2016 Research Day
Faculty of Pharmacy and Pharmaceutical Sciences
November 24, 2016

ABSTRACTS

Categories of Abstracts:
A - Undergrad
B - Graduate students with less than 2 years' experience
C - Graduate students with greater than 2 years' experience
D - Postdoctoral Fellows
E - Other (not judged)

A1
Exploring Patients' Experiences with Asthma Care in Community Pharmacies

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Purpose: To identify how patients in Alberta manage their asthma, what resources are required, and how patients understand pharmacists' roles in asthma management.
Methods: We identified seven patients with asthma via the AHS Respiratory Strategic Clinical Network Asthma Working Group (RSCN-AWG). Semi-structured interviews were conducted with each individual participant about their experiences with medication, pharmacies, and pharmacists. The interviews were recorded and transcribed. Inclusion criteria were adults (18-70) with asthma who take one or more asthma medications who were able to consent, and spoke English. General thematic analysis was used to identify key ideas for exploration.
Results: We identified three overarching themes. First under the umbrella of every day management, patients were found to have their own individualized markers for self-monitoring, individualized non-drug measures, and their own unique medication taking patterns. Second with regards to patients' needs, patients emphasized the need for easy access to medications and their "go-to" healthcare professional. Third was the view of "traditional" pharmacist care who was impersonal, although patients who had pre-established pharmacist relationships and experienced individualized asthma care came to expect more from their community pharmacists.
Conclusions: Patients want and need empowerment and accessibility of medications from their go-to healthcare professional; someone they feel is "their" person. Community pharmacists are poised to fill this role, and although most patients had low expectations, they were generally accepting of the concept of a pharmacist managing their asthma. Future research will purposively expand this sample.
Support: n/a

A2
Antioxidant and Anti-inflammatory Properties of Vitelline Membrane Hydrolysates

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Purpose: This is the first report that evaluates the biological significance and the pharmaceutical potential of Vitelline Membrane, a multi-layer structure surrounding egg yolk, and its hydrolysates (VMH) as antioxidant and anti-inflammatory agents. Antioxidant peptides from natural protein sources can prevent oxidative damage by scavenging free radicals, chelating metal ions, and/or donating electrons. Given its

high protein content (87%), VM has a great potential to become a sustainable source of bioactive peptides and improve the environmental problems associated with the currently wasted egg byproducts.
Methods: VM was isolated from egg residues and digested by various proteases (Alcalase, Flavourzyme, and Trypsin) at enzyme:substrate ratio of 1:100 and optimum pH and temperature for 24h. Degree of hydrolysis (DH) was determined using TNBSA method and M_w distribution of hydrolysates were monitored by SDS-PAGE and MALDI-TOF. Antioxidant activities were measured by free radical scavenging, iron chelating activity, and ferric reducing assays. Cytotoxicity of the VMH was measured by MTT assay and the anti-inflammatory properties were studied by nitric oxide production by RAW264.7 macrophage cells. All samples were analyzed in triplicates and significant differences were determined by Duncan's multiple range test.
Results: Fla-treated VM showed the highest DH (27%).The SDS-PAGE indicated that all enzymes digested the main VM protein bands (100, 75, and 44 kDa) into 37, 28 and 18 kDa. MALDI-TOF spectra showed Alc- and Fla-VMH contain more peptides in the range of 500-1500 Da. The highest free radical scavenging and iron chelating activity was observed in Fla and Try-VMH, while Alc-VMH showed the highest reducing capacity. The VMHs significantly suppressed nitric oxide production by LPS-induced macrophage cells indicating the potential anti-inflammatory effect of VMH.
Conclusions: Enzymatic hydrolysis of VM produced short peptides with several bioactivities that can be used in food supplements and nutraceutical products.
Support: This work was supported by Canadian Food Innovators (CFI-009).

A3
Polymeric Nano-Micelles for Delivery of STAT3 Inhibitors to Multiple Myeloma

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Purpose: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is constitutively activated in many types of human cancer including multiple myeloma (MM), for which there is currently no cure. Despite acceptance of STAT3 inhibition as a promising strategy in cancer treatment, STAT3 inhibition has yet to be successfully translated to clinical settings, mostly due to toxicity and inefficient delivery of STAT3 inhibitors to tumours. S3I-201 and its derivative S3I-1757 are effective inhibitors of STAT3 dimerization that have shown activity against MM, but their further development to drug candidates has been hampered due to their low water solubility and poor tumour selectivity. The aim of this research was to design and develop polymeric micellar nano-formulations for delivery of these STAT3 inhibitors to MM. Polymeric micelles have shown promise in solubilisation and tumor targeted delivery of poorly water soluble drugs.
Methods: Diblock copolymers of poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL_X) or PEO-*b*-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-*b*-$PBCL_Y$) having similar degree of polymerization in PEO segment (114 ethylene oxide units) and different degrees of polymerization in PCL (X= 22, 44, 66) and PBCL (Y = 15, 20, 30) were synthesized by ring-opening polymerization. S3I-201 and S3I-1757 were encapsulated via co-solvent evaporation in the PEO-*b*-PCL or PEO-*b*-PBCL block copolymer micelles. Drug-loaded micelles were characterized for their size, encapsulation efficiency, drug release profile, and cytotoxicity against human U266 MM cell line.
Results: PEO-*b*-PCL and PEO-*b*-PBCL block copolymers were successfully synthesized as evidenced by ^{1}H NMR. Block copolymers self-associated to form micelles in aqueous solution. Successful encapsulation of S3I-201 and particularly S3I-1757 in all micellar structures under study was evidenced by HPLC (~25 and 90% encapsulation efficiency, respectively). Dynamic light scattering revealed micelle diameters of 30-70 nm. The release of S3I-1757 from micelles was slower than S3I-201 (~35% in 24 h versus ~100% in ≤ 8 hours, respectively) irrespective of polymer structure. Despite slower drug release, significantly better cytotoxicity (lower IC_{50}) was observed for S3I-1757 loaded in PEO_{114}-*b*-$PBCL_{15}$ (102 ± 7 μM) and PEO_{114}-*b*-$PBCL_{20}$ (110 ± 13 μM) than free S3I-1757 (131 ± 9 μM) against human U266 cells, *in vitro*.
Conclusion: PEO-*b*-PCL and PEO-*b*-PBCL micelles are promising nano-delivery systems for STAT3 inhibitors S3I-201 and S3I-1757 delivery against MM.

A4
Physical Function and Drugs in the Elderly – A Scoping Review

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Purpose: Over half of older adults use medications long-term to treat a chronic condition. Although side effect profiles are well known, evidence has not been synthesized regarding medications impacting physical function. This evidence gap poses a challenge for clinicians making decisions about medication use in older adults at risk of functional impairment. The purpose of this scoping review was to evaluate the literature regarding medication and function in older adults.
Methods: Databases searched were MEDINE, EMBASE, and CINAHL. Study restrictions included English language, subjects mean age >64 years, medications from top 10 drug classes used by older adults, and having a validated physical function test.
Results: We screened 11,375 titles/abstracts, with 41 articles meeting our inclusion criteria. The studies were divided into two categories, with 21 focusing on physical function, and 20 focusing on falls. For physical function, antihypertensive medications lead to motor decline. Most cardiovascular medications (statins, ACE inhibitors (ACE-I), thiazides) showed no impairment with grip strength or overall muscle strength. Findings of functional status with statins and ACE-I were mixed. Although risk of falling was increased within the first 3 weeks of initiating most cardiovascular medications (ACE-I, beta-blockers, nifedipine, candesartan, and thiazides), no risk was seen with chronic users ($\geq$ 12-month use).
Conclusion: There is limited literature available regarding how medications impact physical function in seniors. Most studies did not include functional measures as primary outcomes.
Support: This project was funded as part of the 2016 Seniors Health Strategic Clinical Network (SCN) Summer Studentship Competition.

A5
Developing Tools to Help Pharmacists Take Action with Asthma

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Purpose: to explore site specific application of Asthma Action Plans into the current workflow of community pharmacies with the support of tools and resources.
Methods: This pilot study was conducted in 9 volunteer pharmacies over 1-2 months and consisted of 3 site visits. At visit 1, the research student documented the pharmacy's workflow, resources, staff, technology; the research student also timed patient-pharmacist interactions and interviewed the pharmacist about barriers and facilitators in their practice. At visit 2, the pharmacists received education regarding the Asthma Action Plan, the "Chat, Check, Chart" Asthma Action Plan Tool, and current asthma guidelines. Pharmacists were also provided with suggestions to overcome barriers they expressed previously. Pharmacists then trialed the Asthma Action Plan with 5-10 patients over 2 weeks. Follow up by emails were sent 5-7 days after Visit 2 providing additional resources from which pharmacist may benefit. At visit 3, the pharmacists were interviewed about their experience with the Asthma Action Plan.
Results: Out of the ten pharmacists enrolled, two were not able to complete the trial, five have fully completed it, and three are still ongoing. Each pharmacist created an asthma action plan with three to five patients resulting in positive experiences for both the patient and pharmacist. Many plans were conducted alongside other services, such as care plans or prescription renewals. Pharmacists indicated that the Asthma Action Plan is feasible, but they were unable to integrate them into routine workflow due to many competing demands for time and the high cost associated with print.
Conclusions: Based on early data, due to a current lack of resources and tools in community pharmacies, implementation of the Asthma Action Plan presents challenges. However, it would be feasible for community pharmacies to implement the Asthma Action Plan if provided with the appropriate support tools and resources.

A6

Pharmacists' Perceptions and Attitudes towards Disease Screening and Prevention Roles Including HIV Point of Care Testing

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Purpose: As highly accessible professionals, pharmacists are becoming more involved in the provision of patient care with a significant role in disease prevention and health promotion. Point of care (POC) technology provides opportunities for pharmacists to expand their role in disease screening, including screening individuals for human immunodeficiency virus (HIV). There is currently limited data in regards to perceptions and attitudes of pharmacists towards performing HIV POC testing in Canada. The purpose of this study is to: (1) Explore pharmacists' current involvement in public health activities; (2) Explore pharmacists' perceptions and attitudes towards providing rapid HIV POC testing and compare this to pharmacists' perceptions of other disease screening and prevention activities; and (3) Identify barriers to implementing HIV POC testing in practice.
Methods: A cross-sectional web based survey was developed from previous literature and refined for face and content validity through expert review and pilot testing. The survey will be sent via email to pharmacists on the Alberta College of Pharmacist's clinical register who have agreed to share their contact information for research purposes. Analysis of the survey will include frequency and descriptive statistics.
Results: Expert review and pilot testing of the survey occurred in September through October 2016. The revised survey will be distributed in early November 2016 and will remain open for 3 weeks.
Conclusions: Results from this study will provide important insight regarding current involvement of pharmacists in health promotion and disease prevention activities, as well as attitudes towards taking on new roles such as HIV POC testing. Results will also help identify barriers to implementing such a service including the need for additional education and training programs.

B1-1

Differential Effect of 19,20-epoxydocosapentaenoic Acid (EDP) in H9c2 Cells Grown in High or Low Glucose Conditions

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Purpose: The importance of dietary polyunsaturated fatty acids in the reduction of cardiovascular disease has been recognized for many years. Epoxydocosapentanoic acids (EDPs) are lipid mediators produced by cytochrome P450 epoxygenase from docosahexaenoic acids (DHA). In this study, we investigated the impact of low and high glucose concentrations toward 19,20-EDP-mediated effects in both normoxic and hypoxia-reoxygenation conditions in H9c2 cells.
Methods: H9c2 cells were cultured in DMEM containing either low (5.5mM) or high (25mM) glucose concentrations supplemented with 10% FBS, 1% penicillin and streptomycin at 37°C (5% CO_2/65% N_2). Cells were treated with 0 or 1µM 19,20-EDP and subjected to 30h normoxic or 24h hypoxic and 6h reoxygenation (H/R). Cellular viability was assessed by the reduction MTT. Cell membrane integrity was determined using a fluorogenic peptide substrate (bis-AAF-R110). Proteasomal activity was measured using an assay kit based on the detection of 7-amino-4-methylcoumarin fluorescence after cleavage of the peptide LLVY-AMC. Cell lysates were assessed for caspase-3/7 activity using a profluorogenic peptide. Extracellular oxygen consumption rates (OCR) were assessed using a phosphorescent oxygen sensitive reagent.
Results: Under normoxic conditions H9c2 cells cultured in high glucose and treated with 19,20-EDP demonstrated significant loss in cell membrane integrity, decreased MTT reduction, increased proteasomal and caspase activity as well as decreased OCR and ATP. The effects caused by 19,20-EDP were worsened following H/R treatment in high glucose conditions. In contrast, H9c2 cells cultured in low glucose conditions and treated with 19,20-EDP demonstrated increased MTT reduction, OCR and ATP. Following H/R treatment, 19,20-EDP preserved cellular membrane integrity and viability as well prevented proteasomal and caspase activation while increasing ATP production.
Conclusion: Our data suggest that cells cultured in high glucose conditions, a more 'aerobic glycolytic' state, are susceptible to 19,20-EDP induced cell death. While in low glucose conditions, reflecting more oxidative phosphorylation, 19,20-EDP protected H9c2 cells.

