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ABSTRACTS:
CANADIAN SOCIETY OF PHARMACOLOGY AND THERAPEUTICS 2014 SCIENTIFIC MEETING PROGRAM

IN CONJUNCTION WITH THE CANADIAN STUDENT HEALTH RESEARCH FORUM (CSRHF)

June 10-13, 2014
University of Manitoba
Brodie Centre, Apotex Centre and
Ambassador Room in Canada Inns
Winnipeg, MB

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1

Effects of cyclic cadherin peptide, Ala-Asp-Thr (cADT) on blood brain barrier (BBB) integrity: Potential applications for enhancing drug delivery to the brain

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Objectives: To examine the effects of a cyclic cadherin peptide, containing the Ala-Asp-Thr (cADT) sequence, on BBB integrity.
Methods: The effect of cADT peptide on BBB permeability was examined in Balb/c mice specifically focusing on both the time frame for modulation of BBB permeability and the magnitude of BBB disruption. Blood-brain barrier permeability was assessed with gadolinium contrast agent (Gd-DTPA; a small hydrophilic permeability marker), under control conditions and following exposure to cADT (0.1 -32 □mol/kg) using magnetic resonance imaging (MRI). Administration of imaging agents and cadherin peptide was done through bolus tail vein injections.
Results: Under control conditions, very little Gd-DTPA entered the brain. Mice treated with cADT displayed a dose-dependent increase in BBB permeability as assessed with Gd-DTPA enhanced MRI with doses of 0.1□mol/kg having a minimal effect on enhancement (4-fold) and 32 mol/kg producing maximal increases (14-fold) in Gd-DTPA entry into the brain. The increase in BBB permeability was rapid, occurring within 6-9 minutes following the administration of the cadherin peptide. While there were regional differences in baseline BBB permeability, the cADT peptide produced similar increases in BBB permeability throughout all regions examined. The cADT peptide produced increases in BBB permeability that lasted for more than 2 hrs following the injection of the peptide. Complete restoration of BBB integrity was observed within 4-6 hrs of cadherin peptide administration.
Conclusions: The cyclized cadherin peptide produced a rapid and reversible increase in BBB permeability. The use of the cadherin peptides in combination with therapeutic agents can enhance drug delivery to the brain.

2

The role of adiponectin deficiency in the development of gestational diabetes mellitus

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Objectives: To observe the relationship between adiponectin deficiency and the development of gestational diabetes using adiponectin knock-out mice and high-fat and sucrose diet induced gestation diabetes mellitus (GDM). Previously we have shown that adiponectin is reduced in rats with GDM, and we propose that adiponectin null mice will be at higher risk for development of GDM.
Methods: To induce GDM in pregnant mice, female mice are fed a high fat and sucrose (HFS) diet and are compared to low fat-fed lean controls. Adiponectin knockout mice (strain B6;129-Adipoqtm1Chan/J) are used as a model of adiponectin deficiency and compared to C57/BL6 wild type control mice. The female mice are bred with males of the same genotype. Weight and food consumption are monitored weekly, and blood was collected at each trimester of pregnancy to measure insulin and adiponectin levels. To assess insulin sensitivity, a glucose tolerance test was performed in the third trimester.
Results: Pregnant adiponectin knockout mice exhibited higher fasting blood glucose and insulin levels compared to their respective wild-type controls. In addition, adiponectin knockout mice had impaired glucose tolerance. Body weights and food consumption during pregnancy were not significantly different between the genotypes.
Conclusions: Pregnant adiponectin knockout mice have high fasting blood glucose, hyperinsulinemia and severely impaired glucose

tolerance relative to wild type controls, which is characteristic of GDM. Our results suggest that adiponectin deficiency contributes to the development of GDM.

3

The influence of shape on cellular uptake and magnetic targeting of iron oxide nanoparticles

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Objectives: Our laboratory is examining iron oxide nanoparticles (IONPs) as a potential platform for increasing drug delivery to the brain. While much effort has focused on grafting ligand onto the IONP surface to enhance cellular uptake in brain endothelial cells, herein we report alteration of IONP shape can influence both the preferential cellular uptake in endothelial cells and the ability to augment cell uptake with application of an external magnetic field.
Methods: Nanosphere and nanoplatelet shaped IONPs with the same size and negative surface charge were synthesized. Cellular uptake profiles of nanosphere and nanoplatelet IONPs were compared in brain and lung endothelial cells, as well as liver, intestine, and kidney epithelial cells. Quantitative determination of cellular IONP was performed using ferrozine assay. Transmission electron microscopy (TEM) was used to confirm the internalization of IONPs. Toxicity of both IONP compositions was determined using MTT assay.
Results: Uptake of nanospheres was minimal in all cells tested with or without magnetic field exposure. In contrast, a 3-fold enhancement in internalized nanoplatelets was observed in endothelial cells compared to nanosphere compositions. Exposure of external magnetic field increased nanoplatelet uptake in endothelial cells by 10 fold. The uptake of nanoplatelets in epithelial cells was 3 times lower than endothelial cells. TEM confirmed preferential uptake of nanoplatelets in endothelial cells. No toxicity was observed in endothelial cells treated with nanoplatelets.
Conclusions: Nanoplatelets have improved cellular uptake profiles compared to nanospheres. The increased cellular uptake of the nanoplatelets was especially apparent in endothelial cells. The increased magnetic properties of the nanoplatelets result in enhanced uptake in the presence of an external magnetic field. These properties may allow for better cellular penetration across capillary beds such as the blood-brain barrier. Support provided by NSERC-CIHR Collaborative Health Research Project.

4

Synthetic CXCR4 chemokine receptor agonists alter the pathway dogma of chemotaxis

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Objectives: The recent discovery of synthetic agonists of the CXCR4 allowed for the first time to identify potent biased ligands (ACS Med Chem Lett. 2011 Aug 11;2(8):597-602). CXCL12, the endogenous agonist ligand of CXCR4, was reputed to induce chemotaxis through CXCR4 by Gi and beta-arrestin recruitment. Synthetic full agonists with low nM affinity were produced through grafting the CXCL12 pharmacophore, its N-terminal peptide portion, onto T140, the cyclopeptide antagonist of CXCR4.
Methods: Figure: Typical synthetic CXCR4 agonist with 4 nM affinity and full agonist properties on chemotaxis and Gi activation.
Results: These agonists displayed chemotactic behaviour like CXCL12, both in vitro and in vivo. Subsequent signalling pathway analysis showed that these agonists couple, like CXCL12, CXCR4 to Gi with nanomolar affinities. These synthetic agonists however do not induce beta-arrestin coupling and consequently, significantly reduce CXCR4 internalization upon CXCL12 stimulation.
Conclusions: The availability of biased agonists for these chemokine receptors will allow dissection of the hitherto not well-understood functional interactions of CXCR4. These agonists are also of interest as tools for structural biology investigations to provide molecular details on the mechanism of CXCR4 activation, which remains unknown despite the CXCR4 crystal structure. Finally, agonistic CXCR4 ligands possess several attractive features as alternatives for CXCR4 targeted drug therapy where antagonists are actually the only option; therefore synthetic transitions to partial and fully peptidomimetic agonistic moieties are under way.

5

Prevalence of heavy prenatal alcohol exposure in Uganda, Africa via analysis of fatty acid ethyl esters in meconium

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Objectives: The focus of this study is to measure the prevalence of heavy prenatal alcohol exposure during pregnancy by meconium fatty-acid ethyl esters (FAEEs) analysis in a population-based setting. Fetal alcohol spectrum disorder (FASD) manifests a continuum of permanent birth defects and neurodevelopmental impairments that originate from maternal alcohol use during pregnancy. Previous literature found that of the Ugandan women who drank regularly prior to pregnancy, only 18.3% reported abstinence, whereas an astonishing 11.1% increased alcohol use, and 68.3% decreased consumption. Thus, the number of FASD cases in parts of the sub-Saharan Africa is growing, which is a cause for concern due to the socio-economic impact.
Methods: Meconium samples were collected from 505 newborns between October-November 2013 at Mbarara Regional Referral Hospital. Each meconium sample was accompanied with a questionnaire containing neonatal/maternal information. FAEEs are non-oxidative metabolites of ethanol produced in the fetus are serves as an established biomarker for in utero alcohol exposure. FAEE meconium concentrations greater than 2.00 nmol/g are considered indicative of heavy prenatal alcohol exposure during the last two trimesters of pregnancy. Samples were frozen and shipped to the Motherisk Laboratory for analysis. FAEEs were analyzed by gas chromatography-mass spectrometry and quantified using deuterated internal standards.
Results: Of the 505 samples, 68 have been analyzed by GC/MS to date, where 2 are inconclusive due to it being transitional with stool. The remaining 66 samples did not show heavy prenatal alcohol exposure. Meconium FAEE analysis is currently ongoing at Motherisk Laboratory.
Conclusions: The identification of specific populations in need of basic epidemiologic research on the prevalence of prenatal alcohol exposure is urgently required in order to create awareness and reduce the devastation of FASD. This is the first population-wide study of an entire neonatal population examining fetal alcohol exposure conducted in Uganda.

