2015 PHARMACY RESEARCH DAY ABSTRACTS

UNDERGRADUATE STUDENTS (GROUP A)

A1
Use of the Compensation Plan for Pharmacy Services is Associated with More Diabetes Activities: A Cross-Sectional Survey of Alberta Pharmacists

Rajan Bharadia, Kathleen Lorenz, Ken Cor, Scot Simpson

Purpose: Although important legislative changes have enhanced the opportunity for Alberta pharmacists to provide clinical services to their patients, one of the known barriers to implementing new programs is a lack of reimbursement. In 2012, a compensation plan was introduced that allowed pharmacists to be reimbursed for their clinical services. As diabetes is considered the leading area of specialization in pharmacy, we wanted to know if there is an association between use of the compensation plan and provision of diabetes management activities in community pharmacies.

Methods: A cross-sectional survey of Alberta pharmacists was conducted in 2015. The survey contained questions about use of the compensation plan and a list of 80 diabetes management activities. Additional questions gathered information about diabetes-specific training, additional prescribing authorization (APA), and practice environment. The number of diabetes management activities provided ‘often’ or ‘always’ were compared between respondents who used the compensation plan and those who did not using analysis of variance.

Results: Of the 256 pharmacists who submitted analyzable data, 168 (66%) indicated they work in a community pharmacy; mean (standard deviation) age was 42.1 (11.6) years, duration of practice was 15.7 (12.6) years, 104 (62%) were women, and 143 (85%) indicated they used the compensation plan. Pharmacists who used the compensation plan were more likely to work in a collaborative practice with physicians and have APA. After controlling for these potential confounding factors, pharmacists who use the compensation plan reported a mean of 42.9 (95% CI 39.4-46.4) diabetes management activities, while those who do not reported a mean of 29.9 (95% CI 21.4-38.4) activities (p=0.016).

Conclusions: After considering other important influencing factors, we observed a significantly higher number of diabetes management activities reported by pharmacists who use the compensation plan compared to those who do not use the plan.

A2
Computational Analysis for the Binding Modes of HCV NS5A Inhibitors

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Non-structural NS5A proteins found within the hepatitis C virus (HCV) plays a critical role in viral replication. In-vitro studies suggest that it is also involved in interferon resistance, transcriptional activation, and regulatory cell growth, making this human pathogen quite persistent and resilient against our immune system. With the discovery of the direct-acting antivirals (DAA) daclatasvir, we are now armed with a new class of drug that can selectively inhibit the NS5A protein, thus, reducing viral propagation.

Purpose: To further investigate the potential of DAAAs, we are presenting the predicted binding modes of four other DAA’s HCV NS5A inhibitors (ABT-267, Ledipasvir, AZD-7295, GSK-2336805) with symmetric and non-symmetric molecular scaffolds to NS5A of the HCV GT-1b viral strain.

Method: We used various modeling techniques and computational programs to identify the interactions of each DAA within the NS5A cleft.
Results: Our data shows that GSK is more energetically favored to bind compared to the other DAAs, likely because it can form certain stable hydrogen bonds with the amino acid residues located in the cleft. Furthermore, it also exhibits persistent hydrogen bonding with L31V and Y93H strains, suggesting that GSK remains relatively potent against wild-type (WT) and mutant strains. A compiled list of EC50 values shows that GSK’s potency towards the WT and mutant strains fluctuates minimally, which supports our results. Conclusions: We believe further investigation on the new line of DAAs can enhance our understanding on the necessities to develop better derivatives in our fight against HCV.

A3
An Evaluation of the Quality of iPhone Applications for Hypertension Self-management

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Purpose: Hypertension (HTN) is a leading risk factor for global disease burden. eHealth interventions using mobile technology are tools healthcare professionals (HCP) and patients can use to facilitate HTN self-management. Patient-oriented HTN applications vary significantly in functionality, from tracking blood pressure (BP) to education. It is critical to identify higher quality HTN self-management applications that could help patients. The primary objective was to review the quality of HTN self-management apps that included BP logs.

Methods: The Canadian Apple App Store was searched between May 22 - July 28, 2015 using the keywords hypertension and blood pressure. HTN apps were given an aggregate quality score (max score: 46) after appraisal of functional characteristics, BP tracking features, data validation, analytical features, and content trustworthiness. Apps with an educational component were given an educational quality score (max score: 29).

Results: Of 902 apps screened, 71 were analysed (n: 36 paid, average cost CAD$1.95 [SD: $1.16]). The mean aggregate quality score was 16.8 (SD: 6.8). Thirteen apps contained an educational component, with quality scores ranging from 1-14 out of 29. Free compared to paid apps scored significantly higher in analytical features, quality assurance, and aggregate quality score. Seventeen apps had a privacy policy, and 38 apps were updated in the past year. Few apps allowed BP goal-setting (n=9), reminders (n=19), tracked exercise (n=9) or diet (n=6). When BP readings were in alert ranges, only 4 apps suggested an appropriate course of action. There was a positive correlation between apps that had medication or weight tracking; BP categories; statistical analysis, with a higher aggregate quality score (P<0.001).

Conclusions: HTN self-management applications range in quality and functionality. The average app was of poor quality and few contained an educational component. Even apps with an educational component often had unreliable quality. Thus, there is opportunity for leaders in hypertension management to develop a high quality, evidence-based application targeted to Canadians.

A4
‘Do Bugs Need Drugs?’ Grade 2 Educational Program - Evaluation of Student Learning

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Purpose: In 2015, antibiotic resistance increasingly threatens world health. ‘Do Bugs Need Drugs?’, an educational program developed for second grade students focuses on 3 key messages: hand-washing, differences between bacteria and viruses and antibiotic resistance. Resources including an educational plan,
stories, songs, poems, a puppet, activities, stickers and ‘Glo-germ®, kit for hand-washing were developed. This program has recently been recognized in the G7 Report, Combating Antimicrobial Resistance 2015 as an important program internationally promoting the responsible use of antibiotics. Our objective was to determine knowledge gains by Grade 2 students about program key messages.

**Methods:** Ethics Approval was received from the University of Alberta. Parental consent and student assent were obtained. Surveys of 73 Grade 2 students in 7 Grade 2 students were conducted pre and post-educational program to assess knowledge gained. Five statements with responses Yes, No, Don’t Know assessed knowledge about the program’s messages: 1. Bacteria are different than viruses. 2. Antibiotics work against bacteria. 3. Antibiotics work against viruses. 4. Washing my hands helps to stop getting colds and the flu. 5. Using antibiotics to treat colds and the flu can cause antibiotic resistance.

**Results:** Grade 2 students showed a 32% overall improvement in average total score for the five questions increasing from 50% to 82% correct responses pre and post presentation. Using a paired Wilcoxon Signed Rank analysis of total scores p<0.001. Individually, questions 1, 3 and 5 showed significant learning p<0.05. Pre-study 98% of grade 2 students knew hand-washing prevents colds and flu increasing to 100% post-study.

**Conclusions:** The Do Bugs Need Drugs? Grade 2 Educational program represents an innovative and effective educational program addressing antibiotic resistance for Grade 2 students and provides healthcare professionals, students and teachers with resources available online to promote important health care messaging to our future generation.

**Support:** Alberta Health

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**A5**

**Polymeric Nano-Micelles for Delivery of a STAT3 Inhibitor to Melanoma**

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**Purpose:** Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in several human cancers. Despite acceptance of STAT3 inhibition as a promising strategy in cancer treatment, it has not been successfully translated to clinic, mostly due to toxicity and inefficient delivery of STAT3 inhibitors to tumors. S3I-1757 is an effective inhibitor of STAT3 dimerization that has shown activity against multiple cancer cell lines, but low water solubility and poor tumor selectivity have hampered its further development. The aim of this research was to develop polymeric micellar nano-formulations for the solubilization and selective delivery of S3I-1757 to melanoma tumors.

**Methods:** Diblock copolymers of poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO<sub>114</sub>-b-PCL<sub>22</sub>) or PEO-b-poly(α-benzyl carboxylate-ε-caprolactone) (PEO<sub>114</sub>-b-PBCL<sub>20</sub>) were synthesized and self-assembled to micelles. S3I-1757 was encapsulated in the micelles using co-solvent evaporation. Drug-loaded micelles were characterized for size, encapsulation efficiency and drug release. Free and micelle-encapsulated S3I-1757 was characterized for cytotoxicity against murine B16-F10 melanoma, and bone marrow-derived dendritic cells (BMDC)s by MTT assay. Inhibition of STAT3 transcriptional activity was assessed through measurement of VEGF in the cell supernatant by ELISA. The effect of free and encapsulated S3I-1757 in reversing the immunosuppressive effect of tumor supernatant on the stimulation of BMDCs was also investigated measuring over-expression of CD40, 86 and 80 on BMDC surface as well as IL-6 and IL-12 secretion by flow cytometry and ELISA, respectively.

**Results:** PEO-PCL and PEO-PBCL micelles reached encapsulation efficiency of > 79% and average diameter of < 55 nm after S3I-1757 loading. Free and encapsulated S3I-1757 showed similar IC50s against B16-F10, and similar dose dependent inhibition of VEGF production. Micellar S3I-1757 had significantly lowered cytotoxicity against BMDCs compared to free drug and resulted in greater BMDC IL-12 production.

**Conclusion:** PEO-b-PCL and PEO-b-PBCL micellar formulations of S3I-1757 are promising systems for the development of tumor targeted anti-STAT3 therapeutics in melanoma.
A6
Evaluating the Content & Development of Decision Aid Tools for the Management of Menopausal Symptoms: A Scoping Review

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**Purpose:** Decision-making during menopause is complex given the variability in women’s risk-benefit perceptions of treatments options, including hormone therapy. Several decision aid tools (DATs) have been developed to assist women in making treatment decisions during menopause. Our objective is to identify and evaluate the content and development of DATs for natural and surgical menopause.

**Methods:** We systematically searched electronic databases, including MEDLINE, EMBASE and CINAHL from inception to October 2015. Our inclusion criteria included published articles on the development of DATs for managing menopausal symptoms. Search terms were derived from two main concepts: menopause and DATs, and were searched as subject headings or keywords. Our search was limited to English language and human studies. Two reviewers independently screened titles and abstracts for eligibility, and extracted data on manuscript characteristics, target population, and DAT characteristics related to content and development. Evaluation was based on International Patient Decision Aid Standards’ (IPDAS) quality elements.

**Results:** Our search yielded 15,478 articles. Of these, 20 met our inclusion criteria and 5 have been analyzed thus far. The majority of DATs (80%) were developed for hormone therapy use in natural peri- and post-menopausal women, and were published after the Women’s Health Initiative (60%). All of the DATs were paper-based. None of the DATs’ content provided information about options in sufficient detail for decision-making. Only one study reported a complete systematic process for DAT development. None of the DATs targeted surgically menopausal women. None of the studies met all of the content and development quality criteria established by IPDAS.

**Conclusions:** This scoping review highlighted many discrepancies in available DATs for managing menopausal symptoms during natural menopause. None were identified for surgical menopause. The need for a comprehensive, evidence-based, up-to-date DAT with direct applicability to surgical menopause is imperative.