B1-2

A Comprehensive Structural Study of the Human KCNQ1 Potassium Ion Channel

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Purpose: KCNQ1 (Kv7.1) is a voltage-gated potassium ion channel and is one of the most important cationic channels responsible for initiating the slow delayed rectifier (I_{Ks}) current in the atrial and ventricular myocytes. Mutations in KCNQ1 have been implicated in a wide range of cardiac diseases, such as long QT syndrome (LQT1/LQT5), congenital atrial fibrillation and short QT syndrome. Therefore, studying the fine structural details will enhance our understanding of different physiologically and pathophysiologic phenomena.
Methods: We constructed a homology model incorporating recent NMR data for the human KCNQ1 channel, followed by several refinement steps using tools such as ModeRefiner, FG-MD tools, and validating the model using PROCHECK. We further investigated the structural details of the voltage-sensing domain with the help of an advanced molecular dynamic (MD) technique called Replica Exchange Molecular Dynamic (REMD) simulations. This was followed by an extensive classical MD simulation for more refinement and extraction of further details.
Results: The REMD simulations were helpful in reproducing the experimentally observed structural details of the VSD. The MD simulation provided further insights into the structural variability and dynamic behavior of different components of the ion channel.
Conclusion: In this study, we are reporting a complete homology model for the open state of the KCNQ1 cardiac ion channel. The current study provides valuable insights into the structure of this ion channel and

opens the door to understanding the conduction events taking place at the molecular level. Further studies will focus on investigating the mechanisms of ion conduction and interaction of small molecules with this macromolecular complex.
Support: This project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

B1-3
Delineating Whether Epigenetic Alterations Controlling Energy Metabolism Contribute to Early-Onset Obesity in Children

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Purpose: There are over 2 million children in Canada who are either overweight or obese, and the vast majority of these children will grow up to become obese adults at increased risk for a number of obesity-associated metabolic disorders such as type 2 diabetes. As such, our goal was to determine whether offspring born to obese parents are at risk of developing early-onset obesity and obesity-related metabolic disorders, and whether this is associated with epigenetic modifications to genes controlling energy metabolism.
Methods: 8-week old C57BL/6J male and female mice were fed either a low-fat or high-fat diet for 5-6 weeks and subsequently used for breeding. All offspring were weaned at 3-weeks of age with magnetic resonance imaging utilized to assess adiposity, and glucose and insulin tolerance testing utilized to assess glucose homeostasis.
Results: Offspring born to an obese mouse were significantly heavier at weaning (3-weeks of age) than offspring born to a lean mouse, and this was associated with a trend to increased adiposity. Upon weaning onto a low-fat diet, this difference in body weight disappeared once the mice were 4-weeks of age, and body weights remained similar between offspring born to obese or lean parents until animal sacrifice at 15-weeks of age. Despite no difference in body weight with age, male offspring born to an obese male mouse appeared less insulin sensitive than male offspring born to a lean male mouse, though we did not observe a similar result in male offspring born to an obese female mouse.
Conclusions: Our findings illustrate that offspring born to an obese parent are at risk of being heavier at weaning, but this difference dissipates if the animals are fed a low-fat diet.
Support: Saudi Ministry of Higher Education, Women and Children's Health Research Institute, Molly Towell Perinatal Research Foundation.

B1-4
The Effect of COX-2-selective Etodolac on the Myocardial, Vascular, Gastrointestinal, and Renal Risks: Systematic Review and Meta-analysis

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Purpose: The objective of this systematic review is to conduct an up-to-date systematic review of COX-2-selective etodolac to assess the cardiovascular (stratified into myocardial and vascular), gastrointestinal (stratified into upper and lower tract) and renal risks.
Methods: We searched the MEDLINE and All EBM databases for observational studies or randomized controlled trials that reporting myocardial or all-cause mortality/ vascular and/or gastrointestinal (upper/lower bleeding, obstructions or perforations) or renal outcomes after etodolac use, published until July 2016. We searched for English-language articles using the following keywords: NSAIDs, etodolac, randomized controlled trial, clinical trial. Titles/abstracts of included studies were retrieved and screened independently by two reviews to identify studies that potentially relevant. A standardized, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. The combined odd ratio values (OR; 95 % CI) were calculated using the random-effect meta-analysis model.
Results: We found 105 potentially relevant studies out of 446 citations. There were 22 eligible studies. The data showed a low increase in myocardial and vascular risk among etodolac users. Gastrointestinal and

renal risks were significantly less than other NSAIDs.
Conclusions: NSAIDs have a divers cardiovascular, gastrointestinal, and renal risks. Etodolac is well tolerated in terms of gastrointestinal and renal risks compared to other NSAIDs. However, it has a trend toward more cardiovascular risk than some safer NSAIDs.

B1-5
Targeting the Oncogenic FOXM1 Transcription Factor in Cancer Treatment

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Purpose: Genome-wide gene expression profiling of human cancers has consistently identified the Forkhead box M1 (FOXM1) transcription factor as one of the most commonly activated genes in cancer cells. Also, abnormal activation of the FOXM1 gene is regarded as one of the hallmarks of chemoresistant cancer cells. Accumulating evidence suggests that targeted FOXM1 inhibition could be a promising strategy to treat many types of cancer. The aim of this project is to validate the FOXM1 transcription factor as a drug target.
Methods: We recently carried out a series of molecular modeling protocols in which we have determined the binding energies of 3,323 FDA-approved drugs within the FOXM1/DNA binding domain and we have identified six promising virtual reference drugs with significant binding energies. In the lab, the MTT assay has been carried out on MDA-MB-231 breast cancer cell line to test the ability of the drugs to inhibit cancer cell proliferation, followed by Western blotting to measure the expression of the protein.
Results: Three of the drugs gave promising primary results including: thiostrepton (control) IC_{50} = 3.788 µM, troglitazone IC_{50} = 74.24 µM, and gliquidone IC_{50} > 100 µM, as they found to inhibit the expression of FOXM1 protein.
Conclusions: We suggest that the drug troglitazone, a known anti-diabetic agent, exerts strong binding interactions in the FOXM1/DNA binding domain, making this molecule the best drug candidate to inhibit the transcriptional activity of this oncogenic protein.

B1-6
Healthcare Student Confidence and Competence in Prescribing - Creating an Interview Tool

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Purpose: The initial objective of the study was to develop and trial an interview tool to study the factors influencing confidence and competence in prescribing. This is part of a larger study investigating the confidence and competence of new graduates from pharmacy and medicine with respect to prescribing.
Methods: Literature regarding pharmacy and medical students' confidence and competence on prescribing from Medline Ovid was reviewed to search for previous interview tools. The respective authors were contacted to request additional information on previously published instruments and permission to adapt questions as needed. Based on these tools, interview questions investigated seven themes including: training in prescribing, experience with prescribing errors, factors that influence confidence in prescribing, factors that influence competence in prescribing, impact on work, impact on patients and impact on self. The interview protocol took a semi-structured format of primarily open-ended questions with a variety of prompts. Pilot interviews were conducted with two medical students and two PharmD students to determine the validity of the drafted questions ability to determine competence and confidence.
Results: Overall, the feedback received from the pilot interviews was positive. None of the interviewees felt that any additional themes needed to be addressed, and that the questions assessed prescribing effectively and were appropriate for the interview. However, the students all found it difficult to verbally make the distinction between confidence and competence. Based on these interviews, it was determined to include case studies related to prescribing to differentiate between confidence and competence.
Conclusions: The developed interview tool appeared to be valid in terms of addressing the main pre-identified areas of interest for the study. Feedback from the pilot interviews resulted in slight modifications to address concerns with a few included questions, in addition to the need of case studies to properly assess competency.

B1-7

Chicken Osteoporosis Part II: *In Vitro* and Cell Culture Study

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Purpose: Osteoporosis is defined as structural loss of bone mineralization in egg laying hens that results in fragile bones, making them more prone to fractures due to Deficiency of calcium and vitamin D, and lack of physical activity. This leads to a huge economic loss in the commercial egg industry. Osteoclasts are responsible for bone breakdown. A cytokine called receptor activator of nuclear factor kappa B ligand (RANKL) is known to play a crucial role in osteoclasts formation and its activity. Specific IgY antibodies against RANKL would be used as a novel treatment to target and neutralize osteoclasts without compromising egg production.

Methods: RAW264.7 cells (a macrophage cell line) and chicken bone marrow cells (chBMC) were used to form osteoclasts after stimulation with RANK ligand. They were grown in DMEM and AMEM culture medium respectively (containing 10% fetal bovine serum (FBS), 50 U/ml penicillin and 50 µg/mL streptomycin) and seeded (0.5×10^6/mL) into a 6-well plate. One group of RAW cells were treated with 35 ng/mL RANK ligand and the other with RANKL and anti-RANK IgY antibodies (35 ng/mL each). ChBMC were treated with chRANKL (20 ng/mL) and MCSF (20 ng/mL), and chRANKL, IgY and MCSF (20 ng/mL each). Culture media was changed (with same concentration of antigen and antibodies) every 3 days. After 6 days, cells were stained with acid phosphatase kit.

Results: Tartrate-resistant acid phosphatase (TRAP) activity being an important cytochemical marker of osteoclasts, appears as purplish to dark red in and around the cell. Some TRAP-positive multinucleated cells were observed when RAW264.7 cells were treated with mRANKL and chBMC with chRANKL and MCSF. As expected, no TRAP-positive cells appeared in the absence of RANKL or in presence of IgY.

Conclusion: We successfully demonstrated that anti-RANKL IgY antibodies block the differentiation of macrophages into osteoclasts.

Support: This work was supported by Canadian Food Innovators (CFI-009).

B2-1

The Applications of Advanced Coarse-graining Simulations in Modeling Protein-protein Interactions

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Purpose: Protein-Protein interactions (PPIs) play key roles in most biological processes and aberrant PPIs have been associated with many human diseases. Therefore, in-depth understanding of the structures and the dynamic interactions of PPIs are important in drug design and discovery. Current all-atom molecular dynamics (AA-MD) is considerably restricted to small system sizes (usually thousands of atoms) and short timescales (~100s of ns). However, to understand certain biological processes, large macromolecular complexes of millions of atoms need to be simulated for micro-seconds timescale, which is not feasible with AA-MD. To address these challenges, an alternate approach, Coarse-Graining molecular dynamics (CG-MD), was developed. In this work, we test the abilities of CG-MD to capture the large-scale conformational changes in an HIV envelope glycoprotein (gp120) and CD4 receptor complex.
Methods: Advanced CG-MD implemented in NAMD program has been employed to model the interactions of the gp120-CD4 complex (PDB: 3J70). The system is initially equilibrated using AA-MD and subsequently subjected to several nanoseconds of CG-MD simulations.
Results: At this stage, the complexes have undergone several iterations of minimization and equilibration, which led to a low-energy complex. This complex is currently undergoing rigorous production simulations using CG-MD. Preliminary results from work will be presented.
Conclusions: Our work describes that a combination of AA-MD and CG-MD could accurately model the interactions and dramatic conformational changes occurring within large macromolecular complexes.

