Submitted Abstracts – Faculty of Pharmacy and Pharmaceutical Sciences Research Day 2017

A. Undergraduate

A1-1 Shaja Chaudhry

CASE FINDING FOR GERIATRIC SYNDROMES IN COMMUNITY PHARMACIES

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Purpose: To identify the prevalence of falls and lower urinary tract symptoms (LUTS) in community dwelling seniors accessing pharmacy services.
Methods: This case finding study involved pharmacy students as research assistants surveying consecutive older adults (aged 65 years and older) in community pharmacies regarding geriatric syndromes. The questionnaire included basic demographics, self-report of falls over the past year, self-report of LUTS (urinary incontinence) in the past 4 weeks, and if the individual had sought health care assistance for these conditions. After completing the questionnaire, the students provided the subjects with brochures on these syndromes, as required. Data was entered into the RedCap Database and analyzed descriptively using SAS 9.4.
Results: A total of 190 subjects were surveyed, of which 166 (62%) were female, and the mean age was 75.1 years (SD 9.2). More than one third of the participants, 70 (37%), reported falling and approximately one third, 66 (35.1%), reported a "near fall". Of those that fell, 37 (53%) sustained an injury, yet most, 86 (46%), did not discuss it with a healthcare professional. More than half of the participants, 99 (55%), reported LUTS, but only 46 (25%) discussed these symptoms with a healthcare professional. When discussing falls the vast majority of the participants, 43 (81%), did so with physicians and for LUTS, 45 (98%).
Conclusions: Falls and LUTS are common geriatric syndromes that older adults do not commonly address with healthcare professionals. This case finding study presents an opportunity for community pharmacists in to start the conversation with patients about these syndromes.

A1-2 Nisreen Chehimi

Engagement of Pharmacy Students in Practice Based Research

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Purpose: Pharmacy students completing community practice placements often have unique opportunities to participate in pharmacy practice research projects which allow them to develop research skills while contributing to practice. Yet, student engagement and sustained involvement are common barriers to practice based research. The goal of this study is to describe a peer led model of pharmacy student engagement in practice based research and subsequent recruitment.
Methods: Fourth year pharmacy students completing their 8-week community practice placement (N=45) were invited to participate in a project with the goal of identifying critical inhaler errors in Albertans. Students attended a project presentation and received data collection forms, list of critical inhaler errors and instructions one week prior to their placement starting. To monitor students' progress and address possible questions, a peer student followed up and tracked participants' recruitment via phone calls, Facebook posts and emails. Students were contacted at weeks 2, 4, 5 and 7 and by individual request. Strategies to overcome commonly reported concerns, questions and motivational messages were addressed in emails.
Results: During the first round of phone calls students had recruited 40 patients and reported barriers including lack of familiarity with data collection process, low confidence in completing the asthma action plan and inability to integrate study procedures into pharmacy workflow. By the end of week 4 and 5, the number of recruited patients were N=86 and N=128 respectively. The approximate time to conduct each round of phone calls was

five hours. Students were enthusiastic and displayed interest and effort in contributing to practice based research.
Conclusion: Peer support in engaging pharmacy students in practice based research was feasible and supported the robust recruitment of Albertans with asthma and COPD. Frequent communication with a peer identified issues that had the potential to jeopardize the study and also create timely solutions.

A1-3 Cassandra Cooper

A Scoping Review to Evaluate the Quality of Mobile Applications to Improve Medication Adherence and Management of Oncology Patients

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Purpose: In the pediatric oncology population, non-compliance to medications has been reported as 10-50%, with high rates among adolescents and young adults ranging from 27-60%. Technologies, such as mobile applications (apps), may support patients with medication and disease management. Despite vast availability, app quality and functionality are highly variable. This scoping review identified and evaluated existing apps for cancer patients with the intent to assess quality and identify gaps in functionality.
Methods: A systematic literature search was conducted, including PubMed and EMBASE, to identify articles related to mobile app use, development, testing or evaluation. A manual mobile app search in the iTunes App Store and Google Play Store was also conducted to identify apps for inclusion and evaluation. Articles and apps were excluded if they focused on non-cancer indications, non-medication-based interventions or if they were designed solely for healthcare providers.
Results: Our search yielded 36 articles and 28 apps which were assessed for quality using the MARS tool. These apps varied considerably in regards to features with MARS mean scores ranging from 2.8-4.3. Pain Squad, exclusively available to Apple users, scored the highest and featured an intuitive and visually appealing user interface with gamification elements included within its design. Overall, most apps received low scores in the MARS Engagement domain and offered minimal medication and symptom management functionality, thus requiring a patient to download more than one app to meet their needs.
Conclusions: Our study assessed the quality of currently available mobile oncology apps and identified gaps in app design and function. These results will inform the conceptualization and design of a new and improved app for pediatric cancer patients.

A1-4 Xinqi Ji

Towards optimization of the core in poly(ethylene oxide)-*b*-poly({-carboxyl-caprolactone-*g*-spermine) micelles for siRNA delivery to colorectal cancer cells

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Purpose: Transient gene silencing through use of siRNA may have application in cancer treatment, diagnosis or anti-cancer drug development. However, due to instability of naked siRNA in serum or its negligible cellular internalization, delivery systems are required for siRNA transfection. The objective of this research is to optimize a previously developed polymeric micellar system for delivery of siRNA to target cells following systemic administration.
Methods: Poly(ethylene oxide)-b-poly(a-carboxyl-caprolactone-g-spermine) (PEO-*b*-PCCL-g-SP), with different PCCL degree of polymerization (17, 13, and 11) were synthesized and used for spermine (SP) conjugation (polymers I, II and III, respectively). The SP attachment was performed through a modified method for activating

the carboxylate groups, using oxalyl chloride. The molecular weight of polymers was determined by 1H-NMR and formation of cross-linking was investigated by gel permeation chromatography (GPC). The size and critical micellar concentration (CMC) were determined by DLS. The viability of HCT-116 luc+ cells after treatment with the prepared polymers was determined by MTT assay. Cells were treated with polymeric micelles carrying siRNA against luciferase. Transfection efficiency of treatments were measured by analyzing luminescence following luciferin addition. The correlation between different micellar properties and their transfection efficiency was investigated.
Results: Using 1H NMR, percent spermine attachment in polymers I, II and III was determined at 50, 55 and 27%. The micelle size was 120, 141, and 196 nm, respectively and the CMC values were 0.0116, 0.0156, and 0.017 mg/mL. The zeta potential of micelles was -4.5, -4.51, and -3.78 mV, respectively. The GPC showed formation of cross links which was the highest for polymer II, followed by polymer III. Among different parameters assessed, a strong statistical correlation was found between the degree of polymer cross linking and complexed siRNA transfection.
Conclusions: The results showed cross-linking of the polymer may contribute favorably to the transfection efficiency of PEO-PCCL-SP siRNA micellar complexes.

A1-5 Harjot Malhi

Recurrence of cytomegalovirus (CMV) infection after secondary antiviral prophylaxis post-lung transplantation.

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Background: Cytomegalovirus infection commonly occurs in lung transplant (LTx) recipients, and prior to prophylaxis regimens, was associated with high rate of morbidity and mortality. Patients now receive universal CMV prophylaxis post-transplant, however a third of patients still go on to develop a CMV infection. In University of Alberta (UofA) LTx Program, use of post-CMV infection antiviral prophylaxis is not protocolized. This study reviews the role of secondary antiviral prophylaxis in reducing occurrence of a subsequent CMV infection in LTx recipients after complete treatment of initial infection.
Methods: Retrospective data analysis of LTx recipients performed between September'05–December'13 in the UofA LTx Program. CMV-related data was collected to two years of follow-up post-transplant. Duration of secondary prophylaxis was calculated as number of days LTx recipients received prophylaxis dosing following treatment of first CMV infection. Primary outcome was recurrence of CMV infection.
Results: 301 LTxs were performed during the study period with 93 (31%) recipients being treated for first CMV infection. Of those treated, 49/93 (53%) recipients received secondary prophylaxis (range 1-605 days), of which 31/49 (63%) recipients developed a second CMV infection compared to 21/44 (48%) recipients who did not receive secondary prophylaxis (p=0.132). In 25/93 (28%) recipients receiving secondary prophylaxis minimum 21 days, there was no difference in incidence of second CMV infection compared to those who did not receive secondary prophylaxis [15 (60%) had second CMV infection, p=0.599]. Duration of secondary prophylaxis was not associated with a second CMV episode in cox regression analysis (p=0.233). Among those developing second CMV infection, 44/54 (85%) had a CMV donor (D)/recipient (R) serological status associated with higher risk of CMV infection [23 D+/R+ and 21 D+/R- patients].
Conclusion: Use of secondary antiviral CMV prophylaxis for a minimum of 3 weeks did not reduce incidence of second CMV infection in LTx recipients.

A2-1 Erica McGinn

The Role of Soluble Epoxide Hydrolase Enzyme on Daunorubicin Mediated Cardiotoxicity

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Purpose: To determine which enzymatic process of arachadonic acid (AA) metabolism plays a critical role in conferring daunorubicin (DNR)–induced cardiotoxicity.
Methods: Sprague–Dawley rats (n=6) were injected intraperitoneally with a single dose of DNR (5 mg/kg i.p.) or saline and the hearts were harvested 24 h post-treatment. Human ventricular cardiomyocytes RL-14 cells treated with 0.1 μM DNR in the presence and absence of 1 μM tAUCB (sEH inhibitor) for 3 h. Hypertrophic and fibrotic markers were measured using real-time PCR. Protein expression levels of CYP1B1, CYP2B, CYP2C, and CYP4A were determined using Western blot analysis. Levels of DHETs/EETs, HETEs were determined using LC–ESI–MS. Protein assay kits were used to measure MAPK and NF-kB.
Results: DNR-induced cardiotoxicity was confirmed in-vivo and in-vitro based on increased hypertrophic and fibrotic markers in DNR treated groups. It was found that DNR-induced cardiotoxicity had lowered cardioprotective EET levels, and increased DHETs levels compared to control. it appears that this imbalance is due to the induction of sEH rather than through the inhibition of CYP. In-vitro, the sEH inhibitor, tAUCB restored hypertrophic markers in DNR treated cells to levels matching control. Therefore, it appears that altering levels of AA metabolites can induce or protect cells from cardiotoxicity. Furthermore, it was shown that tAUCB's mechanism of cardioprotection involved inhibiting p38 in the MAPK pathway, and inducing p50 in the NF-kB pathway. Notably, these cell survival effects were induced only under pathological conditions, such as treatment with DNR. This indicates that these proteins are essential to promoting cardioprotection against DNR.
Conclusions: Decreased levels of EET by DNR was due to increased expression of sEH enzyme. This is the first study that directly accredits DNR-induced cardiotoxicity to sEH metabolic activity.

A2-2 Alyssa Schmode

Quality of online information regarding combined oral contraceptives: A content analysis

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Purpose: Combined oral contraceptives (COC) remain a popular choice among women. The Internet is an accessible and popular source of information on contraception options. The objective of this study was to evaluate the quality of information provided on COC's on the Internet.
Methods: A quantitative content analysis was completed on websites containing patient health information on COC. A Google search was completed in October 2016 using "birth control pill", "oral contraception", "oral birth control", "birth control", and "pregnancy prevention" as search terms. Websites were excluded if they were for health care professionals, forums, personal blogs, videos or news articles, the first 3 pages of results were screened. Websites in English which contained health information on COC for a lay audience were included. The DISCERN instrument was used to determine content quality. Websites were analyzed independently by two coders; discrepancies were resolved by a third coder.
Results: Of the 155 websites identified, 32 were eligible for review. Of the sites 81% mentioned contraceptive benefit, yet only 53% reported effectiveness in preventing pregnancy. Most commonly identified non-contraceptive benefits were dysmenorrhea (88%) and menstrual blood loss (84%). Breakthrough bleeding was the most common side effect listed (97%). Most common risks mentioned were VTE (81%), stroke (56%) and MI (47%), most sites failed to mention factors that increased a woman's risk. Contraindications were listed in over half of the sites. Information gaps were identified as less than half of sites mentioned COC start methods (47%) and the quick start method (25%). As well, only 22% of sites provided sufficient detail for management of missed pills. Mean total DISCERN score was 46.3(±9.37), indicating 'fair' quality.

