repopulate the retina with normal vessels which are affected in the OIR model.
Methods: Two different cell types were isolated from mouse bone marrow: lineage negative (Lin-) and mesenchymal stem cells (MSC). These cells were then injected into the vitreous of OIR mice.
Results: Mouse retinas collected at P17 from both MSC and Lin- injected mice demonstrated cell migration to the inner retina. Furthermore, MSC injected mice retinas showed reduced neovascularization and vaso-obliteration where as Lin- injected retinas did not result in significant revascularization.
Conclusion: MSCs migrate to the retina in mice having undergone the OIR model and promote proper vascular repair. These results suggest that MSCs play a role in angiogenesis and could have a therapeutic use in retinopathy of prematurity.

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Endothelial microparticles promote oxidative stress and inflammation in cultured mouse aortic endothelial cells: role of epidermal growth factor receptor

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Background: Microparticles (MPs), submicron fragments of cellular membranes shed from stressed/damaged cells are found in the plasma of healthy individuals with levels increased in vascular injury. Elevated plasma MP levels correlate with vascular dysfunction and predict future cardiovascular events. However, whether MPs themselves contribute to endothelial dysfunction is unclear.
Objectives: We tested the hypothesis that endothelial MPs, through EGFR, influence endothelial cell (EC) function by increasing EC oxidative stress and stimulating pro-inflammatory responses.
Methods: Endothelial MPs were isolated from the media of cultured mouse aortic ECs by centrifugation and quantified by flow cytometry. ECs were treated with endothelial MPs (10^5/ml) and effects on ROS generation (DHE HPLC), pro-inflammatory signaling (adhesion molecule expression) and inflammatory responses (macrophage adhesion) were examined.
Results: Endothelial MPs significantly increased production of superoxide anion in ECs (~2-fold, $P<0.05$) after 4 hours and expression of the cellular adhesion molecules PECAM (~3-fold, $P<0.05$), and VCAM (~3-fold, $P<0.05$) after 8 hours. Treatment with endothelial MPs (10^5/ml) significantly increased macrophage adhesion to ECs after 8 hours ($P<0.05$). Examination by confocal microscopy suggested a surface interaction between ECs and MPs. Additionally, western blot analysis identified the epidermal growth factor receptor (EGFR) ligand HB-EGF. We therefore tested the hypothesis that MPs promote oxidative stress and inflammation through EGFR activation. Co-treatment with the EGFR inhibitor gefitinib (1 µM), blocked MP-induced oxidative stress and inflammation.
Conclusions: In summary, we demonstrate that endothelial MPs are pro-oxidative and pro-inflammatory in ECs. These effects appear to be mediated through surface interaction and stimulation of EGFR. Thus MPs may be more than just biomarkers of vascular injury and may themselves contribute to endothelial dysfunction.

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Hair analysis as a tool for estimating child exposure to environmentally relevant polybrominated diphenyl ethers (PBDEs)

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Background: Polybrominated diphenyl ethers (PBDEs) are chemicals that are added to a variety of consumer products as flame retardants. They are persistent in the environment and have been detected in wildlife and in humans. The human body burden in North America is among the highest in the world. Some studies have reported higher levels of PBDEs in children, than in their mothers, as measured in serum. PBDEs are structurally similar to other persistent organic pollutants, some of which can potentially interfere with endocrine pathways. This is of concern since childhood is an ongoing period of growth and development.
Objectives: To establish a method to quantify PBDEs in the hair of children (newborn-age 16) as a biomarker of long-term systemic exposure.
Methods: A method has been developed using gas chromatography with mass spectrometry (GC/MS). Fifty mg of children's hair is incubated overnight at 40°C with 4N HCl and hexane (4:1) to extract PBDEs. Samples are eluted from 2g $NaSO_4$: 2g Florosil SPE columns with 8mL of hexane. Samples are then analyzed by GC/MS for PBDE congeners; BDE-28, -47, -99, -100, -153, -154, -183 and -209.
Results: The total amount of PBDEs varied among samples. Several congeners could be detected in newborn hair. BDE-209 was present in some samples. The greatest variability was seen with congeners BDE-47 and BDE-99.
Conclusions: This method offers a noninvasive technique for assessing chronic exposure to PBDEs in children, and to examine correlations between systemic exposure and adverse developmental outcomes.

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Impact of hypertension on endothelial CaMKII isoforms from mesenteric arteries

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Background: Calcium signaling is fundamental for endothelial functions and an alteration in the regulatory mechanism can lead to cardiovascular pathologies. Recently, spontaneous calcium oscillations localized within the myoendothelial junctions have been identified and named calcium pulsars. Calcium/Calmodulin Kinase II (CaMKII) is a potential target for Ca2+ pulsars involved in endothelial function. Indeed, CaMKII has recently been suggested to play an important role in endothelial function and dysfunction. CaMKII is characterized by its unique ability to interpret intracellular calcium oscillations frequency into specific outcomes. Therefore, alteration in endothelial CaMKII functions might be related with intracellular calcium oscillations such as calcium pulsars. The aim of this study is to investigate the relationship between CaMKII and calcium pulsars and it's alteration in hypertension. Acute and chronic stimulation of calcium pulsars with phenylephrine appears to increase CaMKII activation in endothelium from murine mesenteric arteries. Moreover, CaMKII translocates following calcium pulsars stimulation. Although found in clusters in hypertensive mouse, CaMKII is homogeneously distributed in the endothelium from CTRL mice. qPCR experiments revealed that all four CaMKII isoforms expression (α, β, γ and δ) are significantly diminished (25-57%) in mesenteric arteries from hypertensive mice. In summary, the increase of calcium pulsars frequency in hypertensive mice correlates with CaMKII's distribution in endothelial cells, suggesting their activation by calcium pulsars. Therefore, an altered relationship between CaMKII and calcium pulsars can potentially be involved in endothelial dysfunction associated to hypertension.

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Development and pharmacological characterization of a new class of peptidic urotensin II antagonists

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Conflict of Interest: None declared

Background: Known antagonists of the urotensin II (UII) receptor (UT) are of limited utility for investigating the pathophysiological role of UII due to poor potency and limited selectivity.
Objectives: Design, synthesis and pharmacological evaluation of new UII antagonists.
Methods: The pharmacological properties of a novel UT antagonist, [Bip4]URP, was investigated in vitro and in vivo using several bioassays.
Results: Competitive binding assays demonstrated the ability of [Bip[4]]URP to fully displace the radioligands ^{125}I-hUII and ^{125}I-URP from human recombinant UT. In ex vivo studies, various concentrations of [Bip[4]]URP did not produce any significant rightward shift of the hUII concentration-response curve, but the maximal response to hUII was not attainable. Interestingly, a slight but non significant rightward shift, along with a non significant reduction of efficacy, was observed with URP. Supporting these observations, dissociation experiments revealed the propensity of this compound to accelerate the dissociation rate of hUII but not URP, suggesting the presence of two binding pockets in close vicinity. In vivo, [Bip[4]]URP had no effect on the biphasic response induced by URP but significantly reduced the hypotensive activity, while keeping intact the pressor effect induced by hUII. These results demonstrated its selectivity against hUII agonistic effect.
Conclusion: The results demonstrated the ability of [Bip[4]]URP to reduce the efficacy of hUII- but not URP-induced vasoconstriction. Moreover, in vivo studies support the in vitro pharmacological profile described above, making [Bip[4]]URP as the first peptidic analog of a new class of urotensinergic antagonists.

