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:

2015 ANNUAL MEETING

CANADIAN SOCIETY OF PHARMACOLOGY AND THERAPEUTICS

June 7-10, 2015
Auditorium
Peter Gilgan Learning Centre
686 Bay Street,
Toronto, ON

CANADIAN SOCIETY OF PHARMACOLOGY AND THERAPEUTICS 2015 ANNUAL MEETING

JUNE 7-10, 2015
Toronto, Ontario

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Impact of endotoxin on the expression of drug transporters in the placenta of HIV-1 transgenic (HIV-Tg) rats

Ghoneim, Ragia; Kojovic, Dea; Piquette-Miller, Micheline
University of Toronto, Toronto, ON

Objectives: Subclinical endotoxemia has been reported in HIV infected individuals and immune activation may be exacerbated in these patients due to immune dysregulation. As infection-induced inflammation can alter the expression of placental drug transporters, it is plausible that this may be potentiated in HIV pregnant women. Similar to humans the HIV-Tg develops immune disorders and AIDS associated conditions. Therefore, our objective was to examine the impact of low-dose endotoxin on the expression of placental drug transporters in HIV-Tg rats.
Methods: 3-5 month pregnant HIV-Tg rats or wild-type littermates (WT) were treated with low dose endotoxin (0.1 or 0.25 mg/kg) on GD18 (n=4-8/group) and placentas harvested 18 hr later. Gene expression was measured using qRT-PCR and serum cytokine levels were measured using ELISA.
Results: Following endotoxin administration, there was a dose-dependent increase in pro-inflammatory cytokine levels in both HIV-Tg and WT rats, but to a greater extent in HIV-Tg. Endotoxin administration decreased the expression of Abcb1a, Slco2b1 and Slco4a1 in a dose dependent manner in HIV-Tg but not WT rats. Changes in transporter expression significantly correlated to cytokine induction. Endotoxin was associated with higher expression of Abcg2 in HIV-Tg and WT but only reached significance in HIV-Tg. Abcc3 expression was increased in endotoxin-treated WT but not HIV-Tg. Endotoxin administration did not impose significant changes in the expression of Abcb1b, Abcc1, Abcc2 and Abcc4 in HIV-Tg and WT rats.
Conclusions: Our results indicate that the inflammatory response is augmented in HIV-Tg rats following low dose exposure to endotoxin. Immune activation is associated with significant changes in the expression of several drug transporters in the placenta of HIV-Tg rats. Overall, our data suggests that placental transfer of drugs may be altered in the HIV population due to subclinical endotoxemia and other co-existing infections.

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GWAS uncovers a novel gene for susceptibility to anthracycline-Induced cardiotoxicity

Aminkeng, Folefac[1]; Bhavsar, Amit[1]; Visscher, Henk[2]; Rassekh, Shahrad[1]; Lee, Jong[1]; Brunham, Liam[1]; Caron, Huib[3]; van Dalen, Elvira[3]; Kremer, Leontien[3]; van der Pal, Helena[3]; Rieder, Michael[4]; Bernstein, Daniel[5]; Hayden, Michael[1]; Carleton, Bruce[1]; Ross, Colin[1]
[1]The University of British Columbia, Vancouver, BC; [2]Radboud University Medical Center, Nijmegen, The Netherlands; [3]Emma Children's Hospital, Amsterdam, The Netherlands; [4]University of Western Ontario, London, ON; [5]Stanford University, Palo Alto, CA

Objectives: Anthracyclines are used to treat over 70% of childhood cancers but their clinical utility is limited by anthracycline-induced cardiotoxicity. This manifests as asymptomatic cardiac dysfunction in 57% of children and as congestive heart failure in 16-20% of children. Some genetic risk factors have been identified, but much of the variability in the susceptibility to ACT remains unaccounted for, suggesting the existence of additional genetic factors. The goal of the study was to perform a genome-wide association study for anthracycline-induced cardiotoxicity in patients treated for childhood cancers.
Methods: We performed the first ever genome-wide association study - GWAS (740K SNPs) of anthracycline-induced cardiotoxicity in Canadian children of European descent (280 patients), with subsequent replication in Dutch children of European ancestry (96 patients). We then performed additional replications in non-

European populations including African, Hispanic, East Asian and Aboriginal Canadian patients.
Results: We discover a new gene for anthracycline-induced cardiotoxicity involve in cardiac regeneration and remodeling and a nonsynonymous coding variant within the gene predicts the development of anthracycline-induced cardiotoxicity (GWAS – P = 4.1x10-8, odds ratio = 6.0; replication - P = 0.0042, odds ratio = 4.1). This association was replicated in non-European populations.
Conclusions: The novel genetic biomarker is strongly associated with anthracycline-induced cardiotoxicity in children.

3

AST-120 and hepatic transport in chronic kidney disease

Kucey, Andrew; Velenosi, Thomas; Tieu, Alvin; Urquhart, Brad
University of Western Ontario, London, ON

Objectives: Hepatic drug transporter function is decreased in chronic kidney disease (CKD). The objective of this study was to determine if decreasing gut-derived uremic toxins with the spherical carbon adsorbant AST-120 recovers hepatic transporter function.
Methods: In vivo: A 7 week study has been conducted on Wistar rats with CKD induced through 0.7% adenine in standard rat chow. Treatment groups received AST-120 in the final 3 weeks to decrease the circulating levels of gut-derived uremic toxins. Rats also received an intravenous injection of rosuvastatin to evaluate in vivo hepatic transporter function. Rosuvastatin concentration in plasma and liver will be measured with ultra-performance liquid chromatography coupled to mass spectrometry. Transporter mRNA and protein expression will also be determined by real-time PCR and western blotting techniques respectively.
In vitro: Using the human hepatoma cell line Huh7, specific uremic toxins will be tested to see if they will impair hepatic transporter function. Selected uremic toxins will be incubated on cells followed by the addition of tranposrter probe substrates. The amount of probe drug retained in the cell will be determined by mass spectrometry.
Results: Preliminary results have shown significantly higher plasma creatinine and urea in CKD as well as CKD+AST-120 groups. Gut-derived uremic toxins, such as indoxyl sulfate, p-cresyl sulfate, and hippuric acid are significantly higher in CKD rats and restored to near control levels following treatment with AST-120.
Conclusions: Patients with CKD have an increased incidence of adverse drug reactions. Decreases in hepatic uptake transporter activity such as OATPs will result in reduced clearance of substrate drugs. This could lead to toxicity if doses are not adjusted accordingly. This study will help determine the mechanisms of altered hepatic drug clearance in CKD.

4

Preliminary report on the residual effects of cannabis on young drivers' performance of driving-related skills

Pan, Jie Fei[1;] Mann, Robert[2]; Brands, Bruna[3]; Gina, Stoduto[2]; Wickens, Christine[2]; Burston, Jillian[1]; Huestis, Marilyn[3]; Le Foll, Bernard[2]
[1]University of Toronto, Toronto, ON [2]Centre for Addiction and Mental Health, Toronto, ON; [3]Health Canada, Toronto, ON; [3]National Institute on Drug Abuse, Baltimore, Maryland; [4]University of Toronto, Toronto, ON

Objectives: The effects of cannabis on driving abilities may not be limited to the time period immediately after use. The current study examines the residual effects of cannabis on driving-related skills in young drivers using a high-fidelity driving simulator. We hypothesize that impairment on psychomotor functions and driving abilities will be observed at 24 and 48 hours following a single dose of cannabis.
Methods: The study is a randomized, double-blind, placebo-controlled mixed design clinical trial, including regular cannabis-using drivers, between the ages of 19-25, who smoke cannabis 1-4 times per week. Eligible participants undergo a practice session followed by 3 testing days (drug administration and 24 and 48 hours post-drug) consecutively. Measures of simulated driving performance, cognitive and psychomotor functions, and subjective drug effects are collected concurrently with levels of cannabinoids in biological fluids before and after a one-time cannabis administration (approximately 12.5% (active) or<0.01% (placebo) THC). Data analyses conducted focus on comparison of baseline to 24 and 48 hours post-drug.
Results: 40 participants (60% males, age = 22±2) of the target sample size of 142 completed the study and were included in this analysis. Preliminary data suggest that, in the driving only

condition, following distance behind a slow moving vehicle increased in the active group 48 hours after smoking ($p<0.05$). Although not statistically significant, trends were observed in reduction of verbal recall memory, increase in omission and commission error of a sustained attention task, and increase in total collisions in the driving only scenario at both 24 and 48 hours post-dose.

Conclusions: Preliminary data suggest that there may be some residual effects of cannabis on driving but findings are not conclusive. We will yield more definitive results as the sample size increases.

5

Probiotics for infantile colic: A randomized double-blind placebo-controlled trial investigating lactobacillus reuteri DSM 17938

Kim Chau MSc[1,3], Eddy Lau[2,4,5], Saul Greenberg MD[2,4], Sheila Jacobson MD[2,4], Natasha Verma MD[3], Gideon Koren MD[1,2,3,4]

[1]Department of Pharmacology & Toxicology, The University of Toronto, Toronto, ON; [2]Department of Pediatrics, The University of Toronto, Toronto, ON; [3]Division of Clinical Pharmacology, The Hospital for Sick Children, Toronto, ON; [4]Division of Pediatric Medicine, The Hospital for Sick Children, Toronto, ON; [5]Department of Pediatric Medicine, St. Joseph's Health Care, Toronto, ON

Objectives: Evidence exists demonstrating an association between the intestinal microbiota and infantile colic and therefore, the aim of this study is to determine whether supplementation Lactobacillus reuteri DSM 17938 improves infantile colic symptoms compared to placebo.

Methods: This randomized double-blind placebo controlled study involving 52 infants diagnosed with infantile colic (crying and/or fussing for ≥3 hrs/d on ≥3 d/wk for 7 d) were randomly assigned to receive placebo or L. reuteri at a standard dose of 108 colony-forming units once daily for 21 days. Daily crying and/or fussing times were recorded on a maternal diary and changes in the duration of daily crying and/or fussing times and adverse events were collected on days 7, 14 and 21.

Results: Total average crying and fussing times (minutes) throughout the study (day 0 to 21) were significantly shorter among colicky infants in the probiotic group compared to infants in the placebo group: 1719±750 min (29±13 hr) and 2195±764 min (37±13 hr) (P=0.028), respectively [relative risk (RR) 0.78(95% confidence interval (CI) 0.58-0.98)]. Infants administered L. reuteri DSM 17938 showed a significant reduction in daily crying and fussing times (minutes/day) at the end of treatment period compared to those receiving placebo (median [IQR]): 60 (64) and 102 (87), (P=0.045), respectively. On Day 21, a significantly higher proportion of infants in the L. reuteri DSM 17938 group responded to treatment by more than 50% crying time reduction compared to infants administered placebo: 17 vs. 6 (P=0.035) [RR 3.3(95% CI 1.55-7.03)].

Conclusions: The link between the intestinal microbiota to the clinical manifestation of infantile colic provides a sound rationale to conduct this study, as L. reuteri DSM 17938 significantly reduces crying/fussing times in breastfed colicky infants compared to placebo. Furthermore, the good safety profile of L. reuteri DSM 17938 deems it an effective treatment for infantile colic.

6

Use of medications of questionable benefit in Ontario long-term care residents with advanced dementia

Matlow, Jeremy[1]; Bronskill, Susan[1,2]; Bell, Chaim[1,2]; Stall, Nathan[1]; Austin, Peter[1,2]; Seitz, Dallas[3]; Herrmann, Nathan[1]; Wu, Wei[4]; Fung, Kinwah[2]; Fischer, Hadas[2]; Rochon, Paula[4]; Gruneir, Andrea[5]; Gill, Sudeep[3]

[1]University of Toronto, Toronto, ON; [2]Institute for Clinical Evaluative Sciences, Toronto, ON; [3]Queen's University, Kingston, ON, [4]Women's College Research Institute, Toronto, ON; [5]University of Alberta, Edmonton, AB

Objectives: This research aims to determine the extent to which medications of questionable benefit (MQB) are dispensed to older adults with advanced dementia in long-term care (LTC) homes and to characterize the variables that are associated with MQB prescription at time of death.

Methods: Using linked healthcare administrative databases, we conducted a cross-sectional study of MQB dispensation among 18,794 residents of LTC homes in Ontario who had advanced dementia and who died between June 1, 2010 and March 31, 2013. Advanced dementia was defined as a diagnosis of dementia within the last 5 years of life and a score ≥5 on the patient's last Cognitive Performance Scale assessment. MQBs were derived from a previously published list of drug classes developed using a Delphi

consensus panel. Simple logistic regression was used to identify variables independently associated with MQB prescription in the last week of life.

Results: Our decedent cohort included 13,235 (70.4%) women, mean age at death was 87.1 ± 7.2 years, and 4,420 (23.5%) individuals received at least one MQB within the last week of life. Among those receiving at least one MQB within the last week of life, the most common MQB classes prescribed were anti-dementia drugs (60.5%), lipid-lowering agents (45.4%), and antiplatelet agents (18.3%). Younger age, being male, increased number of physicians seen in the last year of life, and urban LTC home were all associated with receiving at least one MQB in the last week of life ($p<0.001$). Of the 10,086 residents dispensed at least one MQB within the last year of life, 43.8% were still receiving MQB dispensation within the last week of life.

Conclusions: Many LTC residents with advanced dementia continue to receive MQB dispensations toward the end of life. Careful and individualized analysis of the benefit of these medications in end of life care is warranted.

7

Effects of fixed or self-titrated dosages of Sativex on cannabis users

Trigo, Jose[1]; Lagzdins, Dina[1], Rehm, Jürgen[1]; Selby, Peter[1]; Gamaleddin, Islam[1]; Fischer, Benedikt[1]; Barnes, Allan J[2]; Huestis, Marilyn A[2]; Le Foll, Bernard[1]
[1]Centre for Addiction and Mental Health, Toronto, ON; [2]National Institutes of Health (NIH), Baltimore, USA

Objectives: There is currently no pharmacological treatment approved for cannabis dependence. In this pilot study, we assessed the feasibility of fixed and self-titrated dosages of Sativex (1:1, Δ9-tetrahydrocannabinol (THC)/cannabidiol (CBD)) on withdrawal from cannabis and craving among nine community-recruited cannabis-dependent subjects.

Methods: Participants underwent an 8-week double-blind placebo-controlled trial (an ABACADAE design) with four smoke as usual conditions (SAU) (A) separated by four cannabis abstinence conditions (B, C, D, E) with administration of either placebo or Sativex (up to 108 mg THC/100 mg CBD).

Results: Sativex significantly reduced cannabis withdrawal, but not craving scores when compared to placebo. Participants self-reported not being "high" following Sativex.

Conclusions: Due to these promising results, further systematic exploration of Sativex, as a treatment option for cannabis dependence, should occur.

8 – WITHDRAWN

Pilot of a clinical neonatal screening program for prenatal alcohol exposure via analysis of fatty acid ethyl esters in meconium

Nightingale, Ira[1], Dickinson, Michael[2]; Gareri, Joey[3]; Kapur, Bhushan[1,2], Koren, Gideon[2,3]
[1]University of Toronto, Toronto, ON [2]Miramichi Regional Hospital, Miramichi, NB; [3]The Hospital for Sick Children, Toronto, ON

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Examining the transplacental passage of insulin detemir in vivo

Bapat, Priya[1]; Suffecool, Katarzyna[2]; Rosenn, Barak[2]; Niederkofler, Eric[3]; Kiernan, Urban[3]; Foroutan, Janelle[4]; Antwi, Kwasi[3]; Ribar, Amanda[3]; Koren, Gideon[1].
[1]The Hospital for Sick Children, Toronto, ON; [2]Icahn School of Medicine at Mount Sinai, New York, NY; [3]Thermo Fisher Scientific; [4]North Shore University Hospital, Manhasset, NY

Objectives: Insulin detemir (Levemir®) is a relatively new long-acting insulin analog with greater stability and duration of action than regular human insulin. Insulin detemir has recently been studied in an open-label randomized controlled trial in 310 pregnant women with type I diabetes. The study found that insulin detemir was non-inferior to NPH insulin in either effectiveness or safety, and treatment with insulin detemir resulted in a lower fasting plasma glucose. Although this study demonstrated similar rates of hypoglycemia between groups, the placental transfer of insulin detemir was not studied. Therefore, the objective of this study was to determine if insulin detemir administered to pregnant women will cross the human placenta.