B2-2

One-pot Synthesis of New α-Aminophosphonates under Microwave Irradiation

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Purpose: The objective of this study was to develop a method to synthesize ten new α-aminophosphonates in a one-pot three-component Kabachnik-Fields reaction, and to characterize these compounds by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy and Mass Spectrometry. Currently, α-aminophosphonates have developed much interest in Medicinal Chemistry due to their biological effects such as anticancer activity.
Methods: We tested robustness of the synthetic procedure by using a series of structurally diverse aldehydes, ethyl 4-aminobenzoate and diphenyl phosphite; we varied the reaction conditions such that all starting materials and products could be handled using "green" protocols involving ethanol as the only solvent and Microwave irradiation as the source of heat, without any catalysts.
Results: We obtained ten α-aminophosphonates following the proposed one-pot Kabachnik-Fields reaction, as anticipated without the use of a catalyst; reaction times ranged from 20-40 minutes and the yields were moderate to excellent (58-97%) for this type of reaction.
Conclusions: Microwave irradiation proved to be an excellent alternative to conventional convection heating for the synthesis of α-aminophosphonates in higher yields with shorter reaction times compared with conventional heating. The structures of the new α-aminophosphonates were designed to exert anticancer activity, based on literature, we would expect these compounds arrest cells cycle in the first stages and therefore induce apoptosis. The biological evaluation of these agents will take place in the upcoming months (Mexican colleagues).
Support: CONACYT Project 180854. Loredo Calderon, Graduate Scholarship 229877.

B2-3

Use of Electronic Medication Administration Records (eMAR) to Support Medication Administration Practices in Long-Term Care (LTC): A Scoping Review of the Quantitative and Qualitative Evidence

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Purpose: Patients who reside in long-term care can have extensive medication regimens. Electronic medication administration records may increase the safety of medication administration in this setting. Our objective was to map the extent, range and nature of research on the effectiveness, level of use, and perceptions about eMAR in LTC, identify gaps in current knowledge and priority areas for future research.
Methods: A search of MEDLINE, CINAHL, Cochrane Library, SCOPUS, Theses Global, and ProQuest Dissertations databases from 2000 to 2016 was completed with the assistance of a medical librarian. Original research relating to eMAR in LTC, nursing homes, residential aged care facilities, assisted living facilities and care homes were eligible for inclusion. Both authors completed two rounds of screening for eligibility of papers. Data regarding country of origin, major themes, design, study methods, outcomes studied, and main results were extracted.
Results: Of the 440 citations identified, 11 met inclusion criteria. An additional 5 were obtained from reference lists. Studies were published between 2007 and 2016 and were from the United States (n=11), Australia (n=3), Sweden (n=1) and the UK (n=1). Research themes explored eMAR prevalence in LTC (n=7), evaluated process outcomes (n=6) and the perceptions of the benefits and limitations of eMAR (n=3). Research designs consist of quantitative (n=10), qualitative (n=2) and mixed (n=4) methodologies which included surveys (n=10), interviews (n=6), direct observation (n=4), chart reviews (n=3) and focus groups (n=2). Main process outcomes consisted of nursing time on medication pass (n=1), design challenges (n=1), internal process barriers (n=1), medication administration error (MAE) prevention (n=1), medication order discrepancies (n=1) and eMAR workarounds (n=1).
Conclusion: There is a lack of high quality studies evaluating eMAR in LTC. Further investigations are required to evaluate the impact eMAR has on MAEs and patient safety, factors influencing uptake, and pharmacists' perceptions of eMAR.

B2-4

Melittin from *Apis Meliffera* Bee Venom Reduces Production of Inflammatory Mediators in Macrophages

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Purpose: Melittin is a major component of honey bee (*Apis meliffera*) venom that lyse cell membrane and trigger inflammatory response in the affected area. However, few reports show that melittin suppresses inflammation by preventing the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), a transcription factor that regulates expression of multiple pro-inflammatory genes. The hypothesis of this study is that melittin neutralizes inflammation at maximum tolerated dose (MTD) in macrophage stimulated by lipopolysaccharide (LPS).
Methods: Bee venom was collected using a bee venom collector at bee hives in Beaumont, AB. Melittin was separated from the bee venom by size exclusion chromatography using Sephacryl S-100 column. Purity of melittin was examined by SDS-PAGE. RAW264.7 murine macrophages were used at uniform cell seeding density (5×10^4 cells/cm^2) in *in vitro* experiments. The cytotoxicity of melittin (0.5–5 μg/mL) on macrophages was measured by MTT viability assay to determine the LD_{50} as well as the MTD. The effect of melittin on production of nitric oxide (NO), 16 cytokines including major pro-inflammatory cytokines (TNF-α and IL-1β) in LPS (1 μg/mL)-stimulated macrophages was measured by Griess assay, cytokine array, and real-time PCR (using $2^{-\Delta\Delta Ct}$ method), respectively. The student *t*-test was used to analyze the significant difference between groups.
Results: Melittin (2.8 kDa) was eluted in fraction III (2–10 kDa) of size exclusion chromatography. SDS-PAGE confirmed the purity of melittin at 2.8 kDa in the fraction III. For macrophages, the LD_{50} and MTD of melittin was 2.5 μg/mL and 1 μg/mL, respectively. Melittin (1 μg/mL) significantly reduced the NO production in LPS-stimulated macrophage from 11.44 to 4.98 μM. Moreover, melittin (1 μg/mL) reduced

LPS-stimulated production of pro-inflammatory cytokine mRNA expression by 4.12-fold and 3.01-fold for TNF-a and IL-b, respectively.
Conclusions: Melittin treatment at MTD neutralizes inflammation in macrophage. This result provides insights on the role of melittin on macrophage polarization and its potential application in treatment of inflammatory disorders.
Support: This work was supported by Canadian Food Innovators (CFI-009).

B2-5
Dose-Dependency of Cardiovascular Risks of NSAIDs

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Purpose: The non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat pain and inflammation associated with rheumatoid arthritis. However, NSAIDs also increase cardiovascular risks. We aimed, first, to identify the therapeutic dosage range of diclofenac, a known cardiotoxic NSAID, in adjuvant arthritis (AA), then, to examine whether the cardiovascular side effects of NSAIDs are diclofenac dose-dependent in AA.
Methods: Male Sprague Dawley rats were used. First, they were injected with *Mycobacterium Butyricum* in squalene and when the signs and symptoms of arthritis appeared they were treated with 0, 2.5, 5, 10 or 15 mg/kg for 7 days and monitored. Second, rats were divided into 4 groups; Healthy; AA Control, AA Treated 5 (5 mg/kg/day diclofenac) and AA Treated 15 (15 mg/kg/day diclofenac). The AA group received *Mycobacterium Butyricum* in squalene. After 12 days, rats in inflamed group developed arthritis and started receiving treatment After 7 days of dosing, rats were euthanized and their blood plasma and heart and kidney were harvested and analyzed for arachidonic acid (ArA) metabolites (reflective of heart function) were using HPLC.
Results: Doses greater than 5 mg/kg/day were effective in controlling signs and symptoms of arthritis. However, only the higher doses (15 mg/kg) caused imbalances in ArA metabolic profile toward cardiotoxicity, e.g., heart 20-HETE: Healthy 0.44±0.31; AA Control, 2.11±1.04; AA Treated 5, 1.84 ±1.20; AA-Treated 15, 8.89 ± 0.77 and heart 20-HETE/14,15-EET: Healthy 1.25±0.99; AA Control, 1.38±0.71; AA Treated 5, 2.57 ±1.56; AA-Treated 15, 6.30 ± 2.12.
Conclusion: Within the therapeutic range, only the examined high doses of diclofenac caused imbalances in ArA metabolic patterns toward cardiotoxicity. This is suggestive of dose-dependency of the cardiotoxicity of the NSAID.

B2-6
The Impact of Diet-induced Obesity on the Metabolism of Amiodarone (AM) to Desethylamiodarone (DEA)

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Purpose: To examine for functional changes in drug metabolism caused by obesity, using the AM as a test substrate.
Methods: Livers and intestines were harvested from male Sprague–Dawley rats given for 14 weeks either a,) control diet with water, b.) Normal rodent chow and high fructose corn syrup water (HFCS), c.) 45% high fat diet(HFD) chow with water, or d.) a combination of HFCS and HFD. AM was incubated with isolated microsomal protein from the tissues in incubation media (microsomal protein, NADPH, $MgCl_2$ in phosphate buffer). After 10 min HPLC was used to assay AM and DEA. DEA formation data were fitted to one and two enzyme system models to determine kinetic constants.
Results: In liver a one enzyme model fit best to the data, whereas for intestine a 2 enzyme model was needed. There was a significant reduction in V_{max} in the HFD group and increase in K_m in HFCS group compared to lean controls. In addition, a significant reduction of CL_{int} in all groups compared to controls in liver, whereas in intestine there were no significant changes in these parameters except increasing K_m in HFD-HFCS compared to all groups.

Parameter	Liver			
	Control	HFD	HFCS	HFD-HFCS
V_{max1} (pmol/mg/min)	150.07±28.74	73.13±6.10[a]	136.57±21.92	135.89±61.40
K_{m1} (μM)	59.76±21.57	44.28±15.34	118.54±12.92[b]	100.93±50.52[c]
CL_{int1} (μL/mg/min)	2.65±0.67[d]	1.78±0.52	1.15±0.15	1.50±0.50
	Intestine			
V_{max1}(pmol/mg/min)	7.74±1.86	6.53±0.61	7.89±3.73	8.22±6.77
K_{m1} (μM)	4.93±1.24	2.58±1.38	1.55±1.02	10.33±2.55[e]
CL_{int2} (μL/mg/min)	0.09±0.024	0.08±0.006	0.15±0.05	0.23±0.12

[a] Different from control, HFCS, and HFD-HFCS ($p<0.050$); [b] Different from control and HFD
[c] Different from HFD; [d] All groups were different from control; [e] Different from other groups

Conclusion: Obesity was associated with a reduction in functional activity of liver drug metabolizing enzymes. If translated to humans this finding might require adjustment of the amiodarone dose in obesity.
Acknowledgements: MA was a recipient of Libyan Government Scholarship.

B2-7
Novel Selective FOXM1 Transcriptional Program Suppressors

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Purpose: Characterizing the FOXM1/DNA binding site and interacting residues greatly aids in understanding and designing new drug moieties capable of suppressing the transcriptional activity of the oncogenic FOXM1. Accumulating evidence suggests that targeted FOXM1 inhibition may be a promising strategy to treat human malignancies, indicating that FOXM1 inhibitors may become clinically useful drugs in the near future.
Methods: We performed a series of molecular modeling and molecular dynamics simulations to determine the structural requirements needed by a drug molecule to interfere with the FOXM1/DNA binding domain. Based on our findings, we designed, synthesized and evaluated a series of compounds capable of selectively inhibiting the FOXM1 binding to the DNA.
Results: Compound 11A was able to inhibit the cell growth with GI50 of ~11.90 µM in MDA-MB-231 cell line and GI50 of ~25 µM in MCF-7 cell lines. The compound was also capable of suppressing the FOXM1/DNA interaction in EMSA (IC50: ~ 20.87 µM). Our molecular modeling study also revealed the details of interaction between the inhibitors and the FOXM1/DNA binding domain.
Support: This project was funded by Dr. Antonie Noujam strategic initiatives pilot program.

B2-8
Development of Anti-CD20 Immuno-micelles for Active Drug Targeting to Hematological Cancer

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Purpose: The aim of this study was to covalently attach the anti CD20 antibody, rituximab, to the surface of the polymeric micelles and assess the effectiveness of this approach in enhancing micellar specific interaction with target cells overexpressing CD20.
Methods: Rituximab, was coupled to Cy5.5 NHS ester at pH=8. The Cy5.5 conjugated antibody was coupled to 3-(N-succinimidyloxyglutaryl) aminopropyl, polyethyleneglycol-carbamyl

distearoylphosphatidyl-ethanolamine (NHS-PEG-DSPE). Cy3-N_3^+ was covalently conjugated to a tribockcopolymer, methoxy poly(ethylene glycol)-b-poly(ε-caprolactone)-b-poly(α-propargyl carboxylate-ε-caprolactone) (MPEG-PCL-PPCL),through click reaction. Mixed micelles were then prepared by incubating Cy5.5 labeled antibody modified PEG-DSPE with MPEG-PCL-PPCL-Cy3 at 1:1 molar ratio overnight. Size, critical micellar concentration (CMC) and kinetic stability of mixed micelles (without any cy3 or cy5.5 probes) were measured by zetasizer (DLS measurement) and compared to that for micelles from the individual polymers. The size and morphology of micelles was also investigated by TEM. Flowcytometry was used to follow the association of plain versus antibody modified mixed micelles with CD20 over expressing PTLD cells using fluorescence at 570 and 707 nm, for Cy3 and Cy5.5, respectively.