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**JUNIOR GRADUATE STUDENTS (GROUP B1)**

B1-1
Polymeric Micelles for Targeted Delivery of Diclofenac and its Ethyl Ester Derivative in Inflammation

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**Purpose:** Inflammatory conditions such as arthritis cause cardiovascular (CV) complications. Ironically, nonsteroidal anti-inflammatory drugs (NSAID)s that are given to treat inflammation, can cause CV complications upon long term use, themselves. Previous studies show high drug distribution to organs such as heart and kidney can play a role in the emergence of CV side effects by NSAIDs. The aim of this study is to develop a polymeric micellar formulation for diclofenac, an NSAID with known CV toxicity that can limit the distribution of the drug to heart and kidneys. Such formulation is expected to reduce diclofenac’s CV side effects.

**Methods:** Diclofenac (DFA) and diclofenac ethyl ester (DFE) were encapsulated in polymeric micelles prepared from a number of block copolymers including methoxypoly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-b-PCL), PEO-b-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-b-PBCL), PEO-b-
poly(α-carboxyl-ε-caprolactone) (PEO-b-PCCL), that with a side chain of N,N-dimethyldipropylendiamine on the PCCL block (PEO-b-P(CL-g-DP)), as well as PEO-poly(lactide) PEO-b-P(LA). Prepared micelles were then characterized for their particle size, polydispersity, encapsulation efficiency (EE), morphology, and in-vitro drug release. The kinetics of enzymatic hydrolysis in rat plasma for DFE loaded polymeric micelles versus free DFE was then examined at 37±0.5°C.

**Results:** The DFA and DFE loaded block polymeric micelles exhibited particle size in the range of 27.9-79.1 nm and narrow size distribution. Micellar formulations of DFA exhibited rapid drug release. The best results was obtained with PEO-b-P(CL-g-DP) micelles that showed an EE of 57.9% (±17.9) at an applied drug:polymer ratio of 1:20 mole/mole, 68.6% drug release in the first 4 hrs followed by a sustained release with near complete release at 24 hr. The DFE micelles showed slower release in comparison to DFA. For DFE, the best results were achieved with PEO-PCL micelles. The PEO-PCL micelles exhibited an EE of 60%, and an in-vitro release of 5.9% of the drug within 4 h followed by a sustained release totaling 9.7% drug release at 24 h. The enzymatic hydrolysis of the plasma after 24 h of incubation of polymeric micellar DFE, revealed negligible appearance of the parent compound (DFA) in the blood, whereas 65% of free DFE was converted to parent DFA upon incubation in plasma, only with 4h incubation.

**Conclusions:** The results show a great potential for polymeric micelles in passive targeting of DFE in inflammation.

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**B1-2**

**Pharmacological Characterization of ADP in the Platelet Nitric Oxide Signalling Pathway**

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**Purpose:** The primary function of platelets is to maintain hemostasis and promote wound healing, but pathologically platelets are also involved in thrombosis. An important negative-feedback mechanism limiting platelet aggregation at sites of vascular injury is mediated by nitric oxide (NO) signalling. NO is generated by endothelial nitric oxide synthase (eNOS) present within platelets, although some research groups question its presence. To address this debate, we chose to investigate why some groups have difficulty in detecting the functional effects of platelet-generated NO. Previous studies have shown that ADP, which is present within and secreted from platelet dense granules, counteracts the platelet inhibitory effects of exogenous NO. In addition, the NOS substrate L-arginine may also influence platelet NO generation, as it is bi-directionally transported in and out of platelets through cationic amino acid transporter-1 (CAT-1). Therefore, we hypothesized that passive release of ADP and reverse transport of L-arginine during platelet preparation may antagonize and limit the inhibitory effects of endogenous NO-signalling.

**Methods:** Prostacyclin-washed platelet aggregometry in the presence of apyrase (ADPase), and/or L-arginine was performed to determine the functional effects on platelet-NO-signalling. Flow cytometry of DAF-FM-stained platelets was utilized to measure NO generation.

**Results:** Apyrase (100μg/ml) enhanced the inhibitory effects of L-arginine (100μM) on platelet aggregation stimulated by collagen (51.88±10.72% apyrase & L-arginine vs. 93.57±4.18% L-arginine alone, N = 7, P<0.05). Preincubation with L-arginine (100μM) during the 1hour rest period inhibited aggregation (66.99±6.76% N=8, P<0.05). Addition of L-arginine (100μM) after the 1hour rest period failed to inhibit aggregation (100μM) (83.21±2.17% N=7, P>0.05); however, aggregation was inhibited by higher concentrations of L-arginine (500μM) (53.57±13.23% N = 7, P<0.05).

**Conclusions:** Platelet-secreted ADP and reverse transport of L-arginine, during the rest period, antagonize the negative-feedback effects of platelet-generated NO. Future experiments will further investigate the role of CAT-1 in platelet L-arginine transport.
B1-3
Hybrid Ligand/Target-Based Virtual Screening Protocol to Identify Inhibitors for the XPA-ERCC1 Protein-Protein Interaction

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**Purpose:** Many chemotherapy agents target the DNA of cancer cells, forcing them to die. Nevertheless, an active DNA repair pathway in cancer cells removes these drug-mediated lesions and thereby reversing the therapeutic benefits of DNA damaging agents. Tumor cells can therefore survive, grow and proliferate. In this context, DNA repair inhibitors opened a new avenue in combination cancer treatment. The rationale of using these adjuvant therapies is to block the DNA repair mechanisms from removing the chemotherapy-induced DNA damage, hence optimizing the effect and reducing the required dose of the treatment. A profound example is the nucleotide excision repair (NER) pathway, which removes the DNA adducts induced by platinum-based chemotherapy. A central part of this pathway is the ERCC1-XPA complex. Expression of ERCC1 an XPA in cancer cells is correlated with the response to platinum-based chemotherapy. The only known cellular function so far for XPA is to recruit the ERCC1-XPF endonuclease to the damaged point. Inhibiting this protein-protein interaction can therefore completely disrupt NER activity, enhancing thus the chemotherapy effect.

**Methods:** Recently, we validated the ERCC1-XPA interaction as a promising target to regulate the activity of the NER pathway. Our earlier small molecule hits were able to specifically disrupt this protein-protein interaction and sensitize cancer cells to cisplatin and UV radiation. Here we continued these efforts to identify more selective and potent inhibitors for this interaction.

**Results:** We employed \textit{in silico} computational drug design methods to: 1) optimize the structures of the previously identified inhibitors; 2) identify novel scaffolds to develop different lead compounds with similar pharmacophore features.

**Conclusions:** We identified several potential novel inhibitors for the XPA-ERCC1 interaction, and three novel lead compounds as well. The findings described here form a milestone in discovering novel inhibitors for the NER pathway to improve the efficacy of current platinum-based therapy.

B1-4
Small Hydrophobe Substitution on Polyethyleneimine for Effective Plasmid DNA Delivery into Breast Cancer Cells

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**Purpose:** Improved delivery systems are needed for intracellular delivery of difficult-to-deliver biologics such as plasmid DNA (pDNA). Cationic polymer such as polyethyleneimine (PEI) has been intensively studied for pDNA delivery. Low molecular weight PEI is less toxic and can be readily eliminated from the circulation when employed \textit{in vivo} as compared to high molecular weight counterparts. However, they are not effective for gene delivery and modification with hydrophobic groups is essential to make them effective.

**Methods:** We modified 1.2 kDa PEI (1.2PEI) with small hydrophobe propionate (PrA; \(-\text{CH}_2\text{-CH}_3\)) via \textit{N}-acylation and explored the features of resultant pDNA polyplexes and delivery efficiency in breast cancer cells; MDA-231 and MCF-7.
**Results:** Substitution efficacy of PrA onto 1.2PEI was increased with feed ratio (PrA/PEI) and pDNA binding efficacy of PEI-PrA was decreased in proportion with PrA substitution as a consequences of –NH₂ consumption and/or steric hindrances. Hydrodynamic size of polyplexes was identical irrespective to PrA substitution, but surface charge initially increased with PrA substitution and later decreased at high PrA substitutions. Cellular toxicity of the polymers was increased with PrA substitution but the polymers still displayed less toxicity compared to 25 kDa PEI. pDNA uptake and transfection efficiency in both MDA-231 and MCF-7 was significantly increased with optimal PrA substitution (0.5-1 PrA/PEI) while polymers with the highest substitution and parent polymer were ineffective. Importantly, transfection efficiency of PEI-PrA1 (PrA/PEI = 0.76) was higher than long chain lipid (C=18) grafted 1.2PEI and comparable to 25PEI. In addition, PEI-PrA1 showed higher transfection than 25PEI at day 7 and 14, showing a relatively stable transfection in MDA-231 cells.

**Conclusions:** Substitution of small hydrophobe PrA onto 1.2PEI enhanced transfection efficiency in breast cancer cells, but excess substitution (>1.2 PrA/PEI) was detrimental, emphasizing the importance of balancing polymer hydrophobicity for effective gene delivery.

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**B1-5**

**Assessing the Effect of MCL-1 Knockdown by siRNA on Sensitivity of Breast Cancer Cells to Doxorubicin**

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**Purpose:** Aberrant expression of proteins that prevent apoptosis and change in the balance between pro- and anti-apoptotic proteins consist on major barriers against effective chemotherapy. MCL-1 has been identified as one of the most up-regulated proteins in human cancers, preventing apoptosis via Bak/Bax neutralization. Its expression has been linked to resistance of cancer cells to chemotherapy. The objective of this study was to investigate the validity of MCL1 silencing by siRNA in sensitization of breast cancer cells to doxorubicin.

**Methods:** Breast cancer MDA435 and MDA231 cells were maintained in RPMI-1640 and exposed to free or liposomal DOX, (Caelyx®) for 24-48h. MTT assay was used to determine the cytotoxicity of DOX in these cell lines. They were exposed to MCL-1 siRNA for 48h using lipofectamine as transfecting agent. RT-PCR was then used to quantify MCL-1 mRNA expression using GAPDH. Cells were then treated with MCL-1 siRNA/lipofectamine complexes for 48h, preceding cell treatment with media or free DOX for additional 24h. Cell viability following combination treatment was assessed by MTT and compared to DOX and MCL-1 siRNA monotherapy.

**Results:** Free DOX presented a lower IC₅₀ in breast cancer cells compared to the liposomal DOX, indicating higher potency since it is instantly available when added into the media, whereas DOX is controlled released in Caelyx. MDA435 cells showed less resistance to DOX compared to MDA231 cells. The IC₅₀ of DOX in MDA-435 cells was ~ 40 times lower. Lipofectamine/MCL-1 siRNA complexes efficiently silenced the expression of MCL-1 mRNA by 60 percent compared to control untreated cells or cells treated with scrambled siRNA. However, MCL-1 silencing did not lead to the sensitization of breast cancer cells to DOX under current experimental conditions.

**Conclusions:** Further investigation is needed to attest any benefit for combination of inhibitors MCL-1 expression with DOX in breast cancer therapy.
Favorable Outcomes in Safety, Symptoms, and Lab Measures in an Anti-Gliadin IgY Antibody (AGY) Clinical Trial in Celiac Disease

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**Purpose:** Approximately 1% of the worldwide population has celiac disease (CD), a permanent intolerance to ingested gluten in genetically susceptible individuals. The gluten free diet (GFD) is the only current treatment and non-adherence is associated with significant morbidity and mortality. However, compliance is difficult and many individuals with CD experience ongoing symptoms, adversely affecting quality-of-life. Additional treatment options are needed. Anti-gliadin IgY antibody (AGY) is a novel treatment using an oral egg yolk antibody designed to neutralize the toxic effects of gluten and improve clinical outcome.