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Ethics involved in termination of a wanted pregnancy in reproductive toxicology

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Background: A serious conflict is created when a woman must decide whether or not to terminate a wanted pregnancy after exposure to a teratogen. She must weigh the fetal/child risks following exposure against ethical, religious, and psychosocial issues of pregnancy termination.
Objective: To identify the medical, ethical, cultural and spiritual aspects involved in the process of decision-making regarding the termination of a wanted pregnancy after exposure to a teratogen and to provide guidelines for optimal management.
Methods: A case discussion took place including Clinical Pharmacology fellows and staff, graduate and post graduate students, counselors, obstetricians, bioethics faculty, and representatives of all religious Chaplains of the Hospital for Sick Children, in order to discuss and find better ways to support women in their decision making process.
Summary: 1) Woman should be explained her legal rights in decision-making, including the fact that abortion is legal and free in Canada. 2) One-on-one counseling on fetal risk following exposure should be provided based on evidence-based information. 3) The role of the father and family members in supporting the woman's decision making should be stressed. 4) Religious scholars should be involved for spiritual support. 5) In the event of termination, long term support from medical, religious, psychosocial, and family members should be provided.
Conclusion: Collaboration of healthcare providers, bioethics and chaplaincy representatives, and family members, is essential to reduce the women's moral distress when faced with a decision of pregnancy termination after teratogen exposure.

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Benzodiazepine use in the Grand-Duchy of Luxembourg from 1995 to 2006: proposed definitions of high-dosage use and abuse

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Background: Prevalence rates of benzodiazepine intake vary among studies, mainly due to definitions of benzodiazepine use, abuse, lengths of observation period and samples of subject.
Objective: To examine short- and long-term use of benzodiazepines in a large national sample.
Methods: A 12-year population-based study was conducted in the country of Luxembourg by looking at benzodiazepine prescriptions for all insured subjects in the national health system from 1995 (n =387,862) to 2006 (n =449,972). Patterns of benzodiazepine use and

characteristics of benzodiazepine users were studied.
Results/Conclusion: The overall number of Defined Daily Dose (DDD) per 1000 insured subjects per day was 82.9; the five most prescribed benzodiazepines were lormetazepam, lorazepam, alprazolam, bromazepam and loprazolam. Alprazolam was the only benzodiazepine showing a threefold increase in its annual prescribed volume during the study period. Subjects having had at least one benzodiazepine prescription (n=236,263) in the 12-year study period, were divided into 3 groups: 1) a "short term delivery" group (34.9%) with benzodiazepine prescription ≤ 3 months; 2) a "discontinuous delivery" group (38.4%); and 3) a "continuous delivery" group (26.7%) of subjects who never stopped taking benzodiazepines once prescribed. High-dose use was defined using a new formula to calculate potential abuse, and a constant number of high dose users were found throughout the study period: 5.3% of all users (0.9% of all subjects) had a yearly benzodiazepine intake higher than the maximum recommended dosage.

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IGF-1 effects on oligodendrocyte survival and proliferation are potentiated through PTEN inhibition

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Conflict of Interest: None declared

Background: Myelin is a multilayer specialized membrane that wraps nerve fibers and insulates them facilitating the communication between neurons. Myelin destruction causes neurological impairments such as those observed in patients with multiple sclerosis. Oligodendrocytes (OLGs) are cells responsible for producing myelin in the central nervous system. Insulin-like growth factor-1 (IGF-1) is essential for OLG growth, maturation and survival. IGF-1 binds to its receptor to activate the PI3K/Akt/mTOR cascade which mediates protein synthesis, important for OLG progenitors (OLPs) growth. PTEN (phosphatase and tensin homologue deleted on chromosome 10) is the major negative regulator of PI3K/Akt/mTOR pathway.
Objectives: Our aim is to assess whether an inhibitor of PTEN, potassium bisperoxo oxovanadate (Phen), could be used as pharmacological tool to increase or potentiate IGF-1 effects on OLP survival and proliferation.
Methods: Primary cultures of OPC were prepared from the brains of newborn Sprague-Dawley rats. Thymidine incorporation and MTT assay were used as an OPC proliferation and cell viability indexes, respectively. OPC protein extracts were resolved by Western Blotting.
Results: We found that Phen alone increased OLP proliferation and potentiated the effects of IGF-1. In addition, IGF-1-stimulated signaling pathways were further increased by Phen treatment including Akt, S6 ribosomal protein and ERK.
Conclusion: Overall, our first results suggest a potential role of IGF-1 on OLG proliferation and survival under PTEN inhibition.

57 WITHDRAWN
Recurrence and long-term outcome of Stevens-Johnson Syndrome and toxic epidermal necrolysis in children

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Conflicting Interests: None declared

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Potential use of synthetic kinin B1 receptor peptide agonists as permeability enhancers for improving drug delivery to malignant brain tumours

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Background: Some evidence suggests that kinin B1 receptors (B1R), an inducible G protein-coupled receptor (GPCR), can regulate blood-brain barrier (BBB) permeability, including that of brain tumours.
Objectives: 1) To analyze expression of B1R in brain tumour of syngeneic F98 glioma-implanted Fischer rats. 2) To determine whether the SarLys[DPhe[8]]desArg[9]-bradykinin (NG29), a stabilized kinin B1R agonist, modulates blood brain barrier

(BBB) function, thereby improving delivery of macromolecules directly into brain tumours.
Methods: Expression of B1R (mRNA, protein) in brain normal and tumour tissues was determined by RT-PCR, Western blot, and IHC. Effects of NG29 were monitored by non invasive MRI with Gadolinium-based contrast agents Gd-DTPA (0.5 kDa) and Gadomer (17 kDa) (T1-weighted imaging), and by IHC of endogenous albumin (~66 kDa).
Results: Preferential expression of B1R at tumour cells and surrounding tumour vasculature were detected. Intracarotid infusion of NG29, in contrast to natural LysdesArg^{9}-bradykinin, elevated brain distribution and uptake profiles of intravenous contrast agents within rat glioma and brain surrounding. These effects were dose-dependent, reversible (lasting< 2h), and were blocked by B1R antagonist R892 and non-selective COX inhibitor indomethacin, but not by B2R antagonist HOE140 and NO-synthase inhibitor L-NA. Consistent with MRI data, immunostaining for extravasated albumin at the invasive tumor edge was increased in NG29-treated rats.
Conclusion: Our results document a novel GPCR signalling mechanism for promoting transvascular delivery into brain tumour, involving possibly COX byproducts. They also underline the potential value of synthetic B1R agonists as selective tumour BBB permeabilizers for local delivery of different-sized therapeutics at (peri)tumoral sites.