6

Barth syndrome and the use of monolysocardiolipin acyltransferase-1 as a potential therapeutic agent

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Objectives: 1.) Examine the effects of tafazzin alteration in vitro and ex vivo. 2.) Determine the effects of MLCL AT-1 expression in healthy and BTHS lymphoblasts.
Methods: Eppstein-Barr virus transformed lymphoblasts from healthy and BTHS patients were transfected with TAZ or MLCL AT-1 RNAi or an MLCL AT-1 carrying plasmid. TAZ and MLCL AT-1 gene expression was analyzed using RT-PCR. Mitochondrial fractions from both cell lines were used to study MLCL AT-1 enzyme activity. Linoleic acid incorporation into CL was also studied by using [14C] Linoleic acid and then radioactivity incorporated into CL determined. CL was also isolated from healthy and BTHS lymphoblasts and CL mass was measured using phospholipid phosphorous analysis. Blue native polyacrylamide gel electrophoresis (BN-PAGE) was used to analyze mitochondrial supercomplexes in healthy and BTHS lymphoblasts, as well as in tissues from wild-type and Taz knockdown mice. Mitochondrial function was analyzed in healthy and BTHS lymphoblasts using a Seahorse XF 24 Extracellular Flux Analyzer.
Results: MLCL AT-1 gene expression increased when TAZ was knocked down. Expression of MLCL AT-1 is able to increase MLCL AT-1 enzyme activity and CL mass in healthy and BTHS lymphoblasts. No significant change in [14C] linoleic acid incorporation into CL was observed. Mitochondrial supercomplex formation is disturbed in BTHS lymphoblasts, however, MLCL AT-1 expression is able to elevate mitochondrial supercomplex (and complex I) formation in these cells. In Taz knockdown mice, supercomplex formation is also disturbed. Our results also indicate the overall disturbance of mitochondrial function in BTHS lymphoblasts, but the use MLCL AT-1 is able to improve this effect.
Conclusions: The use of human lymphoblasts and the Taz knockdown mouse model will help us better understand the complexities of BTHS.

MLCL AT-1 expression may serve as a potential therapeutic approach to treat BTHS.

7

Examination of a protein kinase inhibitor library for alterations in P-glycoprotein expression and activity

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Objectives: ATP Binding Cassette (ABC) transporters play a critical role in absorption, distribution and elimination of a broad range of pharmaceuticals. Little is known about potential influences of kinase pathway modulation on expression and activity of ABC transporters. The present study analyzed a PKI library targeting over 25 different kinases, to determine the effect on activity and expression of P-glycoprotein (P-gp) in different representative cell lines.
Methods: Various cell lines, including MDCK-MDR1, MDCK-wt, Caco-2 and HBMEC were grown to confluency on 96 well plates. Cells were exposed to the PKIs (2 μM) for 24 hours, and uptake studies were performed using rhodamine 123 (R123) as a fluorescent probe for P-gp. Changes in efflux activity (EA) were determined and those PKIs producing a change in EA of 25% or more were considered hits. All PKI hits in the ABC transporter activity assay were examined for alterations in protein expression, using in-cell western analysis with P-gp antibody.
Results: A total of 33 out of the 360 PKIs screened in MDCK-MDR1 and MDCK-wt cells were identified as hits in the R123 P-gp activity assay. Nine of the 33 PKIs targeting GSK-3 resulted in an increase in P-gp EA. Only 5 of the 33 PKIs produced changes in P-gp expression in MDCK. The 33 PKI hits were also examined in Caco-2 and HBMEC cell lines. Only one and five of the 33 PKIs were identified as hits in Caco-2 and HBMEC, respectively. None of the hits from the R123 accumulation assay significantly altered P-gp expression in Caco-2 and HBMEC.
Conclusions: PKIs influenced P-gp activity in a cell line dependent manner. In many instances, changes in P-gp activity were not associated with changes in expression levels. Funding provided by NSERC Canada.

8

In vitro functional analysis of novel single nucleotide polymorphisms in OATP1B1

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Objectives: Statin-induced myopathy is a common adverse reaction experienced by patients on statin therapy. Patients who exhibit elevated plasma concentration of statins are thought to be at a greater risk for myopathy. Statins are transported from the blood to hepatocytes via organic anion transporting polypeptide 1B1 (OATP1B1). Single nucleotide polymorphisms (SNPs) in OATP1B1, primarily OATP1B1*5, have been shown to affect plasma statin concentrations. We hypothesized that there may be other SNPs in OATP1B1 that can also contribute to reduced transport activity and increased plasma statin levels.
Methods: OATP1B1 cDNA packaged in pEF6/V5-His TOPO was used as template, and 6 SNPs — 298G>A (rs144508550), 419C>T (rs147450830), 463C>A (rs11045819, *4), 1007C>G (rs72559747), 1463G>C (rs59502379, *9), and 1738C>T (rs71581941) — were introduced separately to wild-type templates using site-directed mutagenesis. OATP1B1 variant cDNAs were expressed in adenovirus. Transport activity was measured with prototypical substrates estrone-3-sulfate, estradiol-17-β-D-glucuronide, and rosuvastatin.
Results: Total uptake was decreased in 419C>T variant, increased in 1007C>G variant, unchanged in *4 variant, and completely abolished in 298G>A, *9, and 1738C>T variants compared to wildtype. Kinetic experiments showed a decrease in Vmax in 419C>T and *4 and an increase in Vmax in 1007C>G with all 3 substrates. Kinetic parameters could not be calculated for variants 298G>A, *9, and 1738C>T because of near complete absence of transport activity. Western blots showed total cellular expression of OATP1B1 was slightly decreased in 298G>A, 419C>T, *9, and 1738C>T, and slightly increased in 1007C>G compared to wildtype. Cell-surface fraction analysis also showed a similar trend.
Conclusions: Our data support the hypothesis that there are additional loss of function SNPs in OATP1B1. Additional clinical validation studies are ongoing. We also plan on carrying out Next-gen sequencing OATP1B1 among patients who exhibit significantly higher than expected statin levels which are not explained by the currently known SNPs in this transporter.

9

A novel ethambutol prodrug to optimize drug delivery to the central nervous system for the treatment of tuberculosis meningitis

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Objectives: The objectives of this study were to synthesize and structurally characterize a new ethambutol prodrug (ETB-CDS) using a Chemical Delivery System to improve the kinetic disposition of ethambutol for the treatment of tuberculosis meningitis (TM). We evaluated antimycobacterial and hydrolysis activities of ETB-CDS, and compared the pharmacokinetics profiles of ETB and ETB-CDS in rats.
Methods: Ethambutol (ETB) was covalently linked to nicotinic acid using dicyclohexylcarbodiimide as a coupling agent. The reaction product was then quaternized with methyl iodide to generate the pyridinium salt. The final step was the reduction of pyridinium moiety using sodium ditionite to produce ETB-CDS. All synthesized compounds were characterized and analyzed structurally by FTIR, C13NMR, H1NMR spectroscopy. The minimal inhibitory concentration (MIC) of ETB-CDS against M. tuberculosis H37Rv (ATTC 27294) was determined using a Microplate Alamar Blue Assay. Chemical and enzymatic hydrolysis studies of ETB-CDS were carried out using acetate and phosphate buffer solutions and human plasma over 24 hours (pH 1.2 and 7.4, respectively). Male Wistar rats (n=40) received either ETB (25 mg/kg) or ETB-CDS (25 mg/kg) via intravenous administration. Serial blood samples were collected for 120 minutes following drug administration. ETB and ETB-CDS plasma concentrations were determined by HPLC with electrochemical and UV-Vis detection, respectively.
Results: ETB-CDS was synthesized with global yields of 10.7% and its structure was identified using spectral data. The prodrug inhibited M. tuberculosis growth with a MIC of 31.25 µg/mL and had a higher partition coefficient than the parent drug. ETB-CDS remained stable at different pHs in the chemical and enzymatic assays. Compared to ETB, ETB-CDS demonstrated a slower clearance (201 vs 75 mL/min/Kg, $p<0.05$) and longer elimination half-life (11.6 vs 33.3 min, $p<0.05$). ETB-CDS had a larger AUC [0-∞] (296.6 vs 130.5 µg/mL/min, $p<0.05$), and larger Vd than ETB (6.2 vs 3.6 L/Kg, $p<0.05$).
Conclusions: Our results suggest that ETB-CDS can be a promising candidate for the treatment of TBM. ETB-CDS has a lipophilic pharmacokinetic profile, which may improve its ability to penetrate the blood-brain barrier.