Methods: Pregnant women with either gestational diabetes mellitus (GDM) or Type II diabetes who received insulin detemir were enrolled in this prospective observational study. Maternal and umbilical cord blood samples were collected immediately following delivery. Insulin detemir levels were were measured using the Thermo ScientificTM Mass Spectrometric Immunoassay Insulin Workflow, and the limits of

detection for insulin detemir and endogenous insulin were 50 and 2 pM, respectively.
Results: A total of 16 pregnant woman-neonate pairs who were treated with insulin detemir in the third trimester were included in this study. Daily doses of insulin detemir ranged from 10-96 units, and maternal plasma levels ranged between 159-3804 pM. Insulin detemir concentrations were not detected in any of the umbilical cord samples (< 50 pM). None of the 16 infants experienced neonatal hypoglycemia.
Conclusions: Insulin detemir does not appear to cross the term human placenta in vivo, supporting previous studies that have suggested its fetal and neonatal safety.

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OATP2B1 and thyroid hormone interplay in intestinal drug absorption
Schaefer, Anima; Tirona, Rommel
Western University, London, ON

Objectives: Oral levothyroxine (T4) absorption is variable between individuals and can be affected by pharmacokinetic drug-drug interactions. Furthermore, it is known that hypothyroidism affects oral drug absorption. The uptake drug transporter, organic anion transporting polypeptide (OATP) 2B1, is considered to play a central role in the intestinal absorption of drugs. Previously we characterized the expression and function of intestinal and hepatic variants of OATP2B1. We hypothesize that thyroid hormone is absorbed by OATP2B1 in the intestine and that thyroid hormone regulates the expression of OATP2B1.
Methods: Transport studies were performed in cultured HeLa cells using a heterologous gene expression system. After transducing cells with OATP2B1 adenovirus and LacZ adenovirus as a negative control, triiodothyronine (T3), T4 as well as rosuvastatin (positive control) uptake was measured using LC-MS/MS. Cultured intestinal Caco-2 and hepatic Huh-7 cell lines were exposed to T3 and T4 for examination of OATP2B1 gene expression by quantitative real-time polymerase chain reaction.
Results: Overexpression of OATP2B1 was not associated with increased T3 or T4 uptake while rosuvastatin cellular accumulation was increased by 42% (p=<0.0001). T3 and T4 treatment increased the mRNA expression of the liver specific variant of OATP2B1 by 14.3-fold (p=0.0003) and 6.2-fold (p=0.0218), respectively, in Caco-2 cells but not in Huh-7 cells. Similarly, intestinal OATP2B1 variant mRNA expression was induced by 7.9-fold (p=0.0001) by T3 and 3.5-fold (p=0.0281) by T4 only in Caco-2 cells.
Conclusions: Thyroid hormones are not transported by OATP2B1 suggesting that OATP2B1 is not involved in the intestinal absorption of T4. OATP2B1 expression is positively regulated by thyroid hormones in a cell type-dependent manner indicating that thyroid hormone status may influence the intestinal absorption of OATP2B1 substrates.

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Role of Oatp-mediated statin uptake in altered insulin secretion in a murine beta cell model
Kim, Michelle; Schwarz, Ute; Hong, Kevin (Min Hwa); Wang, Rennian
University of Western Ontario, London, ON

Objectives: Statins are prescribed to lower cholesterol through 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibition. Though effective and safe, recent evidence from meta-analyses of major statin trials indicates an association between statin therapy and new-onset diabetes, with risk varying with statin potency. While the exact mechanisms have not been elucidated, a role of ubiquinone (CoQ10) has been proposed. Statin-induced HMG-CoA reductase inhibition is thought to deplete cellular CoQ10, thereby reducing ATP and ATP-sensitive potassium-channel function and impairing calcium signalling-dependent insulin secretion. Organic anion-transporting polypeptides (human OATPs, rodent Oatps) mediate cellular uptake of statins, and some isoforms (OATP1B3, Oatp1, Oatp2, Oatp3) were recently reported in human and rat pancreatic islets. Experimental data from murine beta-cell lines (rat INS-1, mouse MIN6) suggest impaired insulin secretion in response to high-potency statins like rosuvastatin and atorvastatin, whereas enhanced insulin secretion was observed for the low-potency pravastatin. A role of Oatp-mediated pravastatin transport in insulin secretion was recently demonstrated in INS-1 expressing Oatp3, however this mechanism has not been assessed for more potent statins. Moreover, Oatp expression in MIN6 cells has not been explored.
Methods: [3H]-rosuvastatin uptake activity in INS-1 cells was examined in the presence or absence of OATP inhibitors. Intracellular drug accumulation was measured by scintillation

spectrometry. Glucose-stimulated insulin secretion (GSIS) was assessed after 24hr statin treatment (1uM, 10uM) in INS-1, and insulin concentrations determined by enzyme-linked immunosorbent assay (ELISA). Oatp gene expression will be assessed in INS-1 and MIN6 cells with RT-PCR.

Results: We observed intracellular accumulation of [3H]-rosuvastatin in INS-1 cells over time, which was reduced in the presence of rifampicin or indomethacin. Preliminary results further suggest impaired GSIS in INS-1 cells when treated with rosuvastatin and atorvastatin.

Conclusions: Our findings suggest Oatp-mediated statin uptake likely results in altered insulin secretion in INS-1 cells, supporting a potential role of OATPs in statin-induced impairment of beta-cell function.

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Importance ratings for proposed competencies for drug safety and effectiveness researchers optimizing the safe and effective real-world use of medications by society

Dolovich, Lisa[1]; Rodriguez, Christine[1]; Cheng, Yin[2]; Weeratunga, Dinusha[2]; Winslade, Nancy[3]
[1]McMaster University, Hamilton, ON; [2]University of Toronto, Toronto, ON; [3]McGill University, Montreal, QC

Objectives: The Drug Safety and Effectiveness Network and Health Canada are supporting capacity building efforts to develop a national curriculum for drug safety and effectiveness research on the real-world use of medications. The objective of this study was to determine how important each proposed competency is to current researchers aiming to optimize the safe and effective real-word use of medications by society.

Methods: This study used a cross-sectional web-based survey of a convenience sample of drug safety and effectiveness researchers. The survey consisted of 97 proposed competencies divided into categories based on the existing competency frameworks. These categories were Scholar, Leader/Manager, Health Advocate, Collaborator, Communicator, and Professionalism. Competencies were rated on a 4-point scale: Extremely Important, Moderately Important, Of Little Importance, and Of No Importance. Each potential respondent received a personal identifier and one reminder email to help increase response rate. The survey was closed March 13, 2015.

Results: Of 519 emails sent, 152 provided a complete response, with 13 providing a partial response, with a total response rate of 165 of 484 (34.1%). Just over half (53.3%) of respondents were female and 45.6% we between age 40-59. The majority of respondents (56.7%) were from Ontario, and 70.4% reported practice in academia and 23.0% were healthcare professional. All 97 competencies were rated as moderately or extremely important by at least 75% of respondents demonstrating high suitability for inclusion on the list of competencies. Sixteen competencies were rated extremely important by 75% or more of respondents. The domain areas with the highest ratings based on average scores across competencies within domains were Collaborator, followed by Scholar, Communication, and Professionalism domains.

Conclusions: This list of competencies was deemed to be thorough, important and complete by respondents. Ultimately, the results can help create curricula in the future to educate the next generation of DS&E researchers.

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Evaluating therapeutic efficacy of a new lithium microemulsion in a rat model of Alzheimer disease

Wilson, Edward; Iulita, M. Florencia; Do Carmo, Sonia; Ducatenzeiler, Adriana; Cuello, A. Claudio
McGill University, Montreal, QC

Objectives: Alzheimer disease is the leading cause of dementia worldwide and currently no cure exists. However, experimental evidence reveals a possible neuroprotective effect of lithium in those at risk for developing Alzheimer disease and in AD animal models. Yet, the narrow therapeutic window and wide toxic side effect profile make lithium inappropriate for long-term treatment, especially in the elderly. We set out to evaluate whether treatment using a novel low-dose formulation of lithium would hold therapeutic benefit in the absence of the standard side effects of conventional lithium.

Methods: Alzheimer disease transgenic rats and wild type controls were treated for 8 weeks with a low-dose, oil-in-water lithium microemulsion (40ug Li/kg, 1 mL/kg) or vehicle (1 mL/kg) by deposit on the rectal mucosa. Measured were cognitive performance on a battery of spatial and

non-spatial tests of learning and memory, and biochemical markers of Aβ pathology, including β-secretase activity and Aβ levels. Serum lithium levels were also measured.
Results: Following treatment with low-dose lithium, Alzheimer rats showed significant improvement on object recognition, spatial navigation, and fear conditioning when compared to vehicle treated Alzheimer rats. Lithium-treated Alzheimer rats also showed a reduction in the level of toxic Aβ proteins and a reduction in BACE1 hyperactivity. The level of serum lithium in treated rats was below detection limit.
Conclusions: We revealed that this novel, low-dose lithium microemulsion preparation shows therapeutic benefit in a rat model of Alzheimer disease, raising the possibility that it be useful in the treatment in clinical populations.

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Impairment of neuroplasticity by binge drinking of alcohol: a paired associative stimulation study

Loheswaran, Genane; Barr, Mera; Rajji, Tarek; Blumberger, Daniel; Le Foll, Bernard; Jeff, Daskalakis
Centre for Addiction and Mental Health, Toronto, ON

Objectives: The current study was aimed at evaluating the effect of a binge drinking episode of alcohol on long term potentiation (LTP)-like neuroplasticity.
Methods: In a within-subject randomized, cross-over design, fifteen healthy alcohol drinkers were administered paired associative stimulation (PAS) following consumption of an alcohol or placebo beverage. PAS is an experimental paradigm that allows for the induction of associative LTP-like neuroplasticity. Subjects were administered alcohol at a dose of 1.5g/l of body water, producing a peak blood alcohol concentration (BAC) of 26.1mM (0.120% BAC). PAS induced neuroplasticity was measured immediately following PAS, post 15 min, post 30 min, post 60 min and the next day.
Results: The binge drinking episode inhibited LTP-like neuroplasticity, which was significantly different from placebo at 30 and 60 min following the PAS administration. Examination of longitudinal effects revealed no differences between alcohol and placebo beverages on LTP-like neuroplasticity the following day.
Conclusions: Findings suggest that binge drinking impairs neuroplasticity and while these effects are no longer evident the day after a single binge session, repetitive binging may produce long lasting changes in neuroplasticity that contribute to the development of alcohol use disorders.

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Quinine enhances adipogenesis in murine preadipocytes

Parlee, Sebastian; Weisheit, Corinne, Simon, Becky; MacDougald, Ormond; Ning, Xiaomin
University of Michigan, Ann Arbor, MI, USA

Objectives: Diminished adipogenesis under conditions of energy surplus leads to the development of type 2 diabetes by modifying adipocyte function and elevating inflammation and ectopic fat deposition. The mechanisms adipocytes use to “sense” surpluses within the nutritional environment and prompt adipogenesis remain elusive. Recently our laboratory investigated whether preadipocyte sweet taste receptors (T1R2/T1R3) fulfill this role. Indeed the T1R2/T1R3 agonist saccharin enhanced adipogenesis of murine and human preadipocytes by rapidly stimulating phosphorylation of Akt leading to increased expression of downstream targets C/EBPα and PPARγ. Heightened adipogenesis was, however, independent of T1R2/T1R3. Despite saccharin preferentially binding T1R2/T1R3, it binds bitter taste receptors (TAS2Rs) at concentrations that correspond with enhanced adipogenesis. Accordingly, in the current study a role for preadipocyte TAS2Rs in adipogenesis was investigated.
Methods: The expression of TAS2Rs were assessed during 3T3-L1 preadipocyte differentiation and within the stromal vascular and adipocyte fraction of lean and obese mice by QPCR. Preadipocyte TAS2R were subsequently stimulated with the agonist quinine, and analyzed for changes in early adipogenic signaling and downstream targets using immunoblot and QPCR.
Results: A subset of TAS2Rs are expressed transiently through adipogenesis and are elevated in the stromal vascular versus adipocyte fraction of lean but not obese mice. Stimulation of TAS2Rs by quinine enhanced 3T3-L1 differentiation in a concentration- and time-dependent manner marked by elevated neutral

lipids and expression of C/EBPα, adiponectin and FABP4. Quinine alone enhanced 3T3-L1 adipogenesis. When combined with each of the adipogenic stimuli methylisobutylxanthine, dexamethasone, or insulin, quinine displayed synergistic enhancement of differentiation, supporting an independent pathway for quinine signaling. Accordingly, preadipocytes treated with quinine manifest elevated phosphorylation of ERK1/2 compared to controls between 30-60 minutes as well as enhanced expression of adipogenic genes C/EBPα and PPARγ at days 5-8.

Conclusions: Taken together our data suggests that TAS2Rs are novel nutrient sensors that regulate adipogenesis.

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Short-term activation of 5-Hydroxytryptamine type 7 (5-HT7) receptor is neuroprotective against NMDA-induced excitotoxicity

Ahmed, Nawaz; Vasefi; Maryam S; Samarajeewa, Anshula; Razavi, Zahra; Gondora, Nyasha; Beazely, Michael A
University of Waterloo, Kitchener, ON

Objectives: Direct activation of platelet-derived growth factor (PDGF) β receptors in primary hippocampal and cortical neurons inhibits N-methyl-D-aspartate (NMDA) receptor activity and attenuates NMDA receptor-induced toxicity. We have previously demonstrated that long-term (24 h) activation of the serotonin (5-HT) type 7 receptor by small-molecule ligands such as LP 12 increases the expression of PDGFβ receptors and this results in the phosphorylation of downstream effectors and a differential regulation of NMDA receptor subunit expression that provides neuroprotective effects against NMDA excitotoxicity. This study aims to i)investigate if the 24 h 5-HT7 agonist induced neuroprotection against NMDA excitotoxicity occurs over shorter agonist treatment (1-4 h), and ii) confirm if the molecular basis of neuroprotection with short-term agonist exposure is similarly associated with the differential regulation of NMDA receptor subunit expression via increased PDGFRβ expression and basal signalling activity.

Methods: Cell cultures of primary embryonic mouse hippocampal neurons, the human neuroblastoma derived SH-SY5Y cell line, and the mouse hippocampal cell line HT22 were utilized. Excitotoxicity was induced by co-treatment with NMDA and glycine or glutamate alone. Cell viability was measured using the MTT assay. Western blotting was used to measure PDGFβ receptor expression and phosphorylation at PLCγ-binding site (tyrosine 1021), downstream signalling elements, and to measure any subunit changes in the NMDA receptor subunit expression (NR1, NR2A, and NR2B).