Results: The formation of mixed micelles was confirmed by DLS by detecting one peak around 65nm for mixed micelles compared to two separate peaks around 50.4 and 23.1 nm for micelles of individual polymers. Similar results were also found by TEM. The CMC of the mixed micelle was 7.8μg/mL; significantly lower than that of NHS-PEG-DSPE micelle (35.6 μg/mL), and higher than that of MPEG-PCL micelle (3.05 μg/mL). Kinetic stability of the mixed micelle was not significantly different from the MPEG-PCL micelle; however it was significantly higher than that of the NHS-PEG-DSPE micelles. Flowcytometry showed higher association of anti-CD20 micelles with PTLD cells compared to plain micelles and CD20 negative cells (SUP-M2).

Conclusion: The results points to the effectiveness of PEG-DSPE/ MPEG-PCL mixed immune micelles modified on their surface with rituximab in enhancing their association with CD20 overexpressing cells.

C1-1

Multiple siRNA Delivery against Cell Cycle and Anti-apoptosis Proteins in Breast Cancer and Normal Cells

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Background and Purpose: Conventional breast cancer therapies have significant limitations that warrant a search for alternative therapies. Short-interfering RNA (siRNA), delivered by polymeric biomaterials and capable of silencing specific genes critical for growth of cancer cells, holds great promise as an effective, and more specific therapy. Since the reports suggest the conjunctional roles of cell cycle and anti-apoptosis proteins, we hypothesize that the dual silencing of cell cycle and anti-apoptosis proteins might be exceptionally effective and specific for treatment of breast cancer.
Methods and Results: We employed amphiphilic polymers and silenced the expression of two cell cycle proteins, TTK and CDC20, and the anti-apoptosis protein survivin to determine the efficacy of polymer-mediated siRNA treatment in breast cancer cells as well as side effects in nonmalignant cells *in vitro*. We first identified effective siRNA carriers by screening a library of lipid-substituted polyethylenimines (PEI), and PEI substituted with linoleic acid (LA) emerged as the most effective carrier for selected siRNAs. Combinations of TTK/CDC20 and CDC20/Survivin siRNAs decreased the growth of MDA-MB-231 cells significantly, while only TTK/CDC20 combination inhibited MCF7 cells growth. The effects of combinational siRNA therapy was higher when complexes were formulated at lower siRNA:polymer ratio (1:2) compared to higher ratio (1:8) in nonmalignant cells such as normal breast epithelial MCF10A cells, human umbilical vein endothelial cells (HUVEC) and human bone marrow stromal cells (hBMSC). The lead polymer (1.2PEI-LA6) showed differential transfection efficiency based on the cell-type transfected.
Conclusions: We conclude that the lipid-substituted polymers could serve as a viable platform for delivery of multiple siRNAs against critical targets in breast cancer therapy. However, the siRNA therapy has showed side effects in normal cells *in vitro* and more selective therapies might be needed to target cancer cells solely.

C1-2

Mechanistically Elucidating the *In-Vitro* Safety and Efficacy of a Novel Doxorubicin Derivative

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Purpose: Doxorubicin is an effective anticancer drug, however it is cardiotoxic and has poor oral bioavailability. Quercetin is a flavonoid found in plant based food with antioxidant properties, P-glycoprotein (P-gp) and CYP3A4 inhibitory effects. To mitigate doxorubicin's therapeutic barriers, DoxQ, a novel derivative of doxorubicin, was synthesized by conjugating quercetin to doxorubicin. In this study, the *in-vitro* safety and efficacy of DoxQ are mechanistically characterized.
Methods: The drug release *in-vitro* and the cellular uptake by Multi-drug resistant canine kidney cells (MDCK-MDR) were quantified by HPLC. The antioxidant activity, CYP450 inhibition, and P-gp inhibition effects were examined using commercial assay kits. The drug potency was assessed utilizing triple negative murine breast cancer cells and the cardiotoxicity was examined in both adult rat and human

cardiomyocytes (RL-14). Dichlorofluorescein (DCF) assay was used to determine the level of reactive oxygen species (ROS) in RL-14 cells. RT-PCR was used to examine the expression of cardiotoxicity markers, oxidative stress markers, and CYP450 enzymes in RL-14 cells.
Results: DoxQ showed lower cytotoxicity to both rat and human cardiomyocytes and also lower levels of ROS and oxidative stress markers compared to doxorubicin. DoxQ inhibited both the expression and catalytic activity of CYP1B1. Additionally, DoxQ inhibited CYP3A4 and exhibited higher cellular uptake by MDCK-MDR cells than doxorubicin.
Conclusions: DoxQ demonstrates a novel therapeutic approach to attenuate cardiotoxcity and poor bioavailability of doxorubicin. The cardioprotective mechanism of DoxQ likely involves scavenging ROS and CYP1B1 inhibition. DoxQ may potentially enhance the poor oral bioavailability of doxorubicin by inhibiting CYP3A4 and P-gp.

C1-3
Pharmacological Characterization of the Functional Role of Calcium-Activated Potassium Channels in Platelets

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Purpose: In arteries, stimulation of endothelial cell small (SK_{Ca}) and intermediate (IK_{Ca}) conductance calcium-activated potassium channels provides a negative-feedback mechanism to limit agonist-induced vasoconstriction. Additionally, endothelial cell K_{Ca} channels in conjunction with nitric oxide (NO) mediate vasodilation in response to agonists and physical stimuli such as increases in blood flow. Platelets, like endothelial cells, possess K_{Ca} channels and have the capability to generate NO via endothelial nitric oxide synthase (eNOS). NO is known to limit platelet aggregation but the role of K_{Ca} channels in platelet function and NO-generation has not been explored. Our objective was to pharmacologically characterize SK_{Ca} and IK_{Ca} channel function in platelets, and to investigate their role in platelet NO production. Our hypothesis was that pharmacological activation of K_{Ca} channels would inhibit platelet aggregation and enhance platelet NO production.
Methods: Platelets were isolated from the blood of healthy volunteers and aggregometry performed in the presence of SK_{Ca} (CyPPA) and IK_{Ca} (SKA-31) channel activators. DAF-FM flow cytometry was used to measure NO generation. Dense and alpha granule secretion were measured by ATP chemiluminesence and P-selectin flow cytometry, respectively.
Results: CyPPA and SKA-31 inhibited collagen-induced aggregation in a concentration dependent manner. IK_{Ca} selective channel blocker TRAM-34 reversed the anti-aggregatory effects of 10μM SKA-31 but not CyPPA. SK_{Ca} channel-selective blocker apamin did not reverse the effect of either CyPPA or SKA-31. CyPPA and SKA-31 inhibited NO generation back to basal resting platelet levels. CyPPA and SKA-31 demonstrated similar inhibitory effects on platelet dense granule secretion, whereas only SKA-31 significantly inhibited alpha granule secretion.
Conclusions: Activation of SK_{Ca} and IK_{Ca} channels inhibits both platelet aggregation and platelet NO generation. Furthermore, the use of selective blockers suggest that IK_{Ca} is the dominant K_{Ca} channel within platelets. These data indicate that K_{Ca} channels may provide novel targets for therapeutics to inhibit platelet aggregation.

C1-4
The Interplay of HIF-1α and Myc under Hypoxia Promotes the Generation of More Tumorigenic and Resistant Phenotype of MDA-MB 231 Cells

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Purpose: To elucidate the interplay of HIF-1α and Myc under hypoxia in triple negative breast cancer (TNBC) cells.
Methods: Two subsets of MDA-MB 231 cells sorted based on differential responsiveness to a Sox2 regulatory region (SRR2) reporter were used. The cell subset responsive to SRR2 reporter (RR cells) is

significantly more tumorigenic than the reporter unresponsive (RU) cells. The effect of hypoxia (1% oxygen) on the conversion of RU to RR cells was investigated by measuring GFP expression and luciferase activity. Hypoxia induced expression of $CD44^+/CD24^-$ marker, chemoresistance to cisplatin and colony formation in RU, RR and Myc-overexpressing stable RU cells were also measured.
Results: Hypoxia induced stem-like features in both RU and RR subsets, but to different extent. A small proportion of RU cells converted to RR cells under hypoxia. This coincided with moderately higher expression of stem cell markers in hypoxic RU cells. In contrast, RR cells exhibited higher expression of stem-like features, including a higher proportion of $CD44^+/CD24^-$ cells and chemoresistance to cisplatin under normoxic and particularly hypoxic conditions. Hypoxia induced upregulation of HIF-1α, but potently suppressed c-Myc expression in both RU and RR cells, but the residual c-Myc in hypoxic RR cells was still much higher than that in hypoxic RU cells. Enforced expression of c-Myc in RU cells effectively conferred stem-like features particularly under hypoxia. Importantly, the level of stemness in hypoxic Myc-overexpressing RU cells was similar to that of RR cells.
Conclusion: Hypoxia-induced HIF-1α up-regulation leads to moderate acquisition of stem-like features in RU cells perhaps because of c-Myc suppression. RR cells on the other hand retain their higher stemness due to the higher residual c-Myc in hypoxia. Persistent expression of c-Myc is a bona fide marker of stemness in MDA-MB-231 cells, especially after hypoxia insult.

C1-5
Perspectives and Decision Making about Hormone Therapy in Women Who Had an Early Surgical Menopause: A Focus Group Study

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Purpose: To explore the process of decision-making about menopausal treatments in women who have had an early surgical menopause (≤45 years).
Methods: We used a descriptive qualitative research design. Women who had an early surgical menopause were purposefully selected from the Edmonton Menopause Clinics. Focus groups were held, each with 6 to 9 participants and lasted for 1.5 hours. All sessions were audio-recorded and transcribed verbatim. Data was analyzed using qualitative content analysis.
Results: We conducted five focus groups from Jun 30 to Jul 21, 2016 (N = 37). One-third of the women had the surgery within the last 5 years. Almost all women had a concurrent hysterectomy (97%) and were current users of hormone therapy (HT) (70%). Two main themes identified were "inadequate support" and "be my own advocate". Women felt they did not get adequate support for symptoms of surgical menopause. They shared that the experience was worse than their expectations and did not feel they were provided with adequate information to prepare them to make therapy decisions. Many felt that their doctors were not understanding of their needs with surgical menopause. Women had to "be their own advocates" and seek information and support from within the healthcare system and outside, to cope with their health issues. Some decided to go on HT to reclaim their quality of life and some decided not to as they were worried about HT adverse effects. To make an informed decision about treatments post-surgery, women expressed a need to learn more about the symptoms of surgical menopause, treatment options, resources, avenues for support and stories of similar experiences, preferably before the surgery.
Conclusion: We identified several modifiable deterrents to decision-making in early surgical menopause which can help inform the development of a patient decision aid for this context.
Support: WCHRI Seed Grant.