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Evaluating the transfer mechanism of rivaroxaban across the dually perfused human placenta

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Objectives: The objectives of our study were to determine the transplacental pharmacokinetics at term of the oral anticoagulant, rivaroxaban, in order to estimate fetal drug exposure, and determine if passive diffusion was the primary mechanism of rivaroxaban transfer across the human placenta.
Methods: Placentae were obtained with informed consent after caesarean delivery of uncomplicated term pregnancies. The transplacental pharmacokinetics of rivaroxaban were measured using dual perfusion of an isolated placental lobule ex vivo. Rivaroxaban, at a concentration of 250 ng/ml, was added to the maternal or fetal circulation only and samples were taken during the pre-experimental (1 h) and experimental (3 h) phases for measurement of rivaroxaban and markers of placental viability. Additional perfusions were conducted under equilibrative conditions with the addition of rivaroxaban to the maternal and fetal circulations at a concentration of 250 ng/ml. Rivaroxaban levels were detected using liquid chromatography-mass spectrometry (LC/MS).
Results: There was rapid transfer of rivaroxaban across the human placenta in both the maternal-to-fetal and fetal-to-maternal directions, as evidenced by transfer ratios of 0.72 ± 0.12 (n=5) and 0.69 ± 0.03 (n=2) after 3 hours. Under equilibrative conditions, rivaroxaban concentrations remained relatively constant, suggesting that rivaroxaban crosses the placenta down a concentration gradient. Placental viability markers remained within normal physiological ranges.
Conclusions: This is the first direct evidence of rapid transfer of rivaroxaban across the human placenta from the mother to fetus and fetus to mother. The results suggest that rivaroxaban crosses the placental barrier via passive diffusion.

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Cisplatin nephrotoxicity in Mexican children with solid tumors

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Objectives: To evaluate the prevalence and severity of cisplatin nephrotoxicity in Mexican children and too determine the impact of nephrotoxicity in growth.
Methods: Retrospective study in 51 children treated with cisplatin for solid tumors. From the clinical chart the information about height, serum creatinine and electrolytes in each cisplatin cycle and after 12 months of treatment was recorded. Nephrotoxicity was graded as follows, 0: normal renal function, 1: asymptomatic electrolytes disorders in blood work, grade 2: need for electrolyte supplementation and/or increase in serum creatinine 1.5-1.9 times from baseline, grade 3: increase in serum creatinine 2-2.9 times from baseline or need for electrolyte supplementation for more than 3 months after treatment completion, grade 4: renal replacement therapy.
Results: 38 patients had nephrotoxicity –NTX- (74.5%). Hypophosphatemia was found in 34 patients, hypomagnesemia in 21, hypokalemia in 18 patients. Grade 1 NTX was observed in 16 and Grade 2 in 22. NTX patients were younger than patients with no-NTX (mean age 6.1 ± 4.5 years vs. 11.52 ± 5.4 respectively, p=0.001), received higher cisplatin total dose (mean accumulated dose in NTX 455mg/m2 vs. 372mg/m2 in no-NTX, p=0.01). There was no change in z Score for height (baseline vs. 12 months) in no-NTX patients, NTX Grade 1 had a significant worsening of the height (z Score baseline -0.39± 0.2 vs. -1.1 ±0.3, paired t test p=0.006), Grade 2 non-significant (p=0.056). We found a negative significant correlation between cisplatin total dose and serum magnesium levels at 12 months (Spearman r=-0.521, p=0.003).
Conclusions: NTX prevalence was 74.5%. NTX patients were younger and received higher cisplatin dose than those with normal renal function. Patients with untreated electrolyte anomalies (NTX Grade 1) had a significant worsening of height z Score at 12 months.

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Risks of congenital malformations in offspring exposed to valproic acid in utero: Emergence of the signals over the last 30 years

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Objectives: Over the last 30 years, valproic acid (VPA) has been suspected as a human teratogen, based on reports of case-control and cohort studies. Since 2009, three large-scale registry-based studies reported significant association between VPA exposure in utero and the increased risk of major congenital malformations (major CMs) including open neural tube defects. The recent surge of data quantity prompted us to systematically analyze the published data to address: 1) time profiles of emergence of the VPA teratogenicity signals over the last 30 years; and 2) risk estimates of specific CMs associated with VPA.
Methods: A systematic literature search was conducted on Medline, Embase classics plus Embase, and Cochrane Central Register of Controlled Trials between 1946 to November 2013. The search terms were regarding 'congenital malformations', 'pregnancy', and 'valproic acid'. Cumulative and conventional meta-analyses were performed with EZR version 1.21. Pooled relative risk (RR) and 95% confidence intervals of VPA monotherapy-associated risks of major CMs, compared to other antiepileptics, were calculated with fixed effects models.
Results: We identified 55 cohort studies. Cumulative meta-analyses showed that the risk of combined major CMs has been statistically significant since 1990. Signals of significant risks of specific major CMs (neural tube defects, congenital heart defects, cleft lip/palate, genitourinary anomalies and musculoskeletal defects) all emerged between 1992 and 2006. Conventional meta-analyses showed that RR of VPA-associated major CMs were 2 to 7 fold, compared to the patients with other antiepileptic drug exposure. Sensitivity analyses confirmed the stability of the results.
Conclusions: The risk signals of major CMs associated with VPA became significant more than 10 years ago. VPA exposure in utero poses 2 to 7 fold increased risks of major CMs, compared to the other antiepileptic drugs exposure.

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Profound reduction in the tamoxifen active metabolite endoxifen in a patient on phenytoin for epilepsy compared to a CYP2D6 genotype matched cohort

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Objectives: Tamoxifen is a prodrug, requiring cytochrome P450 enzyme-mediated metabolism to form the active metabolite endoxifen. Our Personalized Medicine clinic assessed a 49-year-old woman, genotyped as a cytochrome P450 2D6 (CYP2D6) extensive metabolizer, chronically taking phenytoin for a seizure disorder, who initiated tamoxifen therapy two months prior for estrogen receptor positive breast cancer. Phenytoin would be expected to induce the metabolism of tamoxifen, although there are no previous data demonstrating the ultimate effect on endoxifen levels. We sought to determine whether such a drug-drug interaction would be detrimental by measuring endoxifen and intermediate metabolites in this patient, compared to other patients from our clinic matched for genotype and clinical variables.
Methods: Tamoxifen and metabolite levels including endoxifen levels were measured by liquid chromatography–mass spectrometry/mass spectrometry for all patients seen in our clinic taking tamoxifen, expected to be at steady state. The levels measured for the patient taking phenytoin were compared to all patients with a CYP2D6 extensive metabolizer genotype (n=195), and to patients matched for CYP2D6 *1/*41 genotype as well as age, height, and weight (n=8).
Results: The patient had an endoxifen level of 4.7 nmol/L, the lowest our clinic has seen in an extensive metabolizer, and 6-fold lower than the median of the matched controls. Tamoxifen and intermediate metabolite ratios were also reduced, although to a lesser degree compared to the matched controls, suggesting alteration in pharmacokinetics, and not lack of compliance, led to the reduced endoxifen level.
Conclusions: To our knowledge, this is the first report documenting the consequences of induction in terms of both tamoxifen and endoxifen levels during concomitant phenytoin therapy, and this effect would likely result in loss of therapeutic benefit from tamoxifen. Phenytoin should therefore not be used concurrently with tamoxifen for extended periods of time unless a therapeutic drug (endoxifen) monitoring strategy is utilized.

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The role of equilibrative nucleoside transporter 3 (ENT3) in regulating adenosine levels within brain

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Objectives: Adenosine is a signaling molecule acting via cell surface G-protein coupled receptors. In brain, adenosine has neuroprotective, anticonvulsant and sedative properties. However, the mechanisms which regulate adenosine concentrations are poorly understood. Four members of the equilibrative nucleoside transporter family have been identified (ENT1-4) where ENT1 and 2 have been best characterized to date. The current study was initiated to explore the role of ENT3 in regulating adenosine levels.
Methods: Mouse ENT3 gene sequence was inserted into pIRES puro-flag plasmid. HEK293T cells were transiently transfected then used for western blot analysis with a commercial ENT3 specific antibody and an antibody specific for the flag epitope. Mouse brain was dissected and mRNA was isolated from cortex, cerebellum, striatum and hippocampus. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed using ENT3 specific primers designed to amplify a 245 bp product. The ENT3 specific antibody was used in western blot analysis of proteins isolated from the dissected regions of mouse brain. Rat C6 glioma cells, stably transfected with ENT3 or with vector alone, were exposed to hypoxic conditions and extracellular adenosine levels were quantified.
Results: Western blot analysis performed using ENT3-transfected and wild type HEK293T cells showed a 40kD band in transfected cells only that was detected with either the ENT3 specific or the anti-flag antibody. RT-PCR analysis was positive for expression of ENT3 in all brain regions tested. Western blot analysis of dissected mouse brain regions, using the ENT3-specific antibody, showed a major band at 52kD and a minor band of 30kD. Preliminary results indicate that ENT3 transfected C6 glioma cells released less adenosine in hypoxic conditions than vector transfected cells.
Conclusions: Our results indicate that ENT3 is widely expressed in mouse brain and preliminary results indicate a role for ENT3 in intracellular sequestration of adenosine in hypoxic conditions.