Results: Western blot analysis revealed that 4 h activation of 5-HT7 receptors in SH-SY5Y and differentiated HT22 cells increased tyrosine 1021 phosphorylation on the PDGF β receptor (the PLCγ binding site associated with down-regulating NR2B containing NMDA receptors) but not PDGFβ receptor expression. As a result, cell viability assays performed in differentiated HT22 cells showed that 4 h pre-treatment with LP 12 was indeed neuroprotective against 24h NMDA but not glutamate insults. Western blot analysis of differentiated HT22 cells suggested activation of multiple neuroprotective pathways as cells treated with 4 h LP 12 confirmed increases in tyrosine 1021 and ERK1/2 and phospholipase C (PLCγ1) phosphorylation along with increases in CREB expression. Lastly, MTT viability assays in cell cultures of primary embryonic mouse hippocampal neurons suggested that neuroprotection against NMDA toxicity can be observed between one and four hours.

Conclusions: The findings provide further evidence for the possibility of producing growth factor receptor-dependent neuroprotective effects, against extrasynaptic NMDA receptor activity, using small-molecule ligands of G protein-coupled receptors.

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TRPM7 regulates axonal outgrowth and maturation of primary hippocampal neurons.

Turlova, Ekaterina[1]; Bae, Christine YJ[1]; Deurloo, Marielle[1]; Chen, Wenliag[1], Barszczyk, Andrew[1]; Fleig, Andrea[2]; Horgen, F. David[3]; Feng, Zhong-Ping[1]; Sun, Hong-Shuo[1]
[1]University of Toronto, Toronto, ON; [2]The Queen's Medical Centre, Honolulu, Hawaii; [3]Hawaii Pacific University, Kaneohe, Hawaii

Objectives: TRPM7, a calcium-permeable, ubiquitously expressed cation channel was shown to play a role in several processes such as cell adhesion, cytoskeletal regulation and migration. As these processes are necessary for neurite elongation, we investigated the role of TRPM7 in neuronal outgrowth in culture.

Methods: Primary hippocampal culture, whole-cell patch-clamp, immunocytchemistry in conjunction with confocal microscopy, live-cell calcium imaging, western blotting and mass spectrometry were utilized in this study.
Results: We found that siRNA knockdown and pharmacological inhibition of TRPM7 with specific blocker waixenicin A preferentially enhanced axonal outgrowth in a dose-dependent and calcium-dependent manner at several time points in culture. We found that TRPM7 interacts and colocalizes with two cytoskeletal proteins, F-actin and α-actinin-1. Based on these data, we propose a TRPM7-mediated mechanism of actin-based growth cone protrusion and neurite elongation.
Conclusions: Neurite outgrowth is mediated at least in part through calcium-dependent TRPM7 mediation of cytoskeleton at the growth cone, making TRPM7 a potential therapeutic target for neurodegeneration.

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Beta-blocker dialyzability in chronic hemodialysis patients

Tieu, Alvin; Kucey, Andrew; Weir, Matthew; Urquhart, Brad; Velenosi, Thomas
University of Western Ontario, London, ON

Objectives: There is a paucity of data available to describe the dialytic clearance of beta-blockers. The majority of data available are from studies conducted prior to implementation of high-flux dialysis membranes. This study aims to characterize the dialyzability for four of the most commonly prescribed beta blockers–atenolol, bisoprolol, metoprolol and carvedilol—in patients undergoing conventional high-flux hemodialysis.
Methods: Hemodialysis patients from the London Health Sciences Centre (LHSC) are being recruited for a pharmacokinetic, crossover study. Each of atenolol (50mg), bisoprolol (5mg), carvedilol (6.25mg) or metoprolol (50mg) is administered to each patient on separate hemodialysis sessions, prior to initiation. Blood samples from the arterial and venous ports, and total spent dialysate are collected. Beta-blocker concentrations are measured using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). The dialyzer and recovery clearance methods are used to determine beta-blocker dialytic clearance.
Results: Using the dialyzer clearance method, preliminary dialytic clearance values for atenolol, bisoprolol and metoprolol were determined to be 228.6, 89.7, and 119.6 mL/min, respectively. Following hemodialysis, 3.9 mg of atenolol, 1.2 mg of bisoprolol, and 1.6 mg of metoprolol were recovered in the patient's dialysate. These amounts of dialyzed beta-blocker were then used in the recovery clearance method to produce dialytic clearance values of 129.5, 85.8, and 168.6 mL/min for atenolol, bisoprolol, and metoprolol, respectively.
Conclusions: Contrary to previous literature, our preliminary data suggests moderate bisoprolol dialyzability, while atenolol and metoprolol were extensively dialyzed. Drug dialyzability is critically important to optimize pharmacotherapy in dialysis patients. The therapeutic efficacy of beta-blockers to promote patient survival can be rendered counterproductive if substantial dialytic clearance occurs. This study may have clinical implications for increased risk of cardiovascular events for chronic hemodialysis patients being administered atenolol or metoprolol as opposed to bisoprolol.

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Endotoxin modulates the renal expression of drug transporters in a HIV-1 transgenic rat model

Karimian Pour, Navaz; Piquette Miller, Micheline
University of Toronto, Toronto, ON

Objectives: Kidney transporters impact the renal clearance of many drugs including antiretroviral agents. Bacterial co-infections, low grade endotoxemia and immune activation are common in chronic HIV (+) patients. Inflammation due to endotoxin or HIV may influence the expression and activity of drug transporters in the kidney. The HIV-transgenic (HIV-Tg) rat develops immune dysfunction and AIDS associated conditions similar to humans. Our objective was to study the effect of endotoxin and HIV on the renal expression of drug transporters in an HIV1-Tg rat model.
Methods: Five month male HIV1-Tg rats or wild-type (WT) littermates were treated with endotoxin (i.p.) or saline (n=7/group). The mRNA expression of transporters and cytokines were measured 18 hours after treatment in kidney samples using qRT-PCR. Serum cytokine levels were measured by ELISA.
Results: As compared to WT, basal expression of Octn1 and Mate1 drug transporters found to be significantly lower in the kidney of 5 month HIV-

Tg rats compared to their control littermates. On the other hand, the basal expression of Urat1, Oat2, Ent1 and ENT2 were significantly higher in the HIV-Tg rats. Endotoxin induced the release of inflammatory cytokines in the serum of both HIV1-Tg and WT, however interferon-γ and IL-6 induction was significantly reduced in HIV-Tg. Endotoxin significantly down-regulated the expression of Mdra1, Oct3, Oat2, Urat1, Ent1, Mate1 and Pept2 in both HIV-Tg and WT. A pronounced trend of higher expression of Pept1 was seen in HIV1-Tg and WT but this did not reach significance. Levels of Mdr1b, Mrp4, Oct1, Octn2, Oat1 and Oat3 were not changed after endotoxin challenge.

Conclusions: Our results demonstrate that HIV and endotoxin- induced inflammation imposes alterations in the expression of many clinically important drug transporters in the kidney. Therefore, the renal clearance of drug substrates could be altered in patients with co-existing infections. This may provide new insight to potential drug-disease interactions.

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Liver toxicity of glatiramer acetate: characteristics and outcomes of re-challenged patients in the Adverse Event Reporting System Database of the US Food and Drug Administration

Tanoshima, Reo[1,2]; Yeh, E. Ann[1,2]; Ito, Shinya[1,2]; Mazereeuw, Graham[2,3]
[1]The Hospital for Sick Children, Toronto, ON; [2]University of Toronto, Toronto, ON; [3]Sunnybrook Health Sciences Centre, Toronto, ON

Objectives: In rare cases, glatiramer acetate (GA), an immunomodulatory drug for multiple sclerosis, has been reported to be associated with elevated liver transaminases. Although transaminases may normalize upon discontinuation of the drug, there is no information regarding safety of re-administration. The objective of this study is to describe outcomes in individuals with liver dysfunction due to GA, using the Adverse Event Reporting System (FAERS) of US FDA.

Methods: Reports of all cases related to GA submitted to the FAERS, a database available to the public on request, between December 1996 and May 2013 were assessed. Detailed case reports of the patients who developed signs of liver toxicity were collected, and information was summarized.

Results: We identified 8,559 patients with GA-associated adverse events. Among them, 121 had liver dysfunction. GA was discontinued due to liver dysfunction in all of the patients, but re-administered in 15 patients (12.4%). The duration between discontinuation of GA and re-administration was between 1 month and 50 months (median; 11 months). Outcome data from re-administration were available for 13 of the 15 patients one to 12 months after re-administration: 3 showed no recurrence of liver dysfunction, but in 7, liver dysfunction recurred. The rest 3 patients did not develop liver dysfunction, but had immediate reactions such as urticaria.

Conclusions: A small proportion of patients who develop transaminase abnormalities while on GA may be re-challenged with GA safely. The specific profile of patients in whom re-challenge may be safe is unclear, but should be the subject of further investigations. Our results also demonstrate the utility of the FAERS database for the identification and analysis of unpublished cases of drug toxicity. This method may provide more comprehensive information than that in the published literature in some situations, and may be of specific use in clinical decision making.

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Gender segregation of 24-h ambulatory blood pressure (ABPM) response to switching between differing nifedipine osmotic delivery formulations

Pollak, P. Timothy[1]; Dehar, Navdeep[1]; Herman, Robert[1]; Zarnke, Kelly[1]; Feldman, Ross[2]
[1]University of Calgary, Calgary, AB; [2]University of Western Ontario, London, ON

Objectives: Pharmacy initiated switches between “branded” and generic antihypertensive medications happen frequently. Only non-time-based PK parameters are used in approving generic formulations. Therefore timing of modified drug release is not evaluated. ABPM was used to assess clinical differences in response to Mylan-nifedipine ER (MyN) and “branded” Adalat XL (AdN).

Methods: A randomized cross-over block design studied 20 patients receiving daily morning dosed AdN vs. MyN 60 mg. After each 2-week dosing period, 24-hr ABPM was done. Systolic (SBP), both for 24 h and last 8 h (22:00 h - 06:00 h) was examined.

Results: Mean ± SE 24-h SBP was 133 ± 2.4 mmHg with AdN, and 135 ± 2.3 mmHg with MyN

(p=0.03). For last 8 h, mean ± SE nocturnal SBP was 125 ± 3.3 mmHg with AdN and 129 ± 2.8 mmHg with MyN (p=0.018). However when only the 9 women were considered, SBP for 24-h was 133 ± 4.0 mmHg with AdN, and 137 ± 3.3 mmHg with MyN (p=0.0002) and for last 8-h, was 124 ± 5.4 mmHg with AdN and 132 ± 4.0 mmHg with MyN (p=0.0003).

Conclusions: Mean 24-h and last 8 h SBP were statistically significantly higher in patients when taking MyN, than when taking AdN. This is likely based on distribution of nifedipine delivery from MyN's first-order drug release profile vs. AdN's zero-order drug release. Differences in both extent and timing of delivery of a drug with only a 2-hour half-life, could allow important concentration fluctuations in early and late partial AUC's. Sensitivity to the difference in formulations appears greater in women, especially at the end of the dosing interval. This should be explored further. Arbitrary nifedipine switching by pharmacies may lead to unexplained variability in BP control, with unintended consequences in patient management.

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Utility of longitudinal pulmonary function monitoring for early detection of amiodarone pulmonary toxicity

Pollak, P. Timothy[1]; Tourin, Pavel[2]
[1]Calgary, AB; [2]University of Alberta, Edmonton, AB

Objectives: Amiodarone remains widely used, despite concern about possible adverse effects, the most commonly feared being amiodarone pulmonary toxicity (APT). This may present clinically as dyspnea, cough, fever, and weight loss. Better management of loading and maintenance doses may reduce the incidence of APT. Our objective was to analyze prospectively collected long-term serial lung function results in patients receiving amiodarone.

Methods: Sixty-two patients (63.7 +/- 12.9 y) receiving amiodarone were followed for at least 24 months. Loading with 600-1600 mg/d for 7-14 d was followed by 200-400 mg/d. Serum [amiodarone] and active metabolite, [desethylamiodarone (DEA)], were measured at baseline, 0.5, 1, 2, 3, 4, 6, 9 and 12 months and q 6 months. Coincident Forced Vital Capacity (FVC, L), Forced Expiratory Volume (FEV-1, L) and Diffusion Capacity for Carbon Monoxide (DCO, mL/min/mmHg) were measured. A drop in pulmonary function >30% from baseline on consecutive recordings was scrutinized for possible toxicity.

Results: Mean serum [amiodarone] rose with loading doses, peaking at 1.84 ± 0.94 mg/L by 2 wk, then declined as the drug distributed to peripheral tissues, reaching 1.37 ± 0.65 mg/L by 3 months. Mean serum DEA, rose slowly as amiodarone was metabolized, reaching a peak of 1.25 ± 0.69 mg/L at 12 months. Spirometry (mean FVC and FEV-1) did not change from baseline over 36 mo. Mean DCO decreased from 19.7 ± 6.8 at baseline to 17.9 ± 6.9 by 3 months (see Figure). Only 3 patients (4.8%) fell >30% from baseline and all resolved without intervention. Mean DCO for the population returned to baseline by 36 months.

Conclusions: A weak correlation between pulmonary function and serum [amiodarone] suggests a direct effect on the lipid composition of lung surfactant. There is no evidence of cumulative effects on DCO at stable [amiodarone] over time. [Amiodarone] several times clinically useful range, are required to produce toxicity in lung cell cultures. Maintaining serum [amiodarone] in the range of 1 to 2 mg/L provides efficacy while avoiding toxicity. Routine monitoring of pulmonary function did not provide any predictive information. This suggests that documenting pulmonary function at baseline and annually is sufficient to allow proper assessment of new respiratory symptoms that are detected clinically.

23 –WITHDRAWN

Prevalence of heavy fetal alcohol exposure in Canada: A population based meconium study

Delano, Kaitlyn[1]; Kapur, Bhushan[2]; Koren, Gideon[2]; Pope, Eliza[3]
[1]University of Toronto, Toronto, ON; [2]The Hospital for Sick Children, Toronto, ON; [3]McMaster University, Hamilton, ON

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Optimization of intravenous acyclovir dosing in children using Monte Carlo simulation

Takeuchi, Masanobu; Ito, Shinya
The Hospital for Sick Children, Toronto, ON

Objectives: Dosing of intravenous acyclovir for varicella zoster virus (VZV) in immunocompromised host varies according to age. The current typical dosing guideline is:

60mg/kg/day q8h for age < 1 year of age; and 1500mg/m2/day q8h for those ≧1 year of age. However, it is not known if the recommended dosing guideline achieves expected blood concentration profiles in the majority of the patients. The objective of our study was to assess efficacy and toxicity of our current dosing regimens, using a simulation approach.

Methods: We performed Monte Carlo simulation of plasma acyclovir concentrations in 1000 children per main age groups using various dosing regimens. Means and variations of pharmacokinetics data of the pediatric population were retrieved from the literature. The surrogate efficacy target was a steady state plasma concentration of ≥3 mg/L at a mid-point of the dosing interval. The surrogate safety target was a maximum concentration of <50 mg/L at a steady state. A dosing regimen was considered optimal if the achievement rate of these targets is more than 90% of the patients.

Results: The current dosing regimens were not optimal because it achieved the efficacy targets in 90% of the patients, but the resultant plasma levels were off the safety target in more than 30% of the patients. Our simulation further suggests that a smaller daily dose (30 mg/kg/day) with a shorter dosing interval (6h) results in optimal plasma concentration profiles in all paediatric age groups with target achievement of 98% for efficacy and 99% for safety.

Conclusions: The current dosing regimens are unlikely to achieve optimal plasma acyclovir concentrations in immunocompromised children with VZV.