C1-6

2-Methoxyestradiol Protect Against Isoproterenol-induced Cellular Hypertrophy in the Human Ventricular Cardiomyocytes, RL-14 cells, through MAPK- and NF-κB-dependent Mechanisms

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Purpose: Accumulating data supported the evidence that 2-methoxyestradiol (2ME) is a biologically active metabolite and mediates multiple effects on the cardiovascular systems that are largely independent of estrogen receptor. 2ME is a major cytochrome P450 1B1 metabolite and has been reported to have a vasoprotective and anti-inflammatory actions. However, whether 2ME would inhibit isoproterenol (ISO)-induced cellular hypertrophy has not been investigated yet. Therefore, the overall objectives of the present study are to elucidate the potential anti-hypertrophic effect of 2ME in the human ventricular cardiomyocyte, RL-14 cells, and to explore the mechanism(s) involved.
Method: For this purpose, RL-14 cells were treated with 100 μM ISO in the presence and absence of 200 nM 2ME. Thereafter, the cellular hypertrophy markers and cell volume were determined using real-time polymerase chain reaction and phase contrast imaging, respectively. Phosphorylated mitogen activated protein kinases (MAPK) levels and nuclear factor kappa B (NF-κB) binding activity were determined using a commercially available kits.
Results: Our results showed that 2ME significantly inhibited the ISO-induced cellular hypertrophy in RL-14 cells as evidenced by a decrease in the induction of β-myocin heavy chain /α-myocin heavy chain and cell volume. Mechanistically, the protective effect of 2ME against ISO-induced cellular hypertrophy was mediated through a significant inhibition of the phosphorylated p38 and -Jun NH2-terminal kinases (JNK)-induced by ISO, whereas it normalizes the ISO-mediated inhibition of extracellular-regulated kinases1/2 (ERK1/2) signaling pathway. Furthermore, 2ME inhibited the ISO-induced NF-κB binding activity.
Conclusion: our study provides the first evidence that 2ME attenuate ISO-induced cellular hypertrophy through MAPK- and NF-κB-dependent mechanisms.
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.

C1-7

Forkhead Box O 3 Transcription Factor (FoxO3a) is a Potentially Important Regulator in Osteoblast Differentiation and Bone Mineralization

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Purpose: Forkhead Box O transcription factors play important roles in bone metabolism via their reported ability to defend against oxidative stress and apoptosis[1,2]. FoxO3a is of special interest as it is the predominant isoform in bone cells[2]. In osteoblasts, the administration of $1\alpha,25D_3$ leads to increased FoxO3a expression levels, and altered calcium handling. We therefore queried whether FoxO3a is also necessary for vitamin D-mediated bone metabolism and whether this effect is affected by ROS.
Methods: Pre-osteoblast MC3T3-E1 cells undergo differentiation in the presence of ascorbic acid and β-glycerophosphate to become functional osteoblasts. Differentiated MC3T3-E1 cells were treated with 10^{-7}M active vitamin D ($1\alpha,25D_3$) for 24 hours, 250μM hydrogen peroxide (H_2O_2) for an hour or $1\alpha,25D_3$ and H_2O_2 together. mRNA and protein expression of FoxO3a, VDR, RXR and calcium mediators were assessed by quantitative real-time PCR and immunoblotting, respectively. Calcium uptake was measured by ratiometric live cell imaging using Fura-2 AM in differentiated osteoblast cells.
Results: An increase in FoxO3a expression was observed after the first day of differentiation. At day 3 and 7, FoxO3a levels were significantly higher than predifferentiation. $1\alpha,25D_3$ further enhanced both FoxO3a mRNA and protein expression levels in the 7-day differentiated osteoblast cells. Immunocytofluorescence localization of FoxO3a demonstrated the addition of $1\alpha,25D_3$ led to nuclear localization. 7-day differentiated osteoblast cells incubated with H_2O_2 showed no obvious difference from the control, however, increased FoxO3a expression was attenuated when H_2O_2 was added with $1\alpha,25D_3$ in the combination treatment group. In order to study the direct effect of $1\alpha,25D_3$ on calcium regulation in

osteoblasts, expression levels of calcium mediators were assessed. Increased expression of the calcium channel Cav3.1 and plasma membrane Ca^{2+} ATPase (PMCA-1b) was observed during osteoblast differentiation. Addition of $1\alpha,25D_3$ enhanced expression of calbindin-D9k and the sodium-calcium exchanger (NCX). Ratiometric live cell calcium imaging demonstrated $1\alpha,25D_3$ increased calcium uptake and lanthanum chloride inhibited uptake in differentiated osteoblasts.

Conclusion: FoxO3a mRNA and protein expression levels increase during differentiation, suggesting FoxO3a plays a regulatory role in osteoblast differentiation. $1\alpha,25D_3$ further enhanced FoxO3a expression in differentiated MC3T3-E1 and stimulates calcium uptake, consistent with the involvement of FoxO3a in osteoblast mineralization. Whether FoxO3a is required for this or the changes in expression of calcium mediators in osteoblasts will be determined by over-expressing and knocking down FoxO3a and repeating these studies.

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C2-1
Compatibility Effects of Herb Composition in Reducing Uric Acid Level in Hyperuricemic Mice

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Purpose: Rather than monotherapy, Traditional Chinese Medicine (TCM) uses multi-herb formulas to exert therapeutic action and modulate pharmacological effects. Erding Formula (EF) is a TCM preparation listed in the Chinese Pharmacopeia (ChP) and generally used for heat clearing and detoxifying. In the study, the hypouricemic effects of the aqueous extracts of EF and corresponding individual herbs were evaluated in order to find the characteristics of herbal compatibility.
Method: EF are composed of whole plant of Viola yedoensis Makino (Viola), Taraxacum mongolicum Hand.-Mazz. (Taraxacum), Lobelia chinensis Lour. (Lobelia) and root of Isatis indigotica Fort. (Isatidis). The formula and each plant were extracted following ChP and intragastrically administered to mice for 5 consecutive days. The hyperuricemic animal model was induced by potassium oxonate 1h before the last dose. Blood and liver samples were collected 1h after last drug administration. Serum uric acid (SUA) and xanthine oxidase (XOD) activity were determined. Eight groups of male Kunming mice were used (n=10): untreated group, untreated disease group, drug positive control group, EF group, Viola group, Taraxacum group, Isatidis group and Lobelia goup.
Result: The research illustrated that only EF and Viola significantly reduced SUA as compared with untreated disease group. The EF worked better on reducing SUA, especially compared with Taraxacum, Isatidis and Lobelia. Among these groups, only EF and Isatidis showed inhibition on XOD activity in the liver when compared with untreated disease group.
Conclusion: Combinations of two or more herbs can be more effective in reducing uric acid level than using single herb extracts only.
Support: Scientific Grant of Jiangxi Province, China (No. 20144BDH80005 and 20151BBB70268).

C2-2 Modeling the Human XPF Nuclease Domain for Structure-Based Drug Design of Nucleotide Excision Repair and Interstrand Crosslink Repair Inhibitors

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Purpose: The XPF-ERCC1 endonuclease is crucial for the nucleotide excision repair (NER) and interstrand crosslink repair pathways. The inhibition of these mechanisms represents a promising approach to overcome drug resistance in cancer therapy.
Methods: In order to rationally design novel inhibitors of the endonuclease action, we have built a high-quality model for the XPF nuclease pre-catalytic domain. We performed an extensive sequence similarity search across structures available in the Protein Data Bank database to build a high-quality homology model that was furtherly refined through molecular dynamics simulations. An iterative clustering approach was applied to the trajectory to identify the prevalent conformations of the active site. Molecular docking of a representative set of known inhibitors was performed to determine the modes of binding.
Results: The crystal structure of the nuclease domain of the Hef protein from Pyrococcus furiosus was identified as the best suitable template and was used to build the homology. An ensemble of dominant conformations of the active site was extracted from the simulation. The docking results revealed the key interactions dominating the binding of these molecules to the XPF active site.
Conclusions: This work describes in detail the characteristics of ligand binding to the active site of the human XPF nuclease domain. The results constitute an important starting point for the rational drug design of novel specific inhibitors of chemotherapy-induced lesions of DNA in cancer cells.
Support: This work is supported by an Alberta Cancer Foundation grant for the DNA Repair Consortium project.

C2-3
Multimodal Sensing Device Detects Bacteria and Suggests Apt Antibiotics

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In the fight against drug-resistant bacteria, accurate and high-throughput detection methods are needed to combat infections worldwide.
The purpose of this study is to develop a method that can identify bacteria and measure their susceptibility to antibiotics in very short time. This will help fighting pathogenic bacteria and prevent spreading drug-resistant strains.
Methods in brief: a bimaterial microfluidic cantilever (made from silicon nitride and gold nano layer) was coated internally with anti*listerial* monoclonal antibodies and *Listeria*-specific antimicrobial peptides. The attachment of the ligands inside the levers was made physically and chemically using specific ligand attaching molecules. As artificially contaminated samples flow through the microfluidic cells, the ligands capture the targeted stains and allow the non-targeted ones to escape. The device then sends three different signals according to the captured cells, changes in the resonance frequency (mass), changes in the cantilever deflection (adsorption stress) and a nanomechanical infrared spectra correlate to the adsorbed bacteria.
Results: We were able to see the in-situ detection and discrimination of *Listeria monocytogenes* at a concentration of single cell per μL. In addition, trapped *E.coli* in the microchannel expressed a discrete nanomechanical reaction when exposed to different antibiotics in very short period.
In conclusion, this multimodal approach, which combines enrichment with three different modes of read-out, can serve as a platform for the development of a portable, high-throughput device for detection of bacteria and their response to antibiotics.

C2-4
Biodistribution, Effectiveness and Cardiac Toxicity of Traceable Polymeric Micellar Diclofenac in Inflammation

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Purpose: A fluorescently-tagged polymeric micellar formulation of diclofenac (DF), a model of cardiovascular (CV) toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs), that encapsulates DF ethyl ester (DFEE) was developed and used to test the hypothesis, 'reduced heart exposure to NSAIDs improves CV safety profile'. The pharmacokinetics and biodistribution profile of micelles and released DF in healthy, and the efficacy and cardiotoxicity in adjuvant arthritic rats was then evaluated.
Methods: DFEE was encapsulated in traceable polymeric-micelles (DFEE-P) based on methoxypoly(ethylene oxide)-block-poly(ε-caprolactone)(PEO_{5000}-*b*-PCL_{3000}). Single-dose biodistribution of DF was studied in healthy rats administered DFEE-P or free DF intravenously. Bioavailability, efficacy and cardiotoxicity was assessed in rats with induced adjuvant arthritis (AA) following administration of DFEE-P or DF intraperitoneally (DF equivalent 10 mg/kg/day) for 7 days.
Results: In the healthy rats, the 24 h DF concentration was significantly greater in blood following iv administration of DFEE-P as compared with DF (2.1±0.6 μg/ml vs below detection), but, significantly lower in other tissues (heart, 0.8±0.2 vs 1.4±0.2 μg/g; kidneys, 1.2±1.2 vs 4.5±1.7 μg/g; liver, 1.3±0.6 vs 2.5±0.7 μg/g; spleen, 2.8±0.4 vs 5.0±1.7 μg/g). Near-infrared florescence images showed micellar carrier tissue accumulations in-line with those achieved for DF using HPLC. The intraperitoneal DFEE-P was completely absorbed and was shown to be as effective against arthritis as free DF. Furthermore, in AA rats, DFEE-P yielded significantly lower cardiotoxic metabolic profile of arachidonic acid (ArA) in various tissues when compared to free DF (e.g., 20 HETE in heart 0.20±0.01 vs 0.27±0.02 μg/g; in plasma, 42±7 vs 91±7 μg/L).
Conclusions: DF delivery by PEO_{5000}-*b*-PCL_{3000} micelles has high intraperitoneal bioavailability and provides improved biodistribution of DF including prolonged systemic circulation and reduced

accumulation in the cardiac tissue. Moreover, the micelles reduce the extent of imbalance in eicosanoids of ArA attributed to DF and known to be associated with increased CV risks.