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Acyclovir is a substrate for the human breast cancer resistance protein (BCRP)

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Background: The renal transport mechanisms of acyclovir have not been fully elucidated. The human breast cancer resistance protein (BCRP) is a transporter that is widely expressed in human tissues, including the kidney. Studies illustrate that, in mice, BCRP mediates the transport of acyclovir into breast milk. It is plausible that acyclovir is a substrate for human BCRP, and hence, the transporter may be actively involved in the tubular efflux of acyclovir. The role of human BCRP in the transport of acyclovir has not been previously evaluated.
Objectives: To determine whether acyclovir is a substrate for the human BCRP.
Methods: Cellular accumulation studies were conducted to determine whether acyclovir is a substrate for the human BCRP. Transfected human embryonic kidney (HEK293) cells [containing the full-length human *ABCG2* gene encoding the wildtype ABCG2 amino acid sequence] were exposed to [8-^{14}C] acyclovir in the presence or absence of the BCRP inhibitor, fumitremorgin C (FTC) for 2 hours. Intracellular [8-^{14}C] acyclovir accumulation was then assessed using a liquid scintillation counter.
Results: The results illustrated that acyclovir is a substrate for human BCRP. In the presence of FTC, there was a 5-fold increase ($p<0.05$) in the intracellular accumulation of acyclovir.
Conclusions: The study is the first to illustrate that acyclovir is a substrate for the human BCRP. The results suggest that BCRP may play a significant role in the renal clearance of acyclovir, and contributes to the further understanding of the tubular transport of acyclovir. Future studies are required to determine the affinity of the transporter for the antiviral agent.

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

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Background: It is estimated that 82% of children will survive childhood cancer, up from 50% decades ago. While promising, this increased life span has brought awareness of the high potential that these patients may develop late effects, conditions secondary to cancer therapy. Nephrotoxicity is one such late effect, affecting children treated with the chemotherapy drug

ifosfamide, commonly used to treat pediatric solid tumours. While effective, in children it is associated with a 30% risk of developing nephrotoxicity, with 5% of this group developing Fanconi's syndrome. N-acetylcysteine, as synthetic thiol that is currently used clinically in children, has successfully mitigated this renal toxicity in both cell and rodent models. However, before this treatment can be realized clinically, we must demonstrate that it does not interfere with the antitumour efficacy of ifosfamide. Our objective is to compare the efficacy of ifosfamide with and without concurrent n-acetylcysteine therapy in an immunocompromised mouse xenograft model. Female Swiss nu/nu mice will be injected with Ewing's Sarcoma tumour cells. They will receive 60mg/kg injections of ifosfamide (3 days), with or without 1.2g/kg injections of n-acetylcysteine (concurrently and for + 3 days). Tumour volumes will be measured. Preliminary data assessing the efficacy of ifosfamide during concurrent n-acetylcysteine therapy *in vitro*, in relevant tumour cell lines (rhabdomyosarcoma and neuroblastoma) indicate n-acetylcysteine has no effect. We anticipate similar findings with our rodent model (to be completed before May). Upon anticipated results, these findings will demonstrate the clinical applicability of n-acetylcysteine for ifosfamide-induced nephrotoxicity and strengthen support for a clinical trial.

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The relationship between folic acid supplementation and serum folate level in early pregnancy and pregnancy outcomes: MOCEH (Mothers and Children's Environmental Health) study

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Conflict of Interest: None declared

Background: The currently recommended guidelines to minimize the incidence of NTD are well established; serum/plasma folate >7 ng/mL (15.9 nmol/L) or RBC folate concentrations >906nmol/L. However, there is controversy regarding the relationship between serum folate/folic acid supplement use and pregnancy outcome.

Objective: To evaluate the relationship between serum folate/folic acid supplement use and pregnancy outcome.

Methods: As part of the Prospective Cohort Study of the MOCEH, 1220 pregnant women were enrolled. Information about folic acid supplementation, maternal serum folate levels in the 1st & 3rd trimester of pregnancy, and pregnancy outcomes, were obtained. Comparison of pregnancy outcomes between women who received prenatal folic acid supplementation and those who did not receive supplementation was conducted. A post-hoc analysis was performed comparing pregnancy outcome a) between women with blood folate levels <7 ng/mL vs. >7 ng/mL, and b) between women with <20 ng/mL vs. >20 ng/mL folate levels.

Results: The mean and median level of serum folatel were 11.7 ng/mL and 9.6 (95% CI 3.3-26.9), respectively. Overall 32.9% (n= 401/1220) of early pregnant women were at <7ng/mL folate. Only 29.8% (n= 363/1220) of pregnant women took multivitamins with folic acid during the 1st trimester of pregnancy. Serum folate level was higher in the FA supplement group than in the no-FA supplement group. No differences in pregnancy outcomes were observed a) between women who received folic acid supplementation and those who did not, and b) between women with blood folate levels <7 ng/mL vs. >7 ng/mL. Although most preterm deliveries <34 weeks were observed in the group of serum folate <20 ng/mL, there was no statistical significance. Other pregnancy outcomes such as preeclampsia, gestational diabetes, placenta previa and premature rupture of membrane remained similar between groups.

Conclusions: Although most preterm deliveries was observed in the low serum folate levels (<20 ng/mL), there was no significant relation between serum folate level/folic acid supplementation and pregnancy outcomes. This is possibly due to unsufficient sample size, and should be repeated with larger cohorts.

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NK1 receptor antagonists modulate the effects of immunosuppressive drugs on T cells activation: an *in vitro* study

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Conflict of Interest: None declared

Introduction: Cyclosporin A (CsA) and Tacrolimus (FK506) both inhibit T cell activation to prevent or reduce graft reject. Rapamycin also regulate T cells function by repressing cell proliferation. Aside from their therapeutic efficiency, these molecules have several side effects. An option to reduce their adverse effects is to decrease the dose of immunosuppressive drugs by combining it with another molecule. The NK1 receptor (NK1R) expressed on T cells may be a good candidate because this receptor under activation by its ligand, the substance P (SP), regulates T cells function. The goal of this study is to demonstrate the potential efficacy of combining a NK1R antagonist (L-733,060) with CsA, FK506 or rapamycine.
Methods: Jurkat T cells were incubated with rapamycin (1; 0,5 and 0,1 nM) in presence of a NK1R antagonist L-733,060 (1; 5 and 10 uM). The level of phosphorylation of the S6 ribosomal protein (S6R) was measured by flow cytometry. Jurkat cells were also incubated with CsA (1, 5 and 10 ng/ml) or FK506 (0,01; 0,05 and 0,1 ng/ml) with the L-733,060. The modulation of IL-2 production by T cells was measured by ELISA.
Results: The combination of rapamycin with L-733,060 did not affect the level of phosphorylation of the S6R protein. On the other hand, the combination of CsA or FK506 with L-733,060 significantly reduces IL-2 production in stimulated T cells.
Conclusion: The NK1R signalling pathway is implicated in the production of IL-2 in activated T cells. Combining a NK1R antagonist with immunosuppressive drugs may be an interesting alternative to reduce therapeutic doses without affecting therapy efficacy.

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Pharmacokinetics of obesity in childhood – a review

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Conflict of Interest: None declared

Introduction: The obesity epidemic is widely recognized as one of the biggest health threats of the modern age. It is not uncommon for clinicians in primary or secondary care to treat illness in an obese child, which will require treatment with a drug for which there is no guide regarding which body size metric to use in determining the optimum dose for this group of patients. We conducted a review of available literature to determine availability of pharmacokinetic data in obese children.
Methods: A review of available adult and paediatric obesity pharmacokinetic literature was conducted.
Results: Definitions of obesity are even more challenging in children than in adults. Many measures of body composition are available, but have not been extensively verified. Whereas absorption seems not to be affected, distribution, metabolism and elimination are known to be in various extend. Plasma protein levels and binding are comparable to non-obese, and effects on regional blood flow are unclear. Evidence suggests that hydrophilic drugs whose V_d in normal-weight subjects is small should be administered according to ideal body weight, and not total body weight. CYP450 enzyme activity is altered for the 2E1 isoform, but for others results are conflicting. Glomerular filtration rates are mostly similar, but this is unclear for tubular reabsorption.
Conclusions: There is a lack of available pharmacokinetic data for the determination of optimal dosing schedules for obese children, and adults alike.