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The effect of gut-derived uremic toxins on the expression of hepatic drug metabolizing enzymes in chronic kidney disease

Velenosi, Thomas; Tieu, Alvin; Feere, David A; Kucey, Andrew; Urquhart, Brad
University of Western Ontario, London, ON

Objectives: Previous studies have demonstrated a decrease in hepatic drug metabolism in chronic kidney disease (CKD). The objective of this study was to determine if the removal of gut-derived uremic toxins by the spherical carbon adsorbent, AST-120, will recover hepatic CYP3A and CYP2C enzyme function and expression in rats with CKD.

Methods: Chronic kidney disease was induced in male Wistar rats using 0.5% adenine supplemented into rat chow. Control rats were pair-fed to CKD animals. After 5 weeks, control and CKD animals were further divided and received 8% AST-120 or a control diet. Rats were sacrificed 8 weeks after initiation of the study and plasma and liver tissue were obtained.

Results: Plasma creatinine and urea levels were increased 3-fold and 2.5-fold in CKD rats compared to controls ($P<0.05$). The gut-derived uremic toxins: indoxyl sulfate, p-cresyl sulfate and hippuric acid were significantly increased 4.8-fold, 11.8-fold and 3-fold; respectively, in CKD animals compared to controls. Animals with CKD treated with AST-120 had indoxyl sulfate, p-cresyl sulfate and hippuric acid levels significantly lower than CKD animals and similar to control animals. Hepatic CYP3A2 mRNA expression was significantly decreased in rats with CKD ($P<0.05$); however, rats with CKD given AST-120 had a 25% recovery in CYP3A2 mRNA expression. Rats with CKD had decreased hepatic CYP2C11 mRNA expression that was not recovered by AST-120.

Conclusions: AST-120 given to rats with CKD reduced indoxyl sulfate, p-cresyl sulfate and hippuric acid levels similar to control levels. Hepatic CYP3A2 mRNA expression was decreased in CKD and moderately recovered by AST-120. AST-120 did not affect the downregulation of CYP2C11 in rats with CKD. Therefore, gut-derived uremic toxins may partially mediate the downregulation of hepatic CYP3A in CKD.

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Hypoxia-Inducible Factor-1 contributes to transcriptional regulation of Bcl2-/adenovirus E1B 19KDa-interacting protein in hypoxic cortical neurons

Atoui, Samira; Anderson, Christopher; Lu, Ping
University of Manitoba, Winnipeg, MB

Objectives: Poly ADP-ribose polymerase-1 (PARP-1) causes neuron death in brain hypoxia by inducing mitochondrial permeability and nuclear translocation of apoptosis-inducing factor (AIF). Bcl2-/adenovirus E1B 19KDa-interacting protein (Bnip3) is a pro-apoptotic Bcl-2 protein that is induced in hypoxia and increases mitochondrial permeability and neuronal death. Hypoxia-inducible factor-1 α (HIF-1α) is a key regulator of Bnip3 transcription by binding to hypoxia response elements (HRE) in the genomic Bnip3 upstream promoter but it is

unknown whether HIF-1α influences Bnip3-mediated mitochondrial neurotoxicity in hypoxia. Previously, we found that hypoxic activation of PARP-1 depletes nicotinamide adenine dinucleotide (NAD+) and inhibits NAD+-dependent SIRT1. Since HIF-1α/HRE interactions are sensitive to SIRT-1 mediated acetylation, the objective of this study was to determine whether HIF-1α is a regulator of Bnip3 expression in hypoxia

Methods: Mouse cortical neurons cultures were exposed to 0% O2 in a hypoxic chamber for up to 24 hours. Extracts were analyzed by real time qPCR and Western blot to measure mRNA and protein expression of HIF-1α and Bnip3. Immunoprecipitation with anti-HIF-1α followed by western blot with anti-acetyl-lysine was used to quantify hypoxic changes in acetylated HIF1-α. Chromatin immunoprecipitation with anti-HIF-1α followed by qPCR at Bnip3 HRE flanking sites was used to determine hypoxic changes in HIF-1α to the upstream Bnip3 promoter region.

Results: Neither mRNA nor protein levels for HIF-1α were influenced by 0% O2 , while Bnip3 mRNA and protein levels were increased. Hypoxic Bnip3 induction was attenuated by inhibition or deletion of PARP-1, implicating a PARP-1 dependent process. Bnip3 induction was also inhibited in the presence of lentiviral vectors producing shRNA to reduce HIF-1α expression, indicating hypoxic Bnip3 expression is HIF-1α-dependent. Finally, ChIP analysis revealed that HIF-1α binds Bnip3 HRE in hypoxia in a manner that is sensitive to PARP-1 activity

Conclusions: Data show that hypoxic Bnip3 induction is dependent on PARP-1 activity and HIF-1α-mediated transcriptional regulation

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Neurodevelopment of children following maternal hospitalization for nausea and vomiting of pregnancy

Nulman, Irena[1]; Maltepe, Caroline[1]; Farine, Dan[2]; Koren, Gideon[1]
[1]The Hospital for Sick Children, Toronto, ON; [2]Mount Sinai Hospital, Toronto, ON

Objectives: The study objectives were to determine child long-term neurodevelopment following maternal hospitalization for severe NVP.

Methods: Motherisk NVP Helpline callers from 2006-2012 were identified from our prospectively collected database. Women with NVP treated with doxylamine-pyridoxine (Diclectin®/Diclegis®) or with no pharmacotherapy were included. Diclectin®/Diclegis® dose, severity of NVP, hospitalization for NVP, concomitant medications, severity of maternal depression, and maternal IQ were obtained. Children (ages 36/12 to 611/12) were assessed using standardized psychological tests.

The study cohort was divided into three groups: 1) NVP treated with more than 4 tablets per day of Diclectin®/Diclegis® (above the manufacturer recommended number of tablets) (n=62); 2) NVP treated with the recommended up to 4 tablets per day (n=81); 3) NVP and no pharmacotherapy (n=76).

Results: Twenty-two women were hospitalized for severe NVP. The hospitalized women initiated recommended preventive antiemetics significantly later (6.8vs5.7weeks,p=0.02), experienced more severe NVP (11.1vs7.5,p<0.001) and depression (10.1vs5.1,p=0.03), and needed higher daily doses of Diclectin®/Diclegis® (1.0vs0.4mg/kg/d,p<0.001) and concomitant medications. Children of hospitalized mothers achieved significantly lower IQ scores (verbal 107.2vs112.7,p=0.04; performance 105.6vs112.3,p=0.03; full scale 108.7vs114.2,p=0.05). Duration of hospitalization, maternal depression, and maternal IQ were significant predictors for these outcomes. Daily intake of Diclectin®/Diclegis® was not associated with any adverse outcomes.

Conclusions: Timely preventive antiemetics and depression control may prevent hospitalization and be associated with favorable child neurodevelopment. More research is needed to investigate the effect of severe NVP and confirm these results.

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Development of a mouse smooth muscle cell model for assessing the role of chemerin/ chemokine-like receptor 1 signalling in atherosclerosis

Blundon, Heather; Goralski, Kerry; Sinal, Chris
Dalhousie University, Halifax, NS

Objectives: Chemerin is a fat tissue-secreted protein with cell proliferative, migratory and pro-inflammatory properties. Its presence in human serum correlates with atherosclerosis severity however, its role is unknown. This study aims to develop a protocol to isolate aortic vascular

smooth muscle cells VSMCs from wild-type (WT) and chemerin receptor (CMKLR1) knockout (KO) mice, in order to assess the role of chemerin/CMKLR1 signalling in SMC migration and proliferation (important stages of atherosclerotic plaque formation).
Methods: Aortic VSMCs were harvested from adult WT and CMKLR1 KO mice and were cultured and characterized after 0, 7 and 14 days. BrdU and MTT assays were used to assess proliferation and viability of VSMCs upon treatment with chemerin (0- 30 nM) alone and in combination with the inflammatory cytokine TNFα (0-10nM), in the presence and absence of fetal bovine serum (FBS).
Results: Endothelial-specific gene expression decreased while VSMC-specific gene expression increased with increased WT VSMC confluence. WT VSMC proliferation increased by 100- and 200-fold in the presence of 1% and 5% FBS (positive control), respectively. Chemerin did not significantly affect the proliferation of WT VSMCs in the presence or absence of 1% FBS. Co-treatment with chemerin and 1 or 10 nM TNFα reduced WT SMC proliferation by 50%; however, MTT analysis showed no reduction in cell viability. In preliminary studies, chemerin did not affect the proliferation of CMKLR1 KO SMCs.
Conclusions: Mouse aortic VSMCs were successfully isolated, cultured and characterized. Chemerin does not significantly affect aortic VSMC proliferation, but may attenuate proliferation in the presence of additional inflammatory mediators.

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Bias against the null hypothesis in retrospective registries of gestational drug exposure

Etwel, Fatma[1]; Rieder, Michael[1]; Koren, Gideon[2]
[1]University of Western Ontario, London, ON; [2]Motherisk Program, Toronto, ON

Objectives: Results of retrospective pregnancy registries (collected after pregnancy outcome is known) are commonly reported in regulatory documentations and in the medical literature. The objective of the study is to compare the rates of major congenital malformations reported in retrospective vs. prospective registries of the same drug and in the same registry, in an attempt to quantify the potential bias of retrospective reports.
Methods: We searched for all fetal safety reports where both prospective and retrospective registries were available for the same medication.
Results: In all cases the rates of major malformations after exposure to drugs were significantly higher when determined retrospectively than prospectively. The bias was consistent with retrospective studies reporting 4.18±1.23 (range 2.13-5.97) fold higher rates of malformations than prospective registries of the same drug.
Conclusions: The present study confirms a major and consistent bias against the null hypothesis in retrospective registry studies that needs to be considered when interpreting such data. Spontaneous reporting is highly selective toward adverse events, as families with normal pregnancy outcomes are less likely to report them.

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Occupancy of dopamine D2 and D3 receptors by buspirone: A [11C]-(+)-PHNO PET study in humans

Di Ciano, Patricia; Le Foll, Bernard; Payer, Doris; Guranda, Mihail; Nakajima, Shinichiro; Tong, Junchao; Mansouri, Esmaeil; Wilson, Alan; Houle, Sylvain; Meyer, Jeff; Graff, Ariel Boileau, Isabelle
CAMH, Toronto, ON

Objectives: There is considerable interest in blocking the dopamine D3 receptor (DRD3), versus the D2 receptor (DRD2) to treat drug addiction. However, no selective DRD3 antagonist is available in the clinic. Buspirone, an anxiolytic drug, has been proposed as a potential strategy for addiction as it is suggested that this drug has high in vitro affinity for DRD3, binds to DRD3 in the brain of living non-human primate and also disrupts psychostimulant self-administration in preclinical animal models. No study has explored the DRD3 occupancy by buspirone in humans.
Methods: We used positron emission tomography (PET) and the DRD3 preferring radioligand, [11C]-(+)-PHNO, to test the hypothesis that buspirone occupies (decreases [11C]-(+)-PHNO binding) DRD3 more than DRD2. Eight healthy participants underwent [11C]-(+)-PHNO PET scans after administration of placebo,or 30, 60, and 120 mg of buspirone (four scans) in a single-blind within-subjects design.

Results: [11C]-(+)-PHNO binding in DRD2 and DRD3-rich areas was decreased by the highest (60-120mg), but not the lowest (30mg), doses of buspirone. The maximal occupancy obtained was ~25% in both areas. Plasma levels of prolactin (a DRD2 marker) correlated with occupancy by buspirone. Self-reported dizziness and drowsiness increased after buspirone intake but that did not correlate with receptor occupancy in any region.
Conclusions: Overall, the modest occupancy of DRD2 and DRD3 even at high doses of buspirone, yielding high levels of metabolites, suggests that buspirone may not be a drug suitable to preferentially block DRD3 in humans.

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Chemerin expression and secretion is increased in differentiated adipocytes from obese humans compared to normal weight humans

Goralski, Kerry; Ernst, Matthew; Sinal, Christopher
Dalhousie University, Halifax, NS

Objectives: Obesity is characterized by an excess of dysfunctional adipose tissue and is a risk factor for cardiovascular disease and type 2 diabetes. Different mechanisms linking obesity with these comorbidities have been postulated but remain poorly understood. White adipose tissue secretes a number of bioactive molecules known as adipokines, which regulate various biological functions including insulin sensitivity, lipid metabolism, and inflammation. Chemerin is an adipokine that regulates adipocyte differentiation and metabolism by binding to and activating the G protein-coupled receptor chemokine like receptor-1 (CMKLR1). The objective is to determine if adipocytes from obese humans produce greater amounts of chemerin than adipocytes from normal weight humans.
Methods: Cryopreserved subcutaneous precursor adipocytes from normal weight humans (n=3, BMI <24.99) and obese humans (n=3, BMI >30.0) were differentiated into mature adipocytes. RNA, culture media, and cell samples were isolated at Days 0, 3, 7 and 14. The mRNA levels of chemerin, CMKLR1, and markers of adipocyte differentiation were measured using quantitative PCR. Total chemerin was determined using an ELISA.
Results: Staining for neutral lipids and the mRNA expression of the adipogenesis markers PPARg, adiponectin, and leptin increased with time after initiating adipocyte differentiation. Chemerin mRNA and media total chemerin concentrations were higher in 14-day differentiated adipocytes from obese humans compared to normal weight humans.
Conclusions: Precursor adipocytes were successfully differentiated into mature adipocytes over 14 days. The mature-adipocytes from obese humans were primed to express and secrete higher amounts of chemerin than mature adipocytes from lean humans. This may contribute to increased plasma chemerin concentrations in obese humans.

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Diagnostic value of the lymphocyte toxicity assay for types I, III and IV beta-lactam-induced allergy reactions: Pathophysiological implications

Elzagallaai, Abdelbaset; Chow, Lindsey; Abuzgaia, Awatif M; Rieder, Michael J
University of Western Ontario, London, ON

Objectives: Background: Beta-lactam antibiotics (BLAs) are the drugs most associated with immune-mediated hypersensitivity reactions (drug allergy). They can elicit all types of allergic reactions i.e., types I, II, III and IV. Although the immediate IgE-mediated reaction has been well studied, the pathophysiology of non-immediate HSRs is not well understood. Cross-reactions among BLAs with different side chains are variable. The diagnosis and prediction of BLAs-induced allergic reactions is challenging and based mostly on clinical history. The lymphocyte toxicity assay (LTA) is an in vitro diagnostic test for drug allergy. Its value in diagnosis of reactions to BLAs is still unknown. This work is an attempt to evaluate the test performance for diagnosis of different types of allergy to BLAs and to investigate the their different underlying pathophysiology.
Methods: Methods: One hundred and four individuals (52 drug allergy-suspected patients and 52 healthy volunteers) were included in this study. Patients were identified from clinical records and included using a rigorous inclusion criteria based on their clinical presentation. The LTA tests were performed after resolution of the reaction symptoms. Data was expressed as percentage of cell death of after incubation with the suspected drug in presence of rat microsomes.

Results: Results: Patients were grouped according to their exhibited symptoms into 3 groups constituted of 8 patients type I, 23 patients type III and 21 patients type IV. Patients with type III reactions exhibited higher degrees of cell death than the other 2 groups ($p<0.05$) with type I patients exhibiting the lowest degree of cell death among all groups.
Conclusions: Conclusion: The LTA was able to identify patients susceptible to develop allergy to BLAs. Cells from patients with different types of reactions exhibit variable degrees of cell death indicating possible distinct pathophysiological mechanisms. The LTA can be a useful tool to both diagnose drug allergy and explore its underlying pathophysiology.