C2-5

Transcriptional and Post-Translational Regulation of CYP1A1 by Monomethylarsonous Acid in Human Hepatoma HEPG2 Cells

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Purpose: Arsenic is a human toxicant and carcinogen that has been extensively studied over decades; however, no definitive understanding for underlying mechanisms has been established. Arsenic is capable of modulating the expression of aryl hydrocarbon receptor (AhR)-regulated genes, nevertheless, whether its trivalent organic metabolites have similar effects or not need to be investigated. Therefore, in this study we examined the effects of monomethylarsonous acid (MMA(III)) as compared to its parent compound sodium arsenite (As(III)) on the expression of CYP1A1 in HepG2 cells.
Methods: HepG2 cells were treated with MMA(III) (5 μM) or its parents compound As(III) (5 μM) in the absence and presence of the prototypical AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 1nM). Experiments were conducted at 6 h for gene expression; 24 h for XRE-driven luciferase activity, protein expression, and EROD activity.
Results: Our results showed that both MMA(III) and As(III) decreased CYP1A1 mRNA, protein, and catalytic activity levels; and inhibit the TCDD-mediated induction of CYP1A1 mRNA, protein, and catalytic activity levels. MMA(III) and As(III) significantly inhibited XRE-driven luciferase activity and it inhibited the TCDD-mediated induction of XRE-driven luciferase reporter gene expression. Although MMA(III) and As(III) were not shown to be AhR ligands, both compounds showed inhibition of nuclear accumulation of AhR transcription factor as evidenced by immunocytochemical analysis. MMA(III) and As(III) had no effect on CYP1A1 mRNA stability; however MMA(III), but not As(III), decreased the protein stability of CYP1A1. As(III), but not MMA(III), induced HO-1 mRNA levels. Both MMA(III) and As(III) increased ROS production.
Conclusions: Our results demonstrate for the first time that, MMA(III) down-regulates CYP1A1 mainly through transcriptional and post-translational mechanisms. This modulation of CYP1A1 proves that trivalent metabolites of arsenic are highly reactive and could participate in arsenic toxicity.
Support: NSERC Discovery Grant RGPIN 250139 to A.O.S. O.H.E. is the recipient of ACF and AIHS Graduate Studentships.

C2-6

Effect of Hyperlipidemia on Dronedarone Microsomal Metabolism in the Rat

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Purpose: Dronedarone is a derivative of amiodarone that is used for cardiac arrhythmias. It is lipophilic which might allow it to bind to lipoproteins. The objective of this study was to explore the effect of hyperlipidemia (HL) on the metabolism of dronedarone as a substrate.
Methods: Sprague Dawley rats were given poloxamer 407 (P407) to induce HL. Liver and intestine were harvested. The microsomal proteins were isolated and exposed to dronedarone. The incubation mixture consisted of different concentrations of dronedarone (microsomal protein, NADPH, $MgCl_2$ in phosphate buffer). Incubations were performed over the linear range for the dronedarone metabolite (desbutyldronedarone, DBD) formation (10 min at 37°C). HPLC to assay DBD. Formation data were fitted to equations for one and two enzyme systems to determine kinetic constants for DBD formation.
Results: Mean±SD from 6 rats/group:

	Liver		
	V_{max1} *, pmol/mg protein/min*	K_{m1}*, μM*	CL_{int1}*, μL/min/ mg protein*
Control	210.8±3.4	6.7±2.5	33.6±9.6
HL	359.7±85.2†	567.6±182.4†	0.65±0.1†
	Intestine		
	V_{max1} *, pmol/mg protein/min*	K_{m1}*, μM*	CL_{int2}*, μL/min/ mg protein*
Control	20±12.10	14.40±9.31	0.96±0.15
HL	4.5±1.47	3.55±0.66	0.37±0.01*

In liver microsomes formation data fit best to a one enzyme system. Moreover, control microsomes had the highest formation rate coupled with evidence of DBD self-inhibition/inactivation. Significant differences ($p<0.05$) were present in V_{max}, K_m and CL_{int} values in liver in HL relative to Controls. In intestine, a 2 enzyme system best fit the data (one saturable, the other nonsaturable). A significant difference in CL_{int2} was seen for HL compared to control. The formation rate of DBD was reduced in HL compared to controls.
Conclusions: HL caused a decrease in the functional activity for DBD formation by hepatic and intestinal drug metabolizing enzymes.
Acknowledgements: YBJ was a recipient of a Saudi Arabian Government Scholarship.

C3-1
Teens with Celiac Disease: Difficulties Today but Hope for Tomorrow

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Purpose: Celiac disease (CD) is a gluten-triggered autoimmune disorder of the small intestine, which can occur in genetically susceptible individuals at any age. The inflammatory process associated with CD leads to malabsorption of many macro and micro-nutrients (especially iron, vitamin D, vitamin K, and calcium) and can lead to a wide spectrum of both gastrointestinal and extra-intestinal symptoms, osteoporosis, infertility, and even malignancy. The only current approved treatment for CD is a gluten free diet (GFD). Unfortunately, total avoidance of gluten is extremely difficult. Teens with CD are especially at risk for minimizing the seriousness of their disease and subsequent carelessness with the diet, as they strive for peer acceptance and personal autonomy in the context of managing a chronic disease. In fact, adherence to the diet has been reported as low as 30% among teens, the worst of all age groups. Clearly, additional treatment options are needed. Promising results from a pilot study using AGY, an oral anti-gliadin antibody with high binding affinity to gluten, have led to preparation for a larger study.
Methods: A phase II, double blind, placebo controlled, crossover study is planned. Eligible participants will be age 10+, have medically confirmed CD for at least 1 year, be following a GFD but still have symptoms consistent with gluten exposure. Half of the 120 participants will be under age 18.
Results: The trial is expected to being in January 2017, with results in spring 2018.
Conclusions: Positive outcomes could ultimately lead to product approval for AGY, thereby offering an oral, non-toxic, simple-to-use, treatment option that could dramatically improve quality of life and health outcomes for those with CD, with the greatest potential impact on the teenage population.

C3-2
Identifying Simultaneous Matrix Metalloproteinase/Soluble Epoxide Hydrolase Inhibitors

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Purpose: Matrix metalloproteinase (MMP) and soluble epoxide hydrolase (sEH) are completely unrelated enzymes. Nevertheless, their inhibitors are sharing closely similar chemical structure, as well as, similar effects on biological systems. Therefore, the current study aimed to identify a simultaneous inhibitor for MMP and sEH that is expected to have a synergistic therapeutic effect on cardiovascular diseases.
Methods: Six compounds were identified as potential chemical leads for simultaneous MMP/sEH inhibitors, and were tested for their capacity to inhibit MMP and sEH. Inhibition of MMP and sEH activity were measured by liquid chromatography/mass spectrometry, spectrophotometry and zymography, using their endogenous and exogenous substrates. Structure-activity relationship was studied for the most promising compound, CTK8G1143, by synthesis of a series of urea-thiadiazole derivatives.
Results: Two compounds, CTK8G1143 and ONO-4817, were identified to inhibit both MMP and sEH activity. CTK8G1143 and ONO-4817 inhibited the recombinant human sEH activity by an average of 67.4% and 55.2%, respectively. The IC_{50} for CTK8G1143 and ONO-4817 to inhibit recombinant human sEH were 5.2 and 3.5 μM, respectively, whereas, their maximal inhibition values were 71.4% and 42.8%, respectively. Also, MMP and sEH activity of human cardiomyocytes were simultaneously inhibited by CTK8G1143 and ONO-4817. Regarding other compounds, they showed either MMP or sEH inhibitory activity but not both.
Conclusion: a simultaneous inhibitor for MMP and sEH could provide a promising intervention for the prevention and control of several diseases, especially cardiovascular diseases.
Support: This work was supported by CIHR grant to AOSE; AAE and RB are recipients of AIHS studentship.

C3-3

Thermo-reversible Gels Based on Triblock Copolymers of PEG and Functionalized Caprolactone: The Effect of Polymer Polydispersity on their Gelation Behaviour

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Purpose: The long term objective of this study is to develop biodegradable stimuli-responsive gels with optimal properties for depot and smart drug delivery. In this study, we first developed an optimum method of solution polymerization for the preparation of block copolymers based on poly(ethylene glycol) (PEG) and functionlaized poly(caprolactone) (PCL). We then investigated the effect of polymer polydispersity on the gelation of triblock copolymers prepared by bulk versus solution polymerization.
Methods: Triblock copolymers of poly(α-carboxyl-co-benzyl carboxylate-ε-caprolactone)-*b*-PEG-*b*-poly(α-carboxyl-co-benzyl carboxylate-ε- caprolactone) (PCBCL-PEG-PCBCL) were prepared through ring opening polymerization of α-benzyl- ε-caprolacton (BCL) and PEG (1450 kDa) by either bulk polymerization or an optimized solution polymerization method using biphenyl as solvent; followed by hydrogenation of block copolymer. Prepared block copolymers were characterized for their molecular weight, polydispersity index (PDI) and branching by ^{1}H NMR and gel permeation chromatography (GPC). The sol-gel transition of polymer solutions in water was measured using inverse flow method, differential scanning calorimetry (DSC)and rheometrical analysis.
Results: Optimum condition for the synthesis of triblock copolymers of maximum yield and desired degree of polymerization for the PCBCL block (~14) was achieved when 30%wt of biphenyl was used in the reaction. Copolymers with thermo-responsive behavior at 15 % w/w, have shown higher polydispersity and branching; irrespective of the applied method of polymerization. For these block copolymers at 15 % w/w, the sol-gel transition temperature was 35 °C as measured by inverse flow method, DSC and rheometrical analysis.
Conclusion: The present study illustrates successful use of solution polymerization for the preparation of triblock copolymers based on PEG and functionalized PCL. We also demonstrated that the PDI of block copolymers can affect their sol-gel transition. Tri block copolymers with a broader molecular weight distribution, showed better sol-gel transition.

C3-4

Characterizing Pharmacist Prescribers in Alberta using Cluster Analysis

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Purpose: Pharmacists are practicing different types of prescribing in Canada since 2007. Alberta was the first jurisdiction in Canada, to authorize pharmacist prescribing. Legislative approval of prescribing authority does not assure its adoption in practice instantaneously. Understanding the mode of adoption and facilitating the adoption process is correspondingly significant to translate the law into practice. Therefore, our objectives of this study were to i) characterize Albertan pharmacists by clustering them into different groups according to their prescribing practice, ii) to examine the predominance of these groups in different practice settings and iii) to compare the experience of support from the practice environment among these groups of pharmacists.
Methods: A cross sectional survey was tested for validity and reliability in three stages and administered among random 700 practicing registered pharmacists in Alberta in 2013 to explore adoption of pharmacist prescribing. We ran descriptive analysis to measure the participants' demographic information. We used cluster analysis, to characterize and group the participants based on their prescribing behavior. We used chi-square and ANOVA to compare the groups of pharmacists according to their practice setting and experience of support from practice setting.
Results: We found three types of pharmacist prescriber groups- "Renewal focused prescriber", "Comprehensive adopter", and "Disease focused prescriber" who prescribed by employment of different level of clinical knowledge and liabilities. Renewal focused prescribers and disease focused prescribers were predominant in community and hospital/consultancy setting respectively. Increased level of support from practice environment facilitated comprehensive adoption of prescribing.

Conclusion: Strategies to support adoption of pharmacist prescribing should be explicit in different practice settings for different prescriber groups. Future study can measure shifting of adoption level in these prescriber groups with time in Alberta.

C3-5
A Gadolinium-Doxorubicin Nanoparticle Complex as a Novel Drug-Delivery System

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Purpose: Anthracycline moieties, such as doxorubicin, can form complexes with transition metals. Gadolinium as one of those transition metals has been well investigated as a promising contrast agent for clinical magnetic resonance imaging (MRI). The objectives of this study were to establish a reliable method to fabricate doxorubicin loaded gadolinium nanoparticles and examine the feasibility of the synthesized nanoparticles to deliver doxorubicin to human breast cancer MDA-MB-231 cells and to assess their anticancer efficacy.
Methods: Doxorubicin loaded gadolinium nanoparticles were fabricated by a simple one-step homogeneous precipitation method. Synthesized nanoparticles were further characterized by means of transmission and scanning electron microscope, dynamic light scattering method as well. Confocal fluorescence microscope was used to visualize the cellular uptake of the nanoparticles by MBA-MD-231 cells after 24 hours' incubation. APC-Annexin V was used as fluorescence dye. Flow-cytometry experiments were carried out to evaluate the percentage of apoptotic cells after 3-day incubation.
Results: The synthesized doxorubicin loaded nanoparticles possess desirable morphology (sphere), diameters (average size about 150nm) and zeta potential (+13.8 mV). A high doxorubicin loading (10.1%, w/w) was achieved. Confocal micrographs show that the nanoparticles were actively taken up via endocytosis by MDA-MB-231 human breast cancer cells. A dose-dependent trend in the percentage of apoptotic cells of nanoparticles treated groups was detected by flow-cytometry assay.
Conclusion: A novel doxorubicin loaded spherical gadolinium nanoparticle system was developed with good cellular uptake profile and promising therapeutic efficacy. Gadolinium based nanoparticles and chelates were reported as T1 contrast agent for clinical MRI, which means the doxorubicin gadolinium nanoparticles have a great potential as a multifunctional platform as theranostic medicine.