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Clinical & molecular determinants for optimal factor xa inhibitor therapy

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Objectives: Oral anticoagulants (OACs) are important to stroke prevention for patients with atrial fibrillation. Suboptimal anticoagulation can place patients at risk for a thrombotic event, while excessive anticoagulation can result in serious to fatal bleeding events. Although warfarin had been the main drug of choice for anticoagulation, newer agents that do not require routine monitoring of anticoagulation efficacy are now widely prescribed. Rivaroxaban and apixaban, belong to a new generation of Factor Xa inhibitors (FXIs) that have been approved as alternate therapies to warfarin. However, outside of clinical trials, interpatient variation in OAC blood level or the extent of Factor Xa inhibition has not been assessed. Therefore the objectives of this study are to examine the extent of interpatient variability in the plasma concentration rivaroxaban in patients on this agent, to better delineate the role of clinical as well as pharmacogenetic variables as determinants for identifying patients at risk for excessive as well as subtherapeutic response to these newer OACs.
Methods: We assessed rivaroxaban level in n = 33 patients who were seen in our anticoagulation clinic. Rivaroxaban levels were measured using liquid chromatography-tandem mass spectrometry. Factor Xa activity was measured using the Biophen Direct Factor Xa Inhibitor® (DiXaI) assay. Pharmacogenetic testing for CYP enzymes and transporters will be carried out using TaqMan SNP genotyping assay.
Results: Substantial interpatient variation was observed. Forty-two percent of patients exhibited rivaroxaban level > 95% confidence interval. The pharmacological effect of FXIs is closely correlated to their blood levels.
Conclusions: There is far greater variation in the observed plasma level of FXIs than currently realized. As we enroll additional patients into our study for rivaroxaban as well as apixaban, genetic variation in drug metabolizing enzymes and transporters that predict decreased or increase systemic drug exposure to these agents will also be more fully clarified.

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Regulation of multidrug transporter (BCRP/ABCG2) expression in the mammary gland during lactation

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Objectives: The multidrug transporter ABCG2 is upregulated in the lactating mammary gland where it concentrates drugs/toxins into breast milk. However, the mechanism for this induction is not well understood. Here we sought to explore the role of epigenetics and STAT5 in the regulation of Abcg2 in the lactating mouse mammary gland.
Methods: The expression profile of Abcg2 mRNA isoforms (E1a, E1b, and E1c) in the mouse mammary gland was examined using quantitative RT-PCR. Promoter DNA methylation status was assessed by bisulfite pyrosequencing. Published ChIP-seq datatsets were used to identify regions along the Abcg2 gene that was enriched with the open chromatin histone mark H3K4me2 or bound by the transcription factor STAT5 in the lactating mammary gland. STAT5 recruitment to the mouse Abcg2 gene was further assessed using a forced weaning mouse model in which pups were removed from the lactating mother to quickly turn off STAT5 activity in the mammary gland. These STAT5-binding regions were also investigated for prolactin-induced enhancer activity using luciferase reporter assays.
Results: The E1b isoform was the major isoform expressed in the mouse mammary gland and was induced during lactation. The E1b promoter was hypomethylated and marked with H3K4me2 in both the virgin and lactating mammary gland. However, the E1b/E1c promoter region was more enriched with H3K4me2 during lactation. STAT5 was bound to five regions along the Abcg2 gene during lactation. The recruitment of STAT5 to these regions was significantly reduced after forced weaning. At least one of these STAT5 binding regions, which contain a putative gamma interferon-activated sequence (GAS) motif, was responsive to prolactin treatment.
Conclusions: The mouse Abcg2 gene is already poised for expression in the virgin mammary gland. Therefore, the upregulated expression of Abcg2 in the mammary gland during lactation is likely due to the recruitment of activated STAT5 to the Abcg2 gene.

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Steady-state folate pharmacokinetics in pregnancy: A randomized clinical trial in pregnant women supplementing with 1.1mg vs. 5mg folic acid

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Objectives: Folic acid supplementation during the periconceptional period reduces the risk of neural tube defects. While current prenatal recommendations are based on studies in non-pregnant women of childbearing age, this is the first study aiming to assess steady-state periconceptional and gestational RBC and plasma folate levels in pregnant women supplementing daily with prenatal multivitamins containing 1.1mg (regular dose) vs. 5mg (high dose) folic acid.
Methods: 37 women, between 18-45 years of age, who were early in pregnancy or trying to conceive, and were not previously taking folic acid-containing supplements, were enrolled in this open-label, 2-arm, randomized clinical trial after obtaining informed consent. Participants were randomized to take either 1.1mg or 5mg of folic acid-containing prenatal multivitamins daily till 30 weeks gestational age (g.a.). Plasma and RBC folate levels were measured at baseline, and at g.a.6, g.a.12 and g.a.30 using a chemiluminescent immunoassay.
Results: RBC folate levels significantly increased in both groups from baseline during pregnancy ($p<0.0005$). In the 5mg group, RBC folate levels significantly increased over the course of pregnancy between g.a.6 and g.a.30 ($p<0.0001$), and between g.a.12 and g.a.30 ($p<0.001$). In the 1.1mg group, RBC folate levels increased significantly only between g.a.12 to g.a.30 ($p<0.05$). Plasma folate levels increased in both groups from baseline to g.a.6, and then decreased over the course of pregnancy between g.a.6 and g.a.30, but the decrease was not statistically significant. Plasma levels at g.a.30 in both groups were comparable to their respective baseline levels.
Conclusions: Pregnancy-related physiological changes in folate distribution, metabolism and elimination likely influence steady-state pharmacokinetics of folic acid. Despite supplementation over an extended period of time, steady-state does not seem to be achieved in either dose group. Moreover, the decrease in plasma folate in pregnancy may have clinical relevance for women with poor folate status, and may reflect the increased folate demands during pregnancy.

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A case report of warfarin and carbamazepine drug interaction

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Objectives: To demonstrate the importance of considering carbamazepine and warfarin drug interaction when predicting warfarin dosing, and why the effect should not be underestimated when challenges present in attaining therapeutics INRs for these patients.
Methods: Warfarin is the most frequently prescribed anticoagulant worldwide, and is known to be highly efficacious in the prevention and management of thromboembolisms. Drug interactions remain a challenge in managing patients that require warfarin therapy. Many of these interactions are a result of induction or inhibition enzymes involved in warfarin metabolism. Carbamazepine widely prescribed for epilepsy as well as chronic pain. We report the case of a 44-year-old prescribed warfarin after receiving mechanical mitral and aortic valves three months prior to being assessed by our team. Patient is also on carbamazepine CR 200 mg/day for seizure control. She showed warfarin resistance, as demonstrated by repeated sub-therapeutic INR values. Her observed warfarin maintenance dose of 13 mg/day was significantly higher than the predicted dose 6 mg/day using our genomics-guided dosing nomogram. We then measured S and R warfarin plasma levels. Despite her higher than predicted warfarin dose, her measured S-warfarin levels were within the expected therapeutic range for someone with her VKORC1 genotype. Since INR values were in the target range, it would appear that the higher than predicted dose of warfarin was required to maintain a therapeutically relevant S and R-warfarin levels. The observed phenomenon is consistent with induction of both CYP2C9 and CYP3A4 by carbamazepine.
Results: This patient's S-warfarin levels fall within the expected therapeutic range for someone with her VKORC1 genotype and AF indication. Since INR values were in the target range, it would seem that the cause of warfarin resistance has a pharmacokinetic origin. The therapeutic drug

levels at higher than predicted dose indicates S-warfarin clearance is increased.
Conclusions: Our case illustrates the importance of integrating induction-related drug interactions, as well as pharmacogenomic parameters for optimal warfarin dosing, and such patients may require far higher than predicted dose of warfarin to attain therapeutic benefit.

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Methadone pharmacokinetics in an obese patient

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Objectives: Patients in methadone maintenance treatment (MMT) programs frequently report recurrent withdrawal symptoms and request dose readjustment. Measuring methadone blood levels and half-life (t½) can assist physicians in evaluating if dosing adjustments are warranted. We observed that as patient weight increases, methadone t½ dose as well. Obesity (as indicated by BMI) and its correlation with methadone t½ have not previously been reported in the literature.
Methods: As part of our methadone kinetics service, we obtain patients' pre- and post-dose blood samples, methadone dosing data, height, weight, and medication list. Assays for methadone and its inactive metabolite, EDDP, were done by immunoassay that had previously been validated against both HPLC and GC. We calculated t½, clearance (CL), and volume of distribution (Vd) for both methadone and EDDP. All assays were performed as part of clinical care requests from attending physicians
Results: From June 2002 to January 2014, 268 patient samples were analyzed. All patients were long-term enrollees of an MMT program. Height and weight data were available to calculate BMI for 52 of 268 patients; we report here results from these 52 patients. Mean methadone dose was 1.32 mg/kg (range 0.77-3.61 mg/kg); mean BMI 30.7 (range 19.6-58.5); mean methadone t½ 29.6 h (range 11.8-74.0 h); and mean EDDP t½ 28.7 h (range 7.4-97.2 h). BMI was significantly correlated with methadone t½ ($r = 0.536$, $p < 0.001$). There was no correlation between BMI and CL ($p = 0.41$) or the number of medications received ($p = 0.40$).
Conclusions: We show, for the first time, a significant correlation between obesity (BMI) and methadone t½. We suggest that this phenomenon may play a broader significant role in the management of patients who are obese and receiving lipophilic medications.