**Methods:** This first-in-man, 6-week, open-label, single arm pilot safety study was conducted in adults with biopsy proven CD who follow a GFD but still have periodic symptoms. The primary outcome was safety, measured by adverse events and laboratory tests. A run-in period of 2 weeks determined compliance with questionnaires, including the Celiac Symptom Index (CSI), and evaluation of baseline safety laboratory results. This was followed by a 4-week treatment period with 2 x 500mg capsules AGY, taken just prior to each meal. Adverse events were recorded from the time of consent to study end. Daily CSI assessment, periodic laboratory measures, Health Related Quality of Life (HRQoL), anti-tissue transglutaminase and anti-gliadin IgA/IgG, as well as lactulose mannitol excretion ratio (LMER) were measured.

**Results:** Eleven individuals enrolled, ten completed the study (9 female; mean age 43.4 years; all Caucasian). No safety concerns were identified. Most participants had fewer symptoms, and improved HRQoL, antibody levels, and LMER when taking AGY relative to the run-in period; some changes were statistically significant.

**Conclusions:** AGY is safe and may improve CD related outcome measures. A larger study powered for efficacy evaluation is warranted. **Support:** Project CFI009, Canadian Food Innovators, Ottawa, Canada; Project RES0012109, Alberta Livestock and Meat Agency, Edmonton, Canada; IGY Inc, Leduc, Canada.

Genetic Deletion of Soluble Epoxide Hydrolase Protects Cardiac Mitochondria From LPS-Induced Toxicity

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**Purpose:** Lipopolysaccharide (LPS) is a bacterial wall endotoxin producing many pathophysiological conditions including myocardial inflammation leading to cardiotoxicity. Arachidonic acid, a polyunsaturated fatty acid, can be metabolized to cardioprotective epoxyeicosatrienoic acids (EETs) by cytochrome P450 epoxygenases. EETs are hydrolyzed to less bioactive dihydroxyeicosatrienoic acids (DHET) by soluble epoxide hydrolase (sEH). EETs trigger a wide range of pathways protecting cellular structures, reducing cell death and promoting anti-inflammatory reactions in various cell types. We have recently demonstrated that EETs protect rat neonatal cardiomyocytes against LPS-induced cytotoxicity. The goal of this study is to investigate whether genetic inhibition of sEH influences mitochondrial function following LPS exposure.

**Methods:** Age-matched 2 month old sEH null (KO) and littermate wild-type (WT) mice were injected with LPS (10mg/kg) then sacrificed after 6 or 24 hrs. Hearts and blood were collected to assess inflammatory response and mitochondrial function. Mitochondrial function was evaluated by measuring myocardial levels
of ATP and respiratory activity of isolated cardiac mitochondria using a Clark-type electrode. The levels of glucose and inflammatory cytokines in blood were also assessed.

**Results:** Our data demonstrated that LPS-triggered a massive inflammatory response beginning at 6h and until 24h in WT mice. This coincided with pronounced hypoglycemic response and compromised mitochondrial function in WT mice. In contrast, sEH-KO mice were protected against LPS-induced cardiotoxicity. These animals did not develop hypoglycemia and no loss in body weight was detected. The levels of inflammatory markers (TNFα, MCP-1) were modestly elevated and significantly lower than WT mice treated with LPS. Mitochondrial function was preserved in hearts from sEH KO mice based on higher respiratory control ratios compared to WT.

**Conclusion:** Deleting soluble epoxide hydrolase provides protective effects against LPS-induced cardiotoxicity maintaining mitochondrial function. Our data suggest that inhibiting sEH, elevating endogenous levels of EETs, is a potential therapeutic approach to limiting LPS-induced cardiotoxicity.

**B1-8**

**Pharmacological Inhibition of Soluble Epoxide Hydrolase Preserves Mitochondrial Efficiency and Cardiac Function Post-MI in Aged Mice**

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**Purpose:** Cardioprotective effects of epoxyeicosatrienoic acids (EETs) toward acute myocardial ischemia-reperfusion injury have been recognized; however, it remains unclear whether EET-mediated cardioprotection is sustained in the aged population. Our study investigates the protective effects of EETs by inhibiting soluble epoxide hydrolase (sEH), the enzyme responsible for EET metabolism, following surgical occlusion of left anterior descending artery (LAD) in aged animals.

**Methods:** Age matched 18 month old sEH null (KO) and littermate wild-type (WT) mice were subjected to LAD-ligation to induce myocardial infarction (MI). In parallel, aged C57Bl/6 mice received sEH inhibitor, trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (tAUCB; 10mg/L) or vehicle in drinking water for 4 days prior and 7 days post-surgery. Cardiac structure and function was assessed by echocardiography prior to and 7 days post-surgery. Mitochondrial enzymatic activities of respiratory complexes I, II, IV, and citrate synthase were assessed. Respiratory control ratios were determined using a Clark-type electrode.

**Results:** Hearts from tAUCB-treated mice showed preserved ejection fraction and percent fractional area change compared to WT counterparts. However, no preservation of cardiac function was observed in sEH KO groups. Mitochondrial functions were better preserved following myocardial infarction in hearts from tAUCB-treated and sEH KO mice based on higher respiratory control ratios compared to WT controls. tAUCB treatment increased post-MI enzymatic activity of complex I and II.

**Conclusion:** Our data suggest that while genetic deletion of sEH showed minor protective effects post-MI, pharmacological inhibition of sEH resulted in sustained mitochondrial bioenergetic efficiency and improved cardiac function.
JUNIOR GRADUATE STUDENTS (GROUP B2)

B2-1
Assessing the Effect of Polymer Stereochemistry in Selective Solublization of one Enantiomer from a Racemic Mixture of a Compound with Chiral Carbon

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Purpose: The stereochemistry of the polymer has shown to have a significant impact on its intrinsic properties. The aim of this study was to assess the effect of polymer stereochemistry of the core forming self-associating block copolymers on the solublization of optically active and hydrophobic molecules. Polymeric micelles composed of stereo-active polylactide segments as the hydrophobic part (core-forming block) were selected for this purpose.

Method: Methoxy poly(ethylene glycol)-b-poly(lactides) (PEO-b-PLA) of different stereochemistries, i.e., MPEG-PDLA, MPEG-PLLA and MPEG-PDLLA, were synthesized by ring opening polymerization of D-Lactide, L-Lactide or D,L-Lactide, respectively using methoxy PEG as initiator and stannous octoate as catalyst. H¹NMR (600 MHz) was used to characterize the synthesized polymers and calculate their molecular weight. The α-benzyl carboxylate ε-caprolactone (BCL) was encapsulated into the di-block polymers by solvent evaporation method. UV-VIS Spectrophotometer was used to determine the loading capacity. After separating the loaded polymer from the free monomer by ultrafiltration, the optical activity of the loaded micelles, and the filtrate was measured using polarimeter.

Results: H¹NMR confirmed the successful synthesis of block copolymers at expected molecular weights. Polymeric micelles with stereo regular cores appeared to provide significantly better loading efficiency for BCL than those with non-stereo regular cores. Block copolymer micelles with PDLA appeared to have a significantly better loading capacity for BCL than those with the PLLA core. Optical rotation results showed that polymeric micelles with different stereo configuration under study have no preference for selective solubilization of one enantiomer of BCL from its racemic mixture.

Conclusion: The stereochemistry of the core appeared to have no effect on selective solublization of the optically active model material under study. However, micelles with stereo regular core appeared to have better loading capacity for racemic BCL compared to micelles with optically inactive cores.

B2-2
Comparative Analysis of Chicken RANK-L Protein Production by Escherichia coli (E. coli) and Baculovirus Expression Systems

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Purpose: Osteoporosis is the third most common cause of mortality in commercially caged laying hens. About 90% of caged hens develop the disease. This severe animal welfare problem results from multiple factors, including high rates of egg production, lack of exercise, or perturbed intake of essential minerals (such as calcium and phosphorus) and vitamins (such as vitamin D due to lack of exposure to sunlight). Results are acute and chronic pain, and bone fractures that often cause paralysis or death. Osteoporosis occurs primarily through accelerated loss of the organic and mineral components of bone, such as phosphates and calcium. These bone components are also essential for eggshell formation, leaving laying hens with reduced resources for bone development. We would be specific novel IgY antibodies as a novel treatment to target and neutralize the cells (osteoclasts) responsible for accelerated bone breakdown in chicken osteoporosis without compromising egg production. Preliminary objective was production of functional antigens in different systems with high yield.
Methods: The nucleotide sequence of chRANKL was codon-optimized for E. coli expression and synthesized by GeneArt Inc. (LifeTechnologies). The chRANKL fragments amplified by PCR were digested and ligated into Nco I-Xho I sites of the pET22b(+) expression vector. The E. coli Rosetta 2 (DE3) competent cells were transformed with the pET22b(+)chRANKL expression vectors. The chRANKL expression was induced by isopropyl-beta-D-thiogalactopyranoside (IPTG). The chRANKL expression was optimized by the cell density, IPTG concentration, induction temperature and time and monitored by SDS-PAGE Western blot. chRANKL recombinant proteins were purified from inclusion bodies using affinity column and subsequently refolding was done with Tris Arginine buffer. Similarly for baculovirus expression, the chRANKL DNA was fused to polyhedrin promotor and ligated into the vector and then amplified by PCR. The final PCR product was ligated into restriction-digested pVL1393 to obtain the final baculovirus transfer vector pVL1393-chRANKL. Sf9 cells were initially cultured in monolayer and then suspended in ESF921 medium at 27oC, under shaking at 100 to 120 rpm. Host insect cells Sf9 were co-transfected with the BestBac 2.0 v-cath/chiA deleted DNA and the Baculovirus transfer vector pVL1393-chRANKL. Optimal multiplicity of infection was determined by infecting culture using MOI of 1, 2, 5 and 10. Intracellular proteins were purified by Ni-NTA agarose affinity chromatography whereas secreted proteins were purified by a combination of ion exchange SP-sepharose column and Ni-NTA column. Final protein was dialyzed and characterized.

Results: The chRANKL expression with vector pET22b(+)chRANKL in E. coli was successful in the form of inclusion bodies and purification with column chromatography resulted in high yield. The Baculovirus Expression System was also successfully used because of its ability to process proteins similar to other higher eukaryotes. It gave high-level expression of RANKL with full biological activity. Immunoblotting with anti-6XHis mAb showed that the recombination successfully incorporated the chRANKL-6XHis construct into the Baculovirus genome.

Conclusion: We successfully expressed functional chRANKL proteins in both expression systems both as inclusion bodies and soluble forms. These proteins would be used to generate high affinity and specific chicken IgY for preventing chicken osteoporosis.

Support: Alberta Livestock and Meat Agency

B2-3 Expression of Ebola VP40 Protein and Characterization of Novel Antibodies for Rapid Ebola Diagnostic Assay Development

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Purpose – Ebola Virus belongs to the Filoviridae family. It causes severe haemorrhagic fever and is often fatal for human beings. Clinical diagnosis is difficult during the early stages because of similar symptoms with other infections and absence of an efficient diagnostic system. The aim of our study is to develop a sensitive and quantitative detection system for Ebola infectious disease to be used at clinical settings. We are targeting the Ebola virion protein 40 (VP40) to determine if the viral protein is present in suspected individuals and also the extent of infection. VP40 is the viral matrix protein shed during infection, abundantly present in body fluids during early stages of infection. It is therefore a suitable marker for developing an antigen detection assay. In this study we have expressed codon optimised VP40 in E. coli for higher yield. This VP40 has been used for immunizing chicken and obtaining high affinity IgY antibodies from egg yolk. We have cultured 9 different anti VP40 hybridomas to obtain monoclonal antibodies (MAbs). We will use these MAbs and IgY in combinations for the development of a hetero-sandwich ELISA for early detection of Ebola infection.