C3-6
Genetic Deletion 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. Using mice with a genetic knockdown of soluble epoxide hydrolase (sEH), the enzyme responsible for EET metabolism, our study investigates the preservation of mitochondrial protection in aged animals compared to young counterparts following surgical occlusion of left anterior descending artery (LAD).
Methods: Age-matched sEH null and littermate wild-type (WT) mice averaging 15-18 months old (aged) and 2-4 month old (young) were subjected to permanent LAD ligation. 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. Infarct size was determined through tetrazolium chloride (TTC) assay. Caspase-3, 20S proteasome, aconitase and mitochondrial ETC enzymatic

activities were ascertained using established protocols. Finally, mitochondrial respiration was assessed using a Clark electrode in permeabilized cardiac fibers to obtain respiratory control ratios.
Results: Markers of cell injury, mitochondrial efficiency and overall cardiac function were preserved in aged sEH null mice, although less robustly than in their young counterparts. While aged animals of both genotypes demonstrated a similar overall age-related decline, sEH deletion consistently demonstrated protection from myocardial ischemic injury regardless of age.
Conclusion: Our data demonstrates that aged mice with the whole body deletion of sEH had preserved primary mitochondrial function with increased efficiency of oxidative phosphorylation, which was associated with favourable outcomes following post-MI injury.

D1

Ranolazine Treatment Improves Glycemia and Decreases Body Weight in Obese and Insulin Resistant Mice

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Purpose: Type 2 diabetes (T2D) is a global health problem with high prevalence that is associated with increased cardiovascular (CV) morbidity and mortality. Thus, manufacturers developing new therapies for T2D must now demonstrate CV safety for their agents. On the contrary, it is often not considered that T2D patients often receive co-therapies for their CV disease, and we have limited knowledge on whether these drugs impact glucose control in these individuals. Of interest, ranolazine is an approved antianginal agent that is associated with reductions in glycemia in patients with T2D. Our aim was to further elucidate whether ranolazine improves glycemia in experimental obesity and to identify the associated mechanism(s).
Methods: 10-week old C57BL/6J male mice were fed either a low-fat or high-fat diet for 10 weeks and then treated for 4 weeks with ranolazine (50 mg/kg via daily subcutaneous injection) while remaining on their respective diets. Glycemic control was monitored in mice via glucose and insulin tolerance testing, whereas *in vivo* metabolism was assessed via indirect calorimetry.
Results: Treatment with ranolazine reduced body weight and improved glucose tolerance in obese mice, though we observed no effect on insulin tolerance. The observed improvement in glycemia does not appear to be dependent on body weight reduction, as lean mice treated with ranolazine also showed improved glucose tolerance without changes in body weight. In addition, the ranolazine-mediated reduction in body weight was independent of changes in food intake, but was associated with increases in energy expenditure.
Conclusions: Our data demonstrate that treatment with ranolazine improves glucose homeostasis and reduces body weight in obese mice, suggesting that ranolazine might be an ideal therapy for T2D patients with high CV risk.

D2

Synthesis, Separation, Characterization and Biological Activity of Single Entity Bone-Seeking Parathyroid Hormone-Polyethylene Glycol-Bisphosphonate Conjugates

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Purpose: We have developed and synthesized bone-seeking bisphosphonate (BP) conjugates of Parathyroid Hormone (PTH 1-34), to treat bone conditions such as Osteoporosis. In preclinical rat models of osteoporosis, we have shown that unlike native PTH, our bone-seeking PTH-polyethylene glycol (PEG)-BP conjugates will immediately target to and coat bone mineral after drug administration and significantly build new bone mass. However, the 2 step synthetic strategy employed to synthesize of PTH-PEG-BP results in a mixture of several isomers that requires further separation and characterization in order to determine the most active pharmacological entity in triggering the osteoblast cell PTH receptors.
Method: Using monodispersed PEG and activated Thiol-BP, we synthesized multi-substituted PTH-PEG-BP conjugates. The mixture of different *mono*-, *di*- and *tri*-substituted PTH-PEG-BP were separated using HPLC method and after trypsin digestion, the site of conjugation was determined using MALDI-ToF. The activity of different isomers were tested by cAMP release using the UMR-101 cell line.
Results: PTH-PEG-BP analogues were successfully synthesized and characterized. Theoretically, four primary amines (N-terminus of Ser^{1}, Lys^{13}, Lys^{26}, and Lys^{27}) of PTH can be substituted. *Mono*- and *di*-substituted isomers were the major products. These isomers have shown PTH receptor bioactivity, with *mono*-substituted isomer at primary amine of Lys^{13} most active compared to the others.
Conclusion: Our approach was successful on synthesis of bone-targeting anabolic bone therapeutics. The increased in vitro biological activity of the single *mono*-substituted PTH-PEG-BP isomer, in comparison to commercially marketed PTH, indicates the enormous potential for bone drug delivery to potentially increase efficacy and reduce side effects.
Support: This work was supported by funding from Alberta Innovates – Technology Futures (AITF).

D3

A Computational Analysis on the Mode of Binding of hERG Blockers

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Purpose: Drug-induced blockade of hERG remains a major impediment in delivering safe drugs to the market. Thus, identifying the potential hERG blockers at early stages of lead discovery is fast evolving as a standard in drug design and development. Structure-based *in silico* models of hERG have been developed as a low-cost solution to evaluate drugs for hERG liability. It is now widely agreed that the hERG blockers bind at the large central cavity of the channel. However, the exact binding mode of drugs in the central cavity of the hERG channel is still unknown.
Methods: In this work, we employed a combination of advanced molecular modelling, molecular dynamics and binding free energy methods to identify the most likely binding mode for a panel of known hERG blockers.
Results: Our computational analyses identified some key residues that play important roles in stabilizing the drug-protein interactions and the energetically favourable binding modes for the selected drugs.
Conclusions: The results show that ligands binding in a perpendicular orientation tend to block the channel more efficiently. Further research in this direction should be useful for developing a predictive computational model for hERG liability.
Support: This work has been funded by the Li Ka Shing Applied Virology Institute and the Natural Sciences and Engineering Research Council of Canada (NSERC).

D4

Rational Design and Validation of Small Molecular Inhibitors for the PD-1/PD-L1 Immune-Checkpoint Pathway

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Purpose: Blocking the PD-1/PD-L1 pathway recently emerged as a 'game changing' in cancer immunotherapy and monoclonal-antibodies (MABs) targeting PD-1 has been selected as 'drug of the year' for 2013 [1]. Although these antibodies restored exhausted T cells' function to recognize and kill tumor cells, these MABs have numerous disadvantages. This includes their very high cost and very severe side effects [2]. Our team has been focused on designing small molecule inhibitors for this pathway. Compared to available MAB therapies, our small molecule can offer a more affordable, more easily administered, more easily controlled treatment that could treat a variety of cancers including advanced solid tumors and brain tumors.
Methods: We used state-of-the-art computational modelling techniques to model the interaction between PD-1 and its ligands. This was followed by identifying binding sites that can block their interaction and rationally designing small molecules that can complement these sites. Suggested compounds were synthesized and tested in various biochemical and immunological assays to confirm their activity and specificity to the target the PD-1 pathway.
Results and Conclusions: Here, we summarize our efforts toward this goal and summarize preliminary data on compound #HEKA111, a small molecule inhibitor for the PD-1/PD-L1 pathway that binds to PD-1 and restores the proliferative capacity of exhausted T cell.

Support: The program is generously funded by a $5.4M grant from the Alberta Cancer Foundation and the Li Ka Shing Applied Virology Institute at the University of Alberta.

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D5

Modelling the Interactions of CD28:B7-1 And CTLA-4:B7-1 Complexes: Towards Understanding the Control of Immune Responses against Cancers

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Purpose: Activation of T-lymphocytes (or T-cells) plays a central role in anti-cancer immune responses, which controls tumor initiation and progression. CD28 and CTLA-4 are transmembrane receptors that are responsible for the regulation of the second signal that is crucial for T cell activation. CD28 and CTLA-4 share ~30% sequence identity, and both receptors bind to the same set of ligands, B7-1 and B7-2, from the antigen-presenting cells. Nevertheless, the outcomes of their interactions to their B7-1/2 ligands are contradictory. While the CD28:B7 interaction delivers a co-stimulatory signal to T cells, the interaction between CTLA-4 and B7 ligands trigger an inhibitory signal to T cells. The reasons for such contrasting functional properties of CD28 and CTLA-4, despite their structural similarities and common binding partners, are poorly understood. Furthermore, there is little information about how these receptors interact with the same set of ligands.
Methods: In this work, we performed advanced molecular modeling and extensive molecular dynamics (MD) simulations to characterize the interactions of the CD28 and CTLA-4 receptors to their B7 ligands at the atomic-level. Ensemble-based protein-protein docking and MD-based binding free energy calculations were performed to build the first comprehensive model of CD-28: B7-1 complex.
Results: Our simulations reveal the similarities and differences in the interactions between the two complexes that control the activity of T-cells.
Conclusions: Our results identify the key residues and interactions that cause CTLA-4 bind more strongly to B7-1 when compared to CD-28, as revealed by the earlier experiments.

D6

Characterization of Antioxidant Peptides Released from Casein by Combination of High Hydrostatic Pressure with Enzymatic Hydrolysis

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Purpose: Milk proteins are considered as highly nutritious food component with well-balanced essential amino acid composition and also reported as a good source of bioactive components. This study aimed to determine the effect of an innovative approach, which combines high hydrostatic pressure with enzymatic hydrolysis (HHP-EH) on casein and to investigate the antioxidant and anti-inflammatory properties of casein-derived peptides.
Methods: Bovine casein was hydrolysed using alcalase, elastase, flavourzyme, savinase, thermolysin, and trypsin. Optimum conditions for casein hydrolysis were selected as 100 MPa for 1 hr with 1:50 enzyme:substrate ratio based on the degree of hydrolysis (DH) and M_w distribution of casein hydrolysates. The casein hydrolysates were then evaluated for their antioxidant (DPPH and superoxide scavenging activities), metal chelating and reducing properties (FRAP, iron chelating activity), nitric oxide suppression and effect on cell viability. The hydrolysate with most potent antioxidant properties was subjected to LC-MS/MS analysis to identify the sequences of antioxidant peptides. Data were subjected to ANOVA using statistical analysis software.
Results: Casein hydrolysates produced under HHP-EH had significantly higher DH and higher proportion of smaller peptides compared to atmospheric pressure (AP). Flavourzyme-digested casein at 100 MPa

showed the highest DH, DPPH radical scavenging activity and FRAP values compared to other enzymatic hydrolysates and was significantly higher compared to AP hydrolysates Casein hydrolysates were cyto-compatible and did not adversely influence the macrophage cell growth. Nitric oxide production in macrophage cells was significantly suppressed by HHP-hydrolysates. Peptide characterization by LC/MS-MS revealed that about 59% of the sequence of effective peptides was composed of proline, valine and leucine, which possibly contributed to their antioxidant properties.
Conclusions: These results suggest that compared to AP hydrolysis, HHP-EH can produce a higher yield of shorter peptides with improved bioactivities from casein. HHP-EH could provide a promising new technology to produce easily digestible hydrolysates with bioactive properties from food proteins to be used as functional ingredient in nutraceutical supplements.
Support: This work was supported by Canadian Food Innovators (CFI-009).