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Pharmacogenetics of fatal paediatric obstructive sleep apnea cases in response to codeine: additional cases

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Background: The primary treatment for pediatric obstructive sleep apnea (OSA) is removal of the tonsils and adenoids (adenotonsillectomy). Codeine is prescribed for the pain associated with this procedure. Codeine is a prodrug which requires conversion to morphine via the highly polymorphic cytochrome P450 (CYP) 2D6 enzyme to elicit its analgesic properties. A

functional gene duplication causes increased morphine production in the ultra-rapid metaboliser (UM) phenotype. In 2009, we reported a case of fatal respiratory depression in a 2yr old male post-adenotonsillectomy with toxic morphine levels. Post-mortem analysis revealed bronchopneumonia and a CYP 2D6 UM phenotype. We report now 2 additional Ontario cases. In September 2010 we recorded a near fatal case of respiratory depression in a 3yr old female OSA patient. She arrived in hospital comatose with toxic blood morphine levels despite using recommended codeine doses. Upon mechanical ventilation and standard naloxone treatment she fully recovered. Salivary genetic analysis revealed that she was a CYP 2D6 UM. A third, fatal case was encountered in November 2010, again, with toxic blood morphine levels after taking only recommended doses. This case of respiratory depression occurred in a 4 yr old male. This patient had a previous history of asthma and was identified as a CYP 2D6 UM. We hypothesize that the combination of an UM CYP 2D6 phenotype and an existing respiratory condition in toddlers with OSA increases the risk for central nervous system depression upon standard codeine administration.

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MTHFR C677T polymorphism and the risk of colorectal cancer: a systematic review and meta-analysis

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Conflict of Interest: None declared

Background: Folates are water soluble B vitamins that are cofactors in thymidine and purine synthesis and DNA methylation pathways. Methylenetetrahydrofolate reductase (MTHFR) is key in the remethylation of homocysteine to methionine. In 1995 a variant of the MTHFR enzyme, genotype TT, was identified with reduced activity versus CC, causing an accumulation of homocysteine and higher rates of thymidine synthesis. Individuals with this variant are thought to be at a reduced risk for colorectal cancer.

Objective: This meta-analysis examines whether a relationship exists between the variants of MTHFR C677T gene, folic acid intake and the risk of colorectal cancer.

Methods: A systematic review and meta-analysis were conducted. MEDLINE, Embase and SCOPUS were searched from inception to May 2010 with the following search terms "folic acid", "C677T", methylenetetrahydrofolate reductase", "colorectal cancer", "colonic neoplasms", "rectal neoplasms". Observational studies in adult populations were included that reported MTHFR C677T variants, defined levels of folic acid and the risk of colorectal cancer.

Results: Out of 633 records, 28 studies met our inclusion criteria. Preliminary results indicate that the summary risk estimate for case control studies comparing MTHFR CC to CT was 0.92 (CI 95% 0.83-1.01) with some heterogeneity. The summary risk estimate for case control studies comparing MTHFR CC to TT was 0.82 (CI 95% 0.71-0.95) with some heterogeneity.

Conclusion: This meta-analysis supports the association between the TT genotype and a reduced risk of colorectal cancer. Further analysis is required to provide insight into whether this risk is further modified through folic acid intake levels.

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Transcriptional regulation and function organic anion transporting polypeptide 2B1 splice variants

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Conflict of Interest: None

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. 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/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. Using cell based reporter assays we determined that HNF4α was able to transactivate transcription of the truncated variant but not the full length form. 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.

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Digoxin toxicity precipitated by clarithromycin use: case presentation and review of the literature

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Background: An 83-year-old man with a past history of hypertension, dyslipidemia, myocardial infarction, and left ventricular dysfunction presented with a recent history of fatigue, dyspnea on exertion, cough, nausea, loss of appetite and lightheadedness. Three days prior to his presentation, the patient had been started on clarithromycin 500 mg p.o. b.i.d. for treatment of possible pneumonia. His cardiac medications included digoxin 0.125 mg p.o. q.d., spironolactone 25 mg p.o. q.d., captopril 25 mg p.o. b.i.d., pravastatin 40 mg p.o. q.d., isosorbide mononitrate 30 mg p.o. q.d., furosemide 60 mg p.o. q.d., bisoprolol 2.5 mg p.o. q.d., and potassium 20 mmol p.o. q.d. Due to severe nausea, the patient presented to the Emergency Department following completion of his fifth dose of clarithromycin. On examination, he appeared unwell. Vital signs were stable. Cardiorespiratory examination did not show signs of decompensated heart failure. Blood tests demonstrated a white cell count of 13.6 x 10^9/L, potassium 5.2 mmol/L, creatinine 184 µmol/L (creatinine was normal 3 weeks before), and digoxin levels of 4.6 nmol/L (5 hours post-dose) and 4.7 nmol/L (18 hours post-dose). Ventricular extrasystoles were observed during monitoring. The presentation of this patient was consistent with digoxin toxicity in the context of renal dysfunction and concomitant use of the macrolide antibiotic, clarithromycin, which is known to inhibit P-glycoprotein-mediated efflux mechanisms of digoxin. Both drugs were discontinued. The patient was hospitalized and was discharged ten days later. Health care providers need to be vigilant of this potential drug-drug interaction. A focused literature review will be presented.

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Drug use reviews (DUR) in non institutional settings

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Background/Objective: Drug use review (DUR) has become an increasingly popular component in budget activities conducted by drug benefit programs that operate in the non institutional environment. There is great diversity in the methods used to conduct drug use reviews, and each method has different strengths and weaknesses. This review was designed to determine the circumstances under which these methods are used.
Methods: A literature search was conducted in MEDLINE, EMBASE and CINAHL databases to identify original studies that assessed prescription drug use in a community, published between January 2007 and February 2010. Each article was assessed for (a) data source i.e., patient, pharmacy or prescriber; (b) drug issue of interest; (c) method used for data accrual.
Results: 587 articles involving 632 DUR strategies fulfilled the criteria for inclusion in the analysis. 81% were retrospective studies. The source of the prescription drug use information was obtained from pharmacy or dispensing claims data 52%, the patient 27% and physician records 21%. The most common goal was the assessment of drug use patterns by population demographics (n=151). Measuring adherence and misuse of prescription by patients was second (n=136) and physician adherence to prescription guidelines or recommendations was third (n=120). When data was collected at the patient level the elapsed time between drug use and the survey was more than one month 33% of the time, and in 40% of studies the time period was not specified; drug use information was reported by a proxy 11% of the time.
Conclusion: Most DUR studies were conducted retrospectively using prescription dispensing claims data to address simple quantitative questions. Studies designed to address questions pertaining to the quality of prescribing often used patient reported data to obtain

information that would not be available from administrative databases but these were conducted using weaker methodologies.

