Centennial Research Day

Book of Abstracts

March 7, 2014

Research Day 2014 Committee:
Mo Jamali (Chair), Waheed Asghar, Jenny Carbon, Lisa Guirguis Kamaljit
Kaur, Liam Rourke, Lori Shockey
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<td>8:00 - 9:00</td>
<td>Poster set-up and viewing</td>
<td>Katz Atrium</td>
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<td><strong>Session I</strong></td>
<td>ECHA Rm 2-430</td>
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<td>9:00 - 9:10</td>
<td>Opening remarks / Introduction (Mo Jamali)</td>
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<td>9:10 - 10:10</td>
<td><strong>Keynote speaker:</strong>&lt;br&gt;Greg Eberhart, Edmonton, AB&lt;br&gt;<em>Informing Public Policy through Practice Research</em></td>
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<td>10:10 - 10:30</td>
<td>Coffee</td>
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<td>10:30 - 12:00</td>
<td>Poster Session</td>
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<td>12:05 - 1:50</td>
<td>Lunch / Poster viewing&lt;br&gt;12:05 - Undergraduate student poster judging</td>
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<td><strong>Session II</strong></td>
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<td>1:50 - 2:20</td>
<td>Moderated poster presentations (podium), Part 1</td>
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<td>1:50 - 2:00 Graduate Students, Junior (JA)&lt;br&gt;2:00 - 2:10 Graduate Students, Junior (JB)&lt;br&gt;2:10 - 2:20 Graduate Students, Senior (SA)</td>
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<td>2:20 - 2:30</td>
<td>Coffee Break</td>
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<td>2:30 - 3:15</td>
<td><strong>Speakers:</strong>&lt;br&gt;Fakhreddin Jamali, John Seubert, Nese Yuksel&lt;br&gt;<em>Research in the Faculty of Pharmacy and Pharmaceutical Sciences: Past/Present/Future</em></td>
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<td>3:15 - 3:45</td>
<td>Moderated poster presentations (podium), Part 2</td>
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<td>3:15 - 3:25 Graduate Students, Senior (SB)&lt;br&gt;3:25 - 3:35 Graduate Students, Senior (SC)&lt;br&gt;3:35 - 3:45 Postdoctoral Fellow</td>
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<td>4:00 - 4:15</td>
<td>Award announcements /&lt;br&gt;Closing remarks: Dr. James P. Kehrer, Dean</td>
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Keynote Speaker

Greg Eberhart

Greg Eberhart is the Registrar of the Alberta College of Pharmacists (ACP). He has provided leadership nationally through the Canadian Pharmacists Association (CPhA), the National Association of Pharmacy Regulatory Authorities and the Council of Pharmacy Registrars of Canada. In 2008/09 he participated on CPhA’s “Blueprint Steering Committee” that developed a blueprint for the future of pharmacy in Canada.

Greg led the development of provincial legislation (approved 2007), enabling pharmacists to prescribe and administer drugs by injection. He was a member of the Alberta Pharmacists Association’s “Pharmacy Practice Models Initiative” advisory committee, a demonstration project in 2009/10 focused on innovative reimbursement methods for pharmacists practicing in new practice models.

Greg was a member of the “Minister’s Advisory Committee on Health” in 2010, mandated to lead the development of new umbrella health legislation and is a member of the provincial Health Information Executive Committee. He is a member of the Primary Health Care Strategy Working Group and co-chair of the Cultural Change Expert Advisory Group. In 2008, Greg was presented with a University of Alberta Alumni Honour Award in “recognition of significant contributions made over a number of years to his local community and beyond”. In 2011, he was amongst 103 Albertans awarded the Alberta Pharmacy Centennial Award for Distinction for “having advanced pharmacy in Alberta to the leadership position it holds today”.

Informing Public Policy through Practice Research

In 2007, provincial legislation was amended to enable pharmacists to prescribe and administer drugs by injection. The Alberta prescribing model is the broadest in North America, and parallels authority granted pharmacists in Great Britain through the National Health Service. Since 2007, there has been significant focus on uptake and capacity building; however, there is only anecdotal insight about the impact of this policy decision on Alberta’s health system and the health of Albertans. As pharmacists become increasingly engaged in making prescribing decisions, research is required to better understand how these decisions impact patient care (quality), patient access (accessibility), and Alberta’s health system (sustainability). Additionally, practice-based research is important to facilitating change in pharmacist practices, as the introduction of new human resources (i.e. pharmacy technicians) and technologies (i.e. clinical, information and robotics) necessitate changes in the way pharmacy is practiced, to ensure viability of the profession.
Poster Presentation Instructions

Poster presentation, part I

- Presentation to judges will proceed as follows:
- Groups of up to 8 posters will each be judged by two Faculty members.
- Judging will commence at the time given in the itinerary for the respective poster category. At this time, both judges and ALL students from the respective poster group will meet at poster #1 in the group.
- The first presenter will present his/her poster in a max. of 5 minutes. Presenters may be cut off by the judges after 5 min.
- The poster may then be discussed by the whole poster group. All – i.e. both the judges and the presenters in the group – are invited to ask the presenter questions regarding their poster.
- The whole group (students and judges) will then proceed to the next poster to be discussed.

Poster presentation, part II

- The poster selected by the judges as the best poster of the group will be presented on the podium in the afternoon (see itinerary)
- The podium presentation will be for a maximum of 5 minutes.
- A maximum of ONE powerpoint slide will be allowed for this short presentation. A free presentation without slide will also be possible.
- Judges in the auditorium will select the best overall presentation from these 5 minute talks. (Prizes for the Undergraduate group will be selected only from the poster presentations)
## Poster Groups and Judges

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<tr>
<th>Junior Graduate Students (A)</th>
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<tr>
<td>Poster #</td>
<td>Presenter (Supervisor)</td>
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<tr>
<td>JA1</td>
<td>Yogita Raghuwanshi (Kaur)</td>
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<tr>
<td>JA2</td>
<td>Igor Zlobine (Seubert)</td>
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<td>JA3</td>
<td>Ali Abdussalam (Brocks)</td>
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<td>JA4</td>
<td>Chowdhury F. Faruquee (Guirguis)</td>
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<td>JA5</td>
<td>Samya Elkhatali (El-Kadi)</td>
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<td>Mengjie Yan (Jurasz)</td>
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<tr>
<td>SA1</td>
<td>Alexandra Rodriguez Dimitrescu (Velazquez)</td>
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<td>SA2</td>
<td>Abdullah Alshememry (Unsworth)</td>
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<td>SA3</td>
<td>Abdulrahman Y. Asiri (El-Kadi)</td>
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<td>SA4</td>
<td>Raniah Q. Gabr (Brocks)</td>
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<td>Arash Panahifar (Doschak)</td>
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<td>Waheed Asghar (Jamali)</td>
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<td>SA7</td>
<td>Ali Agahzadeh-Habashi (Jamali)</td>
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POSTER ABSTRACTS
JUNIOR GRADUATE STUDENTS (A)

JA1
NOVEL PEPTIDES FOR TARGETED DRUG DELIVERY TO BREAST CANCER CELLS

Yogita Raghuwanshi, Howe-Ming Yu, Kamaljit Kaur
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: The introduction of various biological ligands like peptides into drug delivery systems has provided the opportunity for the selective delivery of drugs to tumor cells. Peptides possess many advantages such as small size, ease of synthesis, modification, tumor penetrating ability, and good biocompatibility but they are quite unstable and prone to proteolytic degradation, which can be prevented by chemical modifications such as incorporation of D-amino acids or cyclization of the peptide sequence. Kaur group reported earlier that Dox-peptide (WxEAAYQkFL or 18-4a) conjugates specifically target tumor and demonstrate enhanced cytotoxicity against drug resistant cells compared to free doxorubicin (Soudy R., et al J. Med. Chem. 2013). In this study, three analogues of the lead decapeptide 18-4a were designed to further enhance binding affinity to breast cancer cells and stability toward proteases. Five peptides, namely, peptide 18-4a, two linear analogues (18-4b, 18-4c), one cyclic analogue (18-4d), and a control α-peptide (18-4) were synthesized and evaluated. The design strategy involved replacement of either norleucine (x) or lysine (k) amino acid in the lead sequence with corresponding D residues or cyclization of the sequence.

Methods: Peptides were synthesized using Fmoc solid phase peptide synthesis (SPPS) and were purified and characterized using HPLC and mass spectrometry. Peptide stability in human serum and liver homogenate was monitored using RP-HPLC. The peptides were labeled with Fluorescein isothiocyanate (FITC) and the tumor targeting ability was studied using breast cancer cell lines (MDA-MB-435, MDA-MB-231 and MCF-7), while non-cancerous cell lines (HUVEC and MCF-10A) were used as control. A control α-peptide was only used to verify the enzymatic activity of mouse liver homogenate and human serum.

Results: Amongst the three new synthesised analogues, analogues 18-4c and 18-4d both with D-lysine displayed significant increase in cell uptake and specificity for breast cancer cells while maintaining the proteolytic stability. Peptide analog 18-4c (WXEAAYQkFL) showed higher cellular uptake than the parent peptide 18-4a. Interestingly, when the 18-4c linear analog containing D isomer of lysine (k) was cyclized to give 18-4d (cyclicWXEAAYQkFL), it further increased the cellular uptake by the cancer cells while showed significantly lower uptake by the noncancerous control cells.

Conclusion: Peptides identified herein are potentially safe and efficient targeting vectors for developing tumor specific targeted drugs, delivery systems and imaging agents with reduced detrimental off-target effects.

JA2
PPARδ SIGNALING MEDIATES CYTOTOXICITY OF DHA IN H9C2 CELLS

Igor Zlobine1, Victor Samokhvalov1, John M. Seubert1,2
1Faculty of Pharmacy and Pharmaceutical Sciences, 2Department of Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, AB, Canada

Introduction: Dietary polyunsaturated fatty acids (PUFA) have been well established for many years as important mediators in regulating cellular function. Docosahexaenoic acid (22:6n3, DHA) is a n-3 PUFA that is known to evoke differing effects on primary cells compared to immortal cell lines. Treatment with DHA induces cytotoxic effects in immortalized cell lines, while inducing either negligible or protective effects in primary cells. The molecular mechanisms underlying this exclusive cytotoxicity toward immortalized cell lines remains largely unknown. Purpose: The aim of this study was to examine the involvement of PPARδ in the cytotoxicity of DHA on the cardiac H9c2 cell line.

Methods and Results: PPARδ is abundantly expressed in H9C2 cells as analyzed with western blot. Treatment with DHA (100μM) resulted in a significant decline in cell viability and cellular metabolic activity. Furthermore,
treatment with DHA robustly increased total proteasome activities, LDH release and an inflammatory response. While no significant changes to ROS production or lipid peroxidation were observed following DHA treatment, evidence for an apoptotic cell death was inferred by decreased phosphorylation of Akt and activation of caspase-3 activity. Importantly, DHA robustly enhanced PPARδ DNA binding activity in H9c2 cells suggesting that cytotoxic effect of DHA might be mediated via PPARδ signaling. Indeed, co-treatment with GSK 3787, 1uM (specific PPARδ antagonist) abolished the cytotoxic effects of DHA on H9c2 cells. Intriguingly, DHA-mediated cytotoxicity was also greatly reduced by addition of myriocin, a specific inhibitor of ceramide synthesis.

Conclusions: Taken together, these data suggest the involvement of PPARδ signaling in DHA-induced cytotoxicity, which potentially involves formation of ceramide as an integral part of this mechanism.

Support: This work was supported by a grant from the NSERC (JMS).

Key words: docosahexaenoic acid, H9c2 cells, PPARδ, apoptosis.

JA3
USE OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD (HPLC) FOR METFORMIN DETERMINATION IN RAT TISSUES

Ali Abdussalam, Dion R. Brocks
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Purpose: Metformin HCl, a biguanide anti-hyperglycemic agent, is one of the most prescribed oral drugs for the management of Type II diabetes mellitus especially in the overweight and obese. Recent studies also have described a place for it in the treatment of cancer. Most of studies done on metformin were aimed to identify metformin concentrations in plasma, serum or urine samples. However, little information is available on its tissue distribution. Here we adapted a valid reverse phase chromatographic method for assay of the drug in plasma for use in rat tissues.

Methods: Two Sprague Dawley rats were administered 70mg/kg of metformin orally. At 6 hours after the dose, the animals were exsanguinated, with blood and tissues (liver, kidneys and intestine) being collected. Plasma was collected from the blood after centrifugation and the tissues were homogenized in distilled water. To measure metformin concentration, this method uses liquid-liquid extraction procedure in which the extraction was performed using a 1-butanol-hexane (50:50, v/v) mixture under alkaline condition followed by back-extraction into 1% acetic acid. Chromatography was carried out using a C18 column (250 mm*4.6 mm with 5 μm). The mobile phase consisted of Acetonitrile and KH2PO4 6.5 PH buffer (34%: 66%, v/v) plus Sodium dodecyl sulphate (3mM). The flow rate was 0.7 ml/min.

Results: The components eluted within 20 min. The peaks were symmetrical with no interference from endogenous compounds in tissues. The calibration curves were linear (>0.997) over the range of 500-10,000 ng/mL of metformin in rat plasma, liver, kidneys and intestine. The CV of intraday assessments was less than 11%. The percentage of error values were < 10%. The validated lower limit of quantitation of metformin in the tissues was 500 ng/mL.