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Effects of species of origin and inducing agent on the in vitro metabolic activity of isolated liver microsomes

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

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The plasma and serum concentrations of active chemerin are elevated in obese humans

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Objectives: Prochemerin is an adipose-secreted molecule that is cleaved by extracellular proteases to active chemerin. Plasma and serum total chemerin (prochemerin + active chemerin) is increased in obese humans suggesting that chemerin may contribute to obesity-associated diseases. The effect of obesity on the production of active chemerin is unknown, given a lack of assays that specifically measure active chemerin in biological fluids. The objectives were to:1) develop a cell-based bioassay to measure active chemerin in human plasma and serum and 2) determine if obesity specifically increases the formation of active chemerin in fasted and fed states.
Methods: Four normal weight (body mass index (BMI) 20-25) and four obese (BMI >30) subjects were recruited into the study. Two baseline blood samples were collected 1 hour apart after an overnight fast and prior to breakfast. Seven additional blood samples were collected at 30-60 minute intervals in the post-prandial period. A cell-based reporter-gene assay that quantitatively measures chemerin activation of the chemokine like receptor 1was used to determine the active chemerin concentrations.
Results: The average active chemerin concentration over all time points was higher ($P<0.001$) in obese vs. normal weight subjects in serum (8.50 ± 0.59 nM vs. 6.52 ± 0.34 nM) and plasma (6.28 ± 0.59 nM vs. 3.93 ± 0.71 nM). The baseline and postprandial plasma and serum active chemerin concentrations were similar. Plasma and serum active chemerin concentrations more strongly correlated to waist to hip ratio ($r = 0.827$ and 0.877) compared to BMI ($r = 0.568$ and 0.693). The serum active/total chemerin ratio of the normal weight group was higher ($P< 0.001$) than the obese group (59.9 ± 4.4% vs. 47.5 ± 3.5%).
Conclusions: Central obesity contributes to elevated active chemerin concentrations in human plasma and serum supporting the potential for modified chemerin signalling and function in obese individuals with central obesity.

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Cardiopulmonary bypass increases plasma but not cerebrospinal fluid S100B concentrations

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Objectives: S100B is a calcium-binding protein that is primarily produced and secreted by astrocytes in the central nervous system (CNS). It has been proposed to be a marker of CNS injury. Following cardiac surgeries, elevated serum S100B concentrations are commonly observed. It was hypothesized that the increased S100B in serum was originally produced in the brain and then leaked to the blood due to increased blood-brain barrier (BBB) permeability. The objective is to investigate the source of increased S100B in the general circulation following cardiac surgeries.
Methods: We recruited 36 patients who underwent elective aortic aneurysm surgeries in our study. They were divided in two groups (18 per group): cardiopulmonary bypass (CPB) and non-CPB group based on the surgery they received. Plasma and cerebrospinal (CSF) samples were collected before, during and after the surgery. Plasma and CSF S100B concentrations at baseline, pre-CPB, post-CPB, skin closure, arrival at ICU and every 6 hours after the surgery were quantified with an enzyme-linked immunosorbant assay (ELISA).
Results: In the non-CPB group, the concentration of S100B remained at a relatively low and steady level in both plasma (89 ± 16 pg/ml) and CSF (389 ± 89 pg/ml). In the CPB group, most patients showed significantly elevated plasma S100B levels between 0-6 hours after the completion of CPB. The average plasma S100B concentration was significantly higher at the end of CPB versus the average baseline S100B concentration (1200 ±760 pg/ml vs. 80 ± 93 pg/ml), $p<0.0001$, paired t-test. A statistically significant increase in CSF S100B concentration was not observed.
Conclusions: Plasma but not CSF S100B concentrations increased following CPB surgery, suggesting that S100B is also released from extra-cerebral sources and that plasma S100B may not be a reliable biomarker for CNS injury or BBB permeability.

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Creatine improves spatial learning capabilities and mitochondrial function in the 3xTg mouse model of Alzheimer's disease

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Objectives: Alterations in creatine metabolism have been implicated in Alzheimer's disease (AD). Genetic mutations affecting creatine metabolism are associated with deficits in memory, which is impaired in AD. Creatine activates the transcription factor nuclear factor kappa B (NF-κB), which has been shown to be neuroprotective. Gene targets of NF-κB include antioxidants that regulate oxidative stress, which is detrimental to mitochondria. Interestingly, brain NF-κB levels are altered in AD and in the 3xTg mouse model. This study sought to: 1) evaluate the effects of creatine on neuronal mitochondrial function in vitro and hippocampal mitochondrial function in vivo; and 2) determine the effects of creatine supplementation on hippocampal-dependent spatial learning and memory in control and 3xTg AD mice.
Methods: Cortical neurons from embryonic C57BL/6 mice were cultured. Mitochondrial bioenergetics were measured in creatine-treated (1, 5, 10, or 20 mM) vs. untreated neurons. 7-mos-old 3xTg and C57BL/6 control mice received a normal or creatine-supplemented diet (3% w/w) for 8 wks, followed by Morris water maze (MWM) testing. Oxygen consumption rates (OCR) from hippocampal mitochondria in 3xTg and C57BL/6 mice fed a control or creatine-supplemented diet were measured (XF24 Analyzer, Seahorse Biosciences).
Results: In vitro, creatine enhanced OCR in cultured cortical neurons vs. untreated neurons ($p<0.05$). In vivo, creatine supplementation improved spatial learning acquisition in the MWM in control males ($p<0.05$) and 3xTg females vs. control-fed counterparts ($p<0.05$). Creatine supplementation in 3xTg males, however, impaired MWM performance ($p<0.05$) vs control-fed 3xTg males. Basal OCR were decreased in hippocampal mitochondria from 3xTg mice vs control mice ($p<0.05$). Creatine supplementation improved mitochondrial OCR in 3xTg hippocampi ($p<0.05$).
Conclusions: These data demonstrate creatine-induced enhancements in learning and cellular bioenergetics in typical animals and in an AD mouse model. Further research is needed to examined sex-dependent differences with creatine supplementation in the 3xTg AD model.

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The role of Nrf2-Keap1 pathway in cisplatin resistance in neuroblastoma

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Objectives: One of the major causes of treatment failure of neuroblastoma (NB) is drug resistance. Cisplatin plays an important role in the treatment of NB. Nuclear factor erythroid 2-like-2 (Nrf2) is a transcription factor, and regulates anti-oxidative stress enzymes and drug transporters. Nrf2 is negatively regulated by kelch-like ECH-associated protein 1 (Keap1). Loss-of-function mutations of Keap1 gene and subsequently continuous Nrf2 activation were mechanisms of cisplatin resistance in several cancers in adults. The objective of this study was to analyze whether Nrf2-Keap1 pathway is associated with cisplatin resistance mechanism in NB.
Methods: The baseline activity of Nrf2 was examined in 9 NB cell lines (IMR-32, LAN5, CHLA-15, SH-SY5Y, CHLA-20, SKN-FI, SK-N-BE(2), BE(2)C, CHLA-90) by Antioxidant Response Element luciferase reporter assay. In order to investigate whether cisplatin activates Nrf2, quantitative mRNA expressions of Nrf2 and its target genes (Glutamate-Cysteine Ligase, Modifier Subunit: GCLM and NAD(P)H Dehydrogenase, Quinone 1: NQO1) were carried out after 24-hour treatment with cisplatin. Dose-response survival was measured by MTT assay with and without knockdown of Nrf2. Nrf2 was knocked down by transfection of small interfering RNA (siRNA).
Results: No cell lines demonstrated high baseline Nrf2 activities by the reporter assay. Among 9 cell lines, we identified CHLA-90, CHLA-15, and SKN-FI as inducing high Nrf2 target genes with 24-hour cisplatin treatment. Cell viability of CHLA90 by 24-hour cisplatin treatment was assessed by MTT assay with and without Nrf2 siRNA transfection. Down regulation of Nrf2 expression by siRNA did not improve survival rate of CHLA-90 with cisplatin treatment.
Conclusions: Our results showed that Nrf2-Keap1 pathway does not play an important role in cisplatin resistance in NB cells.

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Microvascular endothelial cells from ENT1-null mice are resistant to the effects of oxygen free radicals on nucleobase transport

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Objectives: During ischemia, adenosine released from vascular tissue is ultimately accumulated by vascular endothelial cells via equilibrative nucleoside transporter 1 (ENT1). High intracellular adenosine concentrations lead to the formation of oxygen radicals via xanthine oxidase metabolism of the intermediate nucleobase hypoxanthine. Hypoxanthine levels, and hence oxygen radical production, are regulated by the activity of the nucleobase transporter ENBT1. The goal of the present study was to examine the relationship between ENT1 and ENBT1 activity, and the effect of oxygen radicals thereon.
Methods: Microvascular endothelial cells (MVEC) from ENT1+/+ and ENT1-/- mice were treated with the oxygen radical generators tert-butylhydroperoxide or menadione (100 µM), or subjected to simulated ischemia-reperfusion by incubation in minimal media under an oil layer followed by resuspension. The activities of ENT1 and ENBT1 were measured by the rate of uptake of [3H]2-chloroadenosine and [3H]hypoxanthine, respectively. Catalase and superoxide dismutase activity was measured using commercial kits.
Results: The rate of uptake of [3H] hypoxanthine was similar in the ENT1+/+ and ENT1-/- MVEC (~15 pmol/µl/s). Tert-butylhydroperoxide did not affect ENT1 nor ENBT1 activity. The intracellular superoxide generator menadione, on the other hand, reduced ENT1 and ENBT1 activity in ENT1+/+ MVEC by 65% and 45%, respectively. Simulated ischemia-reperfusion led to a 40% decrease in ENT1+/+ ENBT1 activity, and this effect was attenuated by the superoxide dismutase mimetic MnTMPyP (100 µM). In contrast, ENT1-/- MVEC showed no change in ENBT1 activity upon treatment with menadione or simulated ischemia-reperfusion. ENT1-/- MVEC also showed a significant increase in the activity of catalase (24 ± 4 nmol/min/mg protein) relative to ENT1+/+ MVEC (10 ± 2 nmol/min/mg).
Conclusions: These data suggest that ENBT1 is down-regulated in ENT1+/+ MVEC in response to increased intracellular superoxide production associated with ischemia-reperfusion injury. MVEC isolated from ENT1-/- mice do not show this reduction in ENBT1, likely due to an increase in catalase activity.