Conclusion: Online information on COC's was variable in quality, often missing key information to make informed decisions. Health care professionals should be aware of information gaps to ensure patient counseling is tailored accordingly.

A2-3 Alisha Shivji & Raafi Ali

Real world evidence of abiraterone use post-docetaxel in metastatic castrate resistant prostate cancer

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Purpose: The COU-AA-301 trial demonstrated that in men with metastatic castrate-resistant prostate cancer, using abiraterone post-docetaxel increased overall survival. This study assessed abiraterone's performance in a real world context.
Methods: Retrospective chart review using a provincial pharmacy BDM database and a provincial electronic chart (ARIA). Dispensing data and information on the disease state before and after abiraterone were gathered.
Results: In total, 226 patients were included in this retrospective study with a median follow up of 58 months from the date of abiraterone initiation. Median survival from the start of abiraterone was 17 months [95% CI, 15, 19] for this cohort which was superior to the findings of COU-AA-301 [14.9 months]1 and a real world study done by Clayton et al. [11 months]2. Fourty-four percent of patients had a clinically documented adverse effect. Patients with an adverse effect were shown to have a 24% increased overall survival compared those who didn't [HR 0.76, p=0.048]. There was no statistically significant difference in overall survival when patients were stratified by comorbidities of hypertension and diabetes, PSAdt (prostate specific antigen doubling time), and age.
Conclusion: Abiraterone demonstrated increased overall survival in a real world setting compared to what was seen in a clinical trial. Patients dosed to a level from which they experienced adverse effects showed a better response to the drug. Support: Alberta Health Services, Johnson and Johnson Alberta Health Innovation Project (JAHIP)

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A2-4 Jonathan Thomson

Needs Assessment and Online Program for Physiotherapists in Alberta Regarding Physical Function and Drugs

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Purpose: To identify learning needs related to medications for practicing physiotherapists in Alberta, and to develop online modules to address learning needs related to common medications affecting physical function.
Methods: An online survey was compiled from existing literature regarding self-identified learning needs of health professionals, and a scoping review that was previously conducted by this research team. In addition to learning needs and practice description, demographic questions regarding physiotherapists in Alberta was obtained from the 2015 Alberta Physiotherapists Association Annual Report. The survey was administered through the University of Alberta IST department with one original email and 2 reminders at two week intervals. The survey feedback, scoping review, and Health Canada Drug and Health Products Safety Review all informed the content of the modules for the online program. A total of 4 modules were developed and guided by the

online learning format used by the Faculty of Rehabilitation Medicine, University of Alberta. The modules were presented for peer review to Rehabilitation Medicine. The study was approved by the University of Alberta Health Research Ethics Board.
Results: The survey was distributed to the 2,765 physiotherapists registered with the Alberta Physiotherapists Association. At the time of abstract preparation, there were 284 responses. The majority of responders had over 20 years of clinical experience, worked in general practice in either Edmonton or Calgary, with the most common specialty reported being orthopaedics. The responders reported being unfamiliar with statins, psychiatric medications, antihyperglycemic agents, and medications affecting the stomach (e.g. PPIs). A four module accredited online program was subsequently developed with topics including: (1) drug induced tendinopathy; (2) drug induced myalgias, arthralgias, and rhabdomyolysis; (3) physical function and drugs in the elderly; and (4) antidepressants and post-stroke motor recovery. Each module included a case study, self-assessment questions, and a final assessment quiz in multiple choice format. Conclusion: This project demonstrated that learning needs can be addressed in health professional continuing education through interprofessional collaboration.

A2-5 Xinran Yu

The Promotion and Marketing of Medical Marijuana on the Internet: A Content Analysis

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Purpose: Recent discussion around legalization of marijuana in Canada has provoked greater interest in understanding its use and safety. The Internet is popular and easily accessible for patients seeking health information to guide their decision making. Currently, there are no studies evaluating online information specific to medical marijuana in Canada. The objective of this research is to examine the website content and marketing strategies to promote the sale and use of medical marijuana.
Methods: Websites selling marijuana products in Canada were identified through Google in June 2017. Search terms included “purchase or buy” and “medical” and “marijuana, cannabis, THC, CBD, weed, or pot”. The first 3 pages of each search result were screened based on inclusion and exclusion criteria. A quantitative content analysis was conducted independently by two coders using predetermined criteria; discrepancies were resolved by a third coder.
Results: Of the 183 websites identified, 19 were eligible for review. Only 32% of websites indicated that they were authorized by Health Canada to sell marijuana and 58% required a prescription or medical documentation to purchase. A total of 104 different claims were made for medical use in 89% of websites and 25 different claims made for non-medical effects in 47% of websites. Most common medical claims included pain (79%), insomnia (68%) and anxiety (63%); most common non-medicinal claims included relaxation (47%), euphoria (32%), and increased energy (32%). Less than half of websites (47%) mentioned any side effects. The most common products sold were dried flowers/buds (90%) and oil (63%). Many websites used terms such as “natural,” “organic,” “high-quality,” and “safe” to promote their products.
Conclusion: The availability, quality and consistency of online information is highly variable between websites. Healthcare professionals should have a more active role in guiding patients in making informed decisions regarding the use of medical marijuana.

B. Graduate (less than 2 years)

B1-1 Hamdah Al Nebaihi

Determination of Lidocaine in Human Serum by Using a High Performance Liquid Chromatography

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Purpose: To develop a selective and sensitive high-performance liquid chromatographic method for the determination of lidocaine in human serum.
Methods: Extraction of lidocaine and procainamide (internal standard) from serum (0.25 mL) was achieved using diethyl ether under alkaline conditions. After liquid-liquid extraction, the separation of analytes was accomplished using reverse phase extraction. The mobile phase, a combination of acetonitrile and monobasic potassium phosphate, was pumped isocratically through a C18 analytical column. The UV wavelength was at 277 nm for internal standard, and subsequently changed to 210 for lidocaine.
Results: The assay exhibited excellent linearity (r2>0.999) in peak response over the concentration ranges of 50-5000 ng/mL lidocaine HCl in human serum. The mean absolute recoveries for 50 and 1000 ng/mL lidocaine HCl in serum using the present extraction procedure were 93.9 and 80.42%, respectively. The intra- and inter-day coefficients of variation in serum were <15% at the lowest, and <12% at other concentrations, and the percent error values were less than 9%.
Conclusions: The method displayed high calibers of sensitivity and selectivity for monitoring therapeutic concentrations of lidocaine in human serum.

B1-2 Ahmad Alamarri

Fluconazole protects against angiotensin II-induced cellular hypertrophy through the modulation of cytochrome P450 and their associated arachidonic acid metabolites

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Purpose: Several studies have elucidated the role of cytochrome P450s (CYPs) and their associated arachidonic acid (AA) metabolites in development of cardiac hypertrophy. For example, CYP1A1 has been reported to metabolize AA into a cardioprotective 19-hydroxyeicosatetraenoic acid (HETE) metabolite, whereas CYP3A plays an important role in the formation of cardiotoxic metabolites, mid-chain HETEs. Recently, fluconazole was shown to inhibit the formation of mid-chain HETE metabolites. However, whether fluconazole would be able to attenuate angiotensin II (Ang II)-induced cellular hypertrophy through the modulation of CYPs and their associated AA metabolite has never been investigated before. Therefore, the overall objectives of the present study are to explore the potential antihypertrophic effect of fluconazole and to unravel the molecular mechanism involved.
Methods: For this purpose, rat cardiomyoblasts, H9c2 cell line, and human ventricular cardiomyocytes, RL-14 cell line, were treated with 10 μM Ang II in the absence and presence of 50 μM fluconazole. Thereafter, the formation of AA metabolites was measured using liquid chromatography-electron spray ionization-mass spectrometry (LC-ESI-MS). Whereas, the expression of hypertrophic markers and CYP genes were determined by real time-polymerase chain reaction.
Results: Our results demonstrated that fluconazole significantly attenuated Ang II-induced cellular hypertrophy as evidenced by a significant inhibition of hypertrophic markers, β-myosin heavy chain (MHC)/ α-MHC. Of interest, the protective effect of fluconazole was associated with a significant increase in the formation level of cardioprotective 19-HETE metabolite while it decreased the formation of cardiotoxic mid-chain HETE metabolites through the induction of CYP1A1 and the inhibition of both CYP3A4 in RL-14 cells and CYP3A2 in H9c2 cells genes expression, respectively.
Conclusion: Fluconazole attenuates Ang II-induced cellular hypertrophy through the modulation of CYPs and their associated AA metabolite in vitro. Support: This work was supported by a grant from the CIHR to A.O.S.E.

B1-3 Abdulsalam Alharbi

Development of Novel Polymeric Micellar DACHPt for Enhanced Platinum Based Chemotherapy in Colorectal Cancer

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Purpose: The parent compound of Oxaliplatin, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt) is a potent chemotherapeutic agent with wide spectrum of anticancer activity, lower side effects and no cross-resistance with cisplatin in many cisplatin-resistant cancers. The objective of this project is to develop polymeric micelles of DACHPt with potential for enhanced platinum based chemotherapy of colorectal cancer.
Method: Poly(ethylene oxide)-b-poly-(α-carboxylate-€-caprolactone) (PEO-b-PCCL) diblock copolymer was synthesized. Then, DACHPt was reacted with PEO-b-PCCL to form polymer-metal complexes. The complex was dialyzed in water to prepare DACHPt loaded micelles. Prepared polymeric micelles were then characterized for their average diameter by dynamic light scattering, level of complexed DACHPt by ICP-MS and in vitro release of Pt compared to free drug by ICP-MS. Cytotoxicity of DACHpt micelles against human colorectal cancer cell lines, HCT-116 and SW-620 was evaluated following 24-72 h incubation and compared to free drug cytotoxicity by MTT assay.
Results: Drug loading in polymeric micelles reached high levels of 50.2% w/w on average. Polymeric micellar complexes of DACHPt were found to have a mean diameter of 56 nm. The release of Pt from micelles was sustained. Only 53.6% of Pt was released from micelles after 120 h compared to free drug that released 96.5 % of Pt after 7.5 h. The IC50 of polymeric micellar DACHPt was higher than that of free drug in HCT116 cells in all incubation times (12.86 to 288.7 ug/ml for micelles versus 1.9 to 92.4 ug/ml for free drug at 24-72h incubation). In Sw-620 cells an opposite trend was observed (15.11 to 36.4 ug/ml for micelles versus 94.7 to 120.4 ug/ml for free drug at 24-72 h incubation).
Conclusion: The results point to a great potential for PEO-b-PCCL/DACHPt polyplex micelles for enhanced Pt based chemotherapy in colorectal cancer.
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Arabia. This research was supported by grants from NSERC.