Conclusion: This modified reverse phase chromatographic method was successfully capable of measuring metformin concentrations in rat tissues.

JA4
EFFECT OF GUIDED PEER EVALUATION ON STUDENTS’ SELF-EFFICACY TOWARD REFLECTION

Chowdhury F. Faruquee, Dr. Ken Cor, Dr. Lisa M. Guirguis
Pharmacy Practice division, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Self-reflection is the process of reviewing and analyzing an event to inform future performance. It has been shown to improve patient care and reduce medical errors. Students often lack the self-efficacy (SE) for
We hypothesized that pharmacy students' self-efficacy (SE) toward writing and evaluating reflections would increase over the course of a self-reflection task.

**Method:** A single group pre and post design was used. First year pharmacy students in a communication skill course wrote a reflection on a "patient interview" task. The course instructor guided students as they assessed a draft of a peer's reflection and provided verbal and written feedback. Students used feedback to improve their assignment before submission. Prior to guided peer reflection and after the assignment was returned, students completed an online questionnaire designed to measure SE toward writing and evaluating reflections. The questionnaire contained 4 items measuring SE for writing reflections and 8 items measuring SE for evaluating reflections. Students used a 6 point Likert scale (“not sure at all” to “extremely sure”) to report how sure they could perform specific writing and evaluation tasks. Exploratory factor analysis revealed two distinguishable factors: SE for writing a reflection and SE for evaluating a reflection.

**Results:** The pre and post-test surveys were completed by 119 students (RR = 90.2%). Paired t-tests comparing pre and post test scores for the two scales revealed significant increases in SE for writing reflections (Dif = 0.34, \(t = 3.85, p < 0.05\), two tailed) and SE for evaluating reflections (Dif = 0.50, \(t = 6.07, p < 0.05\), two tailed).

**Conclusion:** Our evaluation revealed improvements in SE beliefs toward writing and evaluating reflections. Future evaluation would benefit from including a control group to make inferences about whether the intervention was the primary driver of these effects.

JA5
ISONIAZID PROTECTS AGAINST ANGIOTENSIN II-INDUCED CARDIAC HYPERTROPHY THROUGH MODULATING CYTOCHROME P450 ENZYMES AND ITS ASSOCIATED ARACHIDONIC ACID METABOLITES

Samya Elkhatali, Osama H. Elshenawy, Ghada Haggag, Ayman O.S. El-Kadi
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

**Purpose:** CYP2E1 is known to selectively form 18- and 19-hydroxyeicosatetraenoic acid (19-HETE). We have recently demonstrated that 19-HETE is the major subterminal-HETE detected in the heart tissue and their formation was decreased during cardiac hypertrophy. Therefore, in the current study we examined whether CYP2E1 inducer, isoniazid protect against angiotensin II-induced cardiac hypertrophy in rats.

**Methods:** Male Sprague-Dawley rats were divided into four experiments groups. First group is the control group and was administered normal saline via miniosmotic pumps while the second group was angiotensin II which was administered by miniosmotic pump (450ng/kg/minute). The third group received isoniazid at a dose of 200mg/kg/day IP daily without angiotensin II and the fourth group received a combination of isoniazid and angiotensin II. At two weeks the rats were euthanized and the heart, liver, and kidney were excised. Cardiac hypertrophy was assessed by echocardiography as well as by measuring the heart weight to tibia length ratio. Cytochrome P450 enzymes and their associated arachidonic acid metabolites were determined by real-time PCR and liquid chromatography-electrospray ionization-mass spectrometry, respectively.

**Results:** Echocardiographic analysis showed that isoniazid improved heart functions. In addition, isoniazide significantly prevented angiotensin II-induced cardiac hypertrophy. The cardioprotective effect of isoniazid was associated with a significant increase in cardiac P450 epoxygenases and their associated metabolites.

**Conclusion:** The present study demonstrates that isoniazid alters the expression of cardiac P450s and their associated arachidonic acid metabolites and partially protects against angiotensin II-induced cardiac hypertrophy. These results further confirm the role of P450s, and their associated arachidonic acid metabolites in the development of cardiac hypertrophy.

**Support:** This work was supported by a grant from the CIHR to A.O.S.E. S.E is the recipient of the Libyan Ministry of Higher Education and Scientific Research scholarship.
ACTIVATED HUMAN PLATELETS PREFERENTIALLY PROMOTE LUNG CANCER STEM CELL MIGRATION

Mengjie Yan¹, Aneta Radziwon-Balicka¹, Paul Jurasz¹,²
Faculty for Pharmacy and Pharmaceutical Sciences¹ and Faculty of Medicine and Dentistry², University of Alberta, Edmonton, AB, Canada

**Purpose:** According to the cancer stem cell theory of cancer origin, a small population of cancer cells within the tumor bulk has stem cell-like characteristics (CSCs) and is responsible for initiating new tumors following metastasis. Numerous studies have shown that platelets contribute to metastasis, in part by stimulating cancer cell migration via release of chemokines from platelet granules. One of the chemokines released from platelet granules is stromal derived factor-1α (SDF-1α), which is known to mobilize both bone marrow and cancer stem cells via increased matrix metalloproteinase expression. Hence, we hypothesize that activated platelets preferentially induce CSC migration by releasing SDF-1α which then binds to its receptor CXCR4 on CSCs leading to increased MMP production and migration.

**Methods:** Platelet releasates were collected from collagen-activated human platelets isolated from healthy donors. CSCs were identified within the A549 human lung carcinoma cell line via flow cytometry according to the Goodell protocol (Hoechst 33342-negative side population). Total and cancer stem cell MMP-dependent migration were measured via a modified Boyden Chamber assay in response to platelet releasates. Pharmacological inhibitors AMD3100 and GM6001 were used to investigate the significance of SDF-1α signalling and MMP-dependence during migration.

**Results:** Platelet releasates preferentially promoted the migration of A549 CSCs (4.3 ± 0.3% pre-migration vs. 7.6 ± 0.7% post-migration side population cells, P < 0.05). Preliminary migration experiments demonstrate AMD3100 and GM6001 decrease total A549 migration, but only GM6001 preferentially inhibits CSC migration.

**Conclusions:** Activated human platelets preferentially stimulate the migration of cancer stem cells within the A549 human lung carcinoma cell line. Further experiments are required to delineate the role of SDF-1α-CXCR4-MMP signalling in platelet-stimulated cancer stem cell migration.
JUNIOR GRADUATE STUDENTS (B)

JB1
REAL-TIME, LABEL-FREE ELECTRO-MECHANICAL DETECTION OF GRAM-POSITIVE BACTERIA USING ANTIMICROBIAL PEPTIDE OF CLASS IIA BACTERIOCIN

Etayash. H1,2, Jiang. K2, T. Thundat2 and K. Kaur1
1Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, T6G 2E1, Canada, 2Department of Chemical and Materials Engineering, University of Alberta, Edmonton, T6G 2V4, Alberta, Canada

Introduction: Food contaminations and emergence of bacterial resistance remain one of the main critical concerns in developed and developing nations due to the lack of hand-held or portable devices for fast detection of pathogenic strains with high sensitivity and selectivity. In this work, we present for the first-time, a real-time, label-free detection of Gram-positive bacteria with high selectivity and sensitivity using interdigitated impedimetric array functionalized with naturally produced antimicrobial peptide of class Ila bacteriocin.

Methods: The antimicrobial peptide was chemically synthesized and covalently immobilized on interdigitated gold microelectrodes via a carboxylic interaction to free amines of a pre-attached thiolated linker. In a gold substrate, the peptide-immobilization was characterized using ellipsometry and grazing angle infrared spectroscopy (FTIR). Detection sensitivity and selectivity was determined by subjecting different bacterial samples (in – PBS and Milk) to impedance readings under a constant flow rate and an optimum frequency range.

Results: Film-thickness and molecular-orientation determined by ellipsometry and grazing angle infrared spectroscopy, respectively, indicated that the peptides were covalently immobilized in a random helical orientation on the gold substrates. Subjecting the interdigitated peptide-sensor to various concentrations of bacteria generated reproducible impedance spectra that detect peptide-bacteria interactions at a concentration of 1 cell per microliter (µL), a clinically relevant limit. Furthermore, the biosensor was capable of distinguishing between closely related bacterial strains and was able to selectively detect very low concentration of L. monocytogenes with limits of detection as low as 10³ cfu mL⁻¹.
The results in overall, offer very sensitive and selective approach in detecting pathogenic Gram-positive bacteria and propose the potential use of this designed system in real-time biosensing applications.

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

JB2
TARGETING CELL CYCLE PROTEINS BY SIRNA USING LIPID-SUBSTITUTED POLYETHYLENIMINE TO BREAST CANCER CELLS

Manoj B. Parmar1, Hamid M. Aliabadi2, Parvin Mahdipoor2, Robert Maranchuk3, Hasan Uludag1,2,4.
1Faculty of Pharmacy and Pharmaceutical Sciences; 2Department of Chemical and Materials Engineering; 3Department of Microbiology and Immunology; 4Department of Biomedical Engineering; University of Alberta, Edmonton, Alberta, Canada

Purpose: The objectives of this study are to find the best cell cycle protein targets to silence their expression by siRNA, and to select the best siRNA delivery system to exert a maximum therapeutic effect specifically on breast cancer cells.

Methods and Results: RNAi has been recognized as a promising approach to control breast cancer growth. For the delivery of siRNA, we synthesized low molecular weights (1.2 and 2.0 kDa) lipid-substituted polyethylenimines (PEI) with different degree of lipid substitutions. Linoleic acid and caprylic acid substituted PEIs have given the most successful silencing of proteins. To explore potential cell cycle proteins as therapeutic targets, we screened an siRNA library in MDA-MB-231 and MDA-MB-435 cells using linoleic acid-substituted 2 kDa PEI to deliver siRNA. Out of 169 cell cycle protein targets, siRNA against cell division cycle protein 20 (CDC20), RAD51, and serine-threonine protein kinase CHK1 diminished the cell growth most significantly in MDA-MB-435 cells. These
identified targets along with another well-studied cell cycle protein, kinesin spindle protein (KSP), were then evaluated in MDA-MB-435 and MCF7 cells using independently prepared siRNAs. The synergistic effect was not seen in combinational siRNA delivery of cell cycle proteins. Surprisingly, the treatment of siRNA against cell cycle proteins stopped cell proliferation in MDA-MB-435, but not in MCF7 cells. However, quantitative-PCR and digital-PCR results indicated down-regulation of mRNA transcripts of cell cycle proteins in both cell-lines. Moreover, flow cytometry results indicated similar uptake of siRNA-polymer complexes in both cells. On the contrary, the uptake study by confocal microscopy suggested that the chosen polymer has delivered more complexes in MDA-MB-435 compared to MCF7 cells.

Conclusions: We speculate that MCF7 cells may have used different survival pathway and escaped the siRNA treatment, and conclude that linoleic acid-substituted PEI is more effective polymer for siRNA delivery to breast cancer cells.

JB3
DEVELOPING NOVEL ASSAYS TO MEASURE THE EFFECTS OF EXOGENOUS NITRIC OXIDE ON THE REACTIVITY OF HUMAN PLATELET SUBPOPULATIONS

Gabriela Lesyk¹, Aneta Radziwon-Balicka¹, Paul Jurasz¹,²
Faculty for Pharmacy and Pharmaceutical Sciences¹ and Faculty of Medicine and Dentistry², University of Alberta, Edmonton, AB, Canada

Background: Platelet activation and thrombus formation within brain or coronary arteries is responsible for ischemic stroke and myocardial infarction, respectively. Nitric oxide (NO) prevents platelet aggregation and thrombosis. Recently, our laboratory identified the existence of human platelet subpopulations based on the presence and absence of endothelial Nitric Oxide Synthase (eNOS+ve and eNOS-ve platelets). Moreover, we have found that eNOS+ve platelets are less reactive and contain more soluble guanylate cyclase (sGC), the NO signal-transducing protein, than their eNOS–ve counterparts. Thus, we hypothesize that exogenous NO will suppress platelet aggregation by eNOS+ve platelets to a greater extent than of eNOS–ve platelets, due to increased sGC signalling.

Methods: Prostacyclin-washed platelets were isolated from the blood of healthy human volunteers. Intracellular flow cytometry was used to detect eNOS thereby distinguishing the platelet subpopulations. A novel platelet aggregation assay under flow conditions was developed utilizing a pizo-electric quartz-crystal microbalance with a collagen-coated sensor.

Preliminary Results: As described before human eNOS +ve and –ve platelets were detected by flow cytometry. In an initial experiment platelet aggregation was detected as a decrease in the vibration frequency of the quartz-crystal sensor. The addition of the NO-donor s-nitrosoglutathione (GSNO), caused a disaggregation of platelets as detected by an increase in the quartz-crystal sensor vibration frequency.

Conclusions and Future Directions: We have been able to replicate the detection of human eNOS-based platelet subpopulations. Further, we have established a novel platelet aggregation assay sensitive to measuring the effects of NO on platelets. As a next step, we will collect platelets that have flowed through the quartz-crystal microbalance in the presence and absence of GSNO, and we will measure the ratios of eNOS +ve to –ve platelets. Future studies will also utilize these novel technologies to determine the effects of anti-platelet agents on the aggregation of eNOS-based platelet subpopulations.