Methods: The recombinant VP40 protein was expressed in E. coli. A 6x His tag was inserted at the C terminus to facilitate purification. The purified protein was recognized in Western blot by MAb specific to
the histidine tag as well as the anti VP40 Ebola hybridomas. In vitro refolded recombinant protein was used to immunize chicken for the development of IgY antibodies at every two weeks. IgY was separated by water soluble method followed by centrifugation at 3000g for 20 min and purification by Sephacryl gel chromatography. Anti VP40 hybridomas are grown in bioreactors (1L) for MAb production. SDS-PAGE, Western Blot and ELISA was conducted to characterise binding and detection efficacy of the different format of antibodies.

**Results:** Successfully expressed the recombinant VP40 antigen with very high yield. Anti-VP40 MAbs and IgY recognized the recombinant protein validating the immunogenicity of the proteins as well as affinity. The ELISA assay showed high affinity between the VP 40 and all the 9 different MAb as well as IgY. Western blot also validated specific binding.

**Conclusion:** As a preliminary study, we have successfully produced VP40, IgY and MAbs for the Ebola virus detection system. The uniqueness of our study is the use of inexpensive chicken IgY antibody in combination with high affinity monoclonal antibodies to increase the sensitivity of the detection system for Ebola infectious disease. The purified Ebola VP40 antigen could potentially be used for developing efficient Ebola diagnostics and also for vaccine development efforts. The Ebola VP40 antigen immunoassay developed could be an efficient and sensitive method of diagnosing Ebola-suspected individuals during a future Ebola disease outbreak.

**Support:** MITACS Graduate Student Internship Program

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B2-4

**Targeting Integrin Beta-1 (CD29) to Reduce the Attachment of Breast Cancer Cells to Bone**

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**Purpose:** The attachment of breast cancer cells with the aid of integrins to extracellular (ECM) proteins such as fibronectin and vitronectin present on bone marrow environment plays a critical role in the metastasis of tumor cells to bone. This attachment acts as a primary site of interaction between cell populations in addition to ECM binding, and allows the tumors cells to remain dormant in bone for a longer period of time and proliferate. Blocking this primary site of attachment might be beneficial in reducing metastasis, which could be achieved by silencing the cell surface integrins present on cancer cells. In this study, siRNA targeting integrin beta-1 (CD29) was delivered using modified polyethyleneimine polymers, with the purpose of reducing breast cancer cells attachment to ECM and bone cells.

**Methods:** Integrin beta-1 silencing experiments were carried out in MDA MB-231 breast cancer cells through immunostaining, qPCR, fibronectin and human bone marrow stromal cell (hBMSC) adhesion assays and cell viability (MTT) assay.

**Results:** Three in-house designed polymers 1.2PEI-taLA6, 1.2PEI-Lau8 and 1.2PEI-LA6 displayed effective silencing at low siRNA concentration (40 nM), as determined by CD29 immunostaining, with 1.2PEI-LA6 exhibiting best silencing. The surface level of integrin beta-1 were reduced until day 6 after treatment, whereas the mRNA levels remained silenced until day 9. The functionality of this silencing was assessed by studying its ability to attach to fibronectin and human bone marrow stromal cells as integrin beta-1 is a primary receptor for fibronectin. In both these assays, significant reduction in the cell attachment was observed in CD29 siRNA treated cells.

**Conclusions:** The mRNA levels of integrin beta-1 gene at three different time points exhibited significant silencing. Both the fibronectin binding and hBMSC adhesion assay revealed that this amount of integrin silencing at the cell surface was adequate to reduce its binding ability thereby revealing the functional benefit of integrin beta-1 reduction in breast cancer cells. Silencing integrin beta-1 decreased the cell number which could be the result of apoptosis or declined cell proliferation and identifying additional targets to reduce the
attachment of breast cancer cells might have better effect on metastasis. Additional studies in animal models will be needed to confirm if metastasis of breast cancer cells will be reduced after integrin beta-1 reduction.

B2-5
A Comprehensive Atomistic Model for the Human Na,1.5 Sodium Ion Channel: Understanding the Molecular Origins Behind Cardiotoxicity

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Purpose: The Na,1.5 Voltage Gated Sodium Channels (VGSCs) are one of the most important cationic channels that are responsible for initiating the myocardial action potential. Mutations in Na,1.5 have been implicated in a wide range of cardiac diseases, such as the long QT syndrome 3 (LQT3), Brugada syndrome 1 (BRGDA1) and sudden infant death syndrome (SIDS). Thus, studying the fine structural details of this important ion channel will enhance our understanding of different physiologically and pathophysiologically observed phenomena.

Methods: Our methodology combined homology modeling of the selected Na,1.5 sequence using the bacterial Na, AB sodium channel as a template for the transmembrane domains. The models were refined using ModeRefiner and FG-MD tools, and validated with PROCHECK. The final model was exposed to molecular dynamic (MD) simulation for 500 ns using NAMD package and the dominant protein conformations were extracted. Some of the known Nav1.5 blockers were then tested on the model by docking and free binding energy calculations.

Results: In this study, we are reporting the most recent and most complete homology model for the inactivated/closed state of Na,1.5 cardiac ion channel based on the Na,1B bacterial ion channel as a template. Our model has been able to retrieve the binding modes of a number of known Na,1.5 blockers and results from several analyses prove its structural accuracy.

Conclusions: At present, no complete models for human cardiac ion channels have been generated, and this is due to several factors mainly related to the scarcity of experimental crystal and/or NMR structures. The current study provides valuable insights into the structure of the Na,1.5 ion channel and can enhance our understanding of the underlying mechanisms involved in this family of ion channels.

B2-6
Effects of Human Platelets on Lung Cancer Stem Cell Invasion

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Purpose: The cancer stem cell theory of cancer origin suggests a small population of cancer cells has stem cell-like characteristics (CSCs) and is responsible for initiating new tumors following metastasis. Studies have shown that cancer cells activate platelets and that platelets contribute to metastasis, in part by stimulating cancer cell invasion. Stromal derived factor-1α (SDF-1α) found and secreted from activated platelets is known to mobilize bone marrow and cancer stem cells via increased matrix metalloproteinase (MMP) expression. We hypothesize that activated platelets release SDF-1α which binds to its receptor CXCR4 on CSCs leading to increased MMP production, and thus preferentially induce CSC invasion.

Methods: A549 lung carcinoma CSCs were identified either as Hoechst 33342-negative side population (SP) and/or sorted using fluorescence activated cell sorting based on CD133 positivity. CSC invasion was compared to total A549 population invasion via modified Boyden Chamber assays in response to collagen-activated human platelet releasates and quantified by flow cytometry and confocal microscopy.
**Results:** Activated platelet relaesates preferentially stimulated invasion by SP-identified CSCs (4.3±0.3% of total population A549 pre- vs. 7.6±0.7% post-invasion, P<0.05). However, the CXCR4 antagonist AMD3100 (10μM) failed to inhibit SP-invasion, but inhibited total A549 invasion (60.85±13.37x10^3 total-population invasion without vs. 44.23±9.88x10^3 with, P<0.05). Preliminary data suggest CD133-positive A549 cell invasion increased compared to CD133-negative cells in response to activated platelet releasates (40.50±10.36% CD133-negative vs. 60.25±9.73% CD133-positive, P=0.2; N=4). Further, CD133 staining demonstrated that the Hoechst negative SP is enriched with CD133-positive cells (1.4±0.59% in total vs. 3.84±1.09% in A549 SP, P<0.05).

**Conclusion:** Activated human platelets preferentially stimulate the invasion of SP-identified CSCs. Identification of CSC based on both CD133 staining and Hoechst negative SP might be more reliable than using Hoechst SP alone. Further experiments are required to delineate the role of SDF-1- CXCR4-MMP signalling in platelet-stimulated cancer stem cell invasion.

**B2-7**  
**A Study on Iron-chelating Capacity of Phosvitin Hydrolysates Produced by High Hydrostatic Pressure combined with Enzymatic Hydrolysis**

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**Purpose:** Phosvitin (PV) is known as a metal binding protein in egg yolk with unique amino acid composition (>55% serine) associated with a large proportion of phosphates. Many attempts to use PV as a potential source of anti-microbial agent were made but it turns out be no feasible due to its insolubility and poor stability in aqueous media. Our preliminary data show the enzyme-treated PV phosphopeptides (PV-P) could be dissolved. On the other hand, the yield of PV-P was extremely low due to high serine-phosphate resistant to enzymatic hydrolysis. The objective of this study was to increase the yield of PV-P by using an innovative processing platform technology of high hydrostatic pressure combined with enzymatic hydrolysis (HHP-EH). Further study was to evaluate the iron-chelating capacity of PV-P.

**Methods:** PV was isolated from egg yolk residues after IgY antibody extraction. PV-P fractions (pH6.0) were produced by HHP-EH with various proteases (Alcalase, Trypsin, Bromelain, Papain, Thermolysin, Elastase, Flavourzyme, Visozyme, and Savinase), in single, double or triple combinations, at E:S ratio of 1:50, under 100 MPa, at 37-50°C for 12-24 h. The optimization of HHP-EH was evaluated by TNBS method for the degree of hydrolysis (DH) of PV. M_ω distribution of PV-P was monitored by SDS-PAGE, MALDI-TOF and HPLC techniques. Iron-chelating capacity of PV-P fractions (> or <3 kDa) vs PV was measured by spectrophotometric detection of ferrous ions. Samples were analyzed in triplicate. The results were plotted in student t-test.

**Results:** Triple combination of Alclase, Elastase and Flavourzyme showed the highest DH (89%) at E:S ratio of 1:50 in each enzyme, under 100 MPa, at 37°C for 24 h. The results of SDS-PAGE and MALDI-TOF showed that PV M_ω bands (48 and 37kDa) were hydrolyzed into PV-P at 30, 23, 17KDa and <3 kDa. The highest iron-chelating capacity was observed in PV-P (<3 kDa fraction) hydrolyzed, compared to PV and PV-P (>3 kDa fraction), regardless of enzymatic and pressure treatment, indicating an efficient iron-chelating capacity of short PV-P.

**Conclusion:** We optimized parameters of HHP-EH processing to increase the yield of PV-P. These short phosphopeptides show high iron-chelating capacity used in anti-microbial agent.

**Support:** MITACS Graduate Internship Program and Canada Food Innovators Grant.
Myeloperoxidase Bioactivates Hydroxylated Metabolites of Non-Steroidal Anti-Inflammatory Drugs (Nsais) into Cytotoxic Specieo

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**Purpose:** NSAIDs use is associated with some idiosyncratic adverse reactions (IADRs), believed to be caused due to their metabolism into reactive metabolites, whose reactivity has not been systematically compared. So, to investigate the reactive metabolites, possibly implicated in NSAIDs’ IADRs, this study probes myeloperoxidase (MPO)-mediated bioactivation of diclofenac, indomethacin and naproxen in comparison with the bioactivation of their hepatic metabolites, 4’-hydroxydiclofenac (4’-OHD), 5-hydroxydiclofenac (5-OHD), O-desmethyl-N-deschlorobenzoyl indomethacin (DMBI), O-desmethylindomethacin (DMI) and O-desmethylnaproxen (ODN), in terms of reactivity and their *in vitro* toxicity.