B1-4 Malak Almutairi

The GLP-1R Agonist Liraglutide Increases Myocardial Glucose Oxidation Rates via Indirect Mechanisms

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Purpose: Type 2 diabetes (T2D) is associated with an increased risk for cardiovascular disease. Of interest, liraglutide, a therapy for T2D that activates the glucagon-like peptide-1 receptor (GLP-1R) to augment insulin secretion, reduces death by cardiovascular causes in patients with T2D. However, it remains enigmatic as to how liraglutide may reduce cardiovascular death in patients with T2D. Importantly, the GLP-1R is not expressed in ventricular cardiac myocytes, so it is likely that indirect actions on peripheral tissues are involved. We hypothesized that augmented insulin secretion is a key factor contributing to liraglutide-induced cardioprotection, which thereby increases cardiac glucose oxidation.
Method: 10-week-old C57BL/6J male mice were treated with either saline or liraglutide (30 □g/kg via subcutaneous injection) 3x over a 24-hr period. 2-hrs following the final injection, all mice were euthanized and had their hearts perfused in the working mode to assess glycolysis and glucose oxidation rates. A separate cohort of mice were euthanized and had their hearts perfused in the working mode under similar conditions, but these hearts were directly treated with either saline or liraglutide (10 nM). At the end of perfusion, all hearts were immediately snap frozen in liquid N2 and subjected to western blot analysis to assess insulin signaling.
Results: Systemic treatment with liraglutide increased myocardial glucose oxidation rates without affecting glycolysis rates. Conversely, direct treatment of the isolated working heart with liraglutide had no effect on

glucose oxidation. Furthermore, systemic treatment with liraglutide augmented insulin signaling in the heart, whereas direct treatment of the isolated working heart was devoid of such an effect.
Conclusion: Our data demonstrates that liraglutide-mediated increases in cardiac glucose oxidation are indirectly mediated. These results are consistent with negligible GLP-1R expression in ventricular cardiac myocytes, and may represent a potential indirect mechanism of GLP-1R agonist-induced cardioprotection.

B1-5 Ahmed Darwesh

Omega-3 Fatty Acids and Ischemia-Reperfusion Injury

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Purpose: Ischemic injury is a leading cause of morbidity and mortality in individuals who suffer from cardiovascular disease (CVD). Evidence has demonstrated dietary supplementation with long-chain n-3 "omega-3" polyunsaturated fatty acids (PUFA) is protective toward CVD, yet the mechanisms remain unknown. Ecosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n3, DHA) are the main n-3 PUFAs derived from food sources. Interestingly, cytochrome P450 (CYP) epoxygenases can metabolize EPA into 5 regioisomeric epoxyeicosatetraenoic acids (5,6-, 8,9-, 11,12-, 14,15-, 17,18-EEQ) and DHA into 6 regioisomeric epoxydocosapentaenoic acids (4,5-, 7,8-, 10,11-, 13,14-, 16,17-, 19,20-EDP). These bioactive lipid mediators are known to affect numerous biological functions and provide cytoprotection, but the molecular and cellular cardioprotective roles remain unknown. The current study investigated the different cardioprotective effects of EPA and DHA, and their metabolites, 17,18-EEQ and 19,20-EDP, against ischemia-reperfusion (I/R) injury.
Methods: Langendorff ex vivo isolated hearts were used to assess and compare the cardioprotective effects. Briefly, hearts were perfused for 40 min of baseline, subjected to 30 min of global no flow ischemia followed by 40 min of reperfusion. Hearts were perfused with vehicle or n-3 PUFAs 20 min before ischemia until the end of the experiment. The percentage of left ventricular developed pressure (%LVDP) at 40 min of reperfusion (R40), as compared to baseline LVDP, was taken as a marker for recovery of contractile function.
Results: Hearts perfused with DHA or 19,20-EDP showed significant cardioprotective effects demonstrated by improved postischemic functional recovery. While hearts perfused with EPA or 17,18-EEQ, did not show any significant improvement in postischemic recovery of LVDP. However, hearts perfused with EPA or 17,18-EEQ demonstrated better cardiac rhythmicity.
Conclusion: Our data indicate there is a differential cardioprotective response between DHA and EPA toward IR injury. Where DHA and 19,20-EDP improve postischemic recovery of LVDP, EPA and 17,18-EEQ suggest anti-arrhythmic properties.

B1-6 Amina Eshreif

The Nutraceutical L-Citrulline Improves Exercise Capacity and Glucose Tolerance in Obese Mice

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Purpose: L-citrulline is an organic α-amino acid nutraceutical that has been shown to augment exercise/muscle performance in animals and humans, which may be due to elevations in mitochondrial function. It has been proposed that enhancing mitochondrial function may mitigate obesity-related insulin resistance, and thus our goal was to determine whether treatment with L-citrulline could improve glycemia in an experimental mouse model of obesity. We hypothesized that L-citrulline treatment would improve glucose homeostasis in obese mice, and this would be associated with elevations in mitochondrial function in skeletal muscle.
Methods: 10-week old C57BL/6J mice were fed either a low-fat (10% kcal from lard) or high-fat (60% kcal from lard) diet, while receiving drinking water supplemented with either vehicle or L-citrulline (100 mg/kg) for 15-

weeks. Glucose homeostasis was assessed via glucose/insulin tolerance testing, while in vivo metabolism was assessed via indirect calorimetry. Mice were run on a forced exercise treadmill to assess endurance, real-time PCR was utilized to measure gene expression, and insulin signaling was assessed via western blot.
Results: As expected, obese mice supplemented with L-citrulline exhibited an increase in exercise capacity, and this was associated with an improvement in glucose tolerance. Consistent with augmented mitochondrial function, we observed an increase in whole body oxygen consumption rates in obese mice treated with L-citrulline. In addition, mRNA expression of mitochondrial transcription factors was increased in muscles from L-citrulline treated obese mice. On the contrary, L-citrulline treatment worsened insulin tolerance, and reduced insulin signaling in both lean and obese mice.
Conclusion: Taken together, L-citrulline supplementation improved both glucose tolerance and exercise capacity in obese mice, supporting the use of L-citrulline as a nutraceutical to augment performance. Nevertheless, the deterioration in insulin sensitivity following L-citrulline supplementation suggests that we should exercise caution with its broad use as a potential nutraceutical.

B1-7 Tianhua Feng

Modeling dynamic structure and investigating properties of the CaV1.2

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Purpose: Voltage-gated calcium channels (CaVs) are widely distributed in the human body and remain essential for normal cardiac functioning. Any abnormalities in the functioning of CaVs results in serious diseases, for example, cardiac arrhythmias and Timothy syndrome. Thus, CaVs are considered as one of the promising targets for curing these diseases. Diverse small molecules, such as Dihydropyridines, Phenylalkylamine, and Benzothiazepine have been identified to modulate the activity of CaVs. However, their mode and site of action are still unknown. In order to understand how the drugs bind and act on CaV, it is necessary to have a high-quality structure of the human CaV channel. However, this has not been resolved yet. Therefore, we have used computational molecular modeling to model the complete three-dimensional (3D) structure of the CaV channel and studied the binding orientation and key interactions that stabilize the known modulators.
Methods: For modeling the 3D structure of the human CaV channel, homology modeling and threading-based techniques were used. The small molecular binding and interactions were identified using molecular docking analysis. Classical molecular dynamics simulations were carried out to predict the binding affinities of the modulators towards the CaV.
Results: The mode-of-binding of Amlodipine and Verapamil were identified. Their relative binding affinities calculated using the MM-PBSA approach shows that both Amlodipine and Verapamil has high potential to block the CaV channel.
Conclusions: The interactions reported from our binding mode analysis will be useful for guiding future drug design and the method employed can be used for screening molecules with the ability to bind CaV.

B1-8 Fizza Gilani

Factors Predicting Pneumococcal Vaccination a Cohort of 2040 Albertans with Type 2 Diabetes

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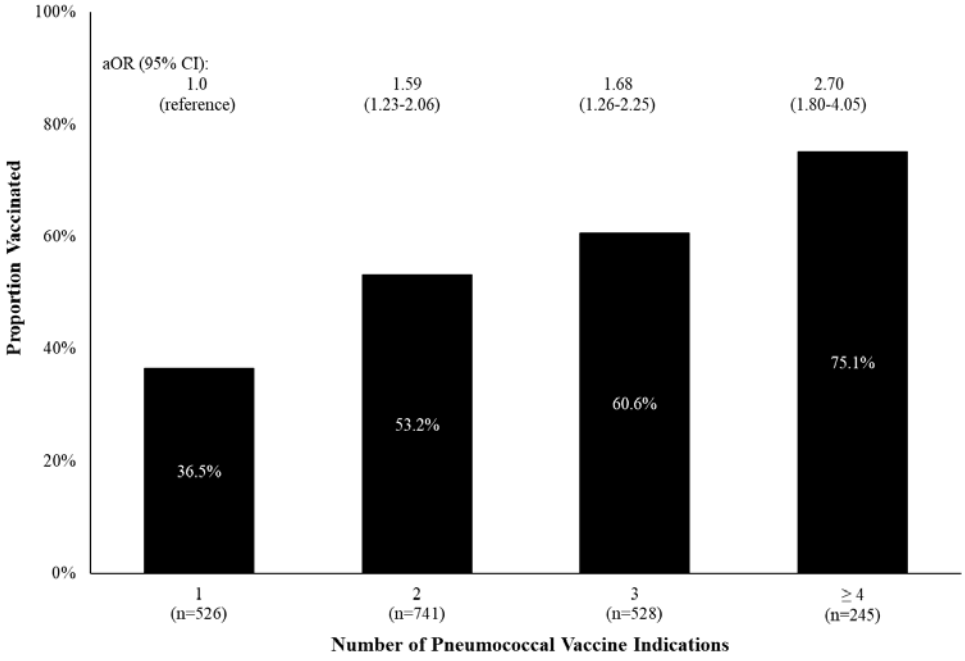
Background: Guidelines recommend pneumococcal vaccination for people with diabetes because they are at high risk of morbidity (e.g. severe pneumonia, meningitis) and mortality from this infection. Although we know vaccine uptake is not ideal, reasons behind the low rate are unknown. We examined data from the Alberta

Caring for Diabetes cohort, which contained information on socioeconomic characteristics, self-management activities, clinical monitoring activities, co-morbidities, health status measures, and health service utilization.

Methods: Multivariable logistic regression analyses were conducted to identify factors associated with self-reported pneumococcal vaccination status. Mean age of the 2,040 participants was 64.4 (±11) years, 45% were women, mean duration of diabetes was 12 (±10) years, and 1,090 (53%) reported receiving a pneumococcal vaccine.

Results: Pneumococcal vaccination rates were strongly related to the number of indications (age ≥65 years, chronic [diabetes, cardiovascular, pulmonary, renal] disease, cancer, smoking, and excessive alcohol use: Figure). After multivariable analysis, age ≥65 years (adjusted odds ratio (aOR) 2.38; 95% CI 1.82-3.11), pulmonary disease (aOR 1.41; 95% CI 1.08-1.84), and cancer (aOR 1.43; 95% CI 1.06-1.93) were independently associated with vaccination. In addition to these factors, women (aOR 1.41; 95% CI 1.14-1.76) and retirees (aOR 1.39; 95% CI 1.09-1.78) were more likely to have been vaccinated.

Conclusion: This information will help shape future programs aimed at improving pneumococcal vaccine uptake in people with diabetes.