JB4
TOXICOLOGICAL IMPLICATION OF PHENYL BUTAZONE AND 6-MERCAPTOPURINE: POSSIBLE ROLE OF FREE RADICAL FORMATION VIA SUPEROXIDE DISMUTASE (SOD-1)

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The main side effect of phenylbutazone, NSAID, and 6-mercaptopurine, active metabolite of azathioprine, is bone marrow suppression. The molecular mechanism of these drugs to cause myelotoxicity is still unknown. Purpose:
First of all, to investigate the effect of superoxide dismutase/peroxidase activity (SOD-1) on the metabolism of phenylbutazone, azathioprine and 6-mercaptopurine.

**Methods:** We used UV spectrometer to monitor the oxidation of phenylbutazone, azathioprine and 6-mercaptopurine. We also utilized direct ESR to detect the free radicals and oxygen electrode to measure the rate of oxygen uptake.

**Results:** The oxidation of phenylbutazone and 6-mercaptopurine was enhanced by a peroxidase activity of SOD-1. However, azathioprine oxidation was not observed. SOD-omitted reactions produced less oxidation of both phenylbutazone and 6-mercaptopurine. The oxidation of phenylbutazone was markedly attenuated upon addition of N,N-dimethyl aniline, which is known as a highly reactive compound with carbonate radical. The carbonate radicals that resulted from reactions of SOD with bicarbonate and hydrogen peroxide were attenuated in the presence of phenylbutazone and 6-mercaptopurine. In the presence of SOD-1, bicarbonate and hydrogen peroxide, the oxygen consumption was enhanced upon addition of phenylbutazone and 6-mercaptopurine.

**Conclusion:** It can be concluded from these data that SOD peroxidase activity may enhance the metabolism of phenylbutazone and 6-mercaptopurine via generating free radicals.

**JB5**

5-HETE-INDUCES CELLULAR HYPERTROPHY IN THE HUMAN VENTRICULAR CARDIOMYOCYTE RL-14 CELLS THROUGH MODULATING THE EXPRESSION OF CYTOCHROME P450 AND ITS ASSOCIATED ARACHIDONIC ACID METABOLITES

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**Purpose:** Recent studies have established the role of midchain-HETE in the development of cardiovascular disease. Among these midchains, 5-HETE has been reported to have vasoconstrictive and pro-inflammatory action. However, whether 5-HETE can induce cardiac hypertrophy has not been reported before. Therefore, the overall objectives of the present study are to elucidate the potential cardiac hypertrophic effect of 5-HETE in the human ventricular cardiomyocyte RL-14 cells and explore the mechanism(s) involved.

**Method:** Human ventricular cardiomyocyte cell line RL-14 was used. The cells were treated with increasing concentration of 5-HETE (2.5, 5, 10 and 20 µM). Thereafter, the cardiac hypertrophy markers, β-myocin heavy chain (β-MHC), α-myocin heavy chain (α-MHC), atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were determined using real-time polymerase chain reaction (RT-PCR). The role of CYP epoxygenases, ω-hydroxylases and soluble epoxide hydrolase in 5-HETE mediated induction of cellular hypertrophy were determined at mRNA, protein and activity levels using RT-PCR, Western blot analysis and liquid chromatography-electron spray ionization-mass spectrometry, respectively.

**Results:** Our results showed that 5-HETE significantly induced the cellular hypertrophy in RL-14 cells as evidenced by increase in cardiac hypertrophy markers, β-MHC, α-MHC, ANP and BNP genes expression. The 5-HETE-induced cellular hypertrophy was associated with proportional increase in CYP4A11, CYP4F11, CYP2J2 and EPHX2 gene expression at mRNA and protein levels. Moreover, 5-HETE significantly increased the formation of the cardiotoxic metabolite, 20-HETE and the degradation products of the cardioprotective metabolites, 8,9-, 11,12- and 14,15-dihydroxyeicosatrienoic acid (DHET) metabolites.

**Conclusion:** The present work provides the first evidence that 5-HETE induces cellular hypertrophy in the human ventricular cardiomyocyte by modulating the expression of cytochrome P450 and its associated arachidonic acid metabolites. Support: This work was supported by a grant from the CIHR to A.O.S.E. Z.H.M. is the recipient of University of Alberta PhD recruiting scholarship.
MYELOPEROXIDASE-DERIVED FREE RADICAL METABOLITES OF AMINOGLUTETHIMIDE ARE ASSOCIATED WITH APOPTOSIS IN HL-60 CELLS: A TOXICOPROTEOMIC APPROACH TO DISCOVERY OF BIOMARKERS OF TOXICITY

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Purpose: Aminoglutethimide (AG), a drug used for the treatment of breast and ovarian cancers, is known to cause toxicities such as agranulocytosis (low neutrophil count) which can increase the incidence of infections. It has been reported that AG utilizes the cell’s most prominent peroxidase, myeloperoxidase (MPO), to be metabolized into a free radical and protein radical formation. Here, we use toxicoproteomics, a branch of toxicogenomics that uses proteomic technology to better understand toxic mechanisms in response to exposure to toxicants, to find potential biomarkers of AG toxicity.

Methods: To investigate AG’s toxicity mechanisms, trypan blue assay was used to assess the toxicity of AG, immunospin trapping SDS-PAGE for identifying protein radicals induced by AG metabolism, quantitative proteomic analysis technique known as stable isotope labelling in cell culture (SILAC) to gain insight into the proteome of Human Leukemia 60 (HL-60) treated with AG, and flow cytometry to identify the type of cell death induced by AG as well as the role of MPO in the cell death mechanism.

Results: We identified 43 proteins that were changed significantly upon AG treatment among which 18 (42%) and 25 (58%) were up and down-regulated, respectively. The quantitative proteomics data showed that AG treatment led to the down-regulation of critical anti-apoptotic proteins responsible for inhibiting the release of pro-apoptotic factors from the mitochondria as well as cytoskeletal proteins such as nuclear lamina. This overall pro-apoptotic response was confirmed with flow cytometry which demonstrated apoptosis to be the main factor of cell death. This response correlated with the intensity of AG-induced protein radical formation in HL-60 cells, which may have an important role in cell death signaling mechanisms.

Conclusions: SILAC provided invaluable information on potential biomarkers of AG toxicity which has greater implication in the evaluation and risk assessment of drug toxicity in the drug development industry.

Support: This work was sponsored by CIHR (Canadian Institutes of Health Research).

Category: Graduate student (More than 2 years of experience)

INHIBITION OF SOLUBLE EPOXIDE HYDROLASE IMPROVES CARDIAC FUNCTION AND LIMITS MITOCHONDRIAL DAMAGE FROM ISCHEMIC INJURY

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Purpose: Cardioprotective effects of epoxyeicosatrienoic acids (EETs) toward acute myocardial ischemia-reperfusion injury have been recognized; however, the precise mechanism(s) are still largely unknown. Our study investigates the protective effects of EETs by inhibiting soluble epoxide hydrolase (sEH), the enzyme responsible for EET metabolism, following surgical occlusion of left anterior descending artery (LAD) of the heart.

Methods: Age matched 2 month old sEH null (KO) and littermate wild-type (WT) mice were utilized in the study, as well C57Bl/6 mice which were administered an sEH inhibitor, trans-4-[4-(3-adamantan-1-y1-ureido)cyclohexyloxy]-benzoic acid (tAUCB; 10mg/L) or vehicle in drinking water for 4 days prior and 7 days post surgery. Mice from all groups were subjected to surgical occlusion of LAD and cardiac function was assessed by echocardiography prior to and 7 days post surgery. Mice were sacrificed on day 7 and heart tissues were dissected
into infarct, peri-infarct (area at risk) and non-infarct (healthy) regions to assess cellular and sub-cellular structure by electron microscopy (EM). Hearts were collected and mitochondrial respiratory enzymes in complexes I, II, III, IV and citrate synthase activities were assayed following IR injury.

**Results:** Hearts from tAUCB treated and sEH (KO) mice showed significantly improved ejection fraction (p<0.05) and fractional shortening (p<0.05) compared to WT counterparts. Echocardiogram revealed less cardiac remodeling in tAUCB treated and sEH KO groups evident by reduced left ventricular internal diameter (p<0.05) during both systole and diastole. Consistently, EM data showed more intact cardiomyocytes with better arrangement of myofibers and mitochondria in the tAUCB treatment and sEH KO group. Inhibition of sEH resulted in better complex I, II and citrate synthase activities compared to control hearts. However, no significant improvements were observed in complex III or IV activities.

**Conclusion:** The inhibition sEH or ablation of sEH gene provides cardiac protection against long-term ischemia, associated with preserved post-ischemic cardiac function and maintaining mitochondrial integrity and respiratory function.

*This work was supported by a grant from CIHR (JMS).*
SA1
HYDROGEN SULFIDE DONATOR (ADT-OH) SHOWS PROMISING ANTICANCER ACTIVITY IN BREAST CANCER CELLS

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The transcriptional factor Forkhead box M1 (FOXM1) is involved in cell proliferation, cycle progression, differentiation, DNA damage repair, apoptosis and drug resistance. Genomic studies have identified FOXM1 to be one of the most highly expressed genes in a wide variety of human tumors, and its suppression inhibits the transcription of genes associated with tumor proliferation and tumor growth, and consequently, FOXM1 has been recently proposed as a new promising target in cancer prevention and cancer treatment. Preliminary results have shown that dual nitric oxide (NO)/hydrogen sulfide (H₂S)-releasing hybrids (NOSH compounds) exert a dose-dependent downregulation of FOXM1 in vitro and in vivo. Our research group is currently investigating the molecular mechanisms involved in NOSH-induced FOXM1 down-regulation. In this regard, the objective is to investigate the role of nitric oxide (NO)- and hydrogen sulfide (H₂S)-release in the in vitro modulation of FOXM1 expression. My work with several dual nitric oxide/hydrogen sulfide-releasing hybrids (NOSH) shows a concentration-dependent cancer cell growth inhibition on breast (SKBR-3) and in liver (Hep G2) but not in colon (HT-29). Interestingly, the same pattern was observed when I tested intermediate compounds containing only the H₂S-releasing moiety, which suggests that this group is essential for the biological activity, because there was no inhibitory response in compounds having the nitric oxide-releasing group. In conclusion, hybrid compounds possessing the ADT-OH group as a hydrogen sulfide-donor have the ability to inhibit cancer cell growth in breast and in liver cancer cells, and this moiety is likely to be responsible for the downregulation of the FOXM1 protein in these cells. In this regard, we are currently evaluating the regulatory response exerted by selective derivatives which release one gas or the other, and the effect it produces in the expression of FOXM1 at the protein and mRNA level.

SA2
DEVELOPING RECOMBINANT PROTEIN THERAPEUTICS FOR TARGETING THE PENUMBRA IN AN ISCHEMIC/ HYPOXIC RAT MODEL: A PLATFORM FOR THERAPEUTIC INTERVENTION IN PERINATAL BRAIN INJURY

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Purpose: Elastin-like polypeptides (ELPs) are an interesting class of tunable, stimulus-responsive biopolymers that reversibly self-assemble in response to various environmental stimuli to form nano- and micron-sized particles. They are composed of multiple repeats of the motif (VPGXG) where X represents any amino acid except proline. ELPs self-assembly behavior varies depending on their sequence composition and chain length. Therefore, we will need to screen an assortment of ELP constructs to find the best sequence ideally suited for targeted drug delivery to the penumbra tissue in perinatal injured brains.

Method: The synthesis of long ELPs sequences can be a significant challenge because of their highly repetitive, GC-rich DNA sequences. Therefore, short gene sequences are seamlessly combined in tandem using a recombinant DNA technique called recursive directional ligation (RDL), which allows a stepwise oligomerization of ELP gene.
Using restriction enzymes to create compatible sticky ends, ELP sequences will be digested, purified with agarose gel electrophoresis then recombined together and cloned into E. coli.

**Results:** Valine and tryptophan were used in guest amino acid position to generate (VPGVG) and (VPGWG) ELPs sequences, respectively. Starting from chemically synthesized pentapeptide made up of 5 repeat of ELPs, we have been able to make multiple protein sequences composed of 10, 20, 40, 80 and 160 repeats using RDL and are currently expressing, purifying and characterizing the resulting proteins.

**Conclusions:** By developing biocompatible recombinantly expressed fusion peptides that self-assemble to form nanoparticles, this will open a new therapeutic window for targeted drug delivery to the penumbra tissue in the injured newborn brain.

SA3
MODULATION OF CYTOCHROME P450 1 (CYP1) BY CHROMIUM IN HEPATIC TISSUE OF C57BL/6 MICE

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**Purpose:** Several studies have examined the toxic effects of individual aryl hydrocarbon receptor (AhR) ligands, yet there are relatively few reports of the combined toxic effects of AhR ligands and other environmental co-contaminants, such as heavy metals. Chromium (Cr^{6+}) is one of the major environmental toxic metal and a potent human toxin, mutagen, and carcinogen. Heavy metals alter the carcinogenicity of AhR ligands by modulating the cytochrome P450 1 (Cyp1) enzyme, however, the mechanism(s) remain unresolved. The objective of the current study was to investigate the effect of Cr^{6+} on Cyp1 expressions and activity in C57BL/6 mice liver.