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Jadomycins B is selectively toxic to triple negative human breast cancer cells in a zebrafish xenotransplantation model

Hall, Steven[1]; Goralski, Kerry[1]; Toulany, Jay[1]; Veinotte, Chansey[2]; Razaghi, Babak[2]; Robertson, Andrew[1]; Martinez-Farina, Camilo[1]; Jakeman, David[1]; Dellaire, Graham[1], Berman, Jason[1,2]
[1]Dalhousie University, Halifax, NS; [2]IWK Health Centre, Halifax, NS

Objectives: Jadomycins are bacterial-derived cytotoxic agents that retain cytotoxicity in multidrug resistant (MDR) MCF7 breast cancer cells that overexpress drug efflux transporters, like P-glycoprotein (P-gp) in vitro. Our objectives are to evaluate the selective cytotoxicity of jadomycins in drug-sensitive and taxol-resistant, P-gp overexpressing triple-negative (TN) breast cancer cells grown in culture and after their xenotransplantation into zebrafish embryos; a preclinical drug discovery model that allows for direct visualization of any jadomycin-induced therapeutic response.
Methods: TN MDA-MB-231 (231-CON) cells were cultured in gradually increasing concentrations (0.05-5 mg/mL) of taxol for 7 months, generating taxol-resistant cells (231-TXL). P-gp expression was determined using quantitative PCR. The effects of drug treatments on cell viability were measured using MTT assays. In vivo jadomycin toxicity was determined in zebrafish embryos, and inhibition of cancer cell proliferation determined in 231-CON cells xenotransplanted into the yolk sacs of embryos.
Results: P-gp gene expression increased 122,000-fold in 231-TXL versus -CON cells. Jadomycins B, S, and F were equipotent in 231-TXL (IC50 values of 2.9-3.1 µM) versus 231-CON (2.7-3.0 µM) cells while the control drugs mitoxantrone and doxorubicin were 19- and 42-fold less potent, respectively. Jadomycin B, S, and F IC50 values were 3.0, 2.9, and 1.6-fold greater in HMECs than in 231-CON cells, respectively. In zebrafish the maximum tolerated doses of jadomycins B, S and F were 40, 55, and 50 µM, respectively. Jadomycin B (20 µM, 48 hours) reduced the proliferation of xenotransplanted 231-CON cells by 72% versus vehicle.
Conclusions: Jadomycins B, S, and F retain their potency in MDR 231-TXL cells and display cancer cell selectivity in vitro and in vivo, warranting further research testing jadomycins against MDR TN breast cancer.

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Risks of autism spectrum disorder in the offspring exposed to selective serotonin reuptake inhibitors in utero: a systematic review and meta-analysis

Tohru; Takeuchi, Matsuyama, Tasuku; Koren, Gideon; Takeuchi, Masanobu, Ito, Shinya
The Hospital for Sick Children, Toronto, ON

Objectives: The objective of our systematic review and meta-analysis is to examine the risk estimates of autism spectrum disorder (ASD) in the offspring exposed to selective serotonin reuptake inhibitor (SSRI) in utero.
Methods: We searched on Medline, Embase classics plus Embase, Cochrane Central Register of Controlled Trials, and PsycInfo up to February 2015. Pooled odds ratio (OR) and associated 95% confidence intervals (CIs) were calculated using the random effect model.
Results: We identified five case-control and three cohort studies. The profile of two Danish cohort studies was almost identical; however, those did not indicate similar risk estimates. The SSRI exposed group had significantly higher risk of ASD than the SSRI non-exposed group (pooled OR 1.43, 95% CI 1.14-1.78). In the subgroup analyses, the risk of ASD was similar between the SSRI exposed group and other antidepressants exposed group (pooled OR 1.14, 95% CI 0.67-1.96). Furthermore, the SSRI exposed group did not show a significantly increased ASD risk (pooled OR 0.96, 95% CI 0.57-1.63) when the analysis was confined to whose mothers had conditions of psychiatric

disorders. There were no significant difference in the pooled risk estimates after replacing the two Danish cohort studies.

Conclusions: Our findings support the association between SSRI exposure in utero and risk of ASD in offspring, but raised several critical concerns about the association. Further study will be warranted especially focusing on the special populations who have indication of antidepressants use.

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Effects of synthetic retinoid, Am80 on inorganic phosphate levels in rats with adenine-induced renal failure

Takitani, Kimitaka; Tamai, Hiroshi
Osaka Medical College, Takatsuki, Osaka

Objectives: Phosphate plays a key role in mineral metabolism, skeletal development, and various cellular functions affecting energy-transfer mechanisms. The serum inorganic phosphate levels are regulated rigorously though intestinal absorption, exchange with intracellular and bone storage, and renal tubular reabsorption. Inorganic phosphate transport in renal proximal tubule is mediated by several sodium-dependent phosphate co-transporter (Npts) genes, which have been classified in three groups: type I (Npt1), type II (Npt2a and Npt2c), and type III (PiT1 and PiT2). Retinoids are a group of compounds that have the equivalent efficacy to retinoic acid, which has a critical role in immunity, cell proliferation/differentiation, reproduction, and morphogenesis. All-trans retinoic acid (ATRA) has an important role in myeloid differentiation of myeloid cells and generally used for the treatment of acute promyelocytic leukemia (APL). Synthetic retinoids, Am80 (4[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carbamoyl] benzoic acid) is an agonist with high specificity for retinoic acid receptor (RAR) alpha and RAR beta. Am80 is used clinically for patients with refractory APL in Japan. ATRA is known to affect inorganic phosphate levels via regulating sodium-dependent phosphate co-transporter genes. We examined the effect of Am80 on inorganic phosphate levels in adenine-induced renal failure rats administered Am80, and investigated the expression of Npt2a and Npt 2c genes.

Methods: Wistar rats (four weeks, male) were assigned to four groups: (1) a control group, (2) an Am80 group (3mg/kg/day), (3) an adenine diet group (0.75% adenine diet), and (4) a combination of adenine diet and Am80 group, and fed for two weeks.

Results: Inorganic phosphate levels of urine and blood in rats with adenine-induced renal failure rats were improved by Am80 administration, and renal expression of Npt2a and Npt 2c in adenine fed rats was increased by Am80.

Conclusions: Synthetic retinoid, Am80 may be a useful agent of modulating phosphate status under the renal failure condition.

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Evaluation of anti-inflammatory effects of different creatine supplements in a canine chondrocytes model

Alraddadi, Eman; Miller, Donald
University of Manitoba, Winnipeg, MB

Objectives: Creatine is a natural ergogenic compound and it is widely used by athletes as an ergogenic aid to enhance performance and muscle mass. The cellular effects of creatine on mitochondrial function suggest there may be additional benefits to creatine supplementation. Little is known about the anti-inflammatory activity of the dietary supplement creatine. The aim of this study is to evaluate the anti-inflammatory effects of creatine supplements in canine chondrocytes.

Methods: Inflammation was examined in primary cultured canine chondrocytes (CCs). Confluent CCs were stimulated with interleukin-1 beta (IL-1B) (10 ng/ml). Culture media was collected from the stimulated cells at various time points (4-72 hours) and analyzed for the appearance of inflammatory mediators, prostaglandin E2 (PGE2) and tumor necrosis factor alpha (TNFa) using enzyme-linked immunosorbent assay (ELISA). Changes in inflammatory response to IL-1B was examined following treatment with various creatine compounds, creatine monohydrate (CM), creatine ethyl ester (CEE) and creatine hydrochloride (CHCl) in addition to the metabolite, creatinine (CRN). In addition, expression of cyclooxygenase 2 (COX-2) in CCs were examined under the various stimulation conditions by western blot.

Results: Exposure to IL-1B, results in a time-dependent increase in both TNFa and PGE2 release from CCs and induction of COX-2 expression. Treatment with COX inhibitor, Rimadyl, substantially reduced PGE2 release, despite increasing both TNFa release and COX-2

expression in the cells. While all the creatine compounds examined significantly reduced PGE2 release, the level of reduction was not as great as Rimadyl. In contrast, all creatine compounds examined reduced TNFa release in stimulated CCs in a time dependent manner.
Conclusions: Creatine related compounds significantly reduce cellular mediators of inflammation in a primary CCs model. As these same cells are involved in joint-related inflammation (i.e. arthritis), creatine based dietary supplements may have a beneficial role in preventing inflammation within the joint and other tissues.

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Evaluation of food effects on the oral pharmacokinetics of rosuvastatin

McLean, Cheynne[1]; Kim, Richard[1]; Gryn, Steven[1]; Morse, Bridget[2]
[1]University of Western Ontario, Granton, ON; [2]Bristol-Myers Squibb, Princeton, NJ

Objectives: Statins are the most commonly prescribed class of drugs for the treatment of hypercholesterolemia and exert their effect through targeted accumulation in the liver through the action of OATP transporters. More recently, its been discovered that the bile acid transporter, NTCP, can also mediate statin hepatic uptake. We hypothesized food ingestion would enhance the activity of NTCP, and thereby lower circulating rosuvastain concentration while increasing its hepatic accumulation.
Methods: This is a prospective, randomized crossover study where rosuvastatin 10 mg was administered under fasting conditions or ingested with a low or high-fat meal. All subjects completed all 3 study days, with a washout period of at least one week between study days. A blood sample was collected at time 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours after drug administration on all study days. Plasma rosuvastatin concentrations were determined using liquid chromatography/mass spectrometry (LC/MS).
Results: Preliminary results from 8 subjects suggest near 30% higher plasma concentration of rosuvastatin when the statin is ingested during fasting state compared to either the low-fat or the high-fat fed state ($p < 0.05$). There was no difference in rosuvastatin plasma level between high fat and low fat fed states.
Conclusions: Current United States and Canadian dosing guidelines indicate no preference for fed or fasting rosuvastatin administration. Our data obtained to date already suggest that rosuvastatin plasma concentrations are lower when ingested with food. Since a common adverse event associated with statin therapy is muscle pain/damage (statin-myopathy), our findings have the potential to serve as a novel and simple strategy for mitigating statin myopathy risk, particularly if we are able to demonstrate that in statin therapy, a lower plasma statin level during the fed state correlates with the similar LDL-cholesterol lowering that is observed in patients who take the statin in a fasted state.

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The impact of variation in rat brain CYP2D activity on codeine analgesia is similar across multiple routes of codeine administration and analgesia assays

McMillan, Douglas; Tyndale, Rachel.
University of Toronto, Toronto, ON

Objectives: Most cytochrome P450 (CYP) drug metabolism occurs in the liver; however, extra-hepatic CYP activity may alter target-tissue drug concentrations and effect. CYP2D is expressed in the brain, and is responsible for metabolizing numerous CNS-acting drugs including codeine, which is activated to morphine. While hepatic CYP2D is un-inducible, higher brain (but not liver) CYP2D is found in smokers, and nicotine administration induces rat and monkey brain (but not liver) CYP2D. Previously, we showed that brain CYP2D activity altered codeine metabolic activation and analgesia following subcutaneous and intraperitoneal administration in rats, through the use of a reflexive analgesic assay. Clinically, codeine is consumed orally, a route with significant absorption barriers and first-pass metabolism. Additionally, pain transmission generally originates in supra-spinal brain areas.
Methods: We assessed the role of rat brain CYP2D in codeine activation and analgesia following codeine administration by oral gavage using direct intracerebroventricular administration of the CYP2D inhibitor propranolol. In addition, we compared results from the spinal-reflex tail-flick assay to the supra-spinal hot-plate assay on codeine analgesia after induction of brain CYP2D with nicotine pretreatment.

Results: Brain CYP2D inhibitor pretreatment resulted in a significant reduction in codeine analgesia when given via oral-gavage (0.30-fold decrease in AUC 0-30min; $p<0.05$ vs. vehicle) as seen with intraperitoneal (0.56-fold; $p<0.05$) and subcutaneous (0.68-fold; $p<0.01$) administration. Furthermore, brain CYP2D induction through seven-day nicotine treatment resulted in a significant increase in codeine analgesia assessed via hot-plate assay (2.46-fold increase in AUC 0-30min; $p<0.01$ vs. vehicle) as seen with the tail-flick assay (1.59-fold; $p<0.001$).
Conclusions: Our research in brain metabolism has focused on modelling a wide range of metabolism phenotypes in preclinical animal models with potential translational relevance to humans. Variation (genetic and/or environmental) in brain CYP2D activity may contribute to clinically relevant differences in individual response to, and abuse liability of, centrally acting CYP2D substrates.

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Effect of genetic variation in CYP2A6, UGT2B10, UGT2B17, OCT2, and FMO3 on nicotine disposition kinetics among African American smokers

Taghavi, Taraneh[1;] St. Helen, Gideon[,2,3]; Benowitz, Neal[2,3]; Tyndale, Rachel[1,4]
[1]University of Toronto, Toronto, ON;[2]Center for Tobacco Control Research and Education, San Francisco, California, [3]University of California, San Francisco, California; [4]Campbell Family Mental Health Research Institute, Toronto, ON

Objectives: Interindividual nicotine metabolism rates vary greatly, even after controlling for genetic variation in the major nicotine metabolizing enzyme, CYP2A6. Nicotine is also metabolized by glucuronidation (by UGT2B10 and UGT2B17) and N-oxidation (by FMO3) and may be transported by OCT2. Here, we present an analysis of the relationship between genotype and disposition kinetics of nicotine among African American smokers, a less-studied population with different allele frequencies and rates of nicotine metabolism.
Methods: Sixty African American smokers received a thirty-minute intravenous infusion of deuterium-labeled nicotine and cotinine. Blood and urine samples were collected and nicotine pharmacokinetic parameters (half-life, Cmax, AUC, non-renal, and total clearance) were estimated. Subjects were genotyped for 18 variants within CYP2A6, UGT2B10, UGT2B17, OCT2, and FMO3, including UGT2B10*2, UGT2B17*2, OCT2 (rs316019), and FMO3 (rs2266782). The association between genetic variation and nicotine disposition parameters was investigated using regression models assuming a dominant effect of variant alleles.
Results: Relative to normal metabolizers, reduced CYP2A6 metabolizers displayed 20% longer half-life (mean=149.9 vs. 124.5 min, respectively; $p=0.010$), accounting for 11% of the variation in nicotine half-life. OCT2 variant genotype was associated with 117% higher Cmax compared to wildtype (mean=20.3 vs. 44.0 ng/ml, respectively; $p=0.001$), accounting for 18.2% of the variation in nicotine Cmax. There was a trend towards lower nicotine non-renal and total clearance with variation in OCT2 ($p=0.082$ and $p=0.078$, respectively), accounting for 5.1% of non-renal and 5.3% of total nicotine clearance variation, respectively. The UGT genes altered glucuronide ratios, however their contribution to nicotine pharmacokinetics was negligible.
Conclusions: Genetic variation in CYP2A6 and OCT2 altered nicotine pharmacokinetics in African American smokers while variation in UGTs and FMO3 genes did not. These findings provide insight into ethnic differences in nicotine disposition kinetics, which could contribute to ethnic disparities in patterns of tobacco use and smoking-related diseases.