B2-1 Kim Ho

Ketone bodies can provide additional energy for the diabetic failing heart without compromising cardiac efficiency

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Purpose: With global excitement surrounding the class effect of SGLT2 inhibitors improving cardiovascular outcomes, it remains unclear how the anti-hyperglycemic agent, empagliflozin, confers its cardiovascular

benefits to the diabetic heart. In addition to decreasing blood glucose levels, empagliflozin also increases circulating ketone (acetoacetate and ß-hydroxybutyrate (ßOHB) levels. Therefore, empagliflozin has been proposed to optimize cardiac energy metabolism and defer the heart's substrate preference from fatty acids and glucose, to ketones. However, it remains enigmatic as to whether increasing myocardial ketone utilization is actually beneficial for the diabetic heart. Consequently, the aim of our study was to assess the metabolic profile and cardiac efficiency of the diabetic heart in response to perfusion with ketones.
Methods: Diabetic (*db/db*) 23-week-old male mice and lean, age-matched C57BL/6J mice were euthanized and had their hearts extracted as isolated working heart preparations. Isolated working hearts were perfused for 60 minutes with appropriately radiolabelled glucose (5mM), palmitate (0.8mM), and ßOHB (0mM or 0.6mM) to assess glycolysis rates, and ketone, fatty acid and glucose oxidation rates, with 100□U/mL insulin added 30 minutes into perfusion. The pulmonary artery was also cannulated during these isolated working heart perfusions, allowing for the evaluation of myocardial oxygen consumption rates and cardiac efficiency.
Results: The inclusion of ßOHB in the perfusate did not affect cardiac function and work in isolated hearts from *db/db* mice. Likewise, db/db hearts perfused with and without ßOHB had similar rates of glucose oxidation, palmitate oxidation and glycolysis. However, the addition of ßOHB increased the total amount of energy (ATP) produced independent of oxygen consumption. Consequently, ketones did not improve cardiac efficiency.
Conclusions: Ketones are not a more or less efficient fuel substrate for the diabetic heart, but can still be used to increase total energy production, which may have clinical utility in cardiovascular disease.

B2-2 Md Harunur Rashid

Mechanisms of Clozapine induced toxicity and oxidative stress.

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Purpose: Clozapine (dibenzodiazepine) is an important antipsychotic drug that is primarily used in patients with treatment-resistant schizophrenia. Generally, clozapine is effective in relieving symptoms in the short term but long-term use of clozapine is associated with side effects such as the risk of moderate neutropenia or severe neutropenia (agranulocytosis) - potentially lethal. As such, patients require bimonthly blood counts in order to be kept on clozapine therapy. A comprehensive understanding of the mechanisms of this side effect would be benefit to these patients. Clozapine is metabolized in neutrophils to a free radical, which then rapidly forms an electrophilic species - nitrenium ion - that generates oxidative stress and may covalently bind to critical targets in neutrophils which may be involved in the toxicity. Dihydronicotinamide riboside (NRH) quinone oxidoreductase 2 (NQO2) 1541AA was found to be present in higher frequency in patients than control. NQO2 is considered an antioxidant enzyme that detoxifies quinones; as such, the mechanistic association with clozapine is unknown. NQO2 and its related enzyme, NQO1, are also known to reduce nitroaromatic compounds, which are also electrophilic to some degree. As the clozapine nitrenium ion is an electrophile, it is not known if it could be a substrate for NQO1/2. Therefore, we hypothesize that the clozapine nitrenium ion can be a substrate for NQO1/2 that will confer cellular protection. This study will involve a combination of in vitro experiments and clinical sample analysis. Firstly, the study will involve biochemical toxicity studies of clozapine using in-vitro cell-based systems by investigating oxidative stress endpoints in cells (HL-60, neutrophils). Next, the studies will involve the determination of biomarkers in schizophrenic patients that are treated with the clozapine. The overall conclusion of this study would be better understanding the clozapine-induced agranulocytosis as well as methods to curtail side effects.

B2-3 Yan Rong

Factors influencing mycophenolic acid exposure in de novo, steroid-free adult kidney transplant recipients

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Purpose: Mycophenolic acid (MPA) is commonly dosed "empirically" in combination with calcineurin inhibitors (e.g. tacrolimus (FK)) and corticosteroids for preventing graft rejection in kidney transplant recipients. Moreover,

there is an increasing trend in prescribing steroid-free regimens for patients with reduced rejection risk. Because traditional MPA pharmacokinetic studies have, in majority, been conducted in the presence of steroids (known to affect MPA disposition), little information is available on patient variables that can influence the pharmacokinetics, hence the dosing, of MPA in steroid-free subjects. The purpose of this study was to determine the associations between commonly collected patient demographic variables/blood biochemistries and MPA exposure (calculated from limited sampling strategy (LSS) for the estimation of area-under the concentration-time curves (AUCs)) in adult kidney transplant patients taking steroid-free regimens.
Methods: Following consent, the following data were collected (N=21, 1-month post-transplant): sex, age, weight, height, serum creatinine (SrCr), albumin level, MPA concentrations at 1, 2, 4 hours post dose (for MPA AUC determination), and FK trough concentrations. Data were analyzed with SigmaStat (version 3.5) and results deemed significant if $p < 0.05$.
Results: The parameter values in our study sample were: sex (N=10 females, 11 males), age (54±12 years old, mean±SD), weight (73.7±21.2 kg), height (169.6±10.2 cm), SrCr (114.1±23.9 µmol/L), albumin (36.8±2.9 g/L), LSS-determined MPA exposure (23.2±6.9 mg·h/L/g), and FK (10.2±1.9 µg/L). Linear regression analyses of all continuous variables indicated that only "weight" exhibited a significant inverse correlation with MPA exposure ($r2=0.51$, $p<0.001$). Male subjects also had reduced MPA AUC compared to females (18.9±4.9 vs. 27.9±5.7 mg·h/L/g, $p<0.001$). Multiple regression analysis incorporating all variables confirmed that weight was the only significant predictor of MPA exposure ($r2=0.68$, $p=0.04$).
Conclusions: Our novel observation of significant correlation between weight and MPA exposure in steroid-free kidney transplant recipients suggests that a "personalized" dosing strategy may be warranted.

B2-4 Sams Sadat

Nano-delivery of novel inhibitors of polynucleotide kinase/phosphatase (PNKP) for targeted inhibition of DNA repair in colorectal cancer: In vitro and in vivo studies

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Purpose: Polynucleotide kinase/phosphatase (PNKP) is a key enzyme involved in repairing DNA following damage by radiation or topoisomerase-I inhibitor treatments. Our research team has identified potent inhibitors of PNKP as potential new drugs for colorectal cancer when combined with chemo or radiotherapy. Specific population of cancer cells with deficiency in phosphatase and tensin homolog (PTEN) expression, are also shown to be sensitive to monotherapy with PNPK inhibitors. Our objective was to develop nano-carriers of lead inhibitors of PNKP that can potentiate their effects on colorectal tumors while alleviating their side effects on normal cells.
Methods: A cellular thermal shift assay (CETSA) was developed to assess the binding capacity of lead PNKP inhibitors (H5 and A83) to intracellular PNKP in wild type (HCT116/PTEN+/+) and PTEN-negative (HCT116/PTEN-/-) colorectal cancer cells. The more potent PNKP inhibitor, A83, was either encapsulated in poly(ethylene oxide)-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-PBCL) nanoparticles or dissolved in water with the aid of Cremophor EL: Ethanol. Maximum tolerable dose (MTD) of free versus nanoparticles of A83 was determined in healthy CD-1 mice following three intravenous (IV) injections at a dose range of 2.5-50 mg/kg (n=4). The anti-cancer activity of both formulations was also determined in HCT116/PTEN-/- xenograft in NIH-III nude mice (n=5) after three IV injections of A83 at a dose of 10 mg/kg.
Results: CETSA showed more potent binding of A83 over H5 to intracellular PNKP. The MTD study revealed no significant loss in weight gain or change in biochemical indicators of toxicity in animals receiving A83 or those receiving saline. The nanocarriers of A83 reduced the rate of HCT116/PTEN-/- xenograft growth in NIH-III mice more efficiently (by 2 folds) than free drug.
Conclusion: The results point to A83, particularly as nanoparticle formulation, as a potential new treatment for colorectal cancer.

B2-5 Sherif Shoieb

R/S enantiomers of 19-hydroxyeicosatetraenoic acid differentially inhibit the formation of mid-chain hydroxyeicosatetraenoic acids in human cardiomyocytes, RL-14 cells

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Purpose: to investigate the differential effects of R- and S- enantiomers of 19-hydroxyeicosatetraenoic acid (19-HETE) on the cardiac cytochrome P450 enzymes as well as their associated arachidonic acid (AA) metabolites.
Methods: Human ventricular cardiomyocytes RL-14 cells were treated with vehicle or 20 μM of 19(R)-HETE or 19(S)-HETE in serum-free media for 24 h. The levels of mid-chain HETEs, terminal/subterminal HETEs and epoxyeicosatrienoic acid were determined using liquid chromatography-mass spectrometry (LC/MS). The level of gene expression was measured using real-time PCR. Thereafter, Western blot analysis was performed to assess the protein levels of different enzymes.
Results: The data showed that both (R)- and (S)-19-HETE significantly decreased the metabolite formation rate of mid-chain HETEs namely 15-, 12-, 8- and 9-HETE compared to the control group while the level of 5-HETE was significantly decreased solely by the S-enantiomer. Gene expression, Western blot analysis and enzymatic catalytic activity assay showed both (R)- and (S)-19-HETE significantly inhibited the catalytic activity of CYP1B1 enzyme, decreased the protein expression levels of 5- and 12-lipoxxygenase (LOX) and significantly decreased the level of cyclooxygenase-2 (COX-2). Notably, the decrease in the level of 15-LOX was only mediated by 19(S)-HETE. Moreover, 19(S)-HETE significantly induced protein levels of both CYP4F2 and CYP4F11 which are implicated in mid-chain HETEs' metabolism.
Conclusions: Our findings provide the first demonstration that both (R)- and (S)-19-HETE significantly decreased the levels of mid-chain HETEs via inhibition of catalytic activity of CYP1B1 enzyme and decreasing protein expression of LOX and COX-2 enzymes. Moreover, the decrease in mid-chain HETEs levels could be attributed to 19(S)-HETE-mediated induction of both CYP4F2 and CYP4F11 enzymes. Our data suggest that 19(S)-HETE has preferential effect in inhibiting the formation of mid-chain HETEs.
Support: This work was supported by a grant from the CIHR to A.O.S.E. S.M.S. is the recipient of Mike Wolowyk Graduate Scholarship.

B2-6 Yeon Kyoung Suh

Role of inflammasome in dipyrone-mediated agranulocytosis

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Purpose: Drug-induced agranulocytosis (DIAG) leads to withdrawal of many clinically important medications from the market. Unfortunately, its idiosyncratic nature and unclear mechanism have made pre-clinical screening very challenging. Many drugs associated with agranulocytosis are known to be oxidized to reactive metabolites by neutrophils. Small reactive molecules can covalently bind to neutrophil membrane proteins, triggering anti-neutrophil antibody production and T-cell responses against neutrophils and neutrophil progenitors. Recent evidence suggests cellular damage inflicted by reactive metabolites of DIAG-associated drugs can cause release of danger-associated molecular patterns (DAMPs) and stimulate inflammasome activation. Inflammasomes are macromolecular complexes that are required for activation of caspase-1 and for the subsequent maturation of pro-inflammatory cytokines IL-1β and IL-18. Dipyrone is a non-opioid analgesic and antipyretic pyrazolone derivative. Currently, the drug has been withdrawn in most developed countries as the risk of blood dyscrasias has become apparent. Dipyrone-induced agranulocytosis is explained by immune-mediated mechanism initiated by modification of the drug to reactive metabolite. However, the role of inflammasome in the mechanistic pathway has not yet been described. Aminopyrine is another analgesic pyrazolone withdrawn due to high risk of agranulocytosis. We postulate that dipyrone and aminopyrine are converted to reactive metabolites by endogenous oxidants, and trigger inflammasome activation in THP-1 human monocytes and HL-60 human neutrophils. The aim of this study is to investigate the implication of inflammasome in dipyrone- and aminopyrine-induced agranulocytosis. IL-1β and IL-18 secretion is a hallmark of

inflammasome activation and will be measured by ELISA. In addition, important steps of intracellular inflammasome pathway will be examined, including cleavage of pro-IL-1β and pro-IL-18 into their mature forms, cleavage of pro-caspase-1 into caspase-1, upregulation of pro-IL-1b and NLRP3 mRNA and proteins, and NF-κB activation. Potential activation of inflammasome by dipyrone and aminopyrine would provide broader insights to the common mechanism of DIAG.