26 WITHDRAWN

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The ontogeny of P-glycoprotein in the human blood brain barrier- Implication for opioid toxicity in neonates

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Objectives: The objective of the study was to quantify the ontogeny of P-gp in the developing blood brain barrier during the fetal, infant and adult stage.
Methods: Postmortem human cortex samples from each of the following ages: 1) gestational age (GA) 20 to 26 weeks, 2) GA 36 to 40 weeks, 3) postnatal age (PNA) 0 to 3 months, 4) PNA 3 to 6 months, and 5) adults were triple immunostained for P-gp, laminin and DAPI. Sections were viewed and analyzed using a spinning disc confocal microscope. P-gp intensity was measured in seven brain microvessels from each section.
Results: The P-gp intensity in adults was significantly higher compared to that at GA 20 to 26 weeks (p=0.0002), GA 36 to 40 weeks (p=0.0002), and PNA 0 to 3 months (p=0.0044). Furthermore, there was a more pronounced decrease in P-gp intensity at GA 20 to 26 weeks (p=0.0011), GA 36 to 40 weeks (p=0.0013), and PNA 0 to 3 months (p=0.0173) compared to at PNA 3 to 6 months.
Conclusions: P-gp expression in the human brain is limited during the fetal period, increases remarkably with postnatal maturation, and reaches adult levels by 3 to 6 months. Given the immaturity of the human brain after birth, morphine may readily cross into the brain. This may explain why young infants are more sensitive to the central effects of morphine during the first couple months of life compared to older infants and adults. Understanding the extent of P-gp expression in the embryo, fetus and infant will facilitate rational drug use during pregnancy and the neonatal period.

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Cardiolipin deficiency changes barrier properties of human cerebral capillary endothelial cells

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Objectives: The blood brain barrier is a restrictively permeable interface that only allow transport of specific compounds into the brain. Cardiolipin (CL) is a mitochondrial phospholipid known to be required for the activity and integrity of the respiratory chain. Reductions in CL content and molecular composition have been associated with a variety of pathological conditions including Barth Syndrome, diabetes and heart failure. The objective of the current research is to investigate the effect of reduction in CL content on the mitochondrial function and the associated changes in barrier properties of human brain capillary endothelial cells.
Methods: Primary human brain microvessel endothelial cells (HBMECs) and HCMEC/D3 cell line were transfected with human cardiolipin synthase (hCLS1) siRNA to reduce cardiolipin (CL) level. Oxygen consumption rate (OCR) were measured to determine the associated changes in mitochondrial function. Paracellular permeability changes were assessed by the rate of fluorescent dextran (FDX) flux (a diffusion marker) across the confluent endothelial monolayer grown on Transwell® inserts. Uptake of 2-deoxy-D-(3H)-glucose was determined as a measure of GLUT1 activity. Uptake of 14C-oleate was used to determine changes in fatty acid transport. In addition, gene expression changes of BBB transporters such as GLUT1, ABCC1-4, P-glycoprotein, BCRP and Creatine transporter (CrT) were also examined.
Results: hCLS1 gene expression was reduced by approximately 75% and 90% in HBMECs and hCMEC/D3 cells respectively upon transfection. Consistently, CLS enzyme activity and mitochondrial function were also reduced. Gene expressions of the creatine transporter showed significant reduction while others such as GLUT1, BCRP, P-glycoprotein and ABCC1-4 were unaltered. Surprisingly, GLUT1 activity was increased even though its gene expression remained unchanged. hCLS1 knockdown did not change the paracellular permeability as well as oleate transport across the endothelial cell monolayer.
Conclusions: CL may play an important role in regulating mitochondrial function, which may control the trans-cellular transport properties of the human brain capillary endothelial cells.

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Prevalence of drug use during pregnancy in Miramichi, NB – Analysis of a routine urine drug screen in the obstetric unit

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Objectives: In recognizing the impact of maternal substance use for child and maternal well-being, the obstetric unit at Miramichi Regional Hospital (MRH) began a routine urine screen for drugs of abuse among all women admitted for labour. The objective of this study was to determine the prevalence of drug use during pregnancy in Miramichi, NB over a six and a half year period and correlate drug screen results with neonatal and maternal outcomes.
Methods: All women admitted for labour at MRH provided a urine sample prior to delivery for analysis of common substances of abuse including amphetamines, opioids, and THC. Women with positive screen results were matched to a randomly selected negative control. An anonymous, retrospective chart review was conducted on cases and controls to obtain maternal demographic information and neonatal and maternal outcomes (ex. birth measurements, complications). All outcomes for cases and controls were statistically analyzed using Chi Square, Fisher Exact, Mann Whitney U test, linear and logistic regressions where applicable.
Results: Between April 2006 and January 2013, there were 2678 deliveries at MRH, with 378 positive drug screens (14.12% rate of positivity). Marijuana, opioids and benzodiazepines were found to be the most commonly detected compounds (45.24%, 33.60% and 13.23% respectively). Cases were more likely to have less education, psychiatric disorders and be smokers (Chi Square; $p< 0.001$) than controls. Neonates of cases were found to be lower in birth length and head circumference (Mann Whitney U; $p < 0.001$) than controls. Drug use was found to be associated with lower neonatal birth weight ($R2=0.138$, $p=0.008$) while opioid and stimulant

use was found to be associated with longer neonatal hospital stay (R2=0.156, p<0.001 and 0.016 respectively).
Conclusions: This study highlights the ongoing problem with substance abuse during pregnancy in Canada with poorer neonatal and maternal outcomes associated with drug use during pregnancy.

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Maternal gestational diabetes mellitus increases the susceptibility of young rat offspring to hepatic steatosis and insulin resistance

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Objectives: Background: Population health data suggests that the development of metabolic disease is influenced by early life events. Gestational diabetes (GDM) is a common complication of pregnancy, but its effects on the offspring are poorly understood.
Hypothesis: Maternal GDM causes obesity, hepatic steatosis and insulin resistance in the offspring.
Methods: Female Sprague-Dawley rats were fed a high fat (45%) and sucrose (HFS) diet prior to mating to cause glucose intolerance and GDM during pregnancy. Lean control pregnant rats received low fat (LF; 10%) diets. The offspring were weaned at 3 weeks of age and randomly assigned to LF or HFS diets for 12 weeks and analyzed for insulin sensitivity and hepatic steatosis. Metabolomic analysis was performed on offspring liver samples to assess changes in lipid soluble metabolite levels.
Results: GDM rats exhibited excessive gestational weight gain, hyperinsulinemia and mild hyperglycemia. The young adult offspring of GDM dams gained more weight than offspring of lean dams. This was accompanied by hepatic steatosis and in vivo insulin resistance compared to the offspring of lean dams. Metabolomic analysis showed a 10-fold increase in levels of the lipotoxic lipid, ceramide, in offspring from GDM dams, regardless of postnatal dietary condition. Increased expression of acetyl-CoA carboxylase-2 and reduced expression of peroxisomal proliferator activated receptor-α and insulin receptor-β in the livers of the young adult offspring of GDM dams could be factors responsible for the development of hepatic steatosis and insulin resistance.
Conclusions: GDM enhances the development of obesity, hepatic steatosis and insulin resistance in the offspring.

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Evaluation of endogenous plasma biomarkers for the prediction of CYP3A4 phenotype as determined by oral midazolam microdose

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Objectives: Cytochrome P450 3A4 (CYP3A4) is involved in the oxidative metabolism of 50% of prescribed drugs. Due to high interindividual variation in hepatic and intestinal CYP3A4 activity resulting from environmental and genetic factors, there has long been interest in the identification and application of predictive endogenous biomarkers of in vivo enzyme function. Indeed, plasma 4-beta hydroxycholesterol, 25-hydroxycholesterol and urinary 6-beta hydroxycortisol are considered useful CYP3A4 biomarkers. However, there remains uncertainty regarding which biomarker is most effective in assessing the combined liver/gut CYP3A4 phenotype. The aim of this study was to evaluate endogenous plasma biomarkers in the predication of CYP3A4 metabolic activity in healthy volunteers phenotyped using an oral midazolam microdose strategy.
Methods: A cohort of fifty-one adult healthy volunteers received a 100 microgram oral dose of midazolam and blood was obtained three hours post-dose. We previously validated this single time-point strategy for prediction of midazolam area under the plasma concentration vs. time curve. Plasma was analyzed by liquid chromatography-tandem mass spectrometry to measure midazolam and biomarker plasma concentrations. Stepwise linear regression was performed to estimate biomarker and covariate contributions to plasma midazolam concentration.
Results: There was 10-fold inter-subject variation in midazolam concentrations. After adjustment for age, gender, and weight, the ratio of plasma 6-beta hydroxycortisol to cortisol level was a significant predictor of midazolam concentrations, responsible for 13% of interindividual variation.
Conclusions: The concentration of the endogenous biomarker, 6-beta hydroxycortisol, in

plasma may be used in prediction of CYP3A4 activity as determined by oral midazolam microdose phenotype. We are performing additional biomarker analysis together with genotype integration to develop quantitative models to predict in vivo CYP3A4 activity as a strategy to provide better and safer pharmacotherapy.