**Methods:** C57BL6 mice were injected intraperitoneally with Cr^{6+} (20 mg/kg) in the absence and presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (15 μg/kg) for 6 and 24 h. The mice were segregated into 4 experimental groups. The first group was control mice and received saline plus corn oil. The second group was Cr^{6+}-treated mice and received Cr^{6+} dissolved in saline plus corn oil. Third group was TCDD-treated mice and received TCDD dissolved in corn oil plus saline. The fourth group was Cr^{6+} plus TCDD–treated mice and received Cr^{6+} dissolved in saline plus TCDD dissolved in corn oil. Moreover, real-time PCR and Western blot has been used to measure mRNA and protein expression, respectively. EROD and MROD have been used to measure the Cyp1a1 and Cyp1a2 activity level, respectively.

**Results:** Cr^{6+} alone did not significantly alter Cyp1a1, Cyp1a2, or Cyp1b1 at mRNA, protein, or catalytic activity levels. Upon co-exposure to Cr^{6+} and TCDD, Cr^{6+} significantly potentiated the TCDD-mediated induction of the Cyp1a1, Cyp1a2, and Cyp1b1 mRNA at 6 h and the protein and catalytic activity levels at 24 h.

**Conclusion:** we demonstrated that Cr^{6+} potentiates the AhR-ligands mediated effect on the carcinogen-activating enzymes Cyp1.

**Support:** This work was supported by the NSERC grant to A.O.S. A.Y.A. is the recipient of Saudi Government Scholarship.

SA4
THE EFFECT OF HYPERLIPIDEMIA ON THE EXPRESSION OF ORGANIC CATION TRANSPORTERS OCT1/2 AND MULTIDRUG AND TOXIN EXTRUSION PROTEIN IN RAT KIDNEY

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**Purpose:** In human, the renal clearance (Clr) of metformin in obese patients is apparently lower than in lean subjects. We hypothesized that this is due to hyperlipidemia (HL), which is prevalent in obese patients. Giving that metformin excretion is transporter-dependent, HL might have a suppressive effect on the expression of SLC kidney transporters responsible for Clr such as organic cation transporters (OCT) 1 and 2, and multidrug and toxin
extrusion protein (MATE) 1. The aim of this study was to investigate the effect of HL on m-RNA and protein expression of these transporters in rats over time.

**Methods:** Male Sprague Dawley rats (~325 g) were administered poloxamer 407 (P407) 1 g/kg intraperitoneal (ip) injection to induce HL. At 36, 72 and 108 hours, kidney tissues were harvested and placed in liquid nitrogen then frozen at -80 °C. Real time PCR and Western blots were assessed for the transporter genes and proteins.

**Results:** There was no significant change in mRNA or protein levels of OCT 1/2 and MATE-1. However, at 72 h after P407 injection, a significant increase in mRNA of OCT-1 and MATE-1 was observed (by 2- and 5-fold, respectively), and a decrease in MATE-1 at 108 h after P407.

**Conclusion:** HL did not affect the transporter expressions at 36 h after dosing. However, chronic HL seems to modulate m-RNA expression of OCT 1 and MATE-1. Further experiments are in need to confirm.

SA5

**BONE-SEEKING NANOPARTICLES FOR EARLY DIAGNOSIS OF OSTEOARTHRITIS**

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**Purpose:** Earlier diagnosis would likely play a vital role in treatment of osteoarthritis (OA). Etiology of OA is still unknown, however, it has been suggested that the primary initiating event(s) occur in subchondral bone, leading to subsequent cartilage degeneration. The purpose of this study was to develop a bone-targeting contrast agent for MRI, to image altered bone turnover at the early stages of OA.

**Methods:** Superparamagnetic Iron Oxide Nanoparticles (SPIONs) were synthesized and conjugated with alendronate (ALN). The structure of tracer (SPIONs-ALN) was characterized with TEM, FT-IR, XPS, and affinity towards hydroxyapatite (HA) was evaluated in-vitro.

Post-traumatic OA was induced in rats surgically. Nanoparticles were administered intravenously to rats (n=3) 3 weeks after the surgery (i.e. early OA) at dose of 2.7mg/Kg Fe. T1 (TE/TR: 13/1250ms) and intermediate T2-weighted (TE/TR: 25/2000ms) MRI were acquired at 20min and 3h after injection.

**Results:** Spherical nanoparticles with diameter of 16nm±5.95 were obtained. Successful conjugation of ALN to SPIONs was concluded by observing amide and phosphonate peaks on FT-IR spectroscopy, and detection of phosphorous on XPS. Bone-targeting nanoparticles showed 65% binding to HA in-vitro.

In-vivo MRI revealed ‘negative enhancement’ at regions of active remodeling 20min after injection, definable as dark hypointense band. These areas included the growth plates (active sites of bone turnover in rats throughout life), the tibial and femoral subchondral bone, and the femoral trochlear groove, known to later develop osteophytes.

**Conclusions:** This study successfully detected altered bone turnover in the early stages of OA, particularly at subchondral bone, long before it becomes sclerotic. This is the first report on imaging of bone remodeling using MRI. The current approach can potentially produce images similar to radioactive ⁹⁵TcTechnetium MDP bone scan, with greater spatial resolution, no ionizing radiation, and the opportunity to also assess cartilage integrity on the same study.
**SA6**

**CYTOCHROME P450 METABOLITES OF ARACHIDONIC ACID IN PLASMA & HEART AS BIOMARKER OF NSAIDS INDUCED CARDIOVASCULAR RISK IN ADJUVANT ARTHRITIS RAT**

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**Purpose:** Elevation of cardiotoxic 20-hydroxyeicosatetraenoic acids (20-HETE, cardiotoxic) and reduced epoxyeicosatrienoic acids (EETs i.e. cardioprotective) are reported in inflammation and cardiovascular diseases. Epidemiological studies have shown that among non-steroidal anti-inflammatory drugs (NSAIDs) rofecoxib and flurbiprofen are more cardiotoxic than meloxicam and celecoxib. We hypothesize that eicosanoids can be used as biomarkers to differentiate between NSAIDs in their cardiovascular risk.

**Methods:** Sprague-Dawley rats were divided into control, inflamed and inflamed-treated groups and the latter was subdivided to rofecoxib 10 mg/kg, meloxicam 0.5 mg/kg, celecoxib 15 mg/kg and flurbiprofen 5 mg/kg. The inflamed group received Mycobacterium butyricum/squalene. After 7 days of treatment, blood and hearts were harvested and analyzed by HPLC-FL method.

**Results:** Inflammation resulted in altered plasma HETE/EET ratio toward cardiotoxicity. NSAIDs further elevated the ratio in the following order: Rofecoxib=flurbiprofen > meloxicam=celecoxib. a, significant vs control, b, significant vs inflamed (p<0.05).

**Conclusion:** The observed plasma HETE/EET ratio agrees with the available epidemiology data so that celecoxib and meloxicam are less likely to cause cardiotoxicity than rofecoxib and flurbiprofen. Plasma eicosanoids may serve as biomarkers of NSAIDs cardiovascular risk.

<table>
<thead>
<tr>
<th>Group (n=4)</th>
<th>Plasma (ng/ml)</th>
<th>Heart (ng/mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-HETE</td>
<td>Total-EET</td>
</tr>
<tr>
<td>Control</td>
<td>24.3 (8.11)</td>
<td>63.6 (7.78)</td>
</tr>
<tr>
<td>Inflamed</td>
<td>44.3 (3.95)a</td>
<td>56.5 (16.9)</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>96.3 (28.04)a,b</td>
<td>20.6 (5.76)a,b</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>102 (6.57)a,b</td>
<td>31.1 (12.0)a</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>59.1 (11.8)a</td>
<td>32.6 (8.58)a</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>65.4 (29.1)a</td>
<td>35.2 (5.20)a</td>
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</table>

**SA7**

**CARDIOPROTECTIVE POTENTIAL OF GLUCOSAMINE IN INFLAMMATORY CONDITIONS THROUGH THE RENIN-ANGIOTENSIN SYSTEM AND ARACHIDONIC ACID PATHWAY**

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**Purpose:** Inflammatory disorders like rheumatoid arthritis and some anti-inflammatory drugs are known to have cardiovascular complication which is likely due to activation of the renin–angiotensin system (RAS) and alteration of arachidonic acid (AA) metabolism. RAS is comprised of two main peptides, angiotensin Ang-II (cardiotoxic) and Ang-1-7 (cardioprotective) that are produced by the angiotensin converting enzyme (ACE) and ACE2, respectively. In the AA pathway, AA metabolism yields 20-hydroxyeicosatetraenoic acid (20-HETE, cardiotoxic) and epoxyeicosatrienoic acids (EETs, cardioprotective). Inflammation alters the ACE2/ACE and 20-HETE/EETs balances. We tested the hypothesis that the anti-inflammatory glucosamine (GlcN) counteract the effects of inflammation on the RAS and AA pathways.

**Methods:** Male Sprague-Dawley rats were assigned to four groups of Cont-placebo, inflamed (INF)-placebo, Cont-GlcN and INF-GlcN (n=4-5/each). On day zero control and INF animals were injected saline or Mycobacterium Butyricum/squalene at the tail base, respectively. The GlcN and placebo groups received daily oral doses of 160
mg/kg GlcN or water, respectively. 16-20 days after, plasma and heart were harvested and analyzed for Ang peptide and AA metabolites using ELISA and HPLC-FL, respectively.

**Results:** Inflammation significantly altered the peptides and metabolites balance toward greater cardiotoxic ratios and GlcN restored the balance.

<table>
<thead>
<tr>
<th></th>
<th>Plasma (fmol/mL)</th>
<th>Heart (fmol/mg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ang 1-7</td>
<td>Ang II</td>
</tr>
<tr>
<td><strong>Cont-placebo</strong></td>
<td>439.7 (56.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.4 (11.7)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>INF-placebo</strong></td>
<td>225.4 (75.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125.3 (13.1)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Cont-GlcN</strong></td>
<td>358.3 (36.2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.0 (6.9)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>INF-GlcN</strong></td>
<td>318.4 (21.9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.8 (4.7)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript character indicates significant differences among the means in the same column.

**Conclusion:** The anti-inflammatory effects of GlcN result in re-establishment of ACE2-ACE and 20-HETE/EETs ratio. This suggests a cardioprotective potential for GlcN in inflammatory conditions such as rheumatoid arthritis.
SENIOR GRADUATE STUDENTS (B)

SB1
EETS TARGET MITOCHONDRIA TO AUGMENT SURVIVAL DURING STARVATION STRESS

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Purpose: During nutrient restriction, mitochondria fuse together into elongated networks, which delays their
collapse and enhances ATP production to sustain cell survival. Previously, we and others have reported the
protective role of epoxyeicosatrienoic acids (EETs) in the heart. EETs are CYP450-dependent metabolites that have
robust but poorly understood anti-apoptotic and cardioprotective properties. EETs protected mitochondria against
fragmentation induced by either hypoxia or oxidative stress. In the present study, we investigate the effect of EETs
on cardiac mitochondria during starvation induced stress.

Methods: Rat neonatal cardiomyocytes (NCM) and HL-1 cardiac cells were treated with 14,15-EET (1 μM) or UA8
(dual acting EET mimetic, 1 μM) in serum free starvation buffer for 24 hours. The putative pan-EET receptor
antagonist, 14,15-EEZE (10 μM), was used as a negative control to confirm EET-mediated effects. Cell survival
was assessed with MTT assay. Western blot analysis was used to assess alterations in mitochondrial dynamics
regulators (DRP1, Fis1, and OPA1) and p62 as a marker for autophagy. Mitochondrial function was assessed by
measuring changes in enzymatic activities and protein expression of key respiratory enzymes. Live-cell imaging
was used to assess alterations in mitochondrial morphology and membrane potential with potentiometric
mitochondrial dye TMRE (0.1 μM). The 3D mitochondrial morphology and network structure was reconstructed
and analyzed by the Filament Tracer module in Imaris software.

Results: Starvation caused a marked activation of autophagy as indicated by consumption of p62 levels. Starvation
caused increased mitochondrial membrane potential and clear mitochondrial elongation, which correlated with
significant reduction in mitochondrial fission proteins DRP1 and Fis1. UA8 treated cell showed further decrease in
both DRP1 and Fis1. Interestingly, UA-8 treated cells had preserved mitochondrial cristae and increased expression
the short form of OPA-1 but no starvation-induced mitochondrial elongation was observed. This coincided with
UA8 enhanced cell survival and maintained mitochondrial respiration during starvation stress.

Conclusions: Together, these initial data suggest that EET-mediated events preserve mitochondrial structure and
minimize the loss ETC enzymatic function, thus enhancing cell survival without the starvation induced
mitochondrial elongation.

SB2
NOVEL SELF-ASSOCIATING POLY(ETHYLENE OXIDE)-B-POLY(EPSILON-CAPROLACTONE)
BASED DRUG CONJUGATE FOR DELIVERY OF STAT3 INHIBITOR JSI-124 TO MURINE B16
MELANOMA

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Purpose: Constitutively activated STAT3 in tumor plays an important role in tumor malignancy. JSI-124 is a
JAK/STAT3 signaling pathway inhibitor with potent antitumor activity. The objective of this research was to
develop a polymeric nanocarrier for the delivery of JSI-124. In this study, JSI-124 was chemically conjugated to
poly(ethylene oxide)-b-poly(α-carboxyl-ε-caprolactone) (PEO-b-PCCL) and its potential antitumor activity was
investigated.