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Investigating the genetic causes associated with incristine-induced neurotoxicity in children

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Conflict of Interest: None declared

Background: Vincristine is a highly effective oncology drug that is considered to be the standard treatment against many types of cancers. However, the clinical utility of vincristine is significantly limited by debilitating adverse drug reactions (ADRs), such as neurotoxicity, and more specifically, peripheral neuropathy.
Objectives: To identify novel predictive genomic markers and/or clinical factors that will determine an individual's susceptibility to vincristine-induced neurotoxicity.
Methods: DNA samples and detailed clinical data were collected through the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), an active ADR surveillance network encompassing 13 Canadian paediatric hospitals. ADRs were characterized according to a modified version of the Common Terminology Criteria for Adverse Events (v4.03), and stratified by severity, location, and type of neuropathy. Patient samples were genotyped for single nucleotide polymorphisms (SNPs) associated with drug absorption, distribution, metabolism, and excretion. A separate panel containing functional and tagging SNPs of genes that are specifically involved in vincristine's mechanism of action was also developed.
Results: Within CPNDS, 26.3% of paediatric cancer patients on vincristine therapy suffered from vincristine-induced neurotoxicity (305 cases and 854 controls). The neurotoxicity cases have been preliminarily divided into categories of peripheral, autonomic, and central neuropathy.
Conclusions: This study aims to establish the causality of vincristine-induced neurotoxicity in order to assess the risk-benefit ratio of utilizing vincristine for each patient. By identifying individuals who would derive a greater benefit from alternative anti-cancer treatments, vincristine-induced neurotoxicity could potentially be avoided. Additionally, the modified neurotoxicity grading scale and accurate symptom classifications serves to facilitate improvements in the monitoring of vincristine-induced neurotoxicity.

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Development of an in vitro system for the functional study of oatp transports of statins

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Background/Objectives: OATP1B1 and OATP1A2 are known transporters of statins such as rosuvastatin. A mutation in these membrane transporters or drug-drug interactions could modify plasma and intracellular concentrations of statins which can lead to cellular toxicity. The goal of this study was to evaluate the uptake of rosuvastatin by its transporters.
Methods: OATP1B1 was cloned into the pIRES-EGFP vector. Site-directed mutagenesis was used to generate the loss-of-function V174A mutant, which was confirmed by sequencing. Stable cell lines were created in HEK293 cells, and selection was done by G418 treatment, followed by selection by Fluorescence Activated Cell Sorting (FACS). OATP1B1 and OATP1B1-V174A expressing cells were then plated on Poly-Lysine treated 6-well plates, and then incubated in the presence of increasing concentrations of rosuvastatin for various time points. OATP1A2 was cloned into the pRetroX-Tight-Pur retroviral vector. HeLa cells were transiently transfected by this plasmid or the empty vector as the control and the uptake assay was performed similarly as above.
Results: The uptake of rosuvastatin was observed in the presence of the wild type OATP1B1 in as little as 5 minutes. However, the uptake of rosuvastatin in OATP1B1-V174A was not observed, confirming that this mutant is non-functional. Also, the uptake of rosuvastatin was greater in OATP1A2 transfected cells than those transfected with the empty vector. Controls were performed to ensure that the background signal of rosuvastatin was minimal.
Conclusions: Stable cell lines as well as transiently transfected cells expressing OATP transporters can be used as in vitro models for functional studies.

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Stereoisomers of naringenin as pleiotropic, selective inhibitors of cytochrome P450 isoforms

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Background: Naringenin is the predominant flavonone in grapefruit, and has been suggested to be a bioactive antioxidant, free radical scavenger and potential adjunct to treatment of Hepatitis C in humans. Interactions between naringenin and the cytochrome P450 system have been of interest since the first demonstration that grapefruit juice reduced cytochrome P450 3A activity. The effects of naringenin on other cytochrome P450 isoforms have been less carefully investigated. In addition, the stereoisomers of naringenin have not been separated and purified before, and so the stereoselectivity of naringenin's effects has not been characterized. We isolated pure naringenin enantiomers and used them to test the ability of S-, R-, and racemic naringenin to inhibit a series of key drug metabolizing cytochrome P450 isoforms *in vitro*. We determined the IC_{50} values for each naringenin preparation using *in vitro* incubations with recombinant human cytochrome P450 isoforms. We also tested the ability and stereoselectivity of naringenin to inhibit cytochrome P450-mediated drug metabolism in human liver microsomes. Naringenin was able to inhibit CYP2C9, CYP2C19 and CYP19 with IC_{50} values below 5µM. No substantial inhibition of metabolism by CYP2B6 or CYP2D6 was observed at concentrations up to 10µM. The S-enantiomer exhibited higher inhibitory potency than the R-enantiomer for CYP2C19 and CYP19, while the R-enantiomer was more potent as an inhibitor of CYP2C9. Chiral flavonones like naringenin are difficult to separate into their enantiomeric forms, but stereoselective effects may be observed that impact clinical activity. Inhibition of specific drug metabolizing enzymes by naringenin observed *in vitro* may be exploited to understand pharmacokinetic changes seen *in vivo*.

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Objective pharmacological properties that may turn a vasoactive hormone into an animal toxin: differences between bradykinin and maximakinin

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Background: Maximakinin, a natural 19-residue peptide from the skin of the amphibian Bombina maxima (DLPKINRKGPRPPGFSPFR), incorporates the full sequence of bradykinin (BK) at its C-terminus with a hydrophilic N-terminal extension. As a putative venom component, it may stimulate BK B_2 receptors (B_2Rs) in a distinct manner relative to the fragile mammalian agonist BK.
Methods: Radioligand binding assays ([^{3}H]BK to B_2R-green fluorescent protein (GFP) conjugate, [^{3}H]enalaprilat to angiotensin converting enzyme (ACE)) were applied in HEK 293(a) cells to compare the affinity of maximakinin to that of BK at characteristic molecular targets. Human umbilical vein contractility and imaging/downregulation/signalling studies of B_2R-GFP in HEK 293 cells were other applied assays of the effects of the peptides at natural or recombinant B_2Rs.
Results: Maximakinin is an agonist of the BK B_2R with a 7-20 fold lesser potency, but a prolonged (≥ 12 h) duration of action relative to BK (ERK MAP kinase activation, c-Fos induction in HEK 293 cells). Maximakinin displaced [^{3}H]enalaprilat binding from recombinant ACE much less effectively than BK. Unlike BK, maximakinin induced the internalization of the fusion protein B_2R-GFP and the downregulation of this construction over a 12-h stimulation period, fully reproducing the effect of synthetic inactivation-resistant B_2R agonists.
Conclusions: Maximakinin has little affinity for ACE, a major ectopeptidase that inactivates BK, and is further resistant to other proteases present in the endosome. It is a natural kinin sequence that elicits a prolonged signalling, a possible basis for a venomous action in nature and for drug development in the laboratory.