B2-7 Cassandra Woit

Healthcare practitioner prescribing: a scoping review about the confidence and competence of pharmacists and physicians

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Purpose: Prescribing is an expected activity for physicians, however it is a relatively new role for pharmacists. The scope of pharmacists in Canada is rapidly expanding to include both supplemental and independent prescribing. Supplemental prescribing is in partnership with physicians and independent prescribing, specifically in Alberta for pharmacists with Additional Prescribing Authorization, includes initial access prescribing and managing ongoing therapy. Both forms of prescribing have been used in many countries including the United States of America, the United Kingdom and Australia. While there are studies investigating the confidence and/or competence of medical students and physicians, there are few investigating the same for pharmacy students or pharmacist prescribers. It is even less well known how the two professions compare in prescribing.
Methods: Sources: Medline from inception to January 2017; reference lists of included studies will also be reviewed. Inclusion criteria: Studies describing either the confidence and/or competence of pharmacist and physician prescribing, including students and recent graduates. Studies describing the views of pharmacists or physicians on prescribing were also included. Two reviewers independently screened titles and abstracts. Full review of potential papers was also done by two independent reviewers. Included studies were reviewed qualitatively for themes.
Results: Out of 1611 unique records, 132 articles were reviewed in full for eligibility. Initial review of data shows the majority of research has been done with the medical profession. In addition, confidence and competence are not always well linked; ie. high confidence can occur with low competence. The pharmacy-related research shows that confidence is generally low.
Conclusions: Preliminary data shows a majority of articles focusing on physician prescribing, often including prescribing errors. This emphasizes the gap in literature surrounding pharmacist prescribing, and the need to evaluate the confidence and competence of new prescribers.

C. Graduate (experienced; more than 2 years)

C1-1 Hanin Aburasayn

Paternal Obesity Differentially Affects Body Weight and Glycemic Control in Male and Female Offspring

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Purpose: As the risk of obesity is increased among children born to obese parents, our aim was to determine if obesity in the father at the time of conception produces an increased risk of obesity and metabolic dysfunction in the offspring.
Methods: 8-week-old C57BL/6J male mice fed a high fat diet (HFD, 60% kcal from lard) for 6-weeks to induce obesity, following which they were mated with age-matched lean females. The offspring were weaned onto either a low-fat diet (LFD, 10% kcal from lard) or HFD and monitored until they reached 15-weeks of age. Magnetic resonance imaging, indirect calorimetry, and glucose/insulin tolerance tests were performed in the offspring to assess adiposity, energy metabolism, and glucose homeostasis.
Results: Offspring fed a LFD and born to an obese father exhibited heavier body weights at weaning compared to their counterparts born to a lean father, though no differences in fat mass or lean mass were observed. In addition, male offspring born to an obese father exhibited worse glucose tolerance despite being more active than their offspring counterparts born to a lean father. Of interest, female offspring born to an obese father and weaned onto a HFD demonstrated significantly heavier body weights and fat mass as they aged versus their counterpart offspring, while indirect calorimetry revealed that they also consumed more oxygen despite being less active. These observations were not recapitulated in male offspring born to an obese father and weaned onto a HFD.
Conclusions: Paternal obesity appears to differentially affect body weight regulation, energy metabolism and glucose homeostasis in male and female offspring, regardless if they are provided a LFD or HFD throughout development.

C1-2 Hanan Al Lawati

Pharmacokinetics and pharmacodynamics of traceable polymeric micellar diclofenac in experimental arthritis

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Purpose: A fluorescently-tagged polymeric micellar formulation of diclofenac (DF), a model of cardiovascular (CV) toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) was developed by encapsulating DF ethyl ester (DFEE) to test the hypothesis that a reduced heart exposure to NSAIDs improves CV safety profile. CYP 450 mediated metabolites of arachidonic acid (ArA) concentrations were measured as markers of cardiotoxicity.
Methods: DFEE was encapsulated in traceable (Cy5.5 labeled) methoxypoly(ethylene oxide)-block-poly(ε-caprolactone) (PEO_{5000}-*b*-PCL_{3000}) micelles (DFEE-TM). Biodistribution, efficacy and cardiotoxicity of DFEE-TM and free DF were compared following multiple ip administration to adjuvant arthritic (AA) rats of 10 mg/kg/day DF equivalent for 7 days (n=6).
Results: Both free DF and DFEE-TM resulted in a rapid reduction in the signs of AA. Moreover, histopathological assessment showed that the DFEE-TM as well as the free DF ameliorated the inflammatory cell infiltration observed in AA heart and kidney. DF was found in significantly lower concentrations in the heart following DFEE-TM administration as compared with free drug (1.5±0.6 vs 2.8±0.6 μg/g). Comparable concentrations were found in the kidneys, liver, and spleen between the two formulations. DFEE-TM yielded significantly lower cardiotoxic metabolic profile of ArA in various tissues when compared to free DF (e.g., 20 HETE in heart 0.20±0.01 vs 0.48±0.07 μg/g; in plasma, 41±6 vs 143±18 μg/L, respectively).

Conclusions: DF delivery by PEO_{5000}-*b*-PCL_{3000} micelles encapsulating DFEE provide improved biodistribution of DF in rats with AA including a reduced accumulation in the cardiac tissue. Moreover, micelles provided an effective therapy with reduced extent of the imbalance in eicosanoids of ArA that are attributed to DF, and are known to be associated with increased CV risks.
Support: HA and SA were supported by the ministries of higher education of Oman and Libya, respectively. Research was supported from a Self-Funded grant from University of Alberta and NSERC.

C1-3 Zuhair Al-Qahtani

Aspirin and Other Non-Steroidal Anti-Inflammatory Drugs Interactions; Systematic Review and Meta-Analysis
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Purpose: There is a controversy regarding the therapeutic relevance of acetylsalycilic acid (Aspirin) and other non-steroidal anti-Inflammatory drugs (NSAIDs) depending on the study design. Many clinical trials and epidemiological studies suggest reduced cardiovascular risk for NSAIDs if used along with low dose Aspirin. In the meantime, in vitro and ex vivo studies suggest that NSAIDs may mask the cardiovascular benefits of Aspirin. We, therefore, carried out a comprehensive systematic search and meta-analysis to assess the cardiovascular risks of concomitant use of NSAIDs and aspirin.
Methods: We searched many databases up to August 2017 using the following keywords: acetylsalicylic acid, aspirin, NSAIDs, nonselective NSAIDs, cyclooxygenase-2, NSAID/aspirin interaction, adverse effects and platelet effects. Titles and abstracts of included studies were retrieved and screened independently by two reviewers to identify potentially relevant studies. The combined odds ratio values (OR; 95% CI) were calculated using the random-effect meta-analysis model.
Results: In total, 47 eligible studies (42 laboratory studies and 7 randomized controlled trials) on the interactions of aspirin and NSAIDs were found. in vitro studies suggest that the antiplatelet effect of aspirin is diminished when combined with some NSAIDs like ibuprofen, naproxen, refecoxib, proxicam and mefenamic acid. Nevertheless, the risk of myocardial infarction or stroke was as compared among patients who used ibuprofen, naproxen, diclofenac, etodolac, or piroxicam with aspirin compared to aspirin alone.
Conclusion: There does not seem to be an increased risk of myocardial infarction or stroke among patients who use long term aspirin and other NSAIDs concomitantly compared with aspirin alone. Support: King Saud University, Riyadh, Saudi Arabia and Self-Funded grants from University of Alberta, Edmonton, Canada.

C1-4 Zaid Almaayah

2-Methoxyestradiol protect against cardiac hypertrophy induced by abdominal aortic constriction
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Purpose: Numerous experimental studies have supported the evidence that 2-methoxyestradiol (2ME) is a biologically active metabolite and mediates multiple effects on the cardiovascular systems that are largely independent of the estrogen receptor. 2ME is a major cytochrome P450 1B1 (CYP1B1) metabolite and has been reported to have vasoprotective and anti-inflammatory actions. However, whether 2ME would prevent cardiac hypertrophy induced by abdominal aortic constriction (AAC) has not been investigated yet. Therefore, the overall objectives of the present study are to elucidate the potential antihypertrophic effect of 2ME and to explore the mechanism(s) involved.

Method: For this purpose, Sprague–Dawley rats were subjected to either sham or abdominal aortic constriction surgery to induce cardiac hypertrophy and treated with 2ME or vehicle. Thereafter, cardiac hypertrophy parameters were determined using echocardiography whereas, protein expression level and the formation of mid-chain hydroxyeicosatetraenoic acid (mid-chain HETEs) were measured using western blot analysis and liquid chromatography-electron spray ionization-mass spectrometry, respectively.
Results: Our results showed that 2ME significantly inhibited cardiac hypertrophy induced by AAC as evidenced by a decrease in the heart weight to tibial length ratio and left ventricular morphology such as an intraventricular septum, left ventricular internal diameter and left the ventricular posterior wall. The antihypertrophic effect of 2ME was accompanied by a significant inhibition of CYP1B1 and its associated cardiotoxic metabolites mid-chain HETEs. Mechanistically, the protective effect of 2ME against AAC-induced hypertrophy was mediated through a significant modulation of the phosphorylated p38 and extracellular-regulated kinases1/2 (ERK1/2) signaling pathway.
Conclusion: Our study provides the first evidence that 2ME prevents cardiac hypertrophy induced by AAC through CYP1B1 and mid-chain HETEs-dependent mechanisms. Support: This work was supported by a grant from the CIHR to A.O.S.E. Z.H.M. is the recipient Izaak Walton Killam and Alberta Innovates-Health solution Scholarships.

C1-5 Valentina Back

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

C1-6 Francesco Gentile

Computational drug design of small molecule inhibitors for the XPF-ERCC1 heterodimer

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Purpose: the ERCC1-XPF heterodimer is a 5'-3' structure-specific endonuclease, essential for the nucleotide excision repair (NER) and inter-strand crosslink (ICL) DNA repair pathways. Its activity is critical to maintain genome integrity and stability and prevent against damage-induced mutations. However, it can counteract the effect of DNA damaging therapies such as platinum-based chemotherapy and radiotherapy. Therefore, the inhibition of the XPF-ERCC1 action in cancer cells using small molecules is a promising strategy to enhance the effect of DNA-damaging cancer therapies.
Methods: we design a computational drug design workflow to rationally design novel analogues of the F06 molecule, a lead inhibitor which targets the dimerization between XPF and ERCC1, essential to create the functional endonuclease, by binding to a binding site on the XPF C-terminal domain. The top hits resulting from our screening were synthesized and tested in protein and cell-based assays.
Results: seven compounds were synthesized, selected based on the *in silico* predicted binding affinities and ligand efficiencies towards the XPF dimerization domain. This approach yielded compound 3 and 4 as potent inhibitors of ERCC1-XPF activity. An *in vitro* ERCC1-XPF endonuclease assay identified compound 4 as the best ERCC1-XPF inhibitor with an IC_{50} value of 0.3 μM. Also, the Kd value of this compound was measured as ~100 nM by fluorescence spectroscopy. Compound 4 also showed a significant inhibition of the removal of cyclobutane pyrimidine dimers (CPDs) compared with control cells after exposure of HCT 116 cells to UV radiation.
Conclusions: our approach led to compound 4, which can potentially be used in combination with existing DNA-damaging therapies to enhance their effects on cancer cells.
Support: Alberta Cancer Foundation – Transformative Program (2015-2018) 26603.