**Methods:** To study the reactivity of the metabolites produced by MPO, kinetic UV-visible spectroscopy was used to determine ascorbate cooxidation rates. Then, glutathionyl radical (GS•) induced by NSAID free radical metabolites was detected using electron spin resonance (ESR) with DMPO (5,5-dimethyl-1-pyrroline-N-oxide) spin trapping, and for *in vitro* toxicity, we used trypan blue exclusion in HL-60 cells, which contain high amounts of MPO. Also, a cytofluorometric glutathione assay was performed in HL-60, and changes in HL-60 mitochondrial membrane potential (ΔΨm) were studied using JC-1 dye.

**Results:** We found that only 4’-OHD, ODN and DMBI cooxidized ascorbate. In addition, diclofenac and naproxen metabolites, 4’-OHD and ODN reaction with MPO gave reactive species that oxidized GSH, forming GS• radical, however, this was not formed with DMBI reactive species, suggesting that the latter is not a free radical species. Even though all the three species shared the ability to oxidize ascorbate, deplete cellular GSH and to decrease mitochondrial membrane polarization, only 4’-OHD-derived species was able to cause significant cytotoxicity.

**Conclusions:** Metabolism of NSAID’s hydroxylated metabolites, namely, 4’-OHD, ODN and DMBI by MPO results in deleterious species that are variable in nature and reactivity, and could potentially be implicated in the development of IADRs associated with the use of their parent drugs.

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SENIOR GRADUATE STUDENTS (GROUP C1)

**C1-1**
The Oxidation of the Anticancer Drug Metabolite, 6-mercaptopurine Ameliorates Cu-Zn Superoxide Dismutase Activity: Potential Involvement of Peroxymonocarbonate

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Azathioprine and its metabolite (6-MP) are mainly effective in the treatment of many inflammatory bowel diseases. At sites of inflammation, reactive oxygen species (ROS) are generated, including hydrogen peroxide. The latter product is known to inactivate superoxide dismutase (in the absence of certain intermediates, such as HCO3−), which enhances oxidative stress. In the presence of HCO3− and H2O2, peroxymonocarbonate, which is known to oxidize electron-rich species such as thiols, can be produced. 6-MP has a thiol moiety in its structure, but it is not known if peroxymonocarbonate can react with this drug.

**Purpose:** To investigate if oxidizing the thiol moiety of 6-MP by peroxymonocarbonate will ameliorate SOD activity.
**Methods:** UV-Vis spectrometry was used to monitor spectral changes (200-500 nm) of 6-MP and other analogs including its parent drug, azathioprine. Electron paramagnetic resonance (EPR) spectroscopy was utilized to detect the effect of thiopurines on carbonate radical, a product of SOD-peroxidase activity (SOD/HCO$_3$/H$_2$O$_2$). LC/MS was used to determine the metabolites of oxidizing 6-MP. SOD activity was measured by using SOD colorimetric assay kit.

**Results:** UV-Vis spectra showed that changes were observed with 6-MP and 6-thioguanine; with less changes observed with 6-thioxanthine and 6-thiouric acid. Kinetic studies demonstrated that the changes in the 6-MP peaks were dependent on HCO$_3$/H$_2$O$_2$ concentrations. Using EPR spectroscopy and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap, we found that the spin adduct of CO$_3$- (DMPO/OH) was attenuated with increasing of 6-MP and 6-thioguanine concentrations. However, DMPO/OH was not significantly attenuated with the presence of azathioprine. Also, oxidation of 6-MP by HCO$_3$/H$_2$O$_2$ resulted in forming a sulfoxide product (C$_6$H$_5$N$_2$O$_2$S; 182.9981 m/z) that was detected by using high resolution LC/MS. Lastly, inactivation of SOD by H$_2$O$_2$/HCO$_3$ was significantly attenuated with the presence of 6-MP.

**Conclusion:** Exposure of 6-MP to both H$_2$O$_2$/HCO$_3$ significantly enhanced its oxidation, and led to protect CuZn-SOD activity. Further cellular studies are necessary to determine the effect of oxidizing 6-MP by H$_2$O$_2$/HCO$_3$ on its pharmacological activity and SOD activity.

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C1-2

**The Role of Cytochrome P450 1B1 and its Associated Mid-chain Hydroxyeicosatetraenoic Acids Metabolites in the Development of Cellular Hypertrophy- induced by Isoproterenol**

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**Purpose:** Despite the substantial progress made during the past two decades of heart research, heart disease remains the leading cause of death in North America and accounts for about 45% of all deaths. Numerous experimental studies have demonstrated the role cytochrome P450 1B1 (CYP1B1) and its associated mid-chain hydroxyeicosatetraenoic acids (mid-chain HETEs) metabolites in the pathogenesis of the heart failure and cardiac hypertrophy. However, the ability of isoproterenol (ISO) to induce cardiac hypertrophy through mid-chain HETEs has not been investigated yet. Therefore, we hypothesized that ISO induce cardiac hypertrophy through the induction of CYP1B1 and its associated mid-chain HETEs metabolites.

**Method:** To test our hypothesis, the human ventricular cardiomyocytes, RL-14 cells, were treated with ISO in the presence and absence of tetramethoxystilbene (TMS), a selective CYP1B1 inhibitor. Thereafter, the cellular hypertrophy markers, cell volume and mid-chain HETEs metabolites were determined using real-time polymerase chain reaction, phase contrast imaging and liquid chromatography-mass spectrometry, respectively.

**Results:** Our results showed that ISO induced cellular hypertrophy in RL-14 cells as evidenced by a significant induction of β-myocin heavy chain /α-myocin heavy chain (β-MHC/α-MHC) and cell volume. Interestingly, the ISO-induced cellular hypertrophy was associated with a proportional increase in the protein expression of CYP1B1 and the formation of mid-chain HETEs metabolites. The direct involvement of CYP1B1 in the ISO-induced cellular hypertrophy was confirmed by the ability of TMS and CYP1B1 siRNA to significantly inhibit the ISO-induced β-MHC/α-MHC and cell volume. Mechanistically, the protective effect of TMS against ISO-induced cellular hypertrophy was mediated through the inhibition NF-κB signaling pathway.

**Conclusion:** The present study provides the first evidence that the inhibition of CYP1B1 and hence mid-chain HETEs attenuate ISO-induced cellular hypertrophy. **Support:** This work was supported by a grant from the CIHR to A.O.S.E. Z.H.M. is the recipient Izaak Walton Killam and Alberta Innovates-Health solution Scholarships.
C1-3
Self-Assembling Elastin-Like Polypeptide Nanoparticles; Effect of Chain Length and Guest Amino Acid Hydrophobicity on Assembly and Disassembly Conditions

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Purpose: As medicine continues to enter the realm of the nanoscale, there is an increasing demand for smart, flexible and safe biomaterials for sophisticated applications. Due to their temperature-induced reversible self-assembly, nanoparticles formed from elastin-like polypeptides (ELPs) may be uniquely suited for drug delivery in conditions where temperature decreases are crucial, viz., hypothermic therapies. ELPs are oligomers of the amino acid sequence VPGXG where X cannot be proline. The work herein describes the synthesis and characterization of medium length ELPs made up of 20-160 repeats as they self-assemble into nanoparticles.

Methods: ELP constructs were made using recombinant methods then expressed in Escherichia coli and purified. The particle assembly and disassembly were characterized using dynamic light scattering (DLS), zeta potential, and scanning electron microscope. DLS studies were performed over a range of increasing and decreasing temperatures.

Results: Conditions under which each ELP construct formed particles and disassociated varied. At temperatures increasing from 5 to 25°C, a leucine-containing ELP made of 20 repeats (L20) showed a particle diameter of approximately 5nm. This increased to about 600nm from 25 to 35°C at which point the particle size decreased to 450nm. Cooling this sample showed the same changes in size, but at lower temperatures than while heating. An L40 ELP exhibited only one increase in size at 15°C while heating and minimal dissolution behaviour upon cooling. A valine-containing ELP (V40) of similar length as L40 also showed only one increase in size with heating but at 25°C. This construct also showed dissolution behaviour at 20°C.

Conclusions: These ELPs do indeed form nanoparticles and variations in their sequences result in differences in their self-assembly and disassembly temperatures. This demonstrates that ELP-based nanoparticles are a robust, customizable biomaterial which can be engineered to behave in specific ways for a wide variety of biomaterial applications.

C1-4
Biodistribution of Dronedarone in the Rat

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Purpose: To determine the biodistribution of dronedarone in Sprague Dawley rats after oral doses.

Methods: Sprague Dawley rats received 55 mg/kg of dronedarone by oral gavage. Under isoflurane anesthesia, rats (4 per time point) were euthanized at 1, 3, 6, 9, 12 and 24 h postdose. Plasma, liver and lung were collected and assayed by a published HPLC method with some modification. The tissues were first homogenized in distilled water (1:3 w/w). Internal standard (ethopropazine) was added to the homogenates, from which components were extracted using liquid-liquid extraction using hexane. A standard curve was constructed with extracted drug from each tissue, for quantification of drug concentration in each tissue. Chromatography was carried out as previously described for assay of dronedarone in rat plasma. The AUC0-24 for dronedarone was determined using the linear trapezoidal rule for dronedarone in plasma, liver and lung.

Results: The mean tissue exposures to dronedarone in rats given 55 mg/kg orally were:
The tissue to plasma ratios for lung and liver were 70 and 43 (AUC), and 94 and 68 (Cmax), respectively. The volume of distribution of dronedarone is >10 L/kg. In line with this we found much higher concentrations of drug in the tissues than were seen in plasma. These findings paralleled our previous findings of the tissue distribution of amiodarone in rats.

Conclusion: The data confirmed that there were substantial concentrations of dronedarone in two of its possible target tissues for toxicity, lung and liver. This may explain in part why dronedarone seems to have some of the same organ-specific toxicities as its forbearer antiarrhythmic, amiodarone.

C1-5
Characterization of eNOS-based Platelet Subpopulations in Transgenic eNOS-GFP Mice

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Purpose: In vitro studies of human platelets in our laboratory have demonstrated existence of two platelet subpopulations based on the presence or absence of endothelial nitric oxide (eNOS) signaling. We also showed that eNOS-negative (eNOS-ve) platelets lacking eNOS are less abundant (20% of total platelets) and initiate haemostatic reactions in response to collagen. Next, we decided to utilize eNOS-GFP transgenic mice to conduct in vivo studies to verify presence of eNOS-based platelet subpopulations and analyze their haemostatic functions. eNOS-GFP mice express functional human eNOS fused to green fluorescent protein (GFP). This fusion protein enables eNOS detection without intracellular immunostaining of platelets. We hypothesize that eNOS-based platelet subpopulations also exist in transgenic eNOS-GFP mice.

Methods: Mice (14-22 weeks old) were genotyped for GFP expression using qPCR and divided into three groups: WT, eNOS-GFP hemizygous and eNOS-GFP homozygous. Mouse whole blood was obtained by cardiac puncture. Flow cytometry was performed on platelet rich plasma (PRP) after staining with anti-mouse CD41-PerCP-Cy5.5 antibodies specific to GPIIb of platelet fibrinogen receptor. GFP mean fluorescence intensity (MFI) was measured only for CD41+ve events. Dot plots of FL3 (CD41) vs. FL1 (GFP) were created and percentage of GFP+ve events among CD41+ve events was calculated.