Methods: JSI-124 was conjugated to the PCCL core through formation of an ester bond in the presence of oxalyl
chloride and triethylamine to form PEO-b-P(CL-JSI-124). PEO-b-P(CL-JSI-124) self-assembled to form micelles
in aqueous solution. The micelles were characterized for their size, CMC, and release of JSI-124 in PBS (pH 7.4) at
37 °C. The cytotoxicity of PEO-b-P(CL-JSI-124) was investigated against B16 melanoma cells using MTT assay. The activity of PEO-b-P(CL-JSI-124) for downregulation of phosphorylated STAT3 (p-STAT3) in B16 cells was investigated by FACS. Effect of PEO-b-P(CL-JSI-124) on B16 cell cycle was analyzed using propidium iodide.

**Results:** The successful conjugation of JSI-124 to PEO-b-PCL was confirmed by TLC and HPLC. The JSI-124 content in the conjugate was 8.8% w/w as confirmed with HPLC. The average hydrodynamic diameter of the micelles by DLS was 41.42 nm. The CMC in aqueous media was 7.97 µM. The release of intact JSI-124 from PEO-b-P(CL-JSI-124) was very slow. By day 14, only 5.5% of JSI-124 was released. Physically encapsulated JSI-124, however, had a similar profile to free JSI-124, showing 100% release within 8h. Treatment of B16 cells with free or conjugated JSI-124 resulted in significant loss of cell viability and suppression of p-STAT3 in a dose-dependent manner. JSI-124 and PEO-b-P(CL-JSI-124) also induced apoptosis and cell cycle arrest in the G2/M phase in B16 cells in a dose-dependent manner.

**Conclusion:** JSI-124 was successfully conjugated to PEO-b-PCL block copolymer while still maintaining its STAT3 inhibitory and anti-cancer activity.

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**SB3**

**DEVELOPMENT OF MOBILE AND WEB-BASED APPLICATIONS FOR SAFER DISPENSING OF ORAL CHEMOTHERAPEUTIC AGENTS**

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**Purpose:** With increasing availability and use of oral chemotherapeutics agents, a greater chance of prescribing error arises, especially when the healthcare professionals involved have limited experience with these medications. We aim to create an innovative solution, for use in the Canadian context, to help non-oncology pharmacists identify problems associated with oral chemotherapy, to achieve timely resolution, and most importantly, avoid patient harm.

**Methods:** We constructed a repository to disseminate information to pharmacists related to oral chemotherapy prescribing: “www.oralchemotherapy.ca”. This website features researched, comprehensive, one-page monographs for each oral chemotherapeutic available in Canada, including risk stratification (high, moderate, low) associated with each medication, as well as drug-drug interaction tables. Additionally, we created a mobile version of this data in a dedicated app called AntiC, for use on iPhones and Android phones. In many cases mobile applications fit better in today’s pharmacy workflow; it is designed to work in the absence of a Wi-Fi connection, allowing for access to information at all times. Both www.oralchemotherapy.ca and the AntiC mobile app are powered by the same data source.

**Results:** The website: www.oralchemotherapy.ca was constructed and is now available. Printable pdf versions of the monographs will be accessible in the near future. The AntiC mobile version of this data is now available for demonstration purposes.

**Conclusions:** It is hoped that this improved access to an additional level of assessment for oral chemotherapeutics available in Canada will empower non-oncology pharmacists and other healthcare professionals in the safer dispensing of oral chemotherapeutic agents, and thus improve patient care. We plan to host workshops to educate pharmacists on potential issues they face when dispensing oral chemotherapy, and introduce them to www.oralchemotherapy.ca and the AntiC app as an easy, rapid and concise resource to improve patient safety.

**Support:** Alberta Innovates: Doctoral Graduate Student Scholarship (Tibor van Rooij).
SB4
PHARMACOKINETICS OF DRONEDARONE IN RATS
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Purpose: Dronedarone is a benzofuran derivative of amiodarone that is used for the treatment of cardiac arrhythmias. To date, there is little published information regarding the pharmacokinetics of the drug in human, and none in rats. Therefore, the aim of this study was to determine the pharmacokinetics (PK) of dronedarone after intravenous and oral administration in rats. Methods: Sprague-Dawley rats were cannulated at the right jugular vein and fasted overnight, food was given 2h post dose. Dronedarone was dosed orally or intravenously as base. After dosing, serial blood samples were collected for 24 h. Samples were assayed for dronedarone using a validated reverse phase HPLC method.

Results: The Pharmacokinetic data were as follows.

Conclusions: This study is the first to report the pharmacokinetic profile of dronedarone in rats. Based on this study, rats seemed to have a lower dronedarone oral clearance, oral volume of distribution and shorter half-life compared to human. After oral administration, dronedarone exhibited low bioavailability in both rat and human.

Support: Y.B.J. is the recipient of Government of Saudi Arabia (Ministry of Higher Education-King Saud University) Scholarship.

*†Fed subjects, Dronedarone biopharmaceutics review, FDA 2009

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a – Vdss; b – Vdarea

SB6
ROLE OF CYTOCHROME P450 ENZYMES IN THE INITIATION AND DEVELOPMENT OF CARDIAC HYPERTROPHY
Hassan N. Althurwi, Ayman O.S. El-Kadi
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Purpose: We have previously shown that seven days of isoproterenol treatment causes significant changes to several cytochrome P450s (P450s) gene expression. However, the cause-effect relationship of P450s during the development of cardiac hypertrophy is still unknown. We hypothesized that P450s play crucial role in the early phases of development of cardiac hypertrophy.

Methods: Human ventricular cardiomyocyte cell line, RL14 was treated with isoproterenol (10µM) for 1, 3, 6, 24 or 48 h. Total RNA was isolated and the expression of hypertrophic markers, different P450s genes and EPHX2 were determined by real time-polymerase chain reaction (RT-PCR). Moreover, cells were incubated with arachidonic acid (AA) to determine P450s metabolites formation.

Results: Isoproterenol caused a significant induction of hypertrophic markers, atrial natriuretic peptide (ANP) and β-myosin heavy chain (β-MHC) at 6 h of treatment and continued thereafter. Interestingly, isoproterenol treatment caused a significant changes in CYP1A1, CYP2C8, CYP2E1, CYP2J2, CYP4F2, CYP4A11 and EPHX2 gene
expression, at 1 and 3 h, before the initiation of cellular hypertrophy. On the other hand, CYP1A2, CYP2B6, CYP2C8, CYP2C19, and CYP2J2 gene expressions were altered, at 24 and 48 h during the development of cellular hypertrophy. Cells incubation with AA reveals that EETs formation and total epoxygenases activity was increased at 48 h whereas sEH activity was decreased at 24 and 48 h suggesting an adaptive response.

**Conclusions:** Our results confirm the role of P450s and their metabolites in the initiation and the development phases of cellular hypertrophy which could reveal novel points of intervention to be exploited in the development of new therapies for the prevention of cardiac hypertrophy at early stages.

**Support:** This work was supported by a grant from the CIHR to A.O.S.E. H.N.A is the recipient of Salman Bin Abdulaziz University scholarship, Saudi Arabia.

**SB7**

**IN SILICO MODELING (GASTROPLUS) TO OVERCOME PHARMACOGENOMIC VARIATION IN HUMAN USING DIFFERENT DEXTROMETHORPHAN DOSAGE FORMS.**

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**Purpose:** The purpose of study was to investigate how modeling can assist the formulation scientist in developing a controlled release dosage form for drugs, which undergo extensive (EM) or poor metabolism (PM). Dextromethorphan (DM) was selected as a model drug because its metabolism is sensitive to pharmacogenomic variations.

**Methods:** A predictive model was generated using various physiological and pharmacokinetic parameters. Simulations were performed using a 30 mg (IR) tablet model with or without co-administration of quinidine as enzyme inhibitor. After validation of the predictive model simulations were performed with zero order release tablets (F1), first order sustained release tablets (F2), an immediate release (35% in 30 min) followed by the zero order release tablets (F3), an immediate release (70% in 30 min) followed by zero order release tablets (F4) and an immediate release (50% in 30 min) followed by pulse release of 50% drug at 2 hours (F5).

**Results:** The IR showed a fast onset, short T_{max} and highest C_{max}, compared to other dosage forms in both the EM and PM. The AUC_{0-24} was similar for all dosage forms for the EM and PM respectively. There was gradual increase in C_{max}, decrease in T_{max} and comparable AUC_{0-24} as the formulation release profile was altered from the F1, F2, F3, F4 and F5 tablets. The formulations were able to alter the drug plasma profiles but did not impact the difference in the observed drug plasma profiles between the EM and PM.

**Conclusion:** In silico modeling was able to predict the drug plasma profiles of different dosage forms. This can assist the formulation scientist to optimize the release properties of a drug from a dosage form to reach a desired drug plasma profile. However, study showed that drug delivery cannot address the differences between EM and PM which were due to pharmacogenomic variations.
SC1
ISONIAZID: NOVEL ROLE AS IMMUNE THERAPY AGAINST TUBERCULOSIS (TB)

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Isoniazid (INH) is one of the oldest and successful anti-tuberculosis drugs used, but its role on the immune system is still unknown. There have been several hypotheses proposed regarding its perplexing side-effects for which the mechanisms are also unclear. We hypothesized that isoniazid has immunomodulatory effects in addition to its well-known bactericidal actions, and this property of INH makes it a revolutionary therapy against TB. Additionally, we hypothesized that immune cells have defensive mechanisms to protect themselves from oxidative metabolites of INH. Human leukemia (HL-60) cells which resemble human promyelocytic immune progenitor cells have peroxidase activity due to their abundant myeloperoxidase (MPO) levels were used as in vitro model system. We treated cells with either INH or INH with glucose/glucose oxidase (G/GO; a source of H2O2 to activate MPO). In this study, we used stable isotope labeling by amino acids in cell culture (SILAC) to quantify the global protein changes which were affected by the treatment of either INH itself or INH with G/GO. We found 49 proteins were significantly altered by the treatment of INH alone. These proteins revealed cellular pathways of HL-60 cell differentiation into immune competent cells; and also activation of defensive mechanisms against putative oxidative stresses. The up-regulating NADPH oxidase activity and monocyte surface antigen CD14 expression analyses confirmed its monocytic differentiation. In contrast, we found 51 proteins were significantly altered in INH-G/GO treated cells. These proteins were involved in the defensive mechanisms through up-regulating the mitochondrial respiratory chain reaction, and enhancing chromosomal as well as cytoskeleton stability. In conclusion, INH induces monocytic differentiation of myeloid progenitor cells which has wide range of defensive mechanisms against putative oxidative stresses. Therefore, INH can be used as immune therapy against multi-drug resistant (MDR) and extensive drug resistant (EDR) TB.

SC2
ROLE OF STEREOREGULARITY IN THE STABILITY OF POLYMERIC MICELLES AND THEIR RELEASE PROFILES

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Purpose: To investigate the effect of polymerization method on the stability and drug release properties of polymeric micelles formed using stereo-active block copolymers.

Methods: Diblock copolymers consisting of methoxy poly ethylene oxide (MePEO) and poly(lactide)s (PLA)s of different stereochemistry were synthesized by bulk or solution polymerization. Polymers and micelles were characterized for their chemical structure by 1H NMR, optical rotation by polarimetry, critical micellar concentration by fluorescence spectroscopy, thermal properties by differential scanning calorimetry, morphology by transmission electron microscopy and size as well as kinetic stability by dynamic light scattering. Release of encapsulated nimodipine from polymeric micelles at different levels of loading was also investigated.

Results: Solution polymerization yielded a higher degree of crystallinity for stereo-regular PLA blocks. Consequently, the related polymeric micelles were kinetically more stable than those prepared by bulk polymerization. At high drug loading levels, the release of nimodipine was more rapid from polymeric micelles with crystalline cores. At lower levels of drug loading, drug release was slower and independent of the stereochemistry of the core.
Conclusions: The results underline the effect of polymerization method in defining core crystallinity in stereoregular block copolymer micelles. It also shows the impact of core crystallinity on enhancing micellar stability and drug release.

SC3
ACTIVATION OF PROCARCINOGEN-ACTIVATING ENZYMES CYP1A1, CYP1A2 AND CYP1B1 BY TRIMETHYL ARSINE OXIDE

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Purpose: Arsenic is a worldwide environmental pollutant that is ranked on the top of priority lists of hazardous substances according to The Agency for Toxic Substances and Diseases Registry (ATSDR) and the Canadian Environmental Protection Act Registry (CEPA). Arsenic compounds are associated with skin and several types of internal cancers, thus classified as human carcinogens. Recent reports revealed that arsenic biomethylation could activate the toxic and carcinogenic potential of arsenic. Therefore, our aim is to investigate the effect of trimethyl arsine oxide (TMAO) on the activation of procarcinogen-activating enzymes cyp1a1, cyp1a2 and cyp1b1.