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Pregnancy outcomes following first trimester exposure to topical Rretinoids: A systematic review and meta-analysis

Kaplan, Yusuf C[1,2]; Ozsarfati, Jak[1,2]; Etwel, Fatma[3]; Nickel, Cheri[1,2]; Nulman, Irena[1,2,]; Koren, Gideon[1,2]
[1]The Hospital for Sick Children, Toronto, ON; [2]University of Toronto, Toronto, ON; [3]University of Western Ontario, London, ON

Objectives: Evaluation of human data regarding the outcomes of topical retinoid-exposed pregnancies is significantly important in terms of counseling pregnant women with an inadvertent exposure.
Our objective is to determine whether exposure to topical retinoids leads to an increase in the risk for adverse pregnancy outcomes.
Methods: We carried out the search in MEDLINE, EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials

databases from inception to December 4th 2014. The selection, review and quality assessment of the studies were carried out by two independent reviewers according to predetermined inclusion criteria. The odds ratios (OR) were calculated by the random effects method.
Results: This meta-analysis, including a total of 654 pregnant women who were exposed to topical retinoids and 1375 unexposed control pregnant women, did not detect significant increases in rates of major congenital malformations (OR 1.22, 95% confidence interval (CI) 0.65-2.29), spontaneous abortions (OR 1.02, 95% CI 0.64-1.63), stillbirth (OR 2.06, 95% CI 0.43-9.86), elective termination of pregnancy (OR 1.89, 95% CI 0.52-6.80), low birth weight (OR 1.01, 95% CI 0.31- 3.27) or prematurity (OR 0.69, 95% CI 0.39-1.23). No significant heterogeneity was detected among the studies for the evaluated outcomes.
Conclusions: The present meta-analysis did not find any association between prenatal topical retinoid exposure and adverse pregnancy outcomes. This result contributes to clinical evidence in reassuring women who were inadvertently exposed to topical retinoids during their pregnancy. However, it does not have the statistical power to justify the use of topical retinoids during the pregnancy.

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Severe central nervous system depression in a neonate breastfed by a mother receiving oxycodone. Case report

Ozsarfati, Yako Jak; Koren, Gideon; Belik, Jaques
The Hospital for Sick Children, Toronto, ON

Objectives: A 9 day old baby boy was brought to the emergency room with poor feeding, excessive sleeping and cyanosis. The baby was born after C/S and uneventful pregnancy of 39 weeks. He had mild TTN (Transient Tachypnea of Newborn) in the first 48 hours and was sent home on the 5th day. He was exclusively breastfed. On the 8th day he developed poor feeding and excessive sleeping and on the 9 th day, was rushed to the hospital. He was admitted to NICU with a tentative diagnosis of sepsis. Only after all routine tests including blood work, blood and CSF cultures returned normal, the NICU team focused on the mother's medications. The mother had received in total of 7-8 tablets of Percocet (acetaminophen and oxycodone) for postpartum pain during the first 5 days after delivery.
Methods: A Motherisk study showed that 20% of babies whose mothers were breastfeeding while taking oxycodone exhibited CNS depression. This can be due to mother's CYP2D6 ultra-rapid metabolizer status or due to low expression and activity of blood brain barrier p-glycoprotein at birth.
Results: The severe CNS symptoms in our patient, such as poor feeding, excessive sleeping and cyanosis were most likely due to toxicity of oxycodone which he received through breastmilk. The baby recovered completely in a few days.
Conclusions: This case report emphasizes that oxycodone is not a safer alternative than codeine and should not be used by breastfeeding mothers.

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Hair cortisol as a biomarker of the HPA axis in pregnant women with asthma

Smy, Laura[1]; Koren, Gideon[1]; Carleton, Bruce[2]; Shaw, Kaitlyn[2]
[1]University of Toronto, Toronto, ON; [2]Child and Family Research Institute, Vancouver, BC

Objectives: Hair cortisol analysis has been used to assess the effect on the hypothalamus-pituitary-adrenal (HPA) axis by a variety of psychiatric and physical stressors. Recently, we observed that hair cortisol of children with asthma was two-fold lower when taking inhaled corticosteroid than prior to the medication. Due to the importance of cortisol in pregnancy, the objective was to examine whether hair cortisol is a sensitive biomarker to assess the effects of asthma on the HPA axis in pregnant women.
Methods: In a prospective case-controlled study, we collected hair samples from pregnant women with and without asthma. Hair samples were segmented, based on the average growth rate of 1 cm/month, and analyzed using a validated ELISA method to provide cortisol results corresponding to pre-conception (PC), trimesters 1-3 (T1-3), and post-partum. The results were compared within and between the two groups of women.
Results: Hair samples for 118 pregnant women, 31 without asthma and 87 with asthma, were analyzed. In healthy controls, there was a statistically significant increase in hair cortisol over the course of pregnancy. This trend was dampened in women with asthma who had

significantly lower median hair cortisol levels in T3 (4.7 ng/g (IQR: 3.0-6.5 ng/g) vs. 7.2 ng/g (IQR: 5.1-9.3 ng/g), p = 0.025).
Conclusions: Hair cortisol successfully detected the expected increase during the course of a healthy pregnancy. In contrast, asthma was associated with a diminished ability to increase cortisol levels in late pregnancy.

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Hair cortisol as a novel biomarker of HPA suppression by inhaled corticosteroids in children

Smy, Laura[1] ; Koren, Gideon[1]; Carleton, Bruce[2] ; Rieder, Michael[3]; Shaw, Kaitlyn[2], Smith, Anne[2] ; Russell, Evan[4]; Van Uum, Stan[3]
[1]University of Toronto, Toronto, ON; [2]Child and Family Research Institute, Vancouver, BC; [3]Univeristy of Western Ontario, London, ON; [4]Queen's University, Kingston, ON

Objectives: Asthma is the most common chronic condition in childhood and the recommended pharmacotherapy for long-term control includes the use of inhaled corticosteroids (ICS). ICS were designed to act at the site of inflammation in the lung, thus decreasing systemic absorption and reducing the risk of adverse effects associated with corticosteroid use (e.g., HPA suppression and its consequent effects). Available data show that measurement of hair cortisol successfully reflects endogenous cortisol levels. We sought to examine whether hair cortisol measurements can be used to identify HPA suppression surrounding ICS therapy in children with asthma.
Methods: Hair samples were collected from the vertex posterior region of the head of 18 children with asthma. Hair was analyzed using a validated ELISA method (Spearman r = 0.9, p < 0.0001, compared to two hair cortisol LC-MS methods). We compared their hair cortisol concentration during ICS use to the concentration prior to ICS use.
Results: During ICS therapy, median hair cortisol levels were twofold lower compared to the period of no ICS use (median 89.8 ng/g vs. 198.2 ng/g, p=0.0015).
Conclusions: Hair cortisol is an effective biomarker of the HPA suppression associated with ICS therapy and can be a sensitive tool for determining systemic effects of ICS use and monitoring adherence. Future research is needed to characterize the effect of untreated asthma on hair cortisol concentrations, if any.

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Determination of predictors of CYP2A6 protein levels and nicotine metabolism in a human liver bank: influence of genetic and non-genetic factors

Tanner, Julie-Anne[1]; Chaudhry, Amarjit[2]; Prasad, Bhagwat[3]; Thummel, Kenneth E[3]; Tyndale, Rachel F[1]
[1]University of Toronto, Toronto, ON; [2]St Jude Children's Research Hospital, Memphis, Tennessee; [3]University of Washington, Seattle, Washington

Objectives: CYP2A6 genetic variation is associated with altered CYP2A6 mRNA, protein expression, and metabolic activity, which in vivo is associated with interindividual differences in the rate of nicotine metabolism and alterations in smoking behaviors. Our aim is to expand on earlier investigations into a larger scale liver bank, ultimately incorporating sequencing and miRNA analyses.
Methods: Human livers (n=361) were genotyped for CYP2A6 reduce/loss-of-function alleles and analyzed for CYP2A6 and POR protein levels, and CYP2A6 activity (nicotine C-oxidation, NCO, in preliminary assessment of 227/361 livers).
Results: Initial investigations indicated that CYP2A6 genotype was associated with CYP2A6 protein expression and rate of nicotine metabolism. Liver samples with variant CYP2A6 alleles had lower CYP2A6 protein levels (P=0.007) and NCO activity (P=0.06); female livers had higher protein levels (P=0.04) and NCO (P=0.02) relative to male livers. Protein levels of cytochrome P450 reductase (POR), a coenzyme necessary for CYP-mediated drug metabolism, were not associated with CYP2A6 genotype (P>0.5). There was a positive association for both CYP2A6 (r=0.81, P<0.0001) and POR protein (r=0.30, P<0.0001) levels with NCO activity. CYP2A6 protein expression accounted for 38.1% of the variation in NCO activity (P<0.001). Together, genotype, gender, and age accounted for only a small portion of the variation in CYP2A6 protein levels (P=0.002) or NCO (P>0.3), suggesting additional unknown CYP2A6 variants and sources of regulation.
Conclusions: CYP2A6 genetic variation significantly influences CYP2A6 protein expression and nicotine metabolism. Variable

nicotine metabolism is associated with altered smoking behaviors, including cigarette consumption, dependence, and ability to quit. Future investigations with this liver bank aim to characterize the wide variability in CYP2A6 expression and activity that remains within genotype groups (protein levels ranging 0-121, pmol/mg) through the assessment, for example, of mRNA levels, miRNA regulation, and the identification of novel CYP2A6 genetic variants.

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OPRM1 A118G and UGT2B7 C802T modulate codeine intake in the postpartum period

Baber, Marta[1]; Chaudhry, Shahnaz[1]; Kelly, Lauren[2]; Ross, Colin[3]; Carleton, Bruce[3]; Berger, Howard[4]; Koren, Gideon[1]
[1]The Hospital for Sick Children, Toronto, ON; [2]The Peter Gilgan Centre for Research and Learning, Toronto, ON; [3]Centre for Molecular Medicine and Therapeutics, Vancouver, BC; [4]St. Michael's Hospital, Toronto, ON

Objectives: In North America, codeine is commonly prescribed for postpartum pain management following cesarean section despite evidence that some women experience inadequate analgesia while others experience excessive sedation and other unpleasant side effects. The spectrum of response to codeine can be, at least in part, accounted for by pharmacogenetic differences that alter the drug's pharmacokinetics and pharmacodynamics. This study's objective was to explore how genetic polymorphisms related to the codeine pharmacological pathway influence codeine intake and reported pain levels among women prescribed codeine for postpartum pain therapy.
Methods: A nested cohort of ninety-eight women who took codeine for the first two days following cesarean section were studied. Participants were instructed to record dosing information and to report their level of pain using the Visual Analog Scale (mm) one hour following each dose of codeine. Participants were genotyped for select polymorphisms of the COMT, ABCB1, CYP2D6, UGT2B7 and OPRM1 genes. The primary endpoints of this study were mean pain score (mm) and mean and cumulative dose intake (mg/kg) for the first two days following cesarean section.
Results: Univariate analysis revealed that maternal age was predictive of mean pain score (p=0.041). Significant differences in mean codeine consumption were seen among the genotypic groups of the OPRM1 A118G (p=0.001) and UGT2B7 C802T (p=0.015) variants. Mean codeine intake also differed between Asians and Caucasians (p=0.048). Multivariate analysis revealed that the OPRM1 A118G and UGT2B7 C802T variants are predictive of mean codeine intake by the cohort overall (p=0.000) and by Caucasians (p=0.001).
Conclusions: Reported pain increases with maternal age. Asians require more codeine than Caucasians. OPRM1 A118G and UGT2B7 C802T variants predict codeine intake in the cohort overall and among Caucasians. These findings may assist in optimizing codeine therapy during the postpartum period.

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Biodistribution of negatively charged iron oxide nanoparticles (IONPs) in mouse and enhanced brain delivery using lysophosphatidic acid (LPA)

Sun, Zhizhi[1;] Worden, Matthew[2]; Wroczynskyj, Yaroslav[1]; Thliveris, James[1]; van Lierop, Johan[1]; Hegmann, Torsten[2]; Miller, Donald[1]
[1]University of Manitoba, Winnipeg, MB; [2]Kent State University, Kent, OH.

Objectives: Effective treatment of brain disorders requires a focus on improving drug permeability across the blood-brain barrier (BBB). Herein, we examined the pharmacokinetic properties of negatively charged iron oxide nanoparticles (IONPs) and capability of using lysophosphatidic acid (LPA) to disrupt the tight junction to allow IONPs to enter the brain.
Methods: Using a mouse model, quantitative determination of IONP in blood and various tissues (liver, spleen, lung, kidney and brain) was performed following IONP administration with or without LPA to transiently disrupt the BBB. Localization of IONPs in tissue was confirmed by transmission electron microscopy (TEM). Potential brain toxicity was evaluated by histological analysis at 2 and 9 days following IONPs treatment.
Results: The half-life of IONPs was 5.9 minute. Liver and spleen were the major organs of IONP deposition. Renal elimination of IONPs was observed. There was limited distribution of IONPs in lung and brain under normal conditions. LPA treatment enhanced brain and spleen accumulation of IONPs up to 3% and 70% of injected dose respectively. Tight junctions in lung

and kidney remained intact and no IONPs were observed suggesting LPA-mediated disruption of tight junctions was localized to the brain. Histological examination of brain slices of LPA and IONPs treated mice revealed no significant toxicity with regard to infiltration of peripheral immune cells or activation of microglia and astrocytes.

Conclusions: LPA facilitated enhanced brain penetration of IONPs. The delivery efficiency in the brain parenchyma following LPA treatment is higher than most of receptor mediated transcytosis delivery of NPs reported in the literature. Transit BBB disruption and enhanced IONP delivery to the brain did not lead to inflammation or toxicity. Our findings suggest enhanced brain delivery via transient disruption of the BBB may be a safe and effective method. Support provided by NSERC-CIHR Collaborative Health Research Project.

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Agranulocytosis induced by anti-tubercular drugs, isoniazid (INH) and rifampicin (R): A rare case report

Bidarimath, Basavaraj; Patil, Banderao; Vardhamane, S H; Sajid, Md; Somani, Roopali
M. R. Medical College, Rajiv Gandhi University of Health Sciences, Gulbarga, Karnataka India

Objectives: Tuberculosis is a chronic granulomatous infection that has caused a high morbidity and mortality over decades. Tuberculosis treatment has many side effects due to long duration and compliance problems that might require cessation of treatment. Agranulocytosis is a serious idiosyncratic drug reaction characterized by severe leukopenia and a very rare side effect of anti-tubercular treatment mainly due to first line drugs namely isoniazid (INH) and rifampicin(R). The current study presents the case of a patient with isoniazid and rifampicin induced agranulocytosis.

Methods: A 55 years old patient with a known case of pulmonary tuberculosis on treatment, presented with dental pain. CT scan was conducted on swelling and revealed a multi-loculated thick walled collection in supraclavicular region and multiple conglumerate lymph nodes over para-tracheal region. Cervical biopsy of granulomatous lesions suggested tuberculous lymphadenitis.

Results: On examination, blood report revealed; WBC:5400/µL, neutrophil:68%, lymphocytes:30%, eosinophils: 3%. Four drug regimen (Isoniazid(H):300mg/day; rifampicin(R):450mg/day; ethambutol(E):1000mg/day; pyrazinamide(Z):1500mg/day) continued according to patient's weight and national guidelines. After 6 weeks, patient developed new onset of fever associated with sore throat. Although blood profile were normal at start of therapy, report (WBC:2600/µL, neutrophil:2%, lymphocytes:72%, eosinophils:0%) revealed agranulocytosis with neutropenia. After stop-start trial, isoniazid and rifampicin were identified as possible cause of neutropenia and were removed. Subsequently, patient was on alternate regimen; ethambutol:1000mg, linezolid:600mg, streptomycin:1gm, ethionamide:250mg with no further isoniazid and rifampicin induced complications with blood count WBC:9200/µL, neutrophil:72%, lymphocytes:20%, eosinophils:0%. One month post-treatment, the sputum smear revealed no acid-fast bacilli.