C1-7 Nasim Ghasemi

Towards Development of Biodegradable Chemically Crosslinked Hydrogels for Stimulus Responsive Drug Delivery

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Purpose: The long-term objective of this study is to develop biodegradable stimuli-responsive gels with optimal properties for depot and smart drug delivery. In this study, we developed the method of polymerization for synthesis of triblock copolymers based on poly (ethylene glycol) (PEG) and functionalized poly(ε-caprolactone) (PCL). We then evaluated the effect of dihydroxy PEG 200 Da as cross linker on the gelation of triblock copolymers.
Methods: Triblock copolymers of poly (α-carboxyl-co-benzyl carboxylate-ε-caprolactone)-b-PEG-b-poly (α-carboxyl-co-benzyl carboxylate-ε- caprolactone) (PCBCL-PEG-PCBCL) were prepared through ring opening polymerization of α-benzyl-ε-caprolactone (BCL) and PEG (1450 kDa) through bulk polymerization method with and without PEG 200 Da as cross linker; this step followed by hydrogenation of block copolymer with palladium on activated charcoal under hydrogen gas stream. Prepared block copolymers were characterized to find out molecular weight, polydispersity index (PDI), and the cross linking degree by gel permeation chromatography (GPC).1H NMR was also used to characterize the structure and molecular weight of the polymer. The sol-gel transition of polymer solutions in water was measured using inverse flow method, differential scanning calorimetry (DSC) and rheometric analysis.

Results: GPC results proved that PEG 200 Da acted as cross-linker during the ring opening polymerization and polymers prepared by pure monomer and PEG 200 Da as cross linker yielded PCBCL-PEG-PCBCL with sol-gel transition temperatures around 35°C at concentration 10-15%.
Conclusion: The present study illustrates successful synthesis of triblock copolymers based on PEG and functionalized PCL with sol-gel transition close to physiological body temperature. The cross linker improved the mechanical property of the gelation state of the gel. It also decreased gel-forming concentration of the triblock copolymer.

C2-1 Amel Hamza

Bisphosphonate Drug Alveolar Bone Burden in Osteoporosis

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Purpose: Osteonecrosis of the jaw (ONJ), a severe bone disorder that leads to bone death is caused mainly by nitrogen-containing bisphosphonate drugs. Recent observations suggest that the nature of ONJ is not an area of avascular necrosis, nor an osteomyelitis, but an inability of the alveolar bone to respond to injury 1. Hence, cytokine factors involved in the regulation of osteoclast differentiation may play an important role with an ONJ episode.
Our study hypothesis was that the identification of new metabolites involved with the pathogenesis of ONJ may serve as a diagnostic mechanism for measuring the extent of ONJ present in the bones of patients following long-term bisphosphonate therapy. We used a metabolomics profiling approach, to observe metabolic changes in plasma and urine in an established rat model of osteoporosis, namely, the surgical ovariectomy (OVX) model in female rats. [2] The commercially available Biocrates kit was used as a tool in order to provide new information on the alteration in metabolite level and to determine the potential therapeutic insight offered by metabolomics profiling following bisphosphonate drug intervention.
Methods: The study subjects were divided into four experimental groups: control rats dosed with vehicle, rats dosed with 0.12mg/kg Alendronate twice Weekly, third group dosed with active vitamin D (100ng/kg) and a combination group receiving both Alendronate and vitamin D. Plasma and urine from the four groups of rats were collected at baseline, 4 week and 8 week study endpoint and subjected to metabolomic analysis and in vivo Micro CT scan measurement of bone volume.
Results: Preliminary results by micro-CT confirmed an osteopenic phenotype developing in the trabecular bone of all OVX rats. A distinct metabolite “fingerprint” was measured following drug treatment between control and treated groups, with key metabolites detected in Alendronate-dosed groups compared to the other groups. In particular, the presence of taurine, sugar, glycerophospholipid, creatinine, and acylcarnitines were in high amounts in the Alendronate-dosed groups, compared to the control group.
Conclusions: These findings suggest that the use of a metabolomic approach would be of value to dentists attempting to alleviate symptoms associated with osteonecrosis of the jaw associated induced by bisphosphonate drug therapy.

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C2-2 Lockhart Jamieson

Alterations in the eicosanoid profile and mitochondrial injury in human ventricular tissue following myocardial infarction

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Purpose: Myocardial infarction (MI) accounts for a significant proportion of death and disability in the ageing population. Certain n-6 PUFAs, linoleic acid and arachidonic acid, have come under investigation for their role in mediating a cardiac response following ischemic injury. CYP450 metabolism of these fatty acids results in formation of numerous metabolites, called eicosanoids, which can be further metabolised by the enzyme soluble epoxide hydrolase (sEH). These lipid mediators exhibit a wide range of cellular effects including alterations to mitochondria structure and function which may impact cardiac performance. This study investigated alterations to CYP-derived eicosanoids and mitochondria in heart tissue obtained from individuals who experienced a previous MI.
Methods: Samples were obtained from male and female individuals included in the Human Explanted Heart Program and correlated to non-failing control hearts (NFC) collected from unused transplant donors through the Human Organ Procurement and Exchange Program at the University of Alberta. Ventricular tissues were harvested from patients who had previously experienced a LAD infarct (≥2yrs), as determined by echocardiography, ECG and coronary angiograms. Protein expression was determined by immunoblotting techniques. Mitochondrial enzymatic activities were assessed by spectrophotometric methods while mitochondrial ultrastructure and cristae density were assessed by electron microscopy. A metabolic profile in ventricular tissue was obtained by LCMS.
Results: Marked differences in both LA and AA metabolic profiles were revealed in post-MI tissues. There were significant increases in cardiotoxic metabolites correlating with decreased cardiac function and injury. Interestingly, no significant alterations were observed in CYP isozyme expression but there was a significant increase in both sEH activity and expression post-MI tissues compared to NFC. Expression of mitochondrial proteins remained unchanged, however enzymatic function declined in post-MI tissues compared to NFC correlating with marked disruption in mitochondrial ultrastructure.
Conclusions: These data provide the first evidence demonstrating a marked shift in eicosanoid metabolism in post-MI hearts that correlates with mitochondrial structural disruption and overall decreased mitochondrial function.

C2-3 Gabriela Lesyk

Characterization of Megakaryocyte Subpopulations based on the Heterogeneity of eNOS Signalling; Effects of Cytokines on Megakaryocyte eNOS and iNOS Expression

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Purpose: Recently, our laboratory has identified in human blood two platelet subpopulations based on the presence or absence of a functional endothelial nitric oxide synthase (eNOS)-signalling pathway. We have also found that eNOS-negative platelets, although less abundant are more reactive than eNOS-positive platelets and initiate aggregate/thrombus formation. Since platelets derive from bone marrow megakaryocytes, we have decided to test the hypothesis that eNOS-negative and eNOS-positive subpopulations of megakaryocytes exist and give rise to their respective eNOS-based platelet subpopulations. Additionally, we have also tested whether pro- and anti-inflammatory cytokines have an effect on eNOS and inducible NOS (iNOS) levels within megakaryocytes as cytokines are known to regulate expression of eNOS and iNOS.

Methods: The megakaryoblastic cell line (Meg-01) was used for experiments in place of megakaryocytes. To validate presence of eNOS within Meg-01 cells, RT-PCR was performed and gel samples were excised and sent for DNA sequencing. Flow cytometry was used to measure eNOS levels via intracellular immunofluorescence and nitric oxide production using cell-permeable fluorescent probe DAF-FM (10μM) in Meg-01 cells. Additionally, Meg-01 cells were treated for 48 hours with IFNγ (10ng/ml) alone and with increasing concentrations of IL-10 (0.1-100ng/ml) and iNOS and eNOS levels were measured by Western Blot and RT-qPCR.
Results: Following RT-PCR and agarose gel electrophoresis a detected cDNA 241 bp band was confirmed to be eNOS via DNA sequencing. Flow cytometry results showed that similarly to platelets Meg-01 cells consist of two eNOS-based subpopulations, eNOS-positive (91.5%±1.2)/nitric oxide-producing (DAF+ve 92.0%±3.34) and eNOS-negative (8.5%±1.2)/nitric oxide-nonproducing (DAF-ve 8.0%±6.68) Meg-01 cells. In experiments with cytokines IFNγ (10ng/ml) increased level of iNOS within Meg-01 cells and higher concentrations of IL-10 (10-100ng/ml) nullified that effect. eNOS levels were not detectable using Western Blot, however qPCR results showed 6.4 fold decrease of eNOS levels following IFNγ and only 2.2 fold decrease with IL-10 (100ng/ml).
Conclusions: Megakaryocyte subpopulations exist within Meg-01 cell line based on the presence or absence of functional eNOS. Pro-inflammatory cytokine IFNγ enhanced iNOS expression and decreased eNOS expression in Meg-01 cells; however, high concentrations of IL-10 attenuated the effect. Our data show that action of pro-inflammatory IFN-γ may promote growth/differentiation of megakaryocytes with decreased levels of eNOS, which may lead to formation of higher numbers of more reactive eNOS-ve platelets leading to higher risk of prothrombotic state. Further experiments are required to confirm whether eNOS+ve and eNOS-ve megakaryocytes give rise to their respective platelet subtypes.

C2-4 Andrew Morgan

An evaluation of MPO- mediated bio-activation of NSAIDs in promyelocytic leukemia (HL-60) cells for potential cytotoxic selectivity.

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Purpose: NSAIDs have been investigated for their utility in cancer therapy. In this study, we have investigated myeloperoxidase (MPO)-mediated metabolism of some NSAIDs and their metabolites. MPO is a highly-expressed peroxidase in acute myeloid leukemia. As bio-activation involves the formation of reactive metabolites, we hypothesized that NSAIDs' metabolites, produced via MPO would be correlated with leukemia cell toxicity.
Methods: We tested the enzymatic peroxidation of three NSAIDs, namely diclofenac, indomethacin, and naproxen in comparison with their hepatic metabolites, 4'- hydroxydiclofenac (4'-OHD), 5-hydroxydiclofenac (5-OHD), O-desmethyl-N-deschlorobenzoylindomethacin (DMBI), O-desmethylindomethacin (DMI) and O-desmethylnaproxen (ODN). Firstly, we used purified peroxidases in kinetic UV–vis kinetic spectrophotometry, and electron paramagnetic resonance (EPR) experiments to determine oxidation of ascorbic acid and glutathione (GSH), respectively. We then used HL-60 cells, as a model of acute myelogenous leukemia to carry out trypan blue exclusion, cellular ATP analysis, mitochondrial membrane potential (MMP) and cytofluorometric GSH assays.
Results: Our results present evidence that diclofenac, 4'-OHD, 5-OHD, DMBI and DMI demonstrated significant cytotoxic effect in the leukemic cells through oxidation by intracellular MPO. In the same vein, only diclofenac and its two metabolites caused a significant drop in the MMP and cellular ATP level; however, the cell death induced by indomethacin metabolites reflected a subtle effect on MMP or GSH content. Interestingly, only diclofenac and 4'-OHD (and not 5- OHD) caused a significant drop in HL-60 cells' GSH content. Among diclofenac compounds, only 4'-OHD also generated GS· radical and caused a significant increase in ascorbate co-oxidation rate. Lastly, even though ODN also generated GS· radical and potently co-oxidized ascorbate, it showed no significant cytotoxicity.
Conclusions: These results provide evidence of a correlation between acute cytotoxicity and MPO-bio-activated NSAIDs, though this was not correlated for all compounds (e.g., ODN). Further studies are required to determine both the MPO-dependent and MPO-independent mechanisms of cytotoxicity.