Methods: C57BL/6 mice were received TMAO (13 mg/kg i.p.) with or without the prototypical AhR ligand, 2,3,7,8-tetrachlorodibenzodioxin (TCDD; 15 μg/kg), then the livers were harvested at 6 and 24 h post-treatment. Thereafter, gene expression of different cyp enzymes and oxidative markers (nqo1, gsta1 and ho-1) were determined by real-time PCR. Protein expression were determined by Western blot, whereas catalytic activities were measured using EROD and MROD assays.

Results: Gene expression results showed that TMAO alone increased cyp1a1, cyp1b1, nqo1, gsta1 and ho-1 at mRNA level. Upon co-exposure to TMAO and TCDD, TMAO significantly potentiated the TCDD-mediated induction of cyp1a1, cyp1b1 and nqo1. Western blotting revealed that, TMAO alone significantly increased cyp1a1 and cyp1a2 protein levels. Furthermore, TMAO significantly potentiated the TCDD-mediated induction of cyp1a1 and cyp1b1 protein level. In addition, TMAO alone significantly increased cyp1a1 and cyp1a2 activities and significantly potentiated the TCDD-mediated induction of cyp1a1 activity.

Conclusion: our results demonstrate for the first time that TMAO, induce the procarcinogen activating enzymes at gene expression, protein, and activity levels. This represents a novel mechanism by which arsenic causes carcinogenicity and would help in rationale designing of treatment to fight against arsenic-induced diseases.

Support: NSERC Discovery Grant, Alberta Cancer Foundation Studentship and Alberta Innovates Technology Futures Scholarship.

SC4
MATRIX METALLOPROTEINASE INHIBITORS ALTER THE ACTIVITY OF SOLUBLE EPOXIDE HYDROLASE

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Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Purpose: Matrix metalloproteinase (MMP) and soluble epxide hydrolase (sEH) are completely unrelated enzymes. Nevertheless, their inhibitors are sharing closely similar chemical structure, as well as, similar effects on biological systems. The only exception is that sEH inhibitors promote cancer, while, MMP inhibitors prevent cancer in vitro, but not in vivo. Therefore, we hypothesized that there is an overlap in the selectivity between MMP inhibitors and sEH inhibitors.

Methods: In the current study, sEH inhibitory activity was evaluated for three MMP inhibitors, MMPI I, ONO-4817, and doxycycline using two methods; 1) the hydrolysis of spectrophotometric substrate, 4-nitrophenyl-2,3-epoxy-3 phenylpropyl carbonate, measured at 405 nm, and 2) the hydrolysis of sEH natural substrate, 14,15-epoxyeicosatrienoic acid, measured by liquid chromatography/mass spectrometry. On the other hand, MMP inhibitory activity was evaluated for three sEH inhibitors, AUDA, tAUCB, and TUPS, using two methods; 1) the degradation of the fluorogenic substrate, OmniMMP™, measured at λ_ex = 328 nm, and λ_em = 393 nm, and 2) zymography. Results: Interestingly, MMPI I, and ONO-4817 significantly inhibited the recombinant human sEH
activity by 60% and 71%, respectively. IC$_{50}$ was 5.1 µM for MMPI I, and 3 µM for ONO-4817. Doxycycline exhibited no inhibitory activity on sEH. Regarding sEH inhibitors, they did not alter the activity of human pro-MMP-2 or MMP-2 catalytic domain, measured by fluorimetry and zymography. Conclusion: MMP inhibitors, such as MMPI I, and ONO-4817, can substantially affect sEH activity in vivo, consequently, their reported in vivo activities should be re-evaluated to include the contribution of sEH inhibition. The potential anticancer effect of MMP inhibitors should be reassessed using more selective inhibitors. Support: This work was supported by a grant from the CIHR to AOSE. AAE is the recipient of Egyptian Government Scholarship and RB is the recipient of AIHS summer studentship.

SC5
VITAMIN D RESCUES FOXO3A EXPRESSION AFTER OXIDATIVE STRESS IN OSTEoblAST-LIKE CELLS

Kathy Tang, Michael R. Doschak
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Purpose: Osteoporosis is a bone disease associated with loss of bone mass, causing fracture in bone. Oxidative stress has been shown to be a contributing factor to the loss of bone mass. We found in our study that vitamin D is capable of rescuing FoxO3a protein expression after exposure to oxidative stress. FoxO3a expression has been shown to play an important role with the increase in bone mass. Osteoblast-like cells have been treated with 100µM hydrogen peroxide, generates reactive oxygen species, leading to decrease in cell viability. After treatment with H$_2$O$_2$, cells were then treated with active vitamin D and demonstrated protein expression of FoxO3a to recover.

Methods: MC3T3-E1 cells were differentiated into osteoblast-like cells then underwent treatment with 100µM hydrogen peroxide to generate oxidative stress. 10µM of active vitamin D was then added to the culture and western blotting employed to analyze FoxO3a expression.

Results: 100µM Hydrogen peroxide decreases cell viability and FoxO3a expression. Active vitamin D is able to increase Foxo3a expression under conditions with or without hydrogen peroxide. Insulin, used as a negative control, shows to decrease in FoxO3a expression. PTH can also increase FoxO3a expression, although not as much as active vitamin D.

Conclusion: FoxO3a expression shown to be rescued after treatment with vitamin D. Hydrogen peroxide decreases FoxO3a expression time-dependently, which can then be recovered with addition of vitamin D.

SC6
UNDERSTANDING HOW ALBERTAN COMMUNITY PHARMACISTS ASSESS THE APPROPRIATENESS OF MEDICATION THERAPY: A MIXED METHODS APPROACH

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Purpose: To characterize how Albertan community pharmacists assess the appropriateness of therapy using a practical communication framework called the Chat, Check, and Chart (CCC) model. In particular, this study will develop a better understanding of how pharmacists communicate to collect information and assess prescription appropriateness.

Methods: Mixed methods convergent design was employed. Twelve pharmacists were audio recorded talking with patients (i.e., consult) as well as thinking aloud (TA) while evaluating medication therapy. Two individuals reviewed recordings in a qualitative way to explore the recording and to quantify how pharmacists collect of patient information and evaluate therapy as per the CCC model.

Results: There were 17 recordings of consults and 15 of TAs. Pharmacists allotted 16% of their TA on clinical related issues for new and chronic medications. In consults, they spent 39% of time discussing clinical issues for new medications and 21% for refills of chronic medications. In consults, pharmacist asked about medication purpose, directions and monitoring for almost 67% of new medications but less than 37% did for chronic refills. We found that all pharmacists checked if the medication was safe, but less than half checked in the prescription was indicated, effective, or useable (i.e., adherence).
Conclusions: This study suggests that pharmacists focus on technical issues particularly for refills and may miss opportunities for patient care beyond medication safety. Pharmacists make almost a complete assessment for new prescriptions and less effort is made for chronic refills. This pilot study highlights the need for further study.
POSTDOCTORAL FELLOWS

PD1
CHARACTERIZATION OF PLATELET SUBPOPULATIONS BASED ON THE HETEROGENEITY OF NOS SIGNALLING

Aneta Radziwon-Balicka1, Bin Dong2, Haitham El-Sikhry1, Barbara Zielnik1, Stephen Ogg2, John Seubert1,2, Ian Winship2, Paul Jurasz1,2
Faculty for Pharmacy and Pharmaceutical Sciences1 and Faculty of Medicine and Dentistry2, University of Alberta, Edmonton, AB, Canada

Purpose: Platelets form thrombi that occlude arteries; however, the molecular mechanisms regulating platelet thrombus formation are poorly understood. One of the most important platelet inhibitory signalling systems is mediated by nitric oxide (NO). Within platelets, NO is synthesized by endothelial nitric oxide synthase (eNOS); however, recent studies have questioned the presence of eNOS in platelets. Hence, we hypothesized that two platelet subpopulations may exist based on the presence and absence of eNOS and that these two subpopulations may have differential roles in hemostatic/thrombotic reactions.

Methods: Platelets were isolated from healthy humans and prostacyclin-washed platelets were prepared. Platelet NO production as determined by DAF-FM fluorescence was measured by flowcytometry and fluorescence microscopy. eNOS, soluble guanylyl cyclase (sGC) and platelet aggregation-mediating receptor (GPIIb/IIIa) were measured in fixed and permeabilized platelets using flow-cytometry. Platelet functionality was assessed using light-aggregometry and flow chamber confocal microscopy followed by flow-cytometry.

Results: Based on DAF-FM fluorescence we identified a platelet subpopulation that produces no or low-levels of NO (12.4±1.3% of total platelets) and a platelet subpopulation which produces NO (87.6±1.6% of total platelets). These two subpopulations corresponded to platelets that lacked or expressed eNOS (17.7%±5.0% eNOS-negative vs. 82.3%±5.2% eNOS-positive). eNOS-positive platelets contained more sGC than eNOS-negative platelets (1.8±0.2 vs. 1.2±0.1 arbitrary units of fluorescence, P<0.05). Upon activation with collagen, more eNOS-negative than eNOS-positive platelets expressed the activated form of GPIIb/IIIa (78.0%±8.5% vs. 21.4%±7.2%). Under flow conditions, eNOS-negative platelets adhered to collagen prior to their eNOS-positive counterparts, which was confirmed by flow-cytometry that showed eNOS-positive subpopulation enrichment after the flow chamber experiment. Finally, the eNOS-negative platelet subpopulation initiated thrombus formation while the eNOS-positive subpopulation limited aggregation.

Conclusions: Human platelet subpopulations exist based on the presence or absence of a functional eNOS-PKG-signalling pathway. eNOS-negative platelets, although less abundant are more reactive than eNOS-positive platelets and initiate aggregate/thrombus formation.

PD2
NF-KB AND MAPK SIGNALLING PATHWAYS MEDIATE BUTHIONINE SULFOXIMINE (BSO)-INDUCED SOLUBLE EPOXIDE HYDROLASE (SEH) LEADING TO CARDIAC HYPERTROPHY

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Purpose: Evidences suggest that up-regulation of soluble epoxide hydrolase (sEH) is associated with the development of myocardial infarction, dilated cardiomyopathy, cardiac hypertrophy, and heart failure. However, the up-regulation mechanism is still unknown.

Methods: In this study, we treated H9c2 cells with buthionine sulfoximine (BSO) to explore whether oxidative stress up-regulates sEH and to identify molecular and cellular mechanisms behind this up-regulatory response. RT-qPCR and Western blot has been used to measure mRNA and protein expression respectively.

Results: We demonstrated that BSO treatment significantly induced sEH at mRNA levels in concentration- and time-dependant manner leading to significant increase in the hypertrophic markers [atrial natriuretic peptide (ANP)]
and brain natriuretic peptide (BNP)]. Furthermore, BSO significantly increased NF-κB DNA-binding activity, this level of binding was paralleled by increase in the nuclear translocation of p50, and p65 subunits, and increase in the cytosolic IkB-α protein levels as measured by western blot analysis. Moreover, our results demonstrated that pre-treatment with NF-κB inhibitor pyrrolidin dithiocarbamate (PTDC) significantly inhibited BSO-mediated induction of sEH and hypertrophic markers in concentration-dependent manner. Moreover, effects of BSO on sEH were abrogated by quercetin, artemisinin and parthenolide, inhibitors of IkB-α phosphorylation, NF-κB nuclear translocation, and NF-κB-p65 binding to DNA respectively. To understand further the role of MAPKs (Mitogen-Activated Protein Kinases) pathway in BSO-mediated induction of sEH mRNA, we examined the role of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK. Indeed, treatment with the MEK1 inhibitor PD98059, JNK1/2 inhibitor SP600125 and the p38 inhibitor SB203580 resulted in significant inhibition in BSO-mediated induction of sEH.

Conclusions: NF-κB signaling pathway involved in BSO-mediated induction of sEH mRNA, and appear to be connected to activation of the MAPK pathway. Our findings further provide a link between sEH-induced cardiac dysfunction and involvement of NF-κB in the development of cardiac hypertrophy.

PD3

BONE-TARGETING PARATHYROID HORMONE ANALOGUES OUTPERFORM UNMODIFIED PTH IN THE ANABOLIC TREATMENT OF OSTEOPOROSIS IN RATS

Yang Yang, Arash Panahifar, Yuchin Wu, Madhuri Newa, Kathy Tang, Krishna H. Bhandari, Michael R. Doschak
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Purpose: Development of PTH conjugates surpassing PTH on anabolic treatment of osteoporosis overcomes short half-life of the latter as a result of rapid in-vivo metabolism and competitive uptakes in non-skeleton tissues.

Methods: After synthesis, bioactivity was confirmed by cAMP generation. In-vitro bone binding affinity was assessed by hydroxyapatite (HA) binding study. Treatment efficiency of either unmodified PTH or PTH-PEG-BP was evaluated by in-vivo micro-CT scans of the proximal tibial metaphysis after systematical administration to ovariectomized rats. Electron probe micro-analysis was used to identify elemental strontium in embedded tibial bone samples, and thin sections stained with H&E and Tetrachrome, for analysis of bone turnover.