Conclusions: In the context of anti-tuberculosis therapy, neutropenia is mostly caused due to isoniazid, but can also occur with rifampicin, ethambutol and streptomycin. In vast majority of cases during therapy, occurrence of neutropenia is due to single agent. By re-challenging patient with each antibiotic individually, the offending drug can be identified, subsequently omitted and therapy completed using an alternative first or second line antibiotics.

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Topical medications containing glycol excipients in burn patients: a case report and systematic review of the literature

Leibson, Tom[1], Davies, Paige[2], Koren, Gideon[1]
[1]The Hospital for Sick Children, Toronto, Ontario; [2]University of Toronto, Toronto, ON

Objectives: We present the case of a toddler who was admitted with a 2nd degree burn of lower limbs after a home accident. Local treatment with Polyethylene glycol (PEG) based antibiotic creams resulted in metabolic acidosis and seizures. The urine was found to be positive for Diethylene glycol. Shortly after application of non-glycol containing topical agents the child's clinical status improved significantly. This study aims to summarize and critically appraise the safety reports of all clinical studies involving burn patients who received glycol based topical treatment.

Methods: A systematic review of MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and ISI Web of Science was conducted. In consultation with a Library Information Specialist, search strategies were developed to ensure that all topical medications containing glycols are included. The paper was included if: (1) the study has reported topical treatment in burn patients, (2) patients were treated with a glycol based product and (3) Study results contained a safety report.
Results: 23 studies were available for appraisal of safety: 2 were retrospective (n=137), 5 were non-controlled prospective (n=422), 7 were controlled non-randomized (n=365) and 9 were randomized controlled trials (n=581). No systemic toxicities were reported in all published studies (n=1505). Total number of fatal cases was 103 (6.8%). Total number of septic patients was 110 (7.3%).
Conclusions: There is an alarming discrepancy between the number of reported cases (some of them fatal) and the lack of any clinical study signal for this adverse response to topical glycol based medications in burn patients. We wish to advocate for careful monitoring of burn patients being treated with glycol based topical products.

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A substitution error leading to central anticholinergic syndrome

De Bruyne, Pauline[1]; Logghe, Karl[2]; Roelens, Filip[2]
[1]The Hospital for Sick Children, Toronto, Canada; [2]AZ Delta Hospital, Roeselare, Belgium

Background: Medication errors can occur at the prescribing, transcription, dispensing and administration stages of drug therapy. An important type of dispensing errors is the dispensing of the wrong drug, i.e. substitution error. Substitution errors account for half of the cases of dispensing errors, giving rise to an overdose in 40% of the patients.
Methods: The importance of possible substitution errors is illustrated in following case report.
Results: We report the case of a 3-year-old girl. She was prescribed 6 mg hyoscine butylbromide (in an intrarectal extratemporaneous preparation) for abdominal cramps in acute gastroenteritis. A few hours after first administration, she showed severe confusion with a Glasgow Coma Scale of 15/15 and no signs of meningeal irritation. Physical examination showed strikingly red cheeks, bilateral mydriasis with poor reaction to light and mild pyrexia of 38.0°C. An electrocardiogram showed a sinus rhythm of 150/min. Routine laboratory investigation was normal. Based on the symptoms, an anticholinergic toxic syndrome was suspected. The Belgian Poison Center suggested an anticholinergic poisoning caused by the substitution of hyoscine butylbromide with hyoscine hydrobromide, which was later confirmed by the pharmacist. The patient was treated with repeated doses of midazolam for agitation. Gradually, her mental status improved. She was discharged after two days.
Conclusions: Although the drug names look similar, hyoscine butylbromide and hyoscine hydrobromide are different in many aspects. Hyoscine hydrobromide is a tertiary amine compound that is easily absorbed and penetrates the blood-brain barrier by passive diffusion. Hyoscine butylbromide is a quaternary amine compound and is poorly absorbed in the gut. As the drug does not readily penetrate the blood-brain barrier, central nervous system effects are rarely reported. Four case reports focusing on the same substitution error were published during the last decade. This illustrates the importance of recognizing possible substitution errors in daily practice.

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How safe is on-label drug use in paediatrics? The case of first generation H_1-antihistamines

De Bruyne, Pauline[1]; Boussery, Koen[2]; Christiaens, Thierry[2]; Mehuys, Els[2]; Van Winckel, Myriam[2]
[1]The Hospital for Sick Children, Toronto, Ontario, Canada; [2]Ghent University, Belgium

Background: Recently, much attention has been paid to off-label prescriptions in paediatrics. However, on-label prescribing can cause health issues too: we discuss the case of first generation H_1-antihistamines (FGAs). These have been in use for over 70 years, for a variety of indications such as relief of allergic conditions, cough and insomnia.
Methods: The available FGAs were listed using their International Nonproprietary Names (INN). For each formulation, the information of the Summary of Product Characteristics issued in five selected European countries (Belgium, France, Germany, the Netherlands and United

Kingdom) was collected. This was plotted against the published evidence on efficacy and safety of each FGA.
Results: 16 different FGAs are currently marketed in single-drug oral preparations in the evaluated countries. When investigating each drug separately, a huge variability in labelled indications, licensing age for paediatric use, and availability characteristics in the different countries is observed. Most of the indications are not supported by evidence from (published) clinical trials.
Conclusions: Both health care professionals and consumers generally assume that all approved H1-antihistamines have been shown to be efficacious and safe (in children), but many in this class - in particular those introduced before 1985 - have not been optimally studied. This might explain the striking number of inconsistencies in indications and licensing ages of the evaluated drugs in 5 neighbouring countries. Moreover, many of the antihistamines are sold over the counter, which may contribute to the impression of antihistamines as being innocent and to overuse. Such overuse can be a serious problem, as sedation is a side effect of all FGAs.

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Safe-Pedrug: a new strategy for paediatric drug research

De Bruyne, Pauline[1], Vande Walle Johan[2]
[1]The Hospital for Sick Children, Toronto, Canada; [2]Ghent University, Belgium

Background: Drug evaluation in children is stimulated by initiatives of the Regulatory Authorities; such as the 'Best Pharmaceuticals for Children Act' and 'the Pediatric Research Equity Act' in the United States and the 'Paediatric Regulation' in Europe. As for the European Paediatric Regulation, Paediatric Investigational Plans must be submitted around the end of Phase 1 of adult trials. However, the proposed paediatric trials tend to be amended frequently and postponed to the end of the drug evaluation process, as they are largely based on extrapolations of results of adult trials.
Methods: Experts in paediatrics, pharmaceutical sciences, veterinary medicine and ethics (of three Belgian universities) collaborated to develop a research consortium that will focus mainly on generating paediatric pharmacokinetic and pharmacodynamic (PK/PD) knowledge before the actual human trials are performed. National and international stakeholders (including Industry, Regulatory Authorities, and Patient Organisations) support this consortium in the valorisation of results.
Results: The above-mentioned networking resulted in the SAFE-PEDRUG project, funded by the Agency for Innovation by Science and Technology (Flanders). This program will explore the value of the porcine juvenile animal model and PK modelling (physiologically-based pharmacokinetic modelling) in providing prior paediatric PK/PD-knowledge. For the evaluation of this approach, three case compounds were selected: desmopressin, lisinopril, and fluoroquinolones. The results of the models are plotted against human paediatric data. Furthermore, PK/PD in neonates and critically ill children will also be explored.
Conclusions: This research initiative illustrates that a close collaboration of experts in the different fields of paediatric pharmacology, together with other stakeholders such as Regulatory Authorities and Patient Organisations), can put a new perspective on the future of paediatric pharmacology. Exchange of ideas and knowledge can help to tailor paediatric clinical trials to the PK/PD-characteristics and needs of children.

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Chronic alcohol exposure alters spatial learning and blood-brain barrier permeability in a zebrafish model

Wagner, Maxine; Miller, Donald W
University of Manitoba, Winnipeg, MB

Objective: Chronic ethanol consumption can influence learning and cognitive function. The present study evaluated the effects of ethanol exposure on spatial learning and the correlation of learning deficits with diminished blood-brain barrier (BBB) function in a zebrafish model.
Methods: Adult zebrafish were randomly assigned into control (regular tank water) or chronic ethanol (0.5% ethanol) treatment groups. Spatial learning was assessed using a plus maze following an 11 day training period where fish were rewarded for entering into the target zone in designated arm of maze. Assessment of BBB permeability was performed after behavioral studies using Rhodamine 800 (P-glycoprotein function) and IRdye 800 (paracellular diffusion marker). The near infrared fluorescence imaging

agents were administered into systemic circulation via intra-orbital injection and imaging of zebrafish tissue was performed 5 minutes after injection.
Results: Fish were maintained in 0.5% ethanol for 24 days with no mortality or morbidity observed. Compared to control fish which showed significant improvements in both the time spent in the target arm and the number of visits to the target arm following the training period, the ethanol treated fish showed no improvement from pre-training values. The deficits in spatial learning observed in the ethanol treated fish were also correlated with increased BBB permeability to Rhodamine 800 (3-fold increase in brain-to-plasma ratio in ethanol group compared to control) and IRdye 800 (1.6-fold increase in brain-to-plasma ratio in ethanol group compared to control).
Conclusions: Zebrafish exposed to chronic ethanol display significant deficits in spatial learning capabilities. Chronic ethanol exposure also affects the BBB with significant increases in both transcellular and paracellular permeability markers detected. As chronic ethanol also impacts learning and cognitive capabilities in humans, zebrafish may be a useful animal model for evaluating both mechanisms of ethanol effects on the brain as well as potential therapeutic targets.

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Salivary melatonin levels in pregnant women with insomnia: A prospective cohort study with two comparison groups

Mirdamadi, Kamelia; Koren, Gideon
Hospital for Sick Children, Toronto ON

Background: Insomnia in pregnancy is associated with depression, hypertension or preeclampsia, gestational diabetes, and increased risk for preterm labor. Studies have shown a pattern of increased maternal plasma melatonin levels as pregnancy proceeds, reaching its peak near term. However, the role of melatonin in insomnia during pregnancy has not yet been investigated.
Objective: The aim of this study is to measure nocturnal saliva melatonin levels in pregnant women with and without insomnia.
Method: Prospective cohort study with three groups: 1- Exposed group; pregnant women with insomnia taking sleep medications. Comparison groups: 2- disease-matched; pregnant women with insomnia not taking any sleep medications, and 3- healthy pregnant women. All groups are matched for gestational age and maternal age. Women collect three Saliva samples one hour before bed in 30 minutes intervals at 12-14 weeks, 24-26 weeks, and 34-36 weeks. ELISA method is used to measure levels.
Results: One-way ANOVA showed significantly lower melatonin levels in the disease-match group ($p<0.04$). Repeated measure analysis showed increased melatonin in the exposed group since after the first trimester ($p<0.01$). Analysis also showed an increased hormone level in the Healthy group in the third trimester ($p<0.002$).
Conclusion: Results of this study so far confirm that pregnant women with insomnia have lower levels of nocturnal melatonin. Increased melatonin levels in the exposed group may be due to improved symptoms of insomnia with pharmacotherapy. Treatment of insomnia with melatonin has been documented with a high safety profile and efficacy in non-pregnant individuals. However, safety of melatonin in pregnancy has not been investigated. In light of the current findings, future studies are needed to evaluate the safety and efficacy of melatonin in pregnancy for the treatment of insomnia. The current study is still in progress for a thorough investigation of the hormone levels in pregnancies with insomnia.

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Safety of varenicline use for nicotine addiction during pregnancy

Tanaka, Toshihiro
Shizuoka Kosei Hospital, Shizuoka, Japan

Background: Mothers' smoking is widely known to be associated with various adverse pregnancy outcomes such as preterm birth and small for gestational age, and to cause various airway infections in infants. Varenicline (Champix® in Canada, Japan, Europe and other countries, Chantix® in the USA, marketed by Pfizer) was developed to treat nicotine addiction and has been on the market since 2006. It is a nicotinic receptor partial agonist, and is expected to be safer than nicotine itself and nicotine replacement therapies by nicotine patches / nicotine gum during pregnancy; however, information on safety of the medication use in pregnant smokers is insufficient.

Objectives: To assess whether varenicline is safe to use during pregnancy.
Methods: Pregnant women on varenicline and their babies after birth were followed up. Blood sampling to measure levels of varenicline was tried.
Results: The outcomes of the pregnancy were as bellow.
Case 1: A 23 year-old female (G2 P1, Tobacco Dependence Screener (TDS): 9 and Brinkman index (BI): 210) started varenicline at 29 weeks gestation. A female baby was delivered at 41 weeks gestation (3446g, Apgar Score: 9 at 1 min / 10 at 5 min) without anomaly.
Case 2: A 25 year-old female (G1 P0, TDS: 7 and BI: 200) started varenicline at 12 weeks gestation. A male baby was delivered at 38 weeks gestation (3302g, Apgar Score: 9 at 1 min) without anomaly.
Case 3: A 25 year-old female (G2 P1, TDS: 8 and BI: 260) started varenicline at 22 weeks gestation. A male baby was delivered 3at 40 weeks gestation (3166g, Apgar Score: 8 at 1 min / 10 at 5 min) without anomaly. In Case 3, blood levels of varenicline at delivery will be analyzed before the conference.
Conclusion: Available data from the 3 pairs of a mother and a baby suggest that varenicline use during pregnancy seems safe.

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Interpatient variation in observed plasma level of new oral anticoagulants, rivaroxaban and apixaban

Gulilat, Markus; Schwarz, Ute; Morgan, Sara; Ross, Cam; LeMay, Sara; Vosper, Heather; Dresser, George, Kim, Richard B
Western University, London, ON

Background: Factor Xa Inhibitors (FXI), rivaroxaban and apixaban have becomewidely available for oral anticoagulant (OAC) therapy. Outside of clinical trials, the interpatient variation in drug response have not been assessed. Our studyobjectives were to examine the extent of interpatient variability in the plasmarivaroxaban and apixaban concentrations of patients with AF, for betteridentifying patients at risk for extreme drug response to FXIs.
Methods: In this cohort study we prospectively enrolled and collected a single blood sample from AF patients prescribed rivaroxaban and apixaban who are followed by our oral anticoagulation clinic. Interim analysis of enrolled subjects to date (rivaroxaban N=26, and apixaban N=33*) were carried out by measuring FXIs plasma levels using liquid chromatography-tandem mass spectrometry.
Results: In contrast to published rivaroxaban levels, in our patient cohort, we observed near 30-fold interpatient variation in rivaroxaban levels with nearly fifty-percent of patients attaining a level greater than predicted 95th percentile. Apixaban plasma concentrations ranged from 57 to 443 ng/ml (7-fold variation) with a mean of 218 ng/ml (SD, 97).
Conclusion: There is far greater variation in observed rivaroxaban plasma levels than currently reported, with a significant proportion of patients attaining higher than predicted plasma level. Observed apixaban plasma concentration appear to be less variable. We are currently enrolling additional patients with a goal of better delineating clinical as well as pharmacogenomic predictors of FXI response. Therapeutic monitoring of FXIs may prove to be an important strategy for OAC selection and dosing. Our findings have major clinical relevance to safe and effective utilization of newer OACs.

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Transient modulation of blood-brain barrier permeability using cadherin binding peptide: a safe and effective approach for acute drug delivery to the brain.