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Ethyl glucuronide as a biomarker of alcohol consumption during pregnancy

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Background: Alcohol consumption during pregnancy can lead to Fetal Alcohol Spectrum Disorder (FASD), which manifests itself in the form of physiological defects, mental retardation and neurobehavioural deficits, the latter of which is rarely diagnosed. Since maternal self-reports are often unreliable, a biomarker of alcohol use during pregnancy is needed to accurately determine fetal exposure and risk for FASD. While Fatty Acid Ethyl Esters (FAEEs) are current biomarkers of exposure, the introduction of a second biomarker that can be tested alongside FAEEs promises to greatly increase the sensitivity and specificity of analytical screens. Ethyl glucuronide (EtG) is a direct metabolite of ethanol that has been detected in the meconium, or first stool of life, of infants born to mothers who consumed alcohol during pregnancy. Whether this detected EtG was formed by the maternal liver and crossed the placenta, by the fetal liver after ethanol crossed the placenta, or by the placenta itself remains unknown.
Objectives: To determine if, and to what extent EtG crosses the human placenta, and to measure placental formation and degradation of EtG.
Methods: Placentae from consenting women undergoing elective Caesarian section at St. Michael's Hospital in Toronto, Ontario will be taken to the on-site perfusion laboratory. One μg/mL EtG will be added to the maternal reservoir and maternal and fetal samples will be taken over a 3 hour period to determine if and to what degree EtG is crossing the human placenta. EtG will be quantified using HS-SPME GC-MS. Placental metabolism of ethanol to EtG and EtG to ethanol will be investigated using placental microsomes. Kinetic parameters (Vmax, Km) will also be determined to help elucidate the role of the placenta in formation and degradation of EtG.
Results: To date the perfusions are being carried out and the level of EtG in the perfusate will be determined using HS-SPME GC-MS with a limit of sensitivity of 1 ng/vial.
Conclusions: We have developed a HS-SPME method for detecting EtG in maternal and fetal perfusate samples. This will allow us to accurately quantify the transfer of EtG across the human placenta.

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Cocaethylene as a biomarker of alcohol and cocaine co-consumption in human hair

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Conflict of Interest: None declared

Background: Cocaethlyene (CE) is a metabolite of cocaine formed only during cocaine and alcohol co-consumption. It is pharmacologically active, prolonging cocaine-related effects.
Objective: To determine if CE can be used as a biomarker in hair testing to indicate alcohol and cocaine co-consumption.
Methods: We used liquid-liquid extraction and solid-phase extraction to isolate cocaine and its metabolites from hair, as well as fatty acid ethyl esters, a direct biomarker of alcohol consumption. The compounds concentrations were analyzed and determined using GC-MS.
Results: Out of 516 individuals who tested positive for cocaine, 336 individuals were confirmed cocaine users. Of these, CE was detected in 73 individuals. From those, only 23 had alcohol testing requested, and 15 tested positive for chronic alcohol abuse. The fraction of cocaine that was converted to CE ranged from 84% at low cocaine concentrations, to about 4% at the higher cocaine concentrations. In a few cases, there were difficulties in identifying CE when compared to benzoylecgonine, another cocaine metabolite, as the two metabolites have similar retention times on GC-MS.
Conclusions: At this stage of our research, about 22% of confirmed cocaine users were positive for CE, indicating alcohol co-consumption. However, other studies have found that, on average, about 62% of confirmed cocaine users also consume alcohol. It is possible that hair accumulation is dose dependent and does not identify low alcohol use.

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Child neurodevelopment following in-utero exposure to maternal azathioprine: preliminary results

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Conflict of Interest: None declared

Background/Objective: Azathioprine (AZA) is an immunosuppressant drug commonly used by pregnant women. Its effects on the fetal central nervous system remain unknown. The objective was to assess child neurocognitive development following in-utero exposure to AZA for maternal Inflammatory Bowel Disease (IBD), and to compare the results to those of three comparison groups.
Methods: *Mother/child pairs*: Group 1-exposed to AZA, Group 2-exposed to corticosteroids; Group 3-exposed to other IBD medications, Group 4-exposed to non-teratogens. Matching criteria: gender and age at testing (children); age (mothers). Using standardized psychological tests, mother/child pairs were assessed on Full Scale, Verbal and Performance IQs.
Results: No significant differences were found among the 4 groups of children (3-12 years) in Full Scale IQ (Group 1=109.2±8.1, Group 2=108.4±12.0, Group 3=109.9±12.9, Group 4=113.9±13.7), Verbal and Performance IQs. Women on corticosteroids experienced more bleeding episodes during gestation (50%vs.3.7%vs.22.9% in AZA group and other medications group, respectively). Children exposed to corticosteroids had significantly shorter gestational ages, lower birth weights and spent more days in NICU. The only significant predictor of these outcomes was corticosteroid group affiliation. Later child health parameters (growth, number of infections and other health problems) did not differ among the groups
Conclusions: There were no associations found between AZA exposure, maternal disease complications during pregnancy, and children's cognitive performance, which is reassuring. However, no statistical differences in IQ may be a power issue. The adverse outcomes related to corticosteroids can be explained by maternal disease activity. The study remains in progress.

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Pharmacokinetic profiles for oral and subcutaneous methotrexate in patients with Crohn's disease

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Background: Methotrexate is a very effective treatment for both the induction and maintenance of remission in Crohn's disease. It is administered subcutaneously due to concerns about drug absorption in this patient population. Furthermore, significant inter-individual variability in its oral bioavailability has been demonstrated in patients with rheumatoid arthritis.
Objectives: To compare the pharmacokinetics of oral and subcutaneous methotrexate in patients with Crohn's disease.
Methods: A total of three patients with stable Crohn's disease have been enrolled thus far. Each patient received an upper endoscopy to assess for evidence of upper gastrointestinal Crohn's disease, followed by two pharmacokinetic (pk) studies – oral (PO) methotrexate and subcutaneous (SC) methotrexate. During each study day, the patients received either PO or SC methotrexate followed by plasma collection at 11 pre-specified time points over a 24 hour period. Methotrexate plasma drug concentrations were then obtained using a sensitive mass spectrophotometer.
Results: Three patients have been enrolled in this ongoing study. All three had normal upper endoscopies with normal duodenal biopsies. The mean half-life was 2.8 hours and 3.2 hours for PO and SC methotrexate, respectively. The mean time to maximum plasma concentration was 1.2 hours and 0.5 hours PO and SC methotrexate, respectively. The area under the curve ratio (PO/SC) was 0.821. There were no adverse events.
Conclusions: This unique study demonstrates that the pharmacokinetic parameters for PO and SC methotrexate were comparable in three patients with Crohn's disease. Oral methotrexate would be a more convenient, safer, and less expensive option for these patients.

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Cardiac effects of hyperthyroidism prevented by concomitant amiodarone administration during mistaken thyroid supplementation

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Conflict of Interest: None declared

Background: Hypo-hyper-thyroid states occur more often in pts taking amiodarone. Its bi-iodinated structure increases iodine stores in pts and inhibits conversion of T4 to T3. It takes >1 year for this iodine to leave the body after stopping drug.
Objectives: To illustrate that not stopping amiodarone when thyroid abnormalities are diagnosed can have beneficial effects.