Results: Both native PTH and PTH-PEG-BP triggered cAMP after incubation with UMR-106 cells containing PTH receptor. HA binding assays indicated up to 41% of PTH-PEG-BP was bound to the mineralized HA pellet, compared to <10% for unmodified PTH. Both PTH and bone-targeting PTH-PEG-BP showed a dramatic reversal in osteopenic bone volume changes by micro-CT. Of particular note, daily PTH-PEG-BP significantly increased bone mass at a greater rate than that of PTH alone. Even once-weekly administration of PTH-PEG-BP was capable of measurable anabolic response suggesting potential for less frequent patient injection with further dosage refinement. Dynamic labeling of mineralizing bone surfaces under each treatment regimen was evidenced by detecting incorporated elemental strontium and confirmed the anabolic increase in newly-formed bone with bone-targeting PTH-PEG-BP.

Conclusions: Ours is the first report of a bone-targeting anabolic bone therapeutic. Compared to currently marketed PTH, PTH-PEG-BP showed significant affinity for bone mineral and significantly improved efficacy in terms of increasing bone volume and BMD in osteoporotic rats. Bisphosphonate-mediated targeting of PEGylated PTH to bone represents a new class of targeted anabolic compound that has not previously been attempted.

Support: Alberta Innovates – Health Solutions Team grant in Osteoarthritis; Mitacs Elevate Strategic Fellowship Program, Canada.
RING OPENING POLYMERIZATION OF α-BENZYL-ε-CAPROLACTONE BY DIHYDROXYPOLY(ETHYLENE GLYCOL): DEVELOPMENT OF OPTIMIZED METHODS FOR THE SYNTHESIS OF ABA TRIBLOCK COPOLYMERS

Mohammad Reza Vakili, Soheila Honary, Afsaneh Lavasanifar
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Introduction of functional groups to poly(ester)s can present opportunities for the development of new biomaterials with versatile applications. Our research group has synthesized a new monomer, i.e., α-benzyl-ε-caprolactone (BCL), that forms poly(ester)s with functional groups upon ring opening polymerization. This monomer (Fig 1) has two esters (endocyclic and exocyclic) in its structure. Reaction of dihydroxy polyethylene glycol (PEG) with the endocyclic ester produces the desired linear polymer structure (Fig 1). However, the reaction of PEG with the exocyclic ester leads to undesired crosslinking. The aim of this study was to optimize the polymerization process towards production of linear polymers while minimizing the cross-linking side reaction.

Methods: A series of poly(BCL)-PEG-poly(BCL) block copolymers were synthesized using different catalyst (stannous octoate) concentrations and reaction times (Table 1). In a typical reaction, BCL (0.3 g) and PEG (0.1) were mixed with catalyst in an ampule, and then heated at 164 °C under vacuum. The product was purified by solvation, precipitation and wash using dichloromethane, hexane and ether, respectively. The degree of polymerization (DP) of BCL was calculated using 1H NMR. The optimum condition was considered to be the condition at which maximum DP was achieved. Factorial design was used to model the experimental data.

Results: The modelling of experimental data (Table 1) showed the following relationship ($r^2=1.000$).

$$DP = 6.125 + 2.275X_1 + 0.575X_2$$

where $X_1$ and $X_2$ are the volume of catalyst and reaction time, respectively. The results showed the main variable defining $DP$ was $X_1$. The interaction parameter ($X_1X_2$) did not affect $DP$. At its best, $DP$ reached 8 to 12.

Conclusions: The factorial design was very useful in understanding the major contributing factor for the optimization of polymer synthesis condition. This analysis showed the volume of catalyst to be the most important factor that should be controlled to minimize the chance of cross-linking.

Table 1: Reaction condition and the results of the synthesis.

<table>
<thead>
<tr>
<th>Cat (mL)</th>
<th>Reaction time (min)</th>
<th>Conversion (%)</th>
<th>$DP$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230</td>
<td>40</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>38</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>230</td>
<td>45</td>
<td>6.1</td>
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<td></td>
<td>210</td>
<td>38</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>230</td>
<td>55</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>46</td>
<td>7.6</td>
</tr>
</tbody>
</table>

$^a$ volume of the catalyst in hexane (100mg/mL)

Figure 1-Scheme for the synthesis of linear PBCL-PEG-PBCL block copolymers
UNDERGRADUATE STUDENTS

U1
NATURAL HEALTH PRODUCT USE IN PATIENTS WITH RHEUMATOLOGICAL CONDITIONS

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Purpose: Natural health products (NHPs) are often used in combination with prescription therapies; however, there is limited data on their benefit and concerns regarding their regulation. Patients with rheumatological diseases have been reported to use more NHPs than the general population and may be at higher risk due to the nature of their condition and concomitant pharmacotherapy. NHP use and adverse effects appear to be under-reported and interactions with conventional therapy can contribute to poor patient outcomes.

Methods: To quantify and describe NHP use in rheumatology patients, we conducted an observational, cross-sectional survey of patients attending the two rheumatology clinics in Edmonton, Alberta. Administrative staff distributed and collected anonymous patient surveys over a 4-week period. Data were analyzed using descriptive statistics.

Results: Of the 500 patients who completed the survey, the majority were women with rheumatoid arthritis attending a follow-up visit. Approximately 60% of patients reported using one or more NHP, with an average of 2.8 NHPs per patient. Physicians were most commonly informed of (67%) and consulted about (52%) NHP use, whereas only ~20% of patients informed or consulted their pharmacist. The majority of patients stated they would not discontinue prescribed medications in favour of NHPs. The most frequently reported products included vitamin D, calcium, omega-3/fish oils, and glucosamine. Patients frequently reported benefit with glucosamine, omega oils, vitamin D, multivitamins, co-enzyme Q10, and two products specifically marketed for arthritis. Few patients reported adverse effects.

Conclusions: This survey demonstrated that NHP use in patients with rheumatological diseases in Edmonton, AB is common and in line with other published data. This data will be utilized to develop NHP monographs specific to this population that will help inform patients and health care professionals of their efficacy and safety.

U2
A CONTENT ANALYSIS OF BLOGS ON BIOIDENTICAL HORMONE THERAPY

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Purpose: About 75% of all users search the Internet for health information. With the recent controversy surrounding the term “bioidentical hormone therapy” (BHT), women may be turning to Internet blogs as a source of health information on BHT. The objectives of this study were to analyze and describe the content and perspectives found in BHT blogs.

Methods: Blogs were identified using Google Blog Search®. Inclusion criteria included: English language, developed or updated between September 2011 and August 2012, and not more than three clicks from the top 200 search results. Videos, discussion forum blogs, and password-protected blogs were excluded. A quantitative content analysis was performed on 105 blogs meeting study criteria. Emerging themes were also identified through thematic analysis. Blogs were analyzed separately by two coders. A third coder resolved any discrepancies among the coders.

Results: The author was indicated in 75% of blogs, with equal representation between male and female. Only 26% of blogs were from health care professionals. About 65% of blogs promoted a product or service. Only one blog was from a professional organization. A fifth of blogs used evidence to support their information. Approximately 57% of blogs defined BHT as compounded formulations, while only 21% indicated that BHT is also commercially available. Bioidentical hormones were categorized mainly as being natural progesterone (50%), testosterone (33%),
estradiol (28%), and estriol (23%). The majority of blogs portrayed BHT positively (82%) and conventional hormone therapy (CHT) negatively (63%). Blogs mentioning safety claimed that BHT was safer than CHT in regards to breast cancer and/or cardiovascular disease. Recurring themes included: “hormone balance”, “natural”, “anti-ageing”, and “individualization.”

**Conclusion:** Individuals seeking health information about BHT on the Internet may encounter information presented in blogs. BHT blogs presented perspectives that were largely commercially based and inconsistent with evidence-based recommendations supported by professional organizations.

**U3**

**CHARACTERIZING ANTIBIOTIC USE IN MANITOBA INFANTS DURING 2010-2013: A CHECK-UP ON PRACTICE**

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**Purpose:** Antibiotic treatment of children has declined over the last decade but utilization of broad-spectrum antibiotics has generally increased. In addition to concerns over antimicrobial drug resistance, antibiotic exposure during infancy is a well-known source of gut microbiota disruption with potential long lasting effects, such as atopic disease and metabolic abnormalities. Therefore we intent to characterize antibiotic exposure in children less than 1 year of age and determine antibiotic use to pertinent clinical practice guidelines.

**Methods:** This descriptive study is based on a medication questionnaire administered to the mothers of 665 infants at approximately 3 months, 6 months and 1 year of their child’s age from January 2010 to May 2013. Mothers were recruited at the Winnipeg, Manitoba site of the Canadian Healthy Infant Longitudinal Development (CHILD) population-based birth cohort. Prescribing data from Manitoba Health was used to determine accuracy of the questionnaire dataset. Concordance of reported infant antibiotic use was determined based on existing guidelines at the time of database collection.

**Results:** Within the first year of life, 27% of infants received at least one course of oral or intravenous antibiotics; when topical antibiotics were included, the antibiotic exposure rate increased to 30%. The overall discordance rate of all antibiotics was found to be 26.4%. Penicillin comprised most of the guideline discordant antibiotic prescriptions at 20%, followed by cephalexin and azithromycin at 15% each. Guideline discordance rate of antibiotics used for acute otitis media was 13.8%.

**Conclusions:** Compared to historical Canadian figures, our results indicate that antibiotic exposure in infants continues to decline. Further, a smaller percentage of antibiotic indications were discordant according to clinical practice guidelines than previously reported in similar pediatric studies. Accordingly, pharmacists have the opportunity to improve the antibiotic treatment for approximately one quarter of Canadian infants regarding drug choice for a given infection.

**U4**

**OPTIMIZING INHALER USE IN HOSPITAL: AN INITIAL LOOK**

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**Purpose:** Advair® (fluticasone/salmeterol) metered-dose inhaler (MDI) use has been steadily increasing, a 30% increase in use from April 2010 to March 2013, in the Edmonton Zone and now ranks among the top ten agents used at the Royal Alexandra Hospital. However, this usage is accompanied by significant wastage as Advair® and several other inhalers are being returned to the dispensary with more than 75% of drug remaining. The aim of this project was to quantify the proportion of inhaler returns in hospital, determine the reasons behind this wastage and implement strategies to minimize inhaler wastage and maximize savings for the hospital.
Methods: In order to estimate inhaler wastage, pharmacy staff were asked to collect all inhalers that were returned to the dispensary either partially used or unused over a two-week period. A root cause analysis was first conducted to obtain feedback on this issue by meeting with pharmacy management and hospital staff.

Results: Based on the findings, intervention strategies were designed accordingly to optimize inhaler usage and minimize wastage, including a 15-minute oral presentation to pharmacy staff and nursing staff on a pulmonary unit as well as creating a handout for hospital staff (technicians, nurses, pharmacists, respiratory therapists) to raise awareness of the issue. Following the intervention the percent of inhalers returned was approximately double that of baseline, whereas the proportion of partially used inhaler returns remained similar to baseline.

Conclusions: Our preliminary intervention was unable to reduce inhaler usage or the proportion of inhalers returning to the pharmacy compared to baseline, suggesting the need for a more focused and extended analysis of inhaler returns to gain better understanding of the long-term implications of inhaler wastage.

U5
ENZASTAURIN POTENTIATES HUMAN PLATELET AGGREGATION AND GROWTH FACTOR SECRETION: EFFECTS ON A549 LUNG CARCINOMA CELLS

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Background: Enzastaurin is a serine/threonine kinase inhibitor relatively selective for Protein Kinase-Cβ (PKCβ) with anti-proliferative and pro-apoptotic effects. Until recently, it was in clinical development by Eli Lilly and Company for the treatment of a variety of cancers including non-small cell lung cancer (NSCLC); however, the primary endpoints in several clinical trials of Enzastaurin were not met. In addition, thrombosis was reported as a major adverse effect of Enzastaurin. While investigating the role of PKC in regulating growth factor release from platelets, we identified that unlike other PKC inhibitors Enzastaurin potentiates platelet aggregation. Therefore, we hypothesized that although Enzastaurin has apoptotic and anti-proliferative effects on cancer cells, it also indirectly promotes their survival via its potentiation of platelet aggregation and subsequent release of growth factors.

Methods: Prostacyclin-washed platelets were isolated from the blood of healthy human volunteers, and platelet aggregation was measured by light-aggregometry. Platelet VEGF release was measured by ELISA. Human A549 lung carcinoma cells were cultured under standard conditions and treated with releasates from Enzastaurin-titrated platelets. A cell death ELISA was performed to measure A549 apoptosis.

Preliminary results: Enzastaurin (10⁻⁸ – 10⁻⁶ M) potentiated platelet aggregation, in response to collagen (1µg/ml), in a concentration-dependent manner. At 10⁻⁶ M, Enzastaurin potentiated VEGF release from platelets during aggregation (P < 0.05 vs. control). Initial experiments reveal that human A549 cells cultured with Enzastaurin-treated platelet releasates demonstrate a trend toward lower apoptosis compared to controls, after treatment with apoptosis-inducing concentrations of Enzastaurin (10⁻⁵ M) (P = 0.1204).

Conclusions: (1) Enzastaurin potentiates platelet aggregation and this likely explains the high incidence of thrombosis in its clinical trials. (2) Potentiation of aggregation enhances the release of growth factors like VEGF from platelets. (3) Further experiments are needed to determine whether enhanced platelet growth factor secretion nullifies Enzastaurin’s apoptotic effects on cancer cells.