32 WITHDRAWN

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A pharmacodynamically based decision rule for individualizing oral amiodarone maintenance dosing in the management of atrial fibrillation

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Objectives: Personalized medicine recognizes that inter-patient differences make "one-dose-fits all" a suboptimal strategy. Pharmacogenomics and drug dosing rate determine drug concentration and that determines therapeutic response. Individualizing drug dosing produces optimal drug concentrations, improving efficacy and minimizing adverse effects. While amiodarone concentrations can be measured directly, an accurate record of the least total dose of drug required to convert a given patient to sinus rhythm provides enough information to calculate their individualized maintenance dose.
Methods: Given that the pharmacokinetic profile of amiodarone varies little in a given patient, the maintenance dose is mathematically proportionate to the accumulated drug required to produce the desired pharmacodynamic (sinus rhythm). This mathematical relationship can be applied to each case using a proposed nomogram.
Results: The formula calculated from pharmacokinetic principles is that maintenance dose = d (1 - 0.5n/N), where d is the total loading dose required to convert to sinus rhythm, n is days to convert, and N is drug half-life. When this is plotted as a nomogram, it provides physicians with assistance in rational dose determination, and a visual perspective regarding the patient's degree of progress towards steady state.
Conclusions: Once the therapeutic decision is made to pursue rhythm control using amiodarone, the proposed nomogram can guide individualized dose calculation. The mathematical relationship used to develop the nomogram provides an early estimate of the patient's optimum long-term amiodarone maintenance dose without requiring precise knowledge of their pharmacokinetic profile. Replacing arbitrary dosing regimens with an individually calculated maintenance dose accounts for most of the biologically determined inter-patient variability in pharmacokinetics, thereby reducing the incidence of therapeutic failure associated with a "one-dose-fits all" strategy.

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Incidence of hyperthyroidism in patients exposed to amiodarone shows large regional variation new abstract

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Objectives: Amiodarone greatly increases exposure to iodine. A 200 mg tablet provides 5 - 7.5 mg of bioavailable iodine, rapidly increasing total body content from the normal 14 mg. Populations exposed to increased environmental iodine have an incidence of hyperthyroidism of 3-5%, the same rate observed in most populations receiving amiodarone. This suggests these patients are reacting to iodine rather than the peripheral pharmacological effect of amiodarone. We studied the rate of hyperthyroidism in patients attending Amiodarone Clinic in Calgary to compare it to known rates.
Methods: Serial thyroid indices (TSH, Free T4 and Total T3), ALT and serum amiodarone concentrations were collected every six months on 115 patients enrolled in clinic for amiodarone dose adjustment and safety monitoring.
Results: During three years of follow-up, 13 patients developed FreeT4 > 30 with an mean onset time at 914 days of therapy and a mean FreeT4 of 44 pmol/L. Mean serum amiodarone was not high at 1.04 mcmol/L (target 1.0 - 2.2 mcmol/L) and there was no evidence of any drug organotoxicity (mean ALT 37 U/L). All cases resolved within 6 months without stopping amiodarone, when treated with methimazole (usually 5 mg p.o. TID).
Conclusions: During three years of follow-up, 13 patients developed FreeT4 > 30 with an mean onset time at 914 days of therapy and a mean FreeT4 of 44 pmol/L. Mean serum amiodarone was not high at 1.04 mcmol/L (target 1.0 - 2.2 mcmol/L) and there was no evidence of any drug organotoxicity (mean ALT 37 U/L). All cases

resolved within 6 months without stopping amiodarone, when treated with methimazole (usually 5 mg p.o. TID).

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Fading antihypertensive effects at end of dosing interval in patients switched from zero order to first order nifedipine osmotic delivery formulations

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Objectives: We observed pts with unexplained increases in BP after switching nifedipine therapy from zero order osmotic delivery as Adalat XL (AdN) to first-order delivery as Mylan-nifedipine XR (MyN). Interchangeability was based on similar AUC averaged over whole dosing interval (12-half-lifes). Differences in drug delivery could manifest as pressure differences at the end of dosing-interval.
Methods: We obtained two 24-h ABPM recordings in patients controlled to target with morning dosing of nifedipine 60 mg after 14 d of accommodation on each of 2 differing nifedipine formulations. Profiles were examined for potential differences between systolic blood pressures (SBP), focusing on nocturnal readings (22:00 to 06:00) when desirable SBP is < 120 mmHg.
Results: Mean ± SE nocturnal SBP from 16 subjects was 119 ± 2.4 mmHg in the AdN group, and 126 ± 2.5 mmHg in the MyN group ($p<0.002$). SBP exceeded 120 mmHg 43% of the time on AdN, and 58% of the time for the MyN ($p<0.04$). Half the patients were insensitive to the formulation switch. When these were removed, mean ± SE SBP was 118 ± 2.4 mmHg in the AdN group, and 129 ± 2.5 mmHg in the MyN group ($p<0.001$). SBP exceeded 120 mmHg 45% of the time on AdN, and 71% of the time for the MyN ($p<0.001$).

Conclusions: Half the subjects studied demonstrated clinically significant differences in SBP at end of dosing interval. This suggests that a first-order pump, MyN, which delivers an amount of drug that is equally effective to AdN during the day, has difficulty maintaining therapeutic effective equal to that of AdN, because first order drug delivery declines over the dosing interval. Larger studies are needed to document the overall impact of switching between the differing pump technologies available once daily oral nifedipine dosing.

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Morphine vs. ibuprofen

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Objectives: In children, sleep disordered breathing is often caused by hypertrophy of the tonsils and/or adenoids and is commonly managed by tonsillectomy with or without adenoidectomy. There is controversy regarding which post-surgical analgesic agents are safe and efficacious. The objective of this study is to evaluate the safety and effectiveness of morphine and ibuprofen in managing post-tonsillectomy pain in children.
Methods: This prospective randomized clinical trial recruited children with sleep disordered breathing, who were scheduled for tonsillectomy +/- adenoid removal. Parents were provided with a pulse oximeter to measure oxygen saturation and apnea events the night before, and the night after surgery. Children were randomized to receive 0.2-0.5 mg/kg oral morphine or 10mg/kg of oral ibuprofen. The Objective Pain Scale (OPS) and visual analog scale were used to assess analgesic effectiveness on postoperative day 1 and day 5. The primary end point was changes in oxygen saturation during sleep after surgery, as compared to pre-operatively.
Results: A total of 91 children aged 1-10 years were randomized. Children receiving ibuprofen showed an improvement in the rate of desaturation events on post-operative day one, whereas children receiving morphine had a significant increase in their rate of desaturation (-1.79 ± 7.57 vs +11.17 ± 15.02, $p < 0.01$). There were no differences seen in analgesic effectiveness, tonsillar bleeding, or adverse drug reactions.
Conclusions: Ibuprofen provides safe and effective analgesia in children undergoing tonsillectomy for sleep disordered breathing. Post-tonsillectomy morphine use should be limited, as it may be unsafe in certain children.

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Determining the oligomeric state of the beta-site APP-cleaving enzyme 1 (BACE1) in natural membranes and detergents

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Objectives: The beta-site APP-cleaving enzyme 1 (BACE1) has a transmembrane sequence (TMS), which is necessary for effective BACE1 cleavage of the amyloid precursor protein (APP). An uncommon sulfur-rich motif, MxxxCxxxMxxxCxMxC, spans the entire TMS of BACE1. The sequence is highly conserved among homologues and is reminiscent of a high-affinity binding site for Cu(I) found in other copper-transporting proteins.
Methods: We designed model peptides of the BACE1-TMS to investigate metal-ion binding and oligomerization uncoupled from the cytoplasmic and the ectodomain. A set of biophysical and colorimetric methods was used to investigate peptide-metal ion complex formation. The role of the metal-ion binding motif in potentially pre-existing oligomers of full-length BACE1 was assessed by Förster resonance energy transfer, automated single-molecule fluorescence counting in living cells, as well as by blue-native and SDS-gel electrophoresis.
Results: We found that the sulfur-rich core motif MxxxCxxxM is involved in metal-ion coordination and oligomerization of BACE1. Addition of Cu(II) facilitated the formation of dimers and trimers of the BACE1 TMS. Importantly, the peptide undergoes a redox reaction with copper ions, resulting in a disulfide bridge involving Cys466 in the center of the conserved MxxxCxxxM motif as the key amino acid. Further, we find peptide trimerization to depend on (i) the presence of monovalent copper ions and (ii) the sulfhydryl group of Cys466. For the full-length protein, FLIM-FRET experiments revealed that BACE1 oligomers are naturally present in living cells, as the oligomeric state of BACE1 remained unchanged in the absence or presence of metal-ions. We determined a stable trimeric assembly of BACE1 in the plasma membrane by accurate single-molecule fluorescence counting. Although the oligomeric state of full-length BACE1 was not altered by the addition of copper in living cells, the addition of monovalent metal-ions was required to visualize di- and oligomers by Western blot analysis.
Conclusions: We propose that BACE1 acts as a bona fide metalloprotein in an oligomeric form in vivo. Additionally, our results demonstrate a novel metal-ion controlled stabilization mediated by the TMS of the BACE1.