Methods: A 53-year-old man treated for atrial fibillation (AF) was monitored regularly (EKG, thyroid, liver, and serum drug monitoring). At 30 mo of therapy, he remained asymptomatic in sinus rhythm, but with elevated freeT4 (42 pmol/L - ULN 25 pmol/L). He was prescribed methimazole 5 mg TID and continued amiodarone.
Results: By mistake, liothyronine 5 µg TID was dispensed without detection for 57 d. Despite this, the pt remained asymptomatic with HR <70 for 53 d before having breakthrough AF when freeT4 reached 93 (3.7 x ULN). Total T3 showed a comparatively minor increase to 3.1 (1.1 x ULN of 2.8 nmol/L).
Conclusion: Although this pt went without prescribed antithyroid treatment, he suffered no hyperthyroid symptoms for 53 d. It appears that amiodarone mitigated the effects of rising T4 by preventing a parallel rise in T3 and maintaining sinus rhythm <70. After 3 mo of methimazole, freeT4 normalized and remains at 20. Hyperthryoidism with amiodarone needs careful monitoring and treatment, but is usually limited to 2-8 mo duration. Stopping amiodarone does not lead to rapid depletion of iodine stores. However, continuing amiodarone at effective doses may prevent many of the cardiovascular consequences of hyperthyroidism.

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Serum antibody titres against influenza virus in pediatric patients after treating with peramivir

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Conflict of Interest: None declared

Background: Influenza virus infection is known to be basically self-limiting; however, severe condition, such as respiratory failure and encephalopathy, can sometimes occur especially in high-risk group of patients, including children, and thorough treatments are required. Peramivir (Rapiacta®, Shionogi & Co., Ltd. / BioCryst), a new neuraminidase inhibitor, administering intravenously, was approved for adults and a clinical trial for children was conducted in Japan during the 2009-2010 influenza season. The data from the trials showed that peramivir is effective and safe enough to use in both adults and children with influenza virus infection.
Objectives: Because it is highly effective and the time to recover from the infection is shortened after the administration of mostly one dose, it was uncertain whether a patient obtains serum antibody against influenza virus after treating with peramivir.
Materials /Methods: A multicenter and open-label clinical trial without a control group was conducted in children with influenza virus infection during the 2009 pandemic A (H1N1) influenza epidemic in Japan to evaluate the efficacy and safety of peramivir. Peramivir was given intravenously at a dosage of 10 mg/kg (600 mg maximum) once daily. Nine patients (4 m/o - 15.5 y/o, median: 1.2 y/o, male/female: 3/6) were enrolled in our facility and were treated with peramivir within 48 hours (5.6 - 28.6 hours, median: 16.1 hours) after developing fever. Eight patients had single dose and one (4 m/o) had second dose due to persistent fever. Blood samples were provided before and after the administration, and serum was separated from each sample and analyzed for the titre of antibody against the 2009 pandemic A (H1N1) influenza virus strain in the hemagglutination inhibition (HI) test.
Results: Ten samples from seven patients were obtained before and next day of the administration (within 60 hours after the onset), and all of them showed negative HI titres less than 1: 10. Four samples from four patients were provided 5 - 7 days after the onset; two showed HI titres 1: 10 and the other two showed less than 1: 10. Nine samples from each patient 1 - 2 months after onset were investigated; all showed positive HI titres (1: 40 – 1: 640, median: 1: 160).
Conclusions: Although peramivir produces rapid recovery from influenza virus infection, it does not seem to interfere with obtaining antibodies in blood.

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Personalizing tamoxifen therapy for breast cancer patients

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Background: Tamoxifen, a standard endocrine therapy for estrogen receptor positive breast cancer, requires hepatic metabolism by cytochrome P450 2D6 (CYP2D6) to be converted to its active metabolite, endoxifen. Genetic variability and drug interactions can cause alterations in CYP2D6 activity. Studies indicate that plasma levels of endoxifen are significantly lower in CYP2D6 poor metabolizers (PMs) and that PMs have worse outcomes including recurrence rates and survival compared to EMs. However, recently there has

been conflicting evidence regarding the usefulness of CYP2D6 genotyping for tamoxifen therapy.
Objectives/Methods: We hypothesize that a patient's CYP2D6 genotype and plasma endoxifen level together will better predict their success on tamoxifen. We are collaborating with the London Regional Cancer Program to refer patients on tamoxifen to provide a blood sample for CYP2D6 genotyping by TaqMan assays and drug level analysis of tamoxifen and endoxifen by LC-MS/MS.
Results: We observe that PMs have the highest tamoxifen plasma concentration compared to intermediate (IMs) and extensive metabolizers (EMs). Endoxifen levels for IMs and PMs are significantly lower than EMs. We observe a 12-fold variability in endoxifen levels among EM patients, suggesting that other factors in addition to CYP2D6 contribute to endoxifen concentration. We are examining the effects of interacting medications by drug level monitoring pre- and post-drug change and dose escalation of tamoxifen in patients within the lowest quartile of endoxifen levels.
Conclusions: Low endoxifen levels irrespective of CYP2D6 genotype may indicate a lack of benefit of tamoxifen therapy and may be a better predictor of therapeutic outcome.

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Pharmacogenetics of post partum management - a single center experience

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Background: Codeine, a common opioid prescribed for pain post c-section, is biotransformed by the highly polymorphic Cytochrome P450 enzyme 2D6 (CYP2D6). Ultrarapid metabolizers (UM), individuals with multiple active copies of CYP2D6, can biotranform up to 50% more codeine into morphine than normal, resulting in adverse reactions to codeine. In contrast, poor metabolizers (PM), individuals who have no active CYP2D6 genes, convert almost no codeine into morphine and as a result may take multiple doses of codeine without attaining analgesia. It would be optimal if we could titrate a mother's codeine dose depending on her level of pain and CYP2D6 genotype.
Objective: To model the pharmacodynamic effect of codeine on pain levels in CYP2D6 retrospectively-genotyped women recovering from c-section.
Methods: Forty-five codeine-prescribed mothers provided a blood sample for CYP2D6 genotyping and recorded their pain level 4x/day for 3 days immediately following a c-section. Genotyping was completed once mothers had been discharged from hospital. Codeine doses and times were recorded, retrospectively adjusted and modeled using the CYP2D6 genotype activity score developed by Gaedigk et al and the pharmacokinetic data from Kirchheiner *et al.*
Results: No correlation (n=45, spearman's rho= 0.034, p=0.827) was found between Area under the VAS-time Curve (AUC) for pain and genotype-adjusted codeine dose for the group. However, women at the genetic extremes reported codeine effects consistent with previous literature. The 2 PMs of codeine reported no analgesia as a result of taking codeine, while two of the three UMs reported immediate pain relief from codeine, but stopped taking it due to adverse affects (i.e. nausea, lightheadedness and constipation).
Conclusion: A model to predict the analgesia affect of codeine is more complex than its CYP2D6 genotype score alone.

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Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children

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Conflict of Interest: None declared

Background: Anthracycline-induced cardiotoxicity (ACT) is a serious adverse drug reaction limiting its use and causing substantial morbidity and mortality in childhood cancer survivors. High cumulative doses are known to increase cardiotoxicity risk, but genetic factors are suspected to be important as well given the high inter-individual variability in tolerated doses.
Objective: Our aim was to identify genetic variants associated with ACT in patients treated for childhood cancer.
Methods: We carried out a genetic association study using 2977 single nucleotide polymorphisms (SNPs) in 220 key drug biotransformation genes in a discovery cohort of 156 anthracycline-treated children from British Columbia, with replication in a second cohort of 188 pediatric oncology patients from across Canada as part of the Canadian Pharmacogenomics Network for Drug Safety (CPNDS).
Results: We identified a highly significant association of a coding variant in a transporter gene with ACT (P=1.0x10^{-4}). We found further evidence (P<0.01) for associations with risk and protective variants in other genes including several other transporters. Combining these variants with important clinical risk factors in a predictive model, we classified patients into three risk groups. In the high-risk group, 75% of patients were accurately predicted to develop ACT, with 36% developing ACT within the first year; whereas in the low-risk group, 96% of patients were accurately predicted not to develop ACT.
Conclusions: We have identified multiple genetic variants associated with ACT. Combined with clinical risk factors, genetic risk profiling can be used to identify high-risk patients who can then be provided with safer treatment options.