U6
PERCEPTIONS OF PHARMACISTS’ ROLE AND PROFESSIONAL DEVELOPMENT NEEDS IN THE ERA OF EXPANDING SCOPES OF PRACTICE

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¹Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; ²Faculty of Extension, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Although the pharmacy profession is moving away from the traditional drug dispensing model towards a patient-centered model, general uptake of the expanded scope of pharmacy practice has been slow. Pharmacists
have identified further training and professional development as a necessity to prepare them for practice change. The purpose of this study is to: (1) understand how pharmacists perceive the pharmacist’s role in the changing health care environment in Alberta, and (2) determine next steps in terms of professional development to support an expanded scope of pharmacy practice.

Methods: Using focus group data from pharmacist and other health care team members as well as previous literature, we developed an on-line survey to evaluate pharmacists’ views of professional development as well as the pharmacist’s role. The survey will be further refined through expert review for face and content validity, focus group interviews and pilot testing.

Results: Expert review, focus group and pilot testing will be completed in February 2014. Following revisions, the survey will be administered to a sample of pharmacists on the Alberta College of Pharmacists’ clinical register.

Conclusions: This study will characterize perceptions of the pharmacist’s role and identify opportunities and needs for pharmacists’ professional development in light of the changing role of the pharmacist. These findings will impact future decisions on development of professional education as well as policy related to professional development. Findings may also be used to formulate recommendations related to entry to practice education.

Support: The Alberta College of Pharmacists.

U7
PREPARATION OF MICELLE-FORMING ABC TRI-BLOCK COPOLYMERS AS NANO-RESERVOIRS FOR CONTROLLED DRUG RELEASE

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Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: The objectives of this study was to: 1) prepare ABC triblock copolymers and their micellar counterparts; and 2) investigate the effect of the three layered micellar structure on drug encapsulation and release, making comparison with two layered core/shell micelles prepared from diblock copolymers.

Methods: AB diblock copolymers consisting of methoxy poly(ethylene oxide) (MePEO) (as the A block) and poly(lactide)s (PLA)s of different stereochemistry (as the B block) as well as ABC triblock copolymers consisting of PEO (as the A block); poly(lactic acid)s with different stereo-chemistries (as the B block) and poly(α-benzylcarboxylate-ε-caprolactone) (PBCL) (as the C block) were synthesized by bulk ing opening polymerization using stannous octoate as catalyst. Di- and triblock copolymers were self-assembled to polymeric micelles. Polymers and/or micelles were characterized for their chemical structure by 1H NMR, optical rotation by polarimetry, thermal properties by differential scanning calorimetry, and sizes by dynamic light scattering. Encapsulation and in vitro release of a model hydrophobic drug, nimodipine, from the self-assembled structures were assessed using UV spectroscopy to measure drug levels.

Results: Successful synthesis of di- and triblock copolymers was confirmed by 1H NMR. The sizes for loaded and empty micelles showed a narrow mono-disperse size distribution within an ideal range of 50-130 nm. Encapsulation of nimodipine reached more than 50 % efficiency in triblock copolymer micellar structures in contrast to 30% efficiency in diblock copolymers. A significant reduction in the burst release of nimodipine incorporated in triblock copolymeric micelles was observed at initial time points compared to diblock copolymer micelles in vitro.

Conclusions: Synthesis of ABC block copolymers based on PEO-PLA-PBCL is a feasible approach. Triblock copolymer micelles were successful in improving the drug loading and release profile compared to diblock copolymer micelles, preventing the initial burst release while achieving a significantly higher drug loaded levels.

U8
USING PEPTIDES TO SYNTHESIZE CANCER TARGETING COMPOUNDS

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Purpose: In order to minimize side effects of chemotherapy, cancer-targeting peptides have been used to selectively deliver chemotherapeutic agents to cancer cells. A previously developed 10-mer peptide, 18-4 (WxEAYQrFL, where x is D-norleucine), was found to have high selectivity and stability but slight toxicity
effects. A novel analogue to this peptide is developed to have similar selectivity and stability but lower toxicity, where a D-arginine is replaced with an L-lysine (WxEAAYKFL, 18-4-2).

**Methods:** This peptide was manually synthesized and coupled to fluorescein isothiocyanate (FITC) through a β-Ala linker. After purification by reverse phase high performance liquid chromatography and characterization by matrix-assisted laser desorption/ionization time of flight mass spectrometry, 10^{-4}M FITC-β-Ala-18-4-2 was incubated with MDA-MB-231 and MDA-MB-435 breast cancer cells for 30 minutes. Cells were then collected and acquired using flow cytometry.

**Results:** Uptake by MDA-MB-231 cells showed a 1.1 fold increase compared to untreated cells, while MDA-MB-435 cells showed a 1.4 fold increase.

**Conclusions:** The 18-4-2 analogue shows affinity for breast cancer cell lines MDA-MB-231 and MDA-MB-435, and could potentially be used as a cancer-targeting peptide. Further investigation towards 18-4-2 uptake in other tumorigenic and non-tumorigenic cell lines and its stability will be carried out.
FACULTY/OTHER POSTERS

F1  
**EETS ATTENUATE LPS-INDUCED PRO-INFLAMMATORY RESPONSE IN HL-1 CARDIAC CELLS VIA INVOLVEMENT OF PPAR-dependent PATHWAYS**

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**Purpose:** Myocarditis or inflammatory cardiomyopathy is inflammation of the heart muscle, which often results in irreversible damage. In this study we investigated whether epoxyeicosatrienoic acid (EETs), cytochrome P450 (CYP) epoxygenase metabolites of arachidonic acid, could reverse a lipopolysaccharide (LPS)-triggered cell death in the mouse atrial HL-1 cell line.

**Results:** Treatment of HL-1 cells with LPS for 24h (1 µg/mL) triggered an inflammatory response, including release of pro-inflammatory cytokines, TNFα and MCP-1, and increased NF-κB DNA binding activity. LPS-induced effects resulted in a significant decrease in cell viability, decline in cell contractility, mitochondrial activity and total antioxidant capacity. A significant increase in caspase-3 activity suggested an apoptotic response was initiated. Activation of peroxisome proliferator-activated receptors (PPAR) such as PPARγ is known to execute anti-inflammatory reactions. Following LPS treatment in HL-1 cells, DNA binding activity of PPARγ significantly decreased suggesting suppression in the anti-inflammatory signal. We utilized 14,15-EET (1 µM) and UA-8 (1 µM), a synthetic EET-analog possessing both EET-mimetic and soluble epoxide hydrolase (sEH) inhibitory effects, to assess the cytoprotective effects of EETs. Both compounds significantly attenuated the pro-inflammatory effects of LPS by limiting the release of TNFα and MCP-1 and reducing NF-κB DNA binding activity. The cytoprotective effect of EETs prevented the decrease in PPARγ DNA binding activity. The EET-mediated cytoprotective effect was abolished by co-treatment with 14,15-EEZE (10 µM), a specific EET antagonist.

**Conclusions:** These preliminary data demonstrate that EETs reduce a LPS-induced pro-inflammatory response in mouse atrial HL-1 cells and highlight a novel intracellular pathway involving PPARγ dependent signaling. Moreover, these data suggest EET-mediated events are a novel therapeutic approach to limiting cardiac inflammation, potentially limiting adverse outcomes. **Support:** JV received funding via the Dr. E. Dekker programme of the Netherlands Heart Foundation (NHF). The research was supported by a grant from NSERC (JMS).

F2  
**IMPACT OF A PHYSICAL ASSESSMENT COURSE ON THE CLINICAL PRACTICE OF PHARMACISTS**

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**Purpose:** New opportunities for skill development are needed to facilitate the pharmacists’ role in medication management. Pharmacists have not traditionally been trained in physical assessment (PA), yet this skill set is valuable for patient assessment and monitoring of drug therapy. The purpose of the study was to assess the impact of a professional development course on the pharmacists’ level of confidence using PA and determine if there were resultant changes to their clinical practices.

**Methods:** Pharmacists attended a 2-day workshop on physical assessment. The course focused on the development of basic PA skills and application to medication monitoring. Surveys were administered before, immediately after, 2 months and 6 months post workshop. Confidence in PA skills and managing drug therapy were assessed using a
4-point scale. Pharmacists were also asked about their integration of PA skills and use of Additional Prescribing Authorization (APA).

**Results:** Ninety-two pharmacists participated in the course, with 86 (93%) consenting to take part in the surveys. Pharmacists’ confidence in performing PA increased between pre-and post-workshop surveys. Of the 48 pharmacists completing the 6-month survey, 48% indicated they had implemented or increased the use of PA within their current practice while 50% felt their patient assessments were enhanced. Measuring vital signs was the most common skill used by the pharmacists after the workshop. The most common barriers identified to using PA included already having access to PA information, over-stepping their professional role, and perceived lack of adequate training. At 6 months 38% had increased confidence with prescribing. Four pharmacists went on to receive their APA in the 6 months after the workshop.

**Conclusions:** Professional development in physical assessment may provide opportunities for pharmacists to integrate new knowledge and skills within their practice. Future research should examine if such educational opportunities will support sustainable practice change.

F3

**IMMUNOMODULATORY ACTIVITY OF ISOMERS BR1UEM-T AND BR2UEM-E: EVIDENCE OF HUMOR- AND CELL-MEDIATED RESPONSES**

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**Purpose:** The immunomodulatory effect of isomers BR1UEM-T and BR2UEM-E was evaluated by studying humor- and cell-mediated immune responses in mice.

**Methods:** The animals were divided in immunosuppressed and immunocompetent groups which were immunized, on the first day, with sheep red blood cells suspension (SRBC) injected intraperitoneally. The subgroups were treated orally with BR2UEM-E (125, 250 and 500 mg/kg) or BR1UEM-T (125, 250 and 500 mg/kg), once a day, for seven days. The immunosuppressed group also received cyclophosphamide (50 mg/kg) intraperitoneally on days 4, 5 and 6 after immunization, one hour after the treatments. The humor- and cell-mediated immune responses were evaluated by the total white blood cells count (WBC), the antibody title (HA) and delayed-type hypersensitivity response (DTH) induced by SRBC, both determined seven days after immunization. The DTH was measured 24 and 48 hours after the induction.

**Results:** About the immunocompetent group, the treatment with BR2UEM-E increased the WBC and decreased the DTH at 48 hours. The treatment with BR1UEM-T increased the WBC, decreased the DTH at 24 and 48 hours and increased the HA. About the immunosuppressed group, the treatment with BR2UEM-E increased the WBC and decreased the DTH at 48 hours. The treatment with BR1UEM-T increased the WBC, decreased the DTH at 24 and 48 hours and increased the HA.

**Conclusions:** Our data showed that the BR2UEM-E presents immunomodulatory activity by increasing the WBC and decrease the DTH. Furthermore, the BR1UEM-T presented anti-inflammatory activity, by decreasing the DTH at 24 hours, and immunomodulatory activity by increasing the WBC, the HA and decreasing the DTH at 48 hours.

**Support:** Grant BEX 11387/13-0, CAPES Foundation, Ministry of Education of Brazil, Brasília – DF 70.040-020, Brazil.
CA 125 TARGETED MOLECULAR IMAGING OF EPITHELIAL OVARIAN CANCER
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Purpose: Epithelial ovarian cancer (EOC) is characterized by over-expression of CA125 that serves as a tumor marker. The present work utilizes an anti-CA125 monoclonal antibody (mAb) and single chain variable fragment (scFv) to develop an immuno-PET (Positron Emission Tomography) strategy for non-invasive diagnosis of EOC.

Methods: Anti-CA125 mAb and scFv were purified by affinity chromatography and evaluated for antigen binding. Bi-functional chelator: pSCN-Bn-NOTA [S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid] was conjugated to anti-CA125 mAb and scFv. ⁶⁴Cu was obtained from Washington University (St. Louis, MO). Ovarian cancer cell lines NIH:OVCAR3 (CA125⁺) and SKOV3 (CA125⁻) were used for in vitro and in vivo studies. BALB/c nu/nu mice were used to develop xenograft models and perform in vivo radiopharmacological evaluation using small animal PET.

Results: Anti-CA125 mAb and scFv were purified in yields of 7 mg/L and 0.6 mg/L. Immunostaining with unmodified, FITC-labeled, NOTA conjugated and radiolabeled anti-CA125 mAb and scFv showed specific binding to OVCAR3 cells and no binding to SKOV3 cells. ⁶⁴Cu-labeling of anti-CA125 mAb and scFv was achieved with isolated radiochemical yields of 65% and 56% respectively with >99% purity. In vivo radiopharmacological evaluation using ⁶⁴Cu-labeled anti-CA125 mAb provided an SUV of 6.90 in OVCAR3 tumors 24 h.p.i, which could be blocked up to 55% by pre-dosing the animal with un-modified anti-CA125 mAb. SUV of 1.8 was seen in SKOV3 tumors attributed to enhanced permeability and retention. ⁶⁴Cu-labeled scFv provided an SUV of 0.63 in OVCAR3 tumors 24 h.p.i versus 0.38 in SKOV3 tumors. Both ⁶⁴Cu-labeled vectors showed expected biological clearance profiles.

Conclusion: ⁶⁴Cu-labeled anti-CA125 mAb and scFv could be prepared successfully with retained in vitro and in vivo immunoreactivity. Both radiolabeled vectors presented targeted tumor accumulation and expected biological clearance profiles. This renders them as potential PET probes for in vivo molecular imaging and targeting of epithelial ovarian cancer.