C2-5 Igor Paiva

Biodistribution of EGFR targeted polymeric micelles in orthotopic colorectal cancer xenografts in mice

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Purpose: Nanocarrier surface modification using ligands interacting with receptors overexpressed on cancer cells is hypothesized to enhance the homing of nano-carrier in tumor. The objective of this study was to test this hypothesis in an orthotopic mice model of colorectal cancer (CC) for polymeric micelles modified on their surface with GE11 peptide that targets epidermal growth factor receptor (EGFR) on CC cells.
Methods: Traceable GE11 modified poly(ethylene oxide)-poly(□-caprolactone) (PEO-PCL) or poly(ethylene oxide)-poly(□-benzyl carboxylate-□-caprolactone) (PEO-PBCL) were developed through peptide attachment to the acetal-PEO and Cy5.5 to PCL or PBCL end (Garg et al., 2017). The uptake of polymeric micelles by HCT-116 cells overexpressing EGFR was measured using flow cytometry. An orthotopic colorectal mice model was developed and used to assess the biodistribution of plain versus GE11 modified micelles following intravenous administration using live animal or ex vivo organ imaging by an IVIS imager.
Results: The results showed micelles with GE11 modification to have a higher in vitro uptake than plain micelles in HCT-116 cells. The cell uptake enhancement seemed to be more prominent for GE11-PEO-PBCL micelles than for GE11-PEO-PCL micelles. The fluorescence intensity in the tumor site from the mice was higher for micelles having PCL core at early time points, whereas micelles with PBCL core accumulated more into the tumor site at later time points (i.e. ~24h). There was a trend both in vivo and ex vivo for a higher micelle tumor accumulation when GE11 peptide was present on micellar surface. The results also showed faster clearance of PEO-PCL based micelles compared to PEO-PBCL ones mostly through mice kidneys leading to lower accumulation of the former nano-carriers in non-target tissues as well as tumor.
Conclusion: Surface decoration of polymeric micelles with the GE11 peptides positively impacted their in vitro CC cell uptake and in vivo accumulation into orthotopic CC tumors.

C2-6 Tasneem Siyam

Developing and evaluating a patient decision aid for managing surgical menopause: The story behind the "SheEmpowers" patient decision aid (PDA)

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Purpose: To systematically develop and evaluate an evidence-based patient decision aid (PDA) to help women decide on hormone therapy (HT) to manage symptoms of early surgical menopause and long-term risks.
Methods: The PDA development was guided by the Ottawa decision support framework and involved 3 phases: an exploratory phase to identify women decisional needs; a development phase to identify evidence related to surgical menopause and treatment options and draft an initial prototype; and an evaluation phase to evaluate the prototype and elicit views on acceptability and usability in a non-clinical setting. For exploratory and evaluation phases, we recruited women from the Edmonton menopause clinics. We searched Medline, TRIP, Dynamed, and others for evidence to inform the content of the PDA. Data on HT outcome probabilities were evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE). All phases were driven by a multidisplinary group of researchers, clinicians and patient representatives to ensure women priorities were met.
Results: Informed by identified needs from the exploratory phase an initial prototype of the PDA was drafted and had 4 components: facts about surgical menopause and HT; HT outcome probabilities to develop realistic expectations; a values clarification section to make trade-offs and clarify values associated with HT; and a

component on guidance in decision-making. We anticipate including supplemental information on other reasonable treatment options for women consideration. We are currently reviewing the tool with our stakeholders to improve content and presentation and gain perspectives on tailoring to women's needs. The evaluation of the PDA is still pending.
Conclusion: Through our adopted, systematic, evidence-based and multidisciplinary approach we hope to develop a PDA than can empower women with early surgical menopause when making therapy decisions and offer them the information, resources, and skills to effectively manage decision-making about HT and other options.

D. Postdoctoral Fellow

D1 Marawan Ahmed

The Too Many Faces Of PD-L1: A Comprehensive Conformational Analysis Study
Marawan Ahmed, and Khaled Barakat

Purpose: In the current study, we focused on the immune-checkpoints PD-1 pathway and in particular on the ligand, PD-L1 [1].
Methods: We studied the conformational dynamics of PD-L1 through principal component analysis (PCA) of existing crystal structures combined with classical and accelerated molecular dynamics simulations. We identified the maximum structural displacements that take place in all PD-L1 crystal structures and in the MD trajectories.
Results: We found that these displacements are attributed to specific flexible regions in the protein. We also investigated the conformational preference for small molecule binding and highlighted a Methionine residue at the binding site, which plays a key role in drug binding [2].
Conclusion: The binding mechanism of PD-L1 to other binding partners is taking place through the conformational selection mechanism. We hope that the data presented here supports the ongoing efforts to discover effective therapies targeting the PD-1 immune-checkpoint pathway.

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D2 Rami Al Batran

Unexpected Improvement in Glucose Homeostasis in Obese Mice Expressing Cre Recombinase Under the Control of the Muscle-Specific Human Skeletal Actin Promoter
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Purpose: The Cre/loxP system for gene targeting has proven to be a powerful tool for understanding gene/protein function in animal models in a tissue-specific manner. In light of recent findings demonstrating that tissue-specific Cre recombinase expression may influence whole-body physiology, the aim of our study was to investigate whether human α-skeletal actin (HSA)-mediated Cre expression, specifically in skeletal muscle, influences glycemia in mice. The acquisition of such data is essential in determining the appropriate mouse

phenotype to use as the control littermate for studying skeletal muscle-specific gene function in studies of insulin resistance and type 2 diabetes (T2D).
Methods: 6-week-old wild-type (WT) littermates or HSA-Cre expressing mice received daily intraperitoneal (IP) injections of tamoxifen (50 mg/kg) suspended in corn oil for 5-days. All mice were allowed a one-week washout post-tamoxifen, following which 8-week-old mice were placed on a standard chow/low-fat diet (LFD, 45% kcal from lard) or high-fat diet (HFD, 60% kcal from lard) for 12-weeks.
Results: lean 16-week-old HSA-Cre mice demonstrated mild improvements in glycemia in response to an IP glucose tolerance test (GTT), which was associated with increases in circulating insulin levels. This improvement in glycemia during an IP-GTT was more pronounced in obese HSA-Cre mice, and once again associated with increases in circulating insulin levels. Conversely, no improvements in glycemia during an ITT, nor changes in insulin signaling, were observed in lean or obese 17-week-old HSA-Cre mice when compared to their WT littermates.
Conclusions: We demonstrate that tamoxifen-mediated liberation of Cre recombinase in skeletal muscle produces marked improvements in glucose tolerance that were associated with increases in circulating insulin levels. Thus, it would appear essential to use the HSA-Cre expressing mouse as the control littermate when investigating the skeletal muscle-specific role of a gene/protein and its potential relevance to skeletal muscle insulin resistance and T2D.

D3 Dinesh Babu

Metabolism of isoniazid by eosinophil peroxidase leads to INH-NAD+ Adduct formation

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Purpose: The formation of isonicotinyl-nicotinamide adenine dinucleotide (INH-NAD+) by the mycobacterial catalase-peroxidase enzyme, KatG, was known to be the major component of the mode of action of isoniazid (INH), an anti-tuberculosis drug. However, there are numerous human peroxidases that may catalyze this reaction. In our previous study, we have reported that neutrophil myeloperoxidase (MPO) is capable of metabolizing INH through the formation of INH-NAD+ adduct, which could be attributed to be a possible mode of action of INH. However, an eosinophilic infiltration of the lungs is more pronounced and characteristic of granulomas in Mycobacterium tuberculosis-infected patients. As such, the role of the eosinophil with respect to isoniazid metabolism is not clear. Thus, the aim of the present study is to investigate the role of eosinophil peroxidase (EPO), a key eosinophil enzyme, during INH metabolism and the formation of its active metabolite, INH-NAD+.
Methods: In our studies, we investigated EPO metabolism of INH using electron paramagnetic resonance (EPR) spin-trapping and UV-Vis spectroscopy, and the resultant INH-NAD+ adduct was characterized using LC-MS. These studies were carried out using purified EPO and eosinophils isolated from asthmatic donors, generously donated by Dr. Paige Lacy.
Results: EPO catalyzed the oxidation of INH and formed several free radical intermediates; the inclusion of superoxide dismutase revealed a carbon-centered radical which is considered to be the reactive metabolite that binds with NAD+. Furthermore, INH oxidation by EPO led to a new product (λmax = 330 nm) in the presence of NAD+. This adduct was confirmed to be isonicotinyl-NAD+ (INH-NAD+) using LC–MS analysis where the intact adduct was detected (m/z = 769).
Conclusions: The findings of this study suggest that eosinophilic EPO may also play a role in INH pharmacological activity through the formation of INH-NAD+ adduct, and supports further evidence that human cells and enzymes are capable of producing the active metabolite involved in tuberculosis treatment.

D4 Aravindhan Ganesan

The effects of protein-protein interactions and ligand-binding on the ion permeation through KCNQ1 channel revealed by computational simulations

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Purpose: The voltage-gated KCNQ1 potassium ion channel interacts with the type I transmembrane protein minK (KCNE1) to generate the slow delayed rectifier (IKs) current in the heart. Off-target interactions of several drugs with that of KCNQ1/KCNE1 ion channel complex have been known to cause fatal cardiac irregularities. Thus, KCNQ1/KCNE1 remains an important avenue for drug-design and discovery research.
Methods: In this work, we present the structural and mechanistic details of potassium ion permeation through an open KCNQ1 structural model using the combined nanoseconds-scale molecular dynamics (MD) and advanced steered MD simulations. We discuss the processes and key residues involved in the permeation of a potassium ion through the KCNQ1 ion channel, and how the ion permeation is affected by (i) the KCNQ1-KCNE1 interactions and (ii) the binding of chromanol 293B ligand and its derivatives into the complex.
Results: The results reveal that interactions between KCNQ1 with KCNE1 causes a pore constriction in the former, which in-turn forms small energetic barriers in the ion-permeation pathway. These findings correlate with the previous experimental data suggesting that the interactions of KCNE1 dramatically slows the activation of KCNQ1. Upon ligand-binding onto the complex, the energy-barriers along ion permeation path are more pronounced, as expected, therefore, requiring higher force in our steered-MD simulations. Nevertheless, the binding of weak blockers into the channel did not impact the ion permeation significantly.
Conclusion: The findings presented here will have some implications in understanding the potential off-target interactions of the drugs with the KCNQ1/KCNE1 channel that lead to cardiotoxic effects. And the methods employed here can also be applied to study other ion channel targets.