Results: GFP MFI for hemizygous and homozygous mice was 9.0±17.45AU (arbitrary units) and 33.8±12.76AU above background fluorescence of WT mouse platelets (N=4, P<0.0114). GFP+ve platelets (CD41+ve events) consisted of 1.41±0.79% in PRP of hemizygous mice and 2.20±0.73% in PRP of homozygous mice.

Conclusions: Preliminary experiments demonstrate presence eNOS-based platelet subpopulations in mice. However, the low amount of GFP+ve (eNOS+ve) platelets in PRP of homozygous mice suggests major species differences in platelet NO-signalling. These differences may in part explain previously reported discrepancies between human and murine platelet NO-signalling data and call into question the utility of mouse models to study platelet eNOS-signalling.
C1-6
Re-Purposing Old Drugs to Modulate Human Cytochrome P450-Mediated Arachidonic Acid Metabolism

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Purpose: Several clinically-approved drugs exhibit a long-considered unwanted effect of inhibiting cytochrome P450 (P450) activity. P450 metabolize arachidonic acid (AA) to several biologically-active epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). Re-purposing clinically-approved drugs to control EETs and HETEs levels could provide new treatment strategies. Accordingly, our aim was to determine how to significantly and selectively modulate P450-AA metabolism in human by clinically-approved drugs.

Methods: Liquid-chromatography-mass-spectrometry was used to determine the formation of 15 AA metabolites by human recombinant P450 enzymes, as well as human liver and kidney microsomes. The effects of P450 inhibition on hepatic P450-AA metabolism by clinically-approved drugs compared with investigational agents were determined. Monte Carlo simulations were performed to calculate specificity and magnitude of modulating P450-AA metabolism after safe doses given to human subjects.

Results: CYP2C19 has the highest EET-forming activity, while, CYP1B1 and CYP2C8 has the highest mid-chain HETE-forming activity. CYP1A1 and CYP4 have the highest subterminal and 20-HETE-forming activities, respectively. Resveratrol and fluconazole produce the most selective and significant modulation of hepatic P450-AA metabolism, comparable to investigational non-clinically-used agents. The IC$_{50}$ and I$_{max}$ of resveratrol inhibition are 3.5-17 µM and 54-79%, respectively, for 16-, 17- and 20-HETEs formation; whereas, the IC$_{50}$ and I$_{max}$ of fluconazole inhibition are 13-27 µM and 60-65%, respectively, for 8,9- and 14,15-EETs formation. Monte Carlo simulations show that 2.5 g daily of resveratrol would produce a decrease in 17- and 20-HETEs by 14-59% in 95% of the subjects without affecting other metabolites; whereas, 50 mg daily of fluconazole would produce a decrease in 8,9- and 14,15-EETs by 13-53%.

Conclusions: Our results show that clinically-approved drugs can provide selective and effective means to modulate P450-AA metabolism, comparable to investigational drugs. Resveratrol and fluconazole are good candidates to be re-purposed as new P450-based treatments. Support: Grant [MOP 106665] from CIHR to AOSE.

C1-7
Novel Roles of Epoxyeicosanoids in Regulating Cardiac Mitochondria

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Purpose: Mitochondria are the primary source of energy in cardiomyocytes playing a key role regulating cell survival and function. We and others have previously reported various cardioprotective effects of epoxyeicosatrienoic acids (EETs). EETs are CYP450 epoxygenase metabolites of arachidonic acid that promote pronounced yet poorly characterized cellular effects. In the present study, we investigate the effect of EETs on cardiac mitochondria during starvation induced stress.

Methods: HL-1 cardiac cells were treated with 14,15-EET (1µM) or UA8 (dual acting EET mimetic, 1µM) in serum free starvation buffer for 24 hours. The putative pan-EET receptor antagonist, 14,15-EEZE (10µM), was used as a negative control to confirm EET-mediated effects. Cell survival was assessed using Trypan Blue exclusion assay. Mitochondrial respiration was measured using a Clark electrode. Live-cell imaging was employed to assess alterations in mitochondrial morphology and membrane potential. The 3D mitochondrial morphology and network structure was reconstructed and analyzed by the FilamentTracer...
Mitochondrial biogenesis was also evaluated.

**Results:** Starvation induced clear mitochondrial elongation, which correlated with significant reduction in mitochondrial fission proteins DRP1. However, starvation inhibited mitochondrial enzymatic activity and energy production. UA8 preserved mitochondrial respiration and cellular ATP levels. Interestingly, UA-8 treated cells had preserved OPA1 oligomers, mitochondrial cristae density and increased expression the short form of OPA-1. Moreover, EET-mediated events induced SIRT1 activity and DNA-binding activity of pCREB(Ser133) and NRF1/2, suggesting improved mitochondrial biogenesis.

**Conclusions:** Together, these data suggest that EET-mediated events preserve mitochondrial structure and function during starvation stress, thus promoting cell survival independently from starvation induced mitochondrial elongation.

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**C1-8**

*Exploring Family Physicians’ Perceptions of Pharmacist Prescribing in Alberta*

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**Purpose:** In 2007, Albertan pharmacists were the first in North America to receive an enhanced range of prescribing options. They can adapt a prescription, prescribe in emergency, and initiate new therapy. Albertan pharmacists reported impact of physician relationship on their prescribing adoption and behavior.

But there is little known about Albertan physicians’ experience and relational dynamics evolving around pharmacists’ prescribing practice. Our objectives of this study are: 1) to explore the perceptions and understanding of Albertan physicians on pharmacist prescribing, 2) to provide information to physicians on the prescribing capacity of Albertan pharmacists, and 3) to translate our findings into practice by developing educational tools.

**Methods:** We are using purposive sampling method and semi-structured face to face or telephone interviews to collect data. We planned to interview 12-14 Albertan family physicians having experiences with pharmacist prescribing in their practice and also interview pharmacists working with them. Interviews are audio recorded and transcribed verbatim for analysis using basic interpretive approach to identify themes, guided by “Relational Coordination” theory. NVivo software is being used to analyze the data. Data collection and analysis are in progress.

**Results:** To date we have interviewed seven physicians and one pharmacist. Relational coordination theory is being used to guide the analysis. Initial themes include partial acceptability, trust development, being in the loop, mixed impact on the practice and patient, professional hierarchy, as well as understanding inter-professional prescribing knowledge and practice.

**Conclusion:** Relational Coordination theory is helping us to explore the changing relational and communication dynamics of physician and pharmacist from the physicians’ experience and perception of pharmacist prescribing. These finding will provide valuable insight into inter-professional communication and will be used to inform strategies to improve collaborative relationship of pharmacists and physicians in future.

**Support:** The study is funded by The Northern Alberta Academic Family Physicians (NAAFP).
C2-1
Isoniazid Induces Monocytic Differentiation via the ERK-MAPK Signaling Pathway

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Introduction: The antibacterial mode of action of INH has been proposed as an inhibitor of the cell wall synthesis; however, numbers of limitations have already been identified; which suggests INH has another additional mechanism(s) of action. From extensive pathophysiological studies, monocytes and macrophages have been identified as the main host immune defense against TB. One study revealed that isonicotinic acid (metabolite of INH) can induced surface marker expression of monocytes, CD38. However, there is no extensive study on INH-induced differentiation. Here we hypothesized that INH induces monocytic differentiation.

Methods: Human promyelocytic leukemia (HL-60) cells, which have the ability to be differentiated, were used here as model system. Nitro blue tetrazolium (NBT) reduction assay and ferriacytochrome C reduction assay were used to detect a marker of differentiation (NADPH oxidase activity). Nonspecific esterase (NSE) activity assay (specific for monocytes) and image-streaming flow cytometry against anti-CD14 and anti-CD16 were deployed to identify the type of differentiated population and subpopulations. Immunoblots against anti-phospho-ERK are proposed to be used to identify the involvement of MEKP-ERK-MAPK signaling pathway. The role of myeloperoxidase (MPO) in catalysing differentiation of INH would also be evaluated here using various MPO inhibitors.

Results: NADPH oxidase activity assays demonstrated the INH-induced differentiation (via the formation of superoxide). NSE activity assay (colorimetric and microscopic) showed that INH can induce significant monocytic differentiation as small as 10 µM of INH. Image-streaming flow cytometry revealed the subpopulations of differentiated cells; at lower concentration (below 100 µM), the subpopulation is classical monocyte (CD14+/CD16−) dominant whereas above those concentration non-classic monocytes (CD14+/CD16+) are also emerged significantly.

Conclusion: This study showed that INH can induce different types of monocytic differentiation in HL-60 cells depending on the concentration. These results may increase our understanding of the therapeutic benefit of INH to combat tuberculosis by increasing immune cell involvement.

C2-2
Modulation of Aryl Hydrocarbon Receptor Regulated Genes by Trimethylarsine Oxide in the Extrahepatic Tissues of C57BL/6 Mice

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Rationale: Arsenic is a human carcinogen that has been extensively studies over decades; however, no definitive understanding for underlying mechanisms has been established. Arsenic is capable of differentially modulating the expression of phase I and Phase II AhR-regulated genes in extrahepatic tissues. However, whether organic arsenicals have similar effects or not need to be investigated.

Experiments: C57BL/6 mice were received trimethylarsine oxide (TMAO; 13 mg/kg ip) with or without the prototypical AhR ligand, TCDD (15 μg/kg). Thereafter, extrahepatic tissues were harvested at 6 h for gene expression or 24 h for protein and catalytic activities determination.

Results: TMAO increased Cyp1a1 mRNA, protein and activity in lung; and increased Cyp1b1 mRNA and protein in lung and kidney. Upon coexposure, TMAO potentiated the TCDD-mediated induction of Cyp1a1 at mRNA, protein as well as activity levels in the lung, and kidney. TMAO potentiated the TCDD-mediated induction of Cyp1a2 at mRNA, protein and activity in the lung. As for Cyp1b1, TMAO potentiated the
TCDD-mediated induction of Cyp1b1 mRNA and protein in the kidney. TMAO induced Nqo1 mRNA in the lung, and kidney, with subsequent increase in Nqo1 protein and activity in the lung. TMAO increased Gsta1/2 protein and Gst activity in the lung and kidney. Upon coexposure, TMAO increased Nqo1 mRNA compared to TCDD in the kidney. TMAO potentiated TCDD-mediated induction of Gsta1/2 protein and Gst activity in the kidney.

**Conclusion:** Our results demonstrate for the first time that, TMAO modulates constitutive and TCDD-induced AhR-regulated genes in a tissue-, and AhR-regulated genes-specific manner. In addition, induction of Cyp1 family in extrahepatic tissues could be crucial for activation of arsenic toxicity and carcinogenicity.

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**C2-3**

**Non-viral (polymeric) Delivery of Combinational siRNAs against Cell Cycle and Phosphatase Proteins to Prevent Metastasis in Breast Cancer**

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**Purpose:** Conventional breast cancer therapies have significant limitations that warrant development of new therapies. The siRNA-mediated silencing of a unique or over-expressed cell cycle proteins could lead to better control of tumor growth. Moreover, several evidences confirmed the role of protein-tyrosine phosphatases in metastasis. We hypothesize that dual siRNA delivery against a cell cycle protein (to decrease tumor cell growth) and a phosphatase protein (to decrease cell migration) may have a drastic impact to treat metastatic breast cancer.