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Neuroinflammation in a phencyclidine-induced schizophrenic model in mice

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Objectives: Schizophrenia is a progressive, neuropsychiatric disorder associated with cognitive impairment, and its etiology remains poorly understood. Growing evidence indicates that neuroinflammation plays a significant role in the pathophysiology of schizophrenia. To test this hypothesis, possible neuroinflammatory responses were evaluated in a phencyclidine (PCP)-induced mouse model of schizophrenia.
Methods: 3-month-old female C57BL/6J mice received daily injections of PCP (20 mg/kg, i.p.) or saline for one week. Behavioural tests, immunohistochemistry, western blot and ELISA were performed to analyze the behavioural and biochemical changes.
Results: PCP-injected mice produced schizophrenia-like behaviours including impaired spatial working memory assessed by the Y-maze task and sensorimotor gating deficits in a prepulse inhibition (PPI) task. Simultaneously, PCP-induced astrocyte and microglial activation was evident in both the cortex and hippocampus on brain specimen analysis. Furthermore, the proinflammatory cytokine interleukin-1β was significantly up-regulated in PCP administrated mice. This glial activation was found associated with a decrease in the phospho-Ser9 epitope of glycogen synthase kinase-3β (GSK3β), which is indicative of increased GSK3β activity.
Conclusions: Our results suggest that there were evident neuroinflammatory changes in the phencyclidine-induced schizophrenia animal model, and indicate that neuroinflammation may play an important role in the pathogenesis of schizophrenia, thus providing a possible therapeutic target for future schizophrenia pharmacology.

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Genome-wide association study identifies genetic variation in a novel gene as a major predictive marker for anthracycline-induced cardiotoxicity in childhood malignancy

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Objectives: Anthracyclines are used to treat over 70% of all childhood malignancies and have significantly improving the 5 – year survival rates in children to at least 80% today. However, their clinical use is significantly limited by anthracycline-induced cardiotoxicity (ACT), which manifests as asymptomatic cardiac dysfunction in up to 65% of treated patients and serious congestive heart failure in 16-20% of treated patients. Understanding the causes and mechanisms, and predicting and preventing ACT is essential to improving long-term outcomes in childhood cancer survivors. Currently, no tests are available to accurately predict the risk of ACT for individual patients. Genetic factors have been hypothesized to contribute to ACT and some of them have been identified but much of the inter-individual variability remains unaccounted for, suggesting the existence of additional and more potent genetic markers. To identify novel predictive genetic factors of ACT, we performed a two-stage genome-wide association study (GWAS) of ACT in children of European descent and subsequent validation in other world-wide populations.
Methods: Childhood cancer patients (=< 18 years of age) who receive anthracycline chemotherapy were recruited from Canada, The Netherlands and USA. A GWAS discovery (740 000 genetic variants) was performed in patients of European descent from Canada (n = 280 patients; 32 cases and 248 controls), with subsequently testing for replication in an independent European population from The Netherlands (n = 96 patients; 22 cases and 74 controls). The world-wide applicability of identified genetic markers was further explored by performing additional testing in 4 independent world-wide populations (Africans = 11 patients, Latinos = 23 patients, East Asians = 31 patients and First Nations = 15 patients).
Results: A novel gene for ACT was identified. A nonsynonymous coding variant in this gene, strongly predicts the development of ACT (discovery - P = 4.12E-08, odd ratio = 4.81; replication - P = 0.00423, odd ratio = 5.15). This genetic marker is also an important biomarker for ACT in other world-wide populations. In this study, the risk of developing ACT for homozygous mutant carriers was 88.83% (high risk genotype, positive likelihood ratio = 24.667), heterozygous carriers was 48.20% (intermediate risk genotype, positive likelihood ratio = 2.788) and homozygous wild type carriers was 16.88% (low risk genotype, negative likelihood ratio = 0.61). Incorporating this marker into a multimarker risk prediction model significantly improves the prediction of risk for ACT beyond established clinical and genetic risk factors (AUC = 0.833, sensitivity = 90.74, specificity = 60.87, P = 4.53E-15).
Conclusions: A novel and potent genetic predictor for ACT has been uncovered. Genetic testing for the non-synonymous coding variant in this gene can be performed as a point-of-care test. With an established role in cardiac development and remodeling, the signaling pathway mediated via this gene is a novel pathway in the pathogenesis of ACT.
Disclosure: We currently conceal the identity of this gene and the identified variant until the manuscript has been submitted for publication.

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Oxidative modification of vesicular neurotransmitter transporters

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Objectives: Brain cells are vulnerable to production of reactive oxygen species (ROS) because the brain consumes about 20% of total body oxygen. Vesicular acetylcholine transporter (VAchT), vesicular monoamine transporter 2 (VMAT2) vesicular glutamate transporter 1 (VGluT1) vesicular glutamate transporter 2 (VGluT2) contain high density of cysteine residues that have significant potential to be oxidatively modified. Therefore we determined whether VAchT, VMAT2, VGluT1 and VGluT2 can be nitrosylated or oxidized by ROS donors.
Methods: Cystein nitrosylation and oxidation of the transporters was measured by biotin-switch method followed by immunoblotting analysis.
Results: We have found that VMAT2, VGluT1 and

VGluT2 can be nitrosylated to different levels by exogenous nitroso-glutathione (GSNO), while only VGluT1 can be oxidized by exogenous hydrogen peroxide (H2O2).

Conclusions: Since vesicular transporters are very important by uploading neurotransmitters into the vesicles, ROS-induced oxidative post-transcriptional modification may regulate the neurotransmission process, which might help explain the pathology of some neurodegenerative diseases.

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Ceftriaxone-induced immune haemolytic anemia: Systematic review and proposed screening strategy

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Background: Ceftriaxone-induced immune hemolytic anemia (CIIHA) is a rare but potentially fatal complication of ceftriaxone therapy. Patients with sickle cell disease (SCD) may be at a higher risk to develop CIIHA due to disease prevalence and common use of ceftriaxone. Thus, there is a need to better characterize the phenomenon. We present a report of a case, followed by a systematic review of the literature and suggestions for screening and prevention of CIIHA.

Methods: EMBASE (1947 to January 13, 2013) and Medline (1946 to January 13, 2013) were searched to identify articles on the topic of CIIHA. The search was expanded to include all third-generation cephalosporin agents in both children and adults. Descriptive statistics were performed to establish a set of clinical and laboratory characteristics.

Results: Thirty-seven cases of CIIHA were identified including our patient. Most patients had underlying conditions, most commonly SCD. Commonly reported features included acute back-pain, dark urine and acute renal failure. Laboratory features included haemoglobinuria, elevated LDH and positive direct antibody testing and/or anti-ceftriaxone antibodies. Thirty-two percent had a preceding, unrecognized, haemolytic episode associated with ceftriaxone. Overall morality was 30% (mostly children). A screening-strategy is proposed.

Impact and Conclusion: A high index of suspicion and routine screening and monitoring of high-risk patients could substantially reduce the morbidity and mortality associated with CIIHA. We therefore suggest repeat urinalysis to detect early, new onset haemoglobinuria, in high risk patients receiving ceftriaxone.

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Increased SSAT1 gene expression is associated with human cancer

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Objectives: Increased urinary levels of N-acetylspermidine have been linked to cancer. We examined the gene expression levels of spermidine/spermine N1-acetyltransferase (SSAT1), the rate-limiting enzyme in polyamine metabolism, in primary patient-derived breast, prostate and lung tumour tissue.

Methods: Total RNA was extracted from normal, human cell lines and tumour tissue using Qiagen QIA Shredder Kit and RNeasy Mini Kit. RNA concentration in each extracted sample was confirmed by nanodrop spectrophotometric measurement. RNA integrity was also assessed. SSAT 1 gene expression was determined by qRT-PCR using cDNA probe specific for SSAT1 and performed using Qiagen QuaniTect SYBR Green RT-PCR kit. The mRNA expression levels of the housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (HPRT1) were measured in parallel using the corresponding PCR primers for these genes. The SSAT1 gene expression levels were normalized with HPRT1 as the internal reference. Normalized SSAT1 gene expression was further analyzed by the ΔΔ ct method.

Results: A 7-fold higher SSAT1 gene expression in breast cancer tissue vs. normal, primary human mammary epithelial cells when normalized with HPRT1 was observed. A 4-fold and 3-fold higher SSAT1 gene expression were seen in prostate and lung cancer tissue vs. normal, primary human

prostate epithelial cells and normal, primary human bronchial/tracheal epithelial cells, respectively, when normalized with HPRT1.

Conclusions: Elevated SSAT1 gene expression levels in cancer tissue may serve as a diagnostic marker for the detection of cancer and that SSAT1 may be a target for anti-cancer drug development.

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