On, Ngoc[1]; Laksitorini, Marlyn[1]; Kiptoo, Paul[2]; Siahaan, Teruna[2]; Miller, Donald[1]
[1]The University of Manitoba, Winnipeg, MB; [2]University of Kansas, Lawrence, Kansas

Objectives: To examine the effects of a cyclic cadherin peptide, containing the Ala-Asp-Thr (cADT) sequence, on BBB integrity.
Methods: The effect 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. BBB permeability was assessed with gadolinium contrast agent (Gd-DTPA; a small hydrophilic permeability marker, and Irdye800 cw PEG, a large hydrophilic permeability marker), under control conditions and following exposure to cADT (0.0001-0.032 mmol/kg) using magnetic resonance imaging (MRI) and near infrared fluorescence imaging. 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 0.0001 mmol/kg having a minimal effect on enhancement (4-fold) and 0.032 mmol/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 hrs of cadherin peptide administration. The presence of cADT also enhanced the accumulation of a large 25kDa hydrophilic permeability marker, Irdye800 cw PEG (5-fold) in the brain as observed with near infrared fluorescence imaging.
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.

LATE BREAKING ABSTRACTS

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Renal organic anion transporter 1 is inhibited by multiple mechanisms

Pelis, Ryan M
Dalhousie University, Halifax, NS

Objectives: Organic anion transporter 1 (OAT1) is important for the renal elimination of numerous drugs and is a potential site of drug-drug interactions. The purpose was to examine the mechanism by which drugs inhibit its activity.
Methods: OAT1 was stably expressed in Chinese hamster ovary (CHO) cells and the kinetics of *para*-aminohippurate (PAH) transport was measured in the absence or presence of a fixed concentration of inhibitor.
Results: Most compounds (10 of 14) inhibited in a competitive manner, lowering the Michaelis constant (K_m) without affecting maximal transport rate (J_{max}). The other compounds were mixed-type (lowering J_{max} and increasing K_m) or non-competitive (lowering J_{max} only) inhibitors. Further studies were conducted with telmisartan, a non-competitive inhibitor. Following brief telmisartan (5 μM) treatment and its removal, PAH uptake was reduced ~50% one hour after washout. Inclusion of 10% fetal bovine serum in the washout buffer led to an almost complete restoration of PAH uptake. Accordingly, [^{3}H]telmisartan accumulated by the cells following brief treatment was slow to leave, and the presence of 10% fetal bovine serum in the washout buffer accelerated its removal. The cellular accumulation of [^{3}H]telmisartan was 30% higher in CHO-OAT1 cells compared to CHO parental cells whether conducted at room temperature or using ice-cold buffers to slow translocation. Telmisartan did not affect cell surface expression of OAT1. Telmisartan was also effective at inhibiting [^{3}H]PAH efflux from CHO-OAT1 cells.
Conclusions: OAT1 is inhibited by multiple mechanisms, and telmisartan, a non-competitive inhibitor 1) binds OAT1 in a reversible manner, 2) is not a translocated substrate, 3) reduces maximal transport rate by lowering substrate turnover number, and 4) is an effective inhibitor of both substrate uptake as well as efflux. Models used to predict drug interactions at OAT1 assume competitive inhibition, and may under-predict drug interaction magnitude with drugs exhibiting other inhibition mechanisms.

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Identification of novel resistance mechanisms of epithelial ovarian cancer to carboplatin

Barghout, Samir; Zepeda, Nubia; Vincent, Krista; Azad, Abul K; Xu, Zhihua; Yang, Christine; Steed, Helen; Postovit, Lynne M; Fu, YangXin
University of Alberta, Edmonton, AB

Objectives: Epithelial ovarian cancer (EOC) constitutes 90 % of all ovarian malignancies. Carboplatin is currently used as a first-line therapeutic agent for EOC in combination with paclitaxel. Despite the initial positive response to carboplatin, recurrence occurs in most advanced EOC patients and the recurrent disease is platinum-resistant. Multiple resistance mechanisms of EOC to platinum-based compounds have been identified, however the inter- and intra-tumor heterogeneity observed in platinum-resistant EOC necessitates the

identification of novel resistance mechanisms to effectively manage this heterogeneous disease.

Methods: EOC cell line A2780s (cisplatin-sensitive) and its derivative A2780cp (cisplatin-resistant) cells were studied using DNA microarray, ingenuity pathway analysis (IPA), quantitative real-time polymerase chain reaction (qRT-PCR), Western blotting, neutral red uptake assay, clonogenic assay and LEF/TCF-driven luciferase reporter assay. RUNX3 expression in human primary EOC cells and primary ovarian surface epithelium (OSE) cells was determined by Western blotting.

Results: A number of genes were found to be differentially expressed in these two cell lines including RUNX3 and genes encoding several components of the Wnt/β-catenin signaling pathway. We selected these genes for further analysis as they have not been previously studied in the context of EOC resistance.

Consistent with our DNA microarray data, subsequent qRT-PCR and Western blotting results showed that RUNX3 expression was higher in A2780cp cells compared to A2780s cells. Further gain- and loss-of-function studies in A2780 cells confirmed the role of RUNX3 in EOC resistance to carboplatin-induced cytotoxicity. Interestingly, our results demonstrate that RUNX3 upregulates the expression of an antiapoptotic protein, suggesting a potential mechanism by which RUNX3 confers resistance to carboplatin.

The gene expression profile analysis also suggested that Wnt/β-catenin signaling was more active in A2780cp cells compared to A2780s cells.We validated these data by qRT-PCR. Using the LEF/TCF-driven luciferase reporter assay, we confirmed that β-catenin transcriptional activity was higher in A2780cp cells than in A2780s cells. Combined treatment of carboplatin and a β-catenin inhibitor was more effective in killing A2780cp cells than either agent alone.

Conclusions: In conclusion, our data suggest that RUNX3 contributes to carboplatin resistance of EOC cells and therefore it could be a potential therapeutic target. In addition, Wnt/β-catenin signaling is more active in resistant EOC cells, suggesting its potential role in EOC resistance.

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Clinical implications of oxidized LDL through modification of regulatory T cells

Kentaro, Sugiyama[1]; Sachiko, Tanaka[1]; Satoshi, Kohsaka[1]; Sayuri, Shirai[1]; Yasuyo, Sudo[2]; Muneharu, Yamada[2]; Noriko, Yoshikawa[2]; Iwao, Nakabayashi[2]; Takashi, Oda[2]; Masaharu, Yoshida[2]; Kenji, Onda[1]; Toshihiko, Hirano[1]
[1]Tokyo University of Pharmacy and Life Sciences, Hachioji, Japan; [2]Hachioji Medical Center, Tokyo Medical University, Japan

Objectives: Nephrotic syndrome is characterized by excessive proteinurea, hypoalbuminemia and hyperlipidemia. One possible explanation for the development of proteinuria is oxidative damage to glomerular cells. Oxidative modifications of LDLs produce a number of neo-self determinants that are highly immunogenic and can elicit strong B cells and T cells responses. Minimal change nephrotic syndrome (MCNS) is caused by T cell dysfunction, which results in an increasing in the permeability of protein in glomeruli. About 30% of initially Glucocorticoid (GC) – responsive MCNS patients will manifest frequent relapse after discontinuance of GC or during the tapering phase of treatment. In the present study, we evaluated the oxLDL levels in plasma and investigate that influence on clinical efficacy of immunosuppressive therapy in MCNS patients.

Methods: This study was approved by the ethic committees of Tokyo Medical University, and written consent was obtained from the patients. Twenty milliliters of venous blood from each subject were obtained and heparinized. We enrolled 41 MCNS patients and 16 healthy subjects. Peripheral blood mononuclear cells (PBMCs) were prepared for analysis of flow cytometry to detect helper T (Th) cells. We evaluated the percentages of CD4+CD25+Foxp3+Treg cells and IL-17+ Th17 cells in CD4+ Th cells. The concentration of oxLDL in plasma was determined using an enzyme-linked immunosorbent assay. The expression of microRNA-146a mRNA was detected by real time RT-PCR.

Results: In this study, we showed that ox-LDL levels in plasma in the MCNS patients were correlated with the expression of microRNA-146a mRNA in PBMCs and significantly higher than those in patients in complete remission or partial remission. The ox-LDL levels in plasma were positively correlated with the albumin levels in plasma. We also found a significant negative correlation between the ox-LDL levels in plasma and the frequency of regulatory T cells in CD4+ Th cells.

Conclusions: We conclude that ox-LDL attenuates the therapeutic efficacy by inhibiting

the differentiation of regulatory T cells in MCNS patients.

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Investigating the mechanism of skin rash caused by trimethoprim

Cao, Yanshan; Uetrecht, Jack.
University of Toronto, Toronto ON

Objectives: We found that nevirapine-induced skin rash is caused by a reactive benzylic sulfate formed in the skin that covalently binds to proteins. This is the first time a reactive sulfate formed in the skin was shown to be responsible for a rash. Trimethoprim (TMP) also causes serious skin rashes and has the potential to form a reactive sulfate metabolite. The objectives are to synthesize the trimethoprim sulfate and test its reactivity, and also to produce an anti-trimethoprim antibody to study potential covalent binding of trimethoprim in the skin.
Methods: The benzylic alcohol metabolite of TMP (OH-TMP) was synthesized by oxidizing TMP to a ketone with manganese dioxide followed by reduction to the alcohol using sodium borohydride. Both products were purified using silica gel chromatography; yield 25% and 95%, respectively. The sulfate conjugate was prepared by reaction of OH-TMP with SO3-triethylamine in tetrahydrofuran. The other objective was to produce antibodies that would recognize TMP-modified proteins. OH-TMP was reacted with PBr3 in dichloromethane followed by addition of mercaptobutyric acid. The TMP-thiobutyric acid adduct was purified. The carboxylic acid will be converted to the reactive hydroxysuccinimide ester and reacted with KLH protein. This TMP-protein adduct will be used to immunize rabbits to produce the desired antibodies.
Results: The half-life of TMP-sulfate was 1.5 hrs in 1 mM formate buffer, pH 4. It did not readily react with simple amino acids. TMP-modified immunogen will be used to immunize rabbits to produce the desired antibodies to study potential covalent binding of TMP.
Conclusions: The proposed TMP sulfate metabolite was synthesized. It is not very reactive but neither was the benzylic sulfate of nevirapine, and it reacted with proteins in the skin resulting in a skin rash. The synthesis of a TMP-protein conjugate that will be used to produce antibodies that recognize TMP-modified proteins is in progress.

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Next generation sequencing technologies improve pharmacogenetic-guided drug dosing

Cohn, Iris; Paton, Tara; Marshall, Christian; Monfared; Nasim; Ray, Peter; Basran, Beveen; Sinajon, Pierre; Cohn, Ronald; Ito, Shinya.
The Hospital for Sick Children, Toronto, ON

Objectives: Pharmacogenetics assists in optimizing drug treatment and prevention of adverse drug reactions. Currently, targeted genotyping strategies (mass spectrometry or fluorescent detection) are used clinically to screen for pharmacogenetic markers with well-characterized drug-gene interactions. Next generation sequencing (NGS) technologies allow detection of well-characterized pharmacogenetic variants and novel/rare variants not covered by existing clinical panels. Here, we determined the accuracy of variant calls from NGS data by coverage and concordance comparison with genotyping data to assess the potential clinical benefit of broader variant detection**.**
Methods: We analyzed 74 pharmacogenetic variant loci in 23 genes in whole genome sequence (WGS, Complete Genomics) and targeted genotyping data generated on mass spectrometry (Agena Biosciences) for 98 pediatric patients of varying ethnicities. Samples from 11 patients in this cohort were also whole-exome sequenced (WES) on the Illumina HiSeq2500 platform**.**
Results: All 74 loci achieve a very good average read depth across the 98 samples that were whole-genome sequenced. WGS data was concordant with all but two genotypes, however, 34 loci have one sample or more with missing data. In contrast, WES dataset exhibit superior coverage of each variant locus (with the exception of four non-coding variants). The expanded analysis identifies 3-4 potentially clinically relevant variants not identified through our targeted genotyping.
Conclusions: Our data demonstrate that NGS identifies most common pharmacogenetic variants detected in targeted genotyping. Remarkably, WGS data misses more variants than WES, although this rarely impact metabolizer status assignment for key enzymes. Furthermore, we find evidence that NGS detects functional variants that directly benefit clinical decision-making.

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The Role of CD8 T cells in Amodiaquine-Induced Liver Injury in PD1-/- Mice cotreated with anti-CTLA-4

Mak, Alastair; Uetrecht, Jack
University of Toronto. Toronto, ON

Objectives: The mechanism of idiosyncratic drug-induced liver injury (IDILI) remains poorly understood, in part due to the lack of a valid animal model. Clinical evidence suggests that most IDILI is immune mediated, and the major factor preventing liver injury in most patients is immune tolerance. Many attempts have been made in the past to develop an animal model of IDILI, but none had characteristics similar to IDILI in humans, and presumably they involved a different mechanism.
Methods: Recently our laboratory reported a model of amodiaquine (AQ)-induced IDILI using PD1-/- mice and an anti-CTLA4 antibody. This may be the first valid animal model of IDILI because it mimics the characteristics of IDILI in humans. The current study extended the duration of AQ treatment to see if this model would lead to liver failure and to further characterize the associated immune response.
Results: Although AQ treatment was extended to 10 weeks and total bilirubin levels were significantly elevated compared to control, there was no further increase from weeks 7 to 10, and the animals did not develop overt liver failure. Mice treated with AQ and anti-CTLA4 had a significant increase in percentage of hepatic CD4, CD8, Th17, and Treg cells after 10 weeks of AQ treatment, as well as significantly decreased NK cells. CD8 T cells have been implicated in several serious idiosyncratic drug reactions, and we used an anti-CD8 antibody to deplete CD8 T cells to study their involvement in this liver injury. We found that depletion of CD8 T cells protected mice from AQ-induced liver injury in this model, which strongly suggests that they are responsible for the liver damage.
Conclusions: This is consistent with the finding of CD8 T cells in liver biopsies of human IDILI and may lead the way to an effective treatment for serious IDILI.

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Sex differences in hepatotoxic and immune responses in a model of chemical-induced liver carcinogenesis in the mouse

Hanna Daniel; Bott, Debbie; Sugamori, Kim S; Grant, Denis M
University of Toronto, Toronto, ON

Objectives: Liver cancer is the third most common cause of cancer-related death worldwide, as a result of exposure to risk factors, many of which are associated with chronic liver inflammation. Liver cancer also shows a sex bias, where men have higher incidence than women even after correcting for differences in risk factor exposure. Mice also show a similar male predominance in liver tumors when exposed to carcinogenic chemicals, such as 4-aminobiphenyl (ABP) and diethylnitrosamine (DEN). Previously, we saw that the postnatal exposure of mice to ABP produces liver tumors only in males, with no differences in ABP-induced DNA damage between sexes. We have also seen a similar relationship in DEN exposed mice. Others have reported, that mature mice exposed to DEN show estrogen-dependent protection from DEN-induced acute hepatotoxicity and inflammation, which may play a role in carcinogenesis. However, standard tumor protocols rely on postnatal exposure of sexually immature mice, and from our findings, are not subject to ABP or DEN-induced acute hepatotoxicity and inflammation. Despite this, it is unknown if these responses develop well after carcinogen exposure, and whether sex hormones modulate these responses.
Methods: Male and female mice were exposed to ABP or DEN postnatally, and sacrificed at a number of time-points that cover the duration in which mice are maintained for tumor studies. Mice were assessed for a multitude of factors to determine whether hepatotoxic and inflammatory responses differ between sexes and if these responses promote hepatic proliferation.
Results: From the mice harvested so far, ABP did not affect overall physiology, increase chronic hepatotoxicity or increase hepatic proliferation. DEN however, caused a significant increase in total body weight only in male mice, but with no increase in chronic hepatotoxicity.
Conclusions: These results suggest that chemical specific factors may be responsible for the observations made so far, but further investigation is required.

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