**Methods and Results:** We initially confirmed the feasibility of delivering siRNA against CDC20, a key protein in cell cycle regulation using a non-metastasizing MDA-MB-435 cells in vitro and in vivo. Here, we performed siRNA delivery studies using metastasizing breast cancer cell-line MDA-MB-231. To deliver CDC20 siRNA effectively to MDA-MB-231, we synthesized a library of lipid-substituted polyethylenimines (PEI), and PEI substituted with linoleic acid (PEI-LA) was found to be the most effective delivery agent based on inhibition of MDA-MB-231 cell growth. To increase the stability of siRNA/PEI-LA complexes, hyaluronic acid (HA) was used as an additive or coating on complexes. HA additive was less toxic and inhibited cell growth significantly higher compared to complexes without HA. To identify siRNAs that were effective against cell migration, we screened siRNA library against 267 phosphatases for inhibition of cell growth and migration, and siRNAs against PPP1R7, PTPN1, PTPN22, LHPP, PPP1R12A and DUPD1 decreased the migration of MDA-MB-231 cells significantly. These identified targets were then validated in vitro using individually prepared siRNAs. Combinational siRNA therapy has successfully decreased the growth as well as migration of MDA-MB-231 in vitro.

**Conclusions:** This study confirmed the importance of CDC20 and several novel phosphatase targets to reduce metastasis of breast cancer. The non-viral delivery system described here could serve as a viable platform for delivery of multiple siRNAs against critical targets.
The Importance of Lysosomal Trapping for Setting Clinically Relevant Product Specifications for Dextromethorphan Immediate Release Dosage Forms

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Purpose: Physiologically based pharmacokinetic (PBPK) in silico models are increasingly used by the pharmaceutical industry and regulatory authorities in the development of new drug products. The study used Dextromethorphan (DEM) as model drug and shows how clinically relevant product specifications can be set using this PBPK model. The PBPK model for DEM and its metabolite dextrorphan (DXO) includes the effect of lysosomal trapping in extensive (EM) and poor metabolisers (PM).

Methods: Gastroplus™ 8.5 (Simulation Plus, Inc.) Advanced Compartmental Absorption and Transit model and PBPK plus modules were used to build the dextromethorphan model for absorption, distribution, and excretion. The model was tested and validated with different pKa, Log P values reported in literature and lysosomal trapping was added to the model. Simulations were performed using a 30 mg immediate release (IR) tablet in EM and PM populations and compared with the reported values in healthy volunteers.

Results: There was a significant difference in DEM plasma profiles between EM and PM simulations. Different Log P and pKa values significantly affected the plasma profile in EM and PM. The model shows that DEM is fast absorbed into the enterocytes but the drug and it metabolite only appear slowly in the plasma due to lysosomal trapping. The PBPK model showed that the absorption of DEM was permeability controlled. Parameter sensitivity analysis showed that a particle size of appropriate range show sufficient dissolution without changing any PK parameters like t\(_{\text{max}}\), C\(_{\text{max}}\), or AUC\(_{0-24}\). Therefore, particle size control together with a disintegration test are suggested as relevant product specifications.

Conclusions: The validated in silico PBPK model was able to simulate the plasma profile of DEM and DXO in PM and EM. If the absorption of a drug is permeability controlled then particle size and disintegration can be used for product specifications to ensure clinical relevant product performance.

The Effect of Hormone Therapy on Quality of Life and Breast Cancer Risk after Risk Reducing Salpingo-oophorectomy: A Systematic Review and Meta-analysis

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Purpose: To identify, evaluate and synthesize evidence on the effect of hormone therapy (HT) on quality of life (QOL) and breast cancer risk, after risk reducing salpingo-oophorectomy (RRSO), in women who are carriers of BRCA mutations.

Methods: We searched electronic databases including MEDLINE, EMBASE, CINHAL, and others, from inception to March 21, 2014, to identify relevant studies. Studies comparing the effect of HT to placebo, non-exposed group or baseline, in women who are carriers of BRCA mutations or who have high risk of breast or ovarian cancer and who have undergone a RRSO, qualified for inclusion. Primary outcomes included QOL and breast cancer. DerSimonian-Laird random effects method was used to calculate pooled weighted mean differences (WMDs). Generic inverse variance method was used to calculate pooled odds ratio (OR).

Results: Of the 829 records identified, eight met our inclusion criteria. All studies were observational. Four studies assessed the effect on QOL. HT use was associated with improved QOL (WMD = 3.26, 95% CI = 0.96-5.56, P = 0.005). The risk of breast cancer was evaluated in 3 studies. Mean duration of follow-up was 2.6 years (range 0.1-19.1). The pooled OR showed a non-significant reduction in breast cancer risk with HT use (OR = 0.63, 95% CI = 0.28-1.40, P = 0.26).
Conclusions: While cumulative evidence from our review suggests that the short term use of HT does not negate the breast cancer-risk reducing benefit of RRSO, there are too few long term studies to draw any strong conclusions. The need for future well designed randomized controlled trials for more established evidence is imperative.

C2-6
Can HIF-1a Silencing Overcome Hypoxia Induced Conversion of Triple Negative Breast Cancer to More Tumorigenic and Drug Resistance Phenotype?

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Purpose: The long-term objective of this study is to assess the role of hypoxia inducible factor alpha 1 (HIF-1α) as a therapeutic target in triple negative breast cancer. For this purpose, we have first investigated the effect of hypoxia on the conversion of MDA-MB-231 cells to more tumorigenic and resistant phenotype. The effect of HIF-1α knock-down by small interfering RNA (siRNA) on this phenomenon was then investigated.

Methods: Parental MDA-MB-231 and its two subsets sorted based on responsiveness to a Sox2 regulatory region (SRR2) reporter were used. The cell subset responsive to SRR2 reporter (RR cells) is found to be significantly more tumorigenic than the reporter unresponsive (RU) cells. Cells were maintained under hypoxia (1-0.1% oxygen) at 37 °C. Lipofectamine complexed HIF-1α siRNA was incubated with cells and the expression of HIF-1α and its downstream proteins were analyzed by immunoblotting. Lastly, the effect of HIF-1α knock down on the conversion of RU to RR cells under hypoxic condition was assessed.

Results: Higher HIF-1α, p-Stat3, BAK and survivin expression were measured under hypoxia compared to normoxia in parental MDA-MB-231 cells and its two subsets (RR and RU cells). Successful knockdown of HIF-1α under hypoxia did not produce any significant effect on the expression of its down-stream proteins, e.g., BAK, MCL1, survivin, cleaved caspase 3, cleaved PARP, but gave rise to the expression of p-Stat3 and c-Myc. RU cells converted to RR cells under hypoxia. Unexpectedly, knockdown of HIF-1α under hypoxia increased RU to RR conversion.

Conclusions: Hypoxic condition led to the over-expression of HIF-1α, generation of more tumorigenic and resistant phenotype in MDA-MB-231 cells. Unexpectedly, HIF-1α knockdown under hypoxia resulted in increased conversion of RU cells to more tumorigenic RR phenotype. This may be attributed to the compensating effect of other transcription factors overexpressed following HIF1-α knockdown.

C2-7
FoxO3a Transcription Factor Activation by 1α,25 vitamin D3 During Osteoblast Differentiation: A Tale of Two Transcription Factors

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Purpose: It is known that active vitamin D (1α,25D₃) plays an important role in mineralization of remodeling bone via direction action on osteoblast cell populations. Conversely, inflammatory cytokines such as TNFα (and glucocorticoids such as prednisolone) induce rapid bone loss, due to increased apoptosis of osteoblasts and osteocytes. Those events are associated with progressively increased levels of Reactive Oxygen Species (ROS) capable of inducing oxidative stress in those cells, which ultimately result in decreases in both osteoblast number and bone mass. On the other hand, FoxO (Forkhead Box O) transcription factors are involved in bone cell proliferation, differentiation, apoptosis and survival from degenerative stimuli such as inflammation and oxidative stress. FoxO3a transcription factor is of special
interest due to its tissue-specific properties. We hypothesized that in the healthy osteoblast, where FoxO3a resides mostly in an inactive phosphorylated form, the administration of 1α,25D₃-vitamin D receptor complex will readily bind RXR (retinoid X receptor) response element and promote bone mineralization. Conversely, in situations of oxidative stress, ROS will enhance retention of unphosphorylated FoxO3a in the nucleus and transcriptional activation, leading to the competitive reduction in available RXR for activation of the vitamin D response element for bone formation.

**Methods:** Undifferentiated and differentiated MC3T3-E1 osteoblastic cells were treated with 10⁻⁷M 1α,25D₃ for 24 hours and subsequent FoxO3a expression was assessed with Western Blotting using specific antibodies. MC3T3-E1 cells were differentiated with the ascorbic acid and β-glycerophosphate. Cells were exposed to oxidative stress to generate ROS and FoxO3a expression was reassessed. Cell proliferation was measured by trypan blue cell counting, MTT assay and mineralization potential was determined by alkaline phosphatase activity and Alizarin Red S Staining followed by spectrophotometric quantification.

**Results:** 1α,25D₃ enhanced FoxO3a expression in MC3T3-E1 osteoblast-like cells during cell mineralization. Basal levels of FoxO3a protein expression were detected in undifferentiated MC3T3-E1 osteoblastic cells, with increased FoxO3a expression following temporal growth in cell culture. In contrast, significantly increased levels of FoxO3a protein expression were measured in differentiated MC3T3-E1 cells treated with 1α,25D₃. Conversely, oxidative stress decreased cell viability and expression of alkaline phosphatase, collagen I and osteocalcin. In the presence of oxidative stress, FoxO3a expression was decreased in the cytoplasm and increased FoxO3a expression was detected in the nucleus.

**Conclusion:** We demonstrated that 24h of treatment with 1α,25D₃ upregulated nuclear FoxO3a activity in MC3T3-E1 cells. The transcription factor FoxO3a is known to play an important role in rescuing cell damage following oxidative stress via its antioxidant actions, and as such, upregulation of FoxO3a activity may serve to reduce osteoblast damage during the course of cellular metabolism, but at the expense of new bone formation.

**C2-8**

**Text Messaging Interventions for Individuals with Mental Health Disorders Including Substance Use: A Systematic Review**

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**Purpose:** To systematically review literature assessing the impact of text messaging interventions on medication adherence or health outcomes in people with mental health disorders including substance use.

**Methods:** We searched four electronic databases (Medline, Cochrane, Embase, Psychinfo) from January 1999 to October 2015 using keyword search terms such as (text message or text messaging or short message service or texting) and (mental disorders or mental illness or substance-related disorders or addiction or drug abuse).

**Results:** Seven studies met our inclusion criteria: three studies evaluated text messaging in patients with schizophrenia or schizoaffective disorder diagnosis, two studies evaluated text messaging in patients with chronic alcohol dependence, and two studies reviewed text messaging in patients with mood disorders. Of the seven included studies, six were randomized controlled trials and one was a prospective pilot study with pre-post intervention design. Frequency of text messaging ranged from once weekly to twelve per day. Five studies measured the impact of text messaging on medication adherence; only one of these reported significant improvements in the text messaging intervention group. In most studies (5/7), the text messaging intervention was found to significantly improve patient scores on a variety of psychiatric and social functioning assessments.