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Study of Natural Health Product Adverse Reactions (SONAR): piloting an active surveillance model in community pharmacies

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Background: Many consumers use natural health products (NHPs) concurrently with prescription medications. As NHP-related harms are under-reported through passive surveillance, the safety of concurrent NHP-drug use remains unknown.
Objective: To assess the feasibility of active surveillance in participating community pharmacies to identify adverse events related to concurrent NHP-prescription drug use.
Methods: Participating pharmacists asked individuals picking up prescription medications about 1) concurrent NHP/drug use in the previous three months and 2) the presence of potential adverse events. If a potential adverse event was identified and the patient agreed, a research pharmacist conducted a guided telephone interview to gather additional information.
Results: Over a total of 112 pharmacy weeks, 2615 patients were screened, of which 1037 (39.7%; 95% CI: 37.8% to 41.5%) reported concurrent NHP and prescription medication use. A total of 77 patients reported a possible AE (2.94%; 95% CI: 2.4% to 3.7%), which represents 7.4% of those using NHPs and prescription medications concurrently (95%CI: 6.0% to 9.2%).
Conclusion: Compared to passive surveillance, this study found active surveillance to markedly improve NHP adverse event reporting rates. Active surveillance is feasible and offers improved quantity and quality of adverse event data, allowing for meaningful adjudication to assess potential harms.

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Adherence measurement methods among HIV positive adolescents in Uganda: a prospective cohort pilot study

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Introduction: The quality of traditional adherence measurements among adolescents is difficult to assess. Antiretroviral (ARV) adherence research among adolescents living with HIV in resource-constrained countries is limited.
Objectives: Our primary objective was determining feasibility of a large-scale, long-term study using electronic adherence monitoring in Uganda. Our secondary objective was to compare accuracy of pill-count (PC) and self-report (SR) adherence with electronic medication vials (eCAPs). eCAP's record compliance in real-time with data downloaded during refills.
Methods: Adolescents receiving care at the Joint Clinical Research Centre in Kampala, Uganda were recruited. ARV's were dispensed in eCAPs for 1 year. Person-pill-days (one day where adherence was measured for one medication in one person) were calculated for each patient and a weighted paired t-test was used to compare the levels of adherence among all subjects for three different adherence methods.
Results: Fifteen patients were included: 40% were female, mean age was 14, mean baseline CD4 count was 244, and average treatment duration was 9 months at study entry. 4721 person-pill-days were observed. Several eCAPs required replacement during the study resulting in some data loss. Consent rate was high (94%) but was slow due to age limit cut-points, indicating that a future study should be multi-site. A longitudinal examination of the eCAP data showed that while most non-adherence among individual subjects was time-dependant (during times of poor adherence all medications were affected), some was drug-dependant (adherent to one medication but not others). Our small pilot sample precluded a regression analysis to further explore this effect. Overall compliance for SR was 99%, PC was 97% and eCAP was 88% ($p<0.05$ for all comparisons). 93%, 67% and 23% of patients had a compliance of greater than 95% among SR, PC and eCAP methods, respectively.
Conclusions: A large-scale adherence study in Uganda is feasible using a more robust electronic monitoring system. Adherence measurements produced by pill counts and self reporting methods appear to overestimate adherence measured electronically. However, overall adherence measured with all methods was still clinically acceptable.

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

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Background: The recent rise in rates of obesity, diabetes and metabolic syndrome among the Western population, has caused a parallel increase in the prevalence of non-alcoholic fatty liver disease (NAFLD). A defining characteristic of NAFLD is steatosis within hepatocytes. Despite the high incidence, little is known regarding the effect of NAFLD on hepatic drug metabolism and its impact on drug response. We hypothesize that hepatic CYP3A activity is altered as a result of fat accumulation, leading to changes in the pharmacokinetics of everyday drugs.
Objective: Establish an *in vitro* model of human NAFLD to study regulation of expression and activity of CYP3A4.
Methods: To better understand the mechanisms involved in the regulation of CYP3A4 in NAFLD, we will use the Huh7 human hepatoma cell line known to natively express CYP3A4. In the cell model, Huh7 cells are incubated with free fatty acids to induce fat overloading similar to that found in steatotic liver. Lipid quantification, cytotoxicity and CYP3A4 mRNA and protein expression will be examined. Furthermore, biotransformation activity will be assessed by measuring the metabolism of midazolam, a probe drug substrate for CYP3A4 enzymes.
Results: Preliminary data from our laboratory indicates that patients with NAFLD have altered CYP3A4 expression and activity.
Conclusions: It is expected that this in vitro model of human NAFLD will provide the platform for further studies aimed to define the signaling pathways involved in regulating CYP3A4 activity. Together with studies in patients, these studies may provide a basis for better and safer pharmacotherapy in NAFLD.

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Hydrogen sulfide mediates the inhibitory effect of sulforaphane on the proliferation of human prostate cancer cells

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Background: Hydrogen sulfide (H_2S) is a novel gasotransmitter that regulate cell proliferation and other cellular functions. Sulforaphane is a sulphur-containing compound that exhibits anticancer properties, and young sprouts of broccoli are particularly rich in sulforaphane. There is consistent epidemiological evidence that the consumption of sulphur-containing vegetables, such as garlic and cruciferous vegetables, may help reduce the occurrence of prostate cancer.
Objectives: We want to determine whether H_2S mediates the anti-survival effect of sulforaphane on prostate cancer and the underlying mechanisms.
Methods: H_2S level, cell viability, and protein expression were measured by methylene blue method, MTT assay, and western blotting, respectively.
Results: A large amount of H_2S is released from sulforaphane when sulforaphane was injected into mice or added into the cell culture medium or mixed with mouse liver homogenates. Both sulforaphane and NaHS (a H_2S donor) decreased the viability of PC-3 cells (a human prostate cancer cell line) in a dose-dependent manner, and supplement of methemoglobin or oxidized glutathione (two H_2S scavengers) reversed sulforaphane-reduced cell viability. NaHS also significantly inhibited PC-3 cell migration. We further found both cystathionine gamma-lyase (CSE) and cystathionine beta-synthase are expressed in PC-3 cells and mouse prostate tissues. H_2S production in prostate tissues from CSE knockout mice was only 20% of that from wild-type mice, suggesting CSE is a major H_2S-producing enzyme in prostate. CSE overexpression enhanced H_2S production and inhibited cell viability in PC-3 cells. In addition, sulforaphane and NaHS activated p38 mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinase (JNK). Pre-treatment of PC-3 cells with methemoglobin decreased sulforaphane-stimulated MAPK activities. Suppression of both p38 MAPK and JNK reversed H_2S- or sulforaphane-reduced viability of PC-3 cells.
Conclusions: Our results demonstrated that H_2S mediates the inhibitory effect of sulforaphane on the proliferation of PC-3 cells, which suggests that H_2S-releasing diet or drug might be beneficial in the treatment of prostate cancer.

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