D7

Fox01-Mediated Regulation of Pyruvate Dehydrogenase and Glucose Oxidation in Diabetic Cardiomyopathy

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Purpose: Cardiovascular disease represents the main cause of death in type 2 diabetes (T2D) patients. This includes diabetic cardiomyopathy (DC), of which there are no approved therapies. Forkhead Box O1 (FoxO1) activity is enhanced in T2D and has been shown to increase expression of PDH kinase 4 (*Pdhk4*), which phosphorylates and inhibits PDH activity. Because the role of FoxO1 on glucose oxidation impairment during DC has not yet been assessed, our aim is to determine whether FoxO1 controls *Pdhk4* transcription and glucose oxidation in the heart.
Methods: Differentiated C2C12 and H9c2 myotubes/myocytes were treated with FoxO1 inhibitor and/or activator, and *Pdhk1/2/4* mRNA, protein expression and PDH phosphorylation were evaluated. In addition, C57BL/6J mice were either fasted or followed by refeeding period, and extracted tissues were evaluated for above mentioned mRNA and proteins. To examine the role of FoxO1 in regulation of the *Pdhk4* promoter, luciferase assays were performed.
Results: FoxO1 inhibition in C2C12 myotubes significantly decreased *Pdhk4* mRNA and PDHK4 protein expression, which correlated with a significant decrease in PDH phosphorylation. Likewise, similar observations were seen in H9c2 myocytes. Dexamethasone induced *Pdhk4* mRNA in both C2C12 myotubes and H9c2 myocytes, which was markedly attenuated with FoxO1-inhibitor, and these findings translated to the appropriate changes in PDHK4 expression and PDH phosphorylation. As fasting is known to activate FoxO1, 16 hrs fast followed by a 4 hr refeed of mice leads to a significant decrease in *Pdhk4* mRNA expression in muscle compared to mice fasted for 20 hrs, which further corroborated by a decrease in PDH phosphorylation. Furthermore, luciferase activity assays showed significant upregulation of *Pdhk4* promoter activity by FoxO1-ADA (active) and downregulation by FoxO1-D256 (dominant-negative) when compared to FoxO1WT.
Conclusions: Our results suggest that FoxO1 controls *Pdhk4* transcription in the heart, and that it may regulate PDH activity and glucose oxidation.

D8

Antioxidant Potential of CORM-2 in Mouse MODE-K Intestinal Epithelial Cells under Oxidative Stress

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Purpose: Mitochondrial complexes (I and II) and NADPH oxidase (NOX) are the major sources of reactive oxygen species (ROS) production contributing to tumor necrosis factor (TNF)-α/cycloheximide (CHX)-

induced apoptosis in mouse MODE-K intestinal epithelial cells (IECs). Carbon monoxide (CO)-releasing molecules (CO-RMs) are novel molecules developed to liberate controlled amounts of CO in biological systems, to therapeutically benefit from the antioxidant and cytoprotective effects of CO. These effects of CO may be linked to its binding to oxidative hemoproteins such as mitochondrial complexes and NOX. At the mitochondrial level, CO can induce a transient burst of superoxide anion ($O_2^{\bullet-}$) production thought to promote a preconditioning state, allowing to counteract subsequent oxidative stress. As the water-soluble CORM-A1 reduced apoptosis and NOX-derived ROS by TNF-α/CHX in MODE-K IECs without inducing mitochondrial $O_2^{\bullet-}$ *per se* or reducing TNF-α/CHX-induced mitochondrial $O_2^{\bullet-}$, the influence of the lipid-soluble CORM-2 was now studied. TNF-α/CHX was compared with hydrogen peroxide (H_2O_2)-, rotenone- and antimycin-A-induced ROS generating systems to investigate whether the lipid solubility of CORM-2 allows it to influence mitochondrial ROS signaling.

Methods: Intracellular total ROS and mitochondrial $O_2^{\bullet-}$ production levels together with cell death were assessed by flow cytometry. Additionally, the influence on TNF-α/CHX-induced changes in mitochondrial membrane potential (Ψ_m) and mitochondrial function was studied.

Results: CORM-2 (40 μM) increased mitochondrial $O_2^{\bullet-}$ production at 2 h after its incubation. CORM-2 abolished TNF-α/CHX-induced total ROS production with partial reduction of cell death. CORM-2 reduced H_2O_2-induced total ROS production. CORM-2 decreased TNF-α/CHX-, rotenone- and antimycin-A-induced mitochondrial $O_2^{\bullet-}$ levels. CORM-2 reduced TNF-α/CHX-induced mitochondrial depolarization and mitochondrial dysfunction.

Conclusions: The lipid solubility of CORM-2 might thus allow its interference with mitochondrial ROS signaling induced by TNF-α/CHX, at least in mouse IECs. CORM-2 could be of therapeutic benefit for cytoprotection of IECs during gastrointestinal disorders involving oxidative stress.

E1

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

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Purpose: LPS is a bacterial wall endotoxin producing many pathophysiological conditions including myocardial inflammation leading to cardiotoxicity. Endotoxemia or sepsis, is a systemic inflammatory response to infection and a major cause of death worldwide. Because specific therapies to treat sepsis are limited, and underlying pathogenesis is unclear, current medical care remains purely supportive. Although an important mediator of endotoxemia is thought to be mitochondrial dysfunction, the underlying molecular mechanisms are remain uncharted. Epoxyeicosatrienoic acids (EETs) are biologically active metabolites of arachidonic acids capable of activating protective cellular pathways in response to stress stimuli. EETs evoke a plethora of pathways limiting impairments of cellular structures, reducing cell death and possess have anti-inflammatory properties in various cell types. Degradation of endogenous EETs mostly occurs through enzyme referred as soluble epoxygenase (sEH). In already published studies, inhibition of sEH has been proved to be beneficial against various physiological stress factors. Furthermore, in our previous studies we showed that pharmacological inhibition of sEH robustly enhanced functional survival of cardiac cells exposed to LPS. However, the whether or not systemic deficiency of sEH protects myocardium in the model of LPS-induced injury remains unknown.
Methods and Results: Using clinically relevant animal models, we examined the role of sEH deficiency in protection of cardiac function in animals exposed to LPS. sEH deficiency robustly reduced detrimental effects of LPS-induced endotoxemia in mice by reducing the levels of overall and cardiac-specific abnormalities. Furthermore, sEH deficiency appeared to robustly preserve mitochondrial function in myocardium, which we propose provides protection against LPS-induced endotoxemia.
Conclusions: Our findings demonstrate a critical role for mitochondria in the pathogenesis of endotoxemia that involves a previously unrecognized role of sEH deficiency in protection against LPS-induced injury.

E2

Expression of Ebola Glycoprotein and Characterization of Monoclonal and Polyclonal Antibodies for Ebola Detection

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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 glycoprotein (GP) to determine if the viral protein is present in suspected individuals and also the extent of infection. GP is the viral envelope 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 GP in Baculovirus expression system. This GP has been used for immunizing chicken and obtaining high affinity IgY antibodies from egg yolk. We have cultured 3 different anti GP 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 GP was expressed in insect species. The purified protein was recognized in Western blot by MAb obtained from GP hybridomas. The recombinant protein was used to immunize chicken for the development of IgY antibodies at every two weeks. 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 GP antigen with very high yield. Anti-GP 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 GP and all the 3 different MAbs as well as IgY. Western blot also validated specific binding.
Conclusion: As a preliminary study, we have successfully produced GP, 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 GP antigen could potentially be used for developing efficient Ebola diagnostics. The Ebola GP 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.

E3

Myeloperoxidase Mediated Bio-activation of NSAIDs in HL-60 cells: A Potential Target Killing Approach in Leukemia Cells

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Purpose: Several lines of evidence pointed at the potential benefit of NSADs in cancer therapy. This study probes the bio-activation of some NSAIDs via Myeloperoxidase (MPO), an overexpressed peroxidase in acute myeloid leukemia (AML) cells. We included three NSAIDs, namely diclofenac, indomethacin and naproxen in comparison with their hepatic metabolites, 4'- hydroxydiclofenac (4'-OHD), 5-hydroxydiclofenac (5- OHD), O-desmethyl- N- deschlorobenzoylindomethacin (DMBI), O-desmethylindomethacin (DMI) and O-desmethylnaproxen (ODN).
Methods: We used a combined biochemical-*in vitro* approach. We used purified peroxidases in kinetic UV-visible spectroscopy and electron spin resonance (ESR) experiments. Then, we used HL-60 leukemia cell line to carry out trypan blue exclusion, mitochondrial membrane potential ($\Delta\Psi_m$) (MMP) and cytofluoremetric glutathione (GSH) assays.
Results: Our results present evidence that diclofenac, 4'-OHD, 5-OHD, DMBI and DMI are oxidized by intracellular MPO showing a significant cytotoxic effect in the leukemic cells. Only diclofenac and its two metabolites caused significant drop in the mitochondrial membrane potential; however the cell death induced by indomethacin metabolites, they did not significantly affect MMP or GSH content. Only diclofenac and 4'-OHD (and not 5- OHD) caused a significant drop in HL-60 cells' GSH content. Among diclofenac family, only 4'-OHD also generated $GS^{\cdot}$ radical and caused a significant increase in ascorbate oxidation rate. Even though ODN also generated $GS^{\cdot}$ radical and cooxidize ascorbate significantly, it showed no significant *in vitro* reactivity.
Conclusion: These results provide the first evidence of the pro-cytotoxic effects of these NSAIDs and their hydroxylated counterparts in AML leukemia cells, and thus their potential therapeutic benefit in treatment of leukemia.

E4

Development of a Liquid Chromatography-Mass Spectrometry (LC/MS) Assay Method for the Quantification of Dronedarone in Rat Plasma

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Purpose: To develop an LC-MS assay for the determination of dronedarone (DR), a Class III antiarrhythmic drug and analogue of amiodarone.
Methods: After adding DR and internal standard (IS, ethopropazine), analytes were extracted from 100 µL rat plasma using liquid-liquid extraction in hexane. The organic layer was transferred into clean tubes and dried *in vacuo*. The dried residue was reconstituted in 150 µL of methanol and up to 50 µL was injected. An isocratic separation was performed on a reverse phase C18 column (Alltima HP, 250 × 2.1 mm) at 25°C using 70% methanol in water with 1 mM ammonium formate (pH=4.5) as mobile phase. Detection was

accomplished by mass spectrometer (Waters Micromass ZQ 4000 spectrometer) in positive-ionization mode using selected ion monitoring: m/z= 557.4 for DR and m/z = 313.2 for IS.
Results: An excellent linear relationship was present between peak height ratios and rat plasma concentrations of DR ranging from 5 to 1000 ng/mL (r^2>0.999). The components eluted at 8 and min for IS and DR, respectively. The intraday and interday coefficients of variation (CV%) were equal or less than 11%, and mean error was <10%. The validated limit of quantification of the assay was 5 ng/mL based on 0.1 mL rat plasma. This method was capable of measuring the plasma concentrations of DR in rat after oral single dose of 55 mg/kg.
Conclusions: This method was found to be fast, sensitive, accurate, specific and applicable for the quantification of DR pharmacokinetics studies. It represented a 5-fold increase in sensitivity compared to our previously published method.
Acknowledgements: YBJ was a recipient of a Saudi Arabian Government Scholarship. AAE is a recipient of AIHS studentship.

E5
Age-Related Alterations in the Level and Expression Pattern of Von Willebrand Factor (VWF)

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Purpose: This study aimed to compare the expression of the endothelial marker CD31, micro vessels marker (Iso lectin IB4), and the procoagulant protein von Willebrand factor (VWF) in Brain, Liver, Heart, Kidney and Lung of young and aged mice tissues, VWF protein levels in different mice tissues, and VWF level in mice and rats Blood.
Methods: This study compared circulating VWF levels in the blood of young and aged mice and rats, using Elisa. Additionally, RT-PCR analyses, Western blot analyses, as well as Immunofluorescent confocal microscopy were used to determine mRNA levels, protein levels, and the expression pattern of the VWF protein, combined with the endothelial marker CD31 and micro vessels marker (Isolectin-GS-IB4), in the livers, brains, lungs, hearts, and kidneys of young and aged mice. Moreover, CD41 a marker of aggregated platelets has been used for Immunofluorescent confocal microscopy.
Results: With age VWF expression at mRNA levels were significantly increased in brains, lungs, and livers, but not in kidney and heart of aged mice compared to young. Also circulating VWF protein levels increased in blood of aged mice and rats compared to young. Moreover, the endothelial staining intensity of VWF increased in micro and macro vessels of brains, lungs, and livers of aged compared to young mice.
Conclusions: With aging, VWF levels are increased in circulation. Furthermore, an altered VWF expression pattern, specifically increased expression in microvasculature of brain, lung, and liver, is observed. Overexpression of VWF in circulation and microvascular vessels of distinct organs as a result of aging may contribute to vascular diseases such as thrombosis.