**Conclusions:** This review suggests that text messaging may be a promising tool to assist in the management of patients with mental illness. To better understand and quantify the effect of text messaging interventions in this population, further research should include theory-based text message constructs in larger samples of patients.
POSTDOCTORAL FELLOWS (GROUP D)

D1
Anthracycline Derivatives: Structures and Cardiotoxicity

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Purpose: Anthracyclines are among the most commonly used chemotherapeutic agents and are effective against a broad spectrum of solid tumors and leukemias. A major limitation for clinical use of anthracyclines as antitumor agents is the dose-limiting cardiotoxicity and rapid drug resistance. In an attempt to defeat these obstacles, various anthracycline analogues have been designed and synthesized over the years, although very few have demonstrated enhanced clinical properties. We therefore studied the associations between the structure and the cardiotoxicity mechanism of clinically used anthracyclines typified by doxorubicin (DOX), daunorubicin (DNR), epirubicin (EPR) and idarubicin (IDR). Answering this question will reveal novel points of intervention to be exploited in a rational design of new anthracyclines with a distinct mode of action.

Methods: In the current study, RL-14 cell line was treated with DOX, DNR, EPR and IDR. Thereafter, cardiotoxicity parameters were determined using gene expression and cell size.

Results: DNR significantly induced cardiotoxicity as evidenced by increase in hypertrophic markers (ANP, BNP, β-myocin heavy chain/α-myocin heavy chain) as well as cell size. Importantly, EPR and IDR treated group were not significantly induced anthracyclines cardiotoxicity; IDR was the least cardiotoxic compound, suggesting that 4-OME play an important role in cardiotoxicity. Interestingly, the cardiotoxicity by DNR was associated with a dramatic change in the soluble epoxide hydrolase (sEH) suggesting a sEH-dependent mechanism. Mechanistically, the DNR-induced cardiotoxicity was mediated through the activation of MAPKs and NF-κB. Interestingly, the induction of cellular hypertrophy was associated with proportional increase in the formation of dihydroxyeicosatrienoic acids (DHETs) parallel to the increase of soluble epoxide hydrolase (sEH) enzyme activity. Blocking the induction of NF-κB, ERK1/2 and sEH signaling pathways significantly inhibited DNR-induced cellular hypertrophy.

Conclusions: Our study provides the evidence for the structure and cardiotoxicity relationship of anthracycline derivatives.

Support: This work was supported by a grant from the Canadian Institutes of Health Research [Grant 106665] to A.O.S.E.

D2
Transdermal Delivery of Bone-Targeting Peptide Hormone Conjugates for Treatment of Tibial Bone Stress Fractures Using Liposomal Cream Base

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Purpose: Stress fractures are common painful bone injuries in athletes or military personnel. The management of pain with systemic NSAIDs are associated with side effects and may interfere with cellular bone repair. Nasal or parenteral parathyroid hormone (PTH) and salmon calcitonin (sCT) trigger bone repair and/or reduce pain burden. Several limitations, such as the invasive route of administration, high dosage requirements and side effects in other tissues preclude their current application. Our hypothesis is that the transdermal delivery (TDD) of sCT or its bone-targeting conjugates will improve bone availability.

Method: For in vitro studies several dosage forms of test compounds were prepared using Diffusimax® cream base and their permeability tested using Franz static cell apparatus. For in vivo studies, radioactive iodinated peptides used in TDD dosage forms and their systemic bioavailability was assessed vs. intravenous or sub-
cutaneous applications. Post-mortem analysis of tibial bone was conducted to gauge the success of TDD and localization of compound to the bone surface.

**Results:** sCT was permeable through synthetic hydrophilic membrane, however, rat skin served as a barrier against hydrophilic peptides. The incorporation of sCT in a TDD system significantly improved its permeability through rat skin. In vivo TDD of $^{125}$I-sCT and $^{125}$I-sCT-PEG-BP resulted in 5.5 and 5.8% systemic availability, respectively. The tissue activity was higher in skin > bone > thyroid ~ liver ~ kidney for both compounds. The activity was significantly higher in treated skin and bone when compared with tissues form untreated limb of the same animal.

**Conclusions:** The results indicate that the liposomal TDD of Diffusimax is able to facilitate permeability of test compounds through synthetic membrane or rat skin. The low systemic exposure indicates that this system can be categorized as topical. This characteristic is favorable when the goal is local delivery with low systemic side effects.

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**D3**

**Comprehensive Computational Characterization for the Interaction Between PD-1 and SHP2**

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**Purpose:** The programmed death 1 (PD-1) pathway is a potent inhibitory mechanism of cytotoxic T cells. Tumors and chronic viral infections can hide from the immune system by overexpressing the PD-1 receptor and its ligands, PD-L1 and PD-L2. [1] [2] Blocking these interactions recently emerged as a ‘game changer’ approach in cancer and antiviral immunotherapy. Despite the significant therapeutic potential of targeting the PD-1 pathway, very little is known about how these proteins interact and what are the subsequent events that take place following their binding. Recent studies confirmed that following PD-1 binding to either of its ligands, PD-1 recruits the src-homology 2 domain-containing phosphatase 2 (SHP2) to its cytoplasmic ITSM domain. This interaction is responsible for delivering the downstream inhibitory signal resulting from PD1/PD-L1 interaction and ultimately inhibiting T cells activation [3].

**Methods:** Here, we build upon our recent success [4,5] and continue our efforts toward a complete atomistic model for the full PD-1 pathway. Our modelling protocol involved comprehensive protein-protein docking search, exceptionally long molecular dynamics simulations combined with binding energy calculations to explore all potential binding poses between PD1 and SHP2.

**Results:** The present study describes, for the first time, a complete homology model for the PD-1 receptor and characterizes its interaction with SHP2.

**References**

E1
Conjugation of CD20 Antibody on Polymeric Micelles for Active Drug Targeting to Haematological Cancer Cells

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**Purpose:** In recent years, accumulating evidence has suggested that the use of nano-carriers can be more effective than free drugs in delivering anti-cancer agents to their target site while avoiding drug delivery to normal organs. The efficiency of nanocarriers as a drug delivery tool can be further improved through modification of nanocarrier surface with various ligands that can interact with receptors overexpressed on cancer cells. To date, various ligands including antibodies, small organic molecules, carbohydrates, peptides and aptamers have been successfully coupled to various forms of nanocarriers. Our goal in this study was to develop monoclonal antibody (mAb) modified polymeric micelles for active drug targeting in haematological caners. The mAb against CD20, rituximab, which is commercially available and in clinical use for different tumors was used as a model mAb.

**Methods:** A malimide functionalized polyethylene glycol-poly caprolactone (PEG-PCL) block copolymer was synthesized and used for the conjugation of CD20 mAb on PEG-PCL micelles. To prepare this polymer, malimide-polyethylene glycol reacted with caprolactone in the presence of stannous octoate as catalyst. The copolymer was characterized by \textsuperscript{1}H NMR spectroscopy. Micelles were formed by solvent evaporation method and characterized for their size and polydispersity using dynamic light scattering (DLS) technique. CD20 antibody was thiolated by 2-iminothiolane. Thiolated CD20 and the micelles reacted in PBS medium. The rest of malimide functional groups were neutralized by 2-mercaptoethanol. The CD20 antibodies on micelles were reacted with NHS-Cy5.5 to measure the level of mAb conjugation and also track the mAb modified micelles for uptake in SP-53 and PTLD cancer cells.

**Results:** The NMR spectrum of Malimide-PEG-PCL showed successful preparation of the block copolymer and was used to measure the degree of polymerization of the PCL block. DLS measurement indicated formation of micelles at about 100 nm in diameter. The successful reaction of thiolated CD20 with micelles was confirmed with DLS measurement. The size of particles after reaction increased about 50 nm in diameter.

**Conclusions:** In this study, an amphiphilic copolymer (Malimide-PEG-PCL) was successfully synthesized for the first time. Our results also confirmed successful reaction of thiolated CD20 and the surface functionalized micelles. Cell uptake studies are currently underway to confirm the functionality of CD20 mAb in enhancing the uptake of polymeric micelles by SP-53 and PTLD cells that overexpress CD20.

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Development and Evaluation of an Educational Program about FASD for Pharmacy Students, Pharmacists and Healthcare Professionals

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Purpose: FASD describes a wide-range of disabilities associated with permanent brain damage caused by prenatal alcohol exposure costing $1.8 million/affected person. Pharmacists and healthcare professionals can play important roles in education and prevention of FASD. Our goal was to develop and evaluate an educational program to educate pharmacists, pharmacy students and health care professionals about FASD.

Methods: An evidenced-based FASD educational program was developed by our team (a pharmacist, physician and patient). A video modeling a pharmacist counseling patients about FASD and pamphlet support practice. Pre and post-program surveys, 43 questions in length, assessed changes in knowledge and attitudes about FASD and comfort in counseling their patients about FASD.

Results: Approximately 70% of community pharmacists and 85% of pharmacy students were interested in playing a role in prevention of FASD, but felt they needed more education. Post-program, students felt more knowledgeable about FASD, showed increased knowledge about the physical signs of FASD, FASD treatment, and increased comfort in discussing FASD with patients (p<0.001). Professionals felt more knowledgeable about FASD treatment and comfort in answering questions about FASD (p<0.001), and had a greater concern about FASD in the community (p<0.05). Both groups showed increased knowledge based on 8 questions p<0.05. Pre-program, 4% of pharmacy students felt knowledgeable about FASD increasing to 60% post-program; awareness of FASD increased from 25% to 73%. Only 27.7% correctly identified signs associated with FASD pre-program vs 71.4% post-program.

Conclusions: A unique FASD educational program was developed for pharmacy students and healthcare professionals providing increased knowledge and awareness of FASD as well as increased comfort in discussing FASD and prevention of FASD with patients. The program was presented live to multidisciplinary health care professionals and by webinar to ~2,000 national and international sites. We are now collaborating with Faculty of Pharmacy, Cedarville University, Ohio.

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Knowledge of the CANRISK Survey Score Does Not Alter Diabetes Screening Practice in a Seniors Population: A Randomized Controlled Study

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Purpose: Diabetes prevalence increases with age, with an estimated 25% of our seniors diagnosed with this chronic condition. Population estimates also suggest one in three people have undetected diabetes. If true, this can have serious implications for seniors and the healthcare system. The CANRISK survey is a simple, non-invasive way to identify people at risk of developing diabetes; however, effectiveness of this screening tool in seniors is unknown. This study investigated if healthcare practitioner knowledge of the CANRISK survey score influences diabetes screening practice.

Methods: Residents of Edmonton seniors’ care facilities were eligible for this randomized controlled trial. Participants completed the CANRISK survey and those with medium or high risk scores were included in the study. Participants were randomly allocated to have their survey score sent immediately (intervention group)
or after a 3-month delay (controls) to their family physician. The primary outcome was performance of any diabetes-related blood test during the 3-month period after survey completion. Between-group differences for the primary outcome were examined using Chi square and logistic regression analyses.

**Results:** A total of 130 seniors participated in the study, mean age 84.8 (± 6.7) years, and 98 (75%) were women. Baseline characteristics were balanced between groups except the control group had more women and a lower mean CANRISK score. Diabetes-related blood tests were conducted in 12 (18%) of 66 immediate notification group participants and 13 (20%) of 64 controls (p=0.76). After controlling for observed differences in baseline characteristics, the odds of having a diabetes-related blood test remained similar between groups (aOR 0.82; 95% CI 0.33–2.03; p=0.67).

**Conclusions:** Practitioner knowledge of CANRISK score did not alter diabetes screening practice. As use of this tool will increase administrative burden, we recommend against using the CANRISK survey to streamline diabetes risk assessment in seniors’ living facilities.