D5 Subha Kalyaanamoorthy

Binding site and interaction analysis of Kv7 modulators on KCNQ1 and KCNQ3 channels

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Purpose: Ion channel proteins and their modulators are being recognized to play a significant role in several diseases, including cancer. Kv7 is a voltage-gated potassium channel family that comprises of five subtypes, KCNQ1 to KCNQ5. Suppression of different Kv7 (KCNQ/M) voltage-gated potassium channels has been reported in various cancers and their physical effects. For example, suppression of KCNQ1 was found in gastrointestinal cancer, while low-level expression of KCNQ2 and KCNQ3 were identified to be the cause of bone cancer pain in animal models. Therefore, small molecule modulators that activate these channels have been of interests for therapeutic applications. Diverse classes of channel activators have been identified, but the knowledge on their sites of action is still limited. A clinically tested drug, retigabine (RTG) is known to activate all Kv7 channels from KCNQ2 to KCNQ5 except KCNQ1. On the other hand, Zinc pyrithione (ZnPy) another Kv7 activator, activates all KCNQ channels except KCNQ3. The knowledge of molecular determinants and isoform-specific potency of RTG and ZnPy would help in the development of new structures with isoform specificity.
Methods: We generated three-dimensional structural models of KCNQ1 and KCNQ3 channels using molecular modelling methods. Potential binding sites were predicted from these models, and the interaction of RTG and ZnPy in these sites were analyzed using molecular docking and binding free energy methods.
Results: The binding sites, key interactions that stabilize RTG and ZnPy will be presented.

Conclusions: Our results will be useful for understanding the isoform specificity of these molecules and therefore, can aid in the development of novel compounds specific to KCNQ subtypes as potential cancer therapeutics.

E. Unjudged

E1 Marawan Ahmed

Discovery and Validation of New Small Molecule Inhibitors for Dengue Envelope Protein
Marawan Ahmed, Anil Kumar, Tom C. Hobman, and Khaled Barakat

Purpose: Despite decades of research efforts, no effective antivirals are available against dengue virus with the exception of recently developed vaccine whose efficacy remains to be determined [1-2]. For these reasons, development of small molecule inhibitors that curb the replication of dengue virus is urgently needed.
Methods: In the current study, we employed state-of-the-art molecular modelling simulations to identify novel inhibitors of the dengue virus envelope protein. The binding modes of all compounds within the conserved β-OctylGlucoside (β-OG) pocket were studied using a combination of docking, molecular dynamics simulations and binding free energy calculations.
Results: Here, we describe ten new compounds that significantly reduce production of dengue virus as determined using standard cell-based virological assays. We began our analyses by confirming the interaction modes between a set of nine control compounds and the E protein. In this regard, a long MD simulation was carried out to confirm the binding site of R1, one of the most potent E protein inhibitor reported in the literature. We then used our model to search for new E protein inhibitors.
Conclusions: We identified a class of Benzothiazole derivatives that showed promising activity profiles as well as being amenable for further structural optimization. Decomposing the MMGBSA binding free energy revealed that the lipophilic interaction is the predominant force for interaction in the identified classes of compounds. We also showed that the electrostatic interaction should be carefully monitored to avoid the loss of activity due to the high desolvation penalty.

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E2 Marawan Ahmed

A Comprehensive Structural Model For The Human KCNQ1/KCNE1 Ion Channel
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Purpose: The voltage-gated KCNQ1/KCNE1 potassium ion channel complex forms the slow delayed rectifier (IKs) current in the heart, which plays an important role in heart signaling [1-2]. A critical step to understand the functional regulation of this ion channel is to build a structural model and study how the model interacts with other binding partners.
Methods: Using a combination of comparative modeling and enhanced MD simulations, we built robust model for the KCNQ1 protein, which accommodated the most recent experimental findings. The inclusion of experimental data was done using an enhanced MD simulation; REMD to impose structural details that were reported in the literature. The biological complex that was formed between KCNQ1 and KCNE1in this study was modeled in order to mimic the physiological open state of this ion channel in complex with the KCNE1 auxiliary protein in a 4:2 stoichiometry.

Results: Amongst the two complexes that were chosen for deeper analysis, we ended up selecting complex #154 in which the N-terminal of KCNE1 proteins form more interactions with the KCNQ1 channel. This was based on an extensive analysis of the interactions of the two proteins as well as based on the analysis of their MD simulations. In this final complex, the two proteins satisfied all experimental constraints and exhibited several favourable contacts that had been retained throughout the MD.
Conclusions: In general, the association of KCNQ1 with KCNE1 significantly reduced the magnitude of the VSD fluctuations.

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E3 Aravindhan Ganesan

How do the co-stimulatory CD28/B7-1 interactions differ from the co-inhibitory interactions of CTLA/B7-1 for orchestrating T-cell immune responses?

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Purpose: Activation of T lymphocytes (or T-cells) plays a central role in anti-cancer immune responses, which controls tumor initiation and progression. CD28 and CTLA-4 are transmembrane receptors that are responsible for the regulation of the second signal that is crucial for T cell activation. These T-cell receptors share some extents of sequence and structural conservation; and they also bind to the same B7 ligands expressed on the antigen-presenting cells. Nevertheless, CD28/B7 interactions trigger a co-stimulatory signal for T-cell activation; while, CTLA-4/B7 interactions generate an inhibitory signal that inactivates the T-cells. Thus, understanding the molecular bases of these key protein-protein interactions at the immunological synapse are important for targeted immune checkpoints-based cancer therapy. Although, the X-ray crystal structure of CTLA-4/B7-1 has been reported earlier, however, the complex structure of CD28/B71 has not been resolved experimentally.
Methods: In this work, we employed a combination of advanced molecular modelling and extensive molecular dynamics (MD) simulations to model the CD28/B7-1 complex and characterize the key interactions that stabilize the complex.
Results: The ensemble protein-protein docking and MD-based binding-free energy calculations were useful to obtain the structure of the CD28/B7-1 complex, which was validated with various mutation-based experimental data (from the literature) and binding assay experiments performed here. Later, extended MD simulations of CTLA:B7-1 and CD28-B7-1 complexes revealed the similarities and differences in their interactions at molecular level.
Conclusion: The results presented in this work will, on a long-run, lead to significant insights for designing novel inhibitors to specifically target CTLA-4 and unleash the immune system against cancer. Support: Alberta Cancer Foundation, Alberta, Canada and Li Ka Shing Applied Virology Institute at the University of Alberta, Canada.

E4 Aravindhan Ganesan

Mathematical modelling and analyses of antibody-induced changes in the co-stimulatory interactions

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Purpose: The human T-cell-based immune responses are orchestrated by two opposing signals: a stimulatory signal, generated by the interactions of CD28 (on T-cells) with the B7 ligands expressed on the antigen presenting cells; and an inhibitory signal, triggered by CTLA-4/B7 interactions. Recently, monoclonal antibodies (mAbs) blocking CTLA-4 have demonstrated exceptional therapeutic benefits in clinical trials, which is transforming human cancer treatment. However, the effects on antibody-mediation on the complex formation at the immunological synapse have not been well understood.
Methods: In this work, we have developed a novel mathematical framework, based on the ordinary differential equations and experimental binding kinetics data, for quantitatively exploring the effects of anti-CTLA-4 mAbs on the co-stimulatory (CD28/B7) and the inhibitory (CTLA-4/B7) interactions at the synapse. We have particularly focused on two potent anti-CTLA-4 mAbs, tremelimumab (from AstraZeneca) and ipilimumab (from Bristol-Myers Squibb), which are currently in clinical trials and the market, respectively, for targeting multiple tumors.
Results: Our simulations have been validated with different experimental data. Overall, our results show that different factors, such as multivalent interactions, mobility of molecules and competition effects, could impact the effects of antibody-mediation.
Conclusion: Therefore, we preset our model as a valuable predictive tool to analyze the dose-dependent effects of anti-CTLA-4 mAbs on the co-stimulation by the CD28 pathway, which can complement and drive biochemistry and immunology experiments in the immune checkpoints research. Support: Alberta Cancer Foundation, Alberta, Canada and Li Ka Shing Applied Virology Institute at the University of Alberta, Canada.

E5 Lockhart Jamieson

Differential effect of 19,20-epoxydocosapentaenoic acid (EDP) in H9c2 cells grown in high or low glucose conditions

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Purpose: The importance of dietary polyunsaturated fatty acids (PUFAs) in the reduction of cardiovascular disease has been recognized for many years. Epoxydocosapentanoic acids (EDPs) are lipid mediators produced by cytochrome P450 epoxygenase from docosahexaenoic acids (DHA). In this study, we investigated the impact of normal and low glucose concentrations toward 19,20-EDP-mediated effects in normoxic conditions in H9c2 cells.
Methods: H9c2 cells were cultured in DMEM containing either normal (25mM) or low (5.5mM) glucose concentrations and supplemented with 10% fatal bovine serum (FBS), 1% penicillin and streptomycin at 37□C (5% CO2/65% N2). Cells were treated with 0 or 1μM 19,20-EDP, 100μM DHA, 1μM Myriocin and subjected to 24h normoxic. Cellular viability was assessed by the reduction of a luciferase substrate by metabolically active cells. Cell lysates were assessed for caspase-1/3 activity using a profluorogenic peptide Ac-WEHD-AMC and Ac-DEVD-AMC. Extracellular oxygen consumption rates (OCR) were assessed using a phosphorescent oxygen sensitive reagent. Mitochondrial respiration was measured using Clark oxygen electrode connected to Oxygraph Plus recorder. Autophagy was detected using monodansylcadaverine (MDC), the fluorescent marker for lysosomal/ autophagic vacuoles. Different fractionation methods were performed to measure the ceramide level by LC/MS.
Results: H9c2 cells cultured under normal (25mM) glucose conditions and treated with 19,20-EDP demonstrated significant loss in cell membrane integrity, decreased MTT reduction, increased proteasomal, caspase activity, autophagy, ceramide level as well as decreased OCR and ATP. In contrast, H9c2 cells cultured in low glucose (5.5mM) conditions and treated with 19,20-EDP demonstrated increased MTT reduction and ATP production.

Conclusion: Our data suggest that cells cultured in high glucose conditions, a more 'aerobic glycolytic' state, are susceptible to 19,20-EDP induced cell death with autophagy. While in low glucose conditions, reflecting more oxidative phosphorylation, 19,20-EDP protected H9c2 cells.

E6 Subha Kalyaanamoorthy

Molecular determinants for blocking the hERG channel

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Purpose: Cardiotoxicity is one of the severe side effects that challenge the drug discovery and development process. Most cardiotoxic effects are induced by the unwanted interactions of the drug with voltage-gated ion channels in the heart, particularly the human ether–à-go-go related gene (hERG) channel. Several drugs were withdrawn from the market due to their severe cardiotoxic side effects triggered by this off-target interaction with hERG. Thus, identifying the potential hERG blockers at early stages of lead discovery is fast evolving as a standard in drug design and development.
Methods: The near-atomic structure of the hERG channel was resolved recently using the cryo-EM technique. By using this structural data, we have developed an in silico workflow that implements multiple receptor conformations to dock the small molecules and post-process the docked structures using binding free energy calculations.
Results: The results from our test data reveals the essential molecular interactions that account for blocking the hERG channel.
Conclusions: Our integrated screening approach can predict the hERG liability of a molecule. Support: Natural Sciences and Engineering Research Council of Canada (NSERC Canada)

E7 Keshav Gopal

GENERATION OF A CARDIAC-SPECIFIC PYRUVATE DEHYDROGENASE DEFICIENT MOUSE MODEL TO USE FOR THE STUDY OF DIABETIC CARDIOMYOPATHY

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Purpose: Cardiovascular disease represents the main cause of death in type 2 diabetes (T2D) patients. This includes diabetic cardiomyopathy (DC), of which there are no approved therapies. Of interest, a number of studies have demonstrated that myocardial glucose oxidation rates are impaired in preclinical models of obesity and T2D. As pyruvate dehydrogenase (PDH) is the rate-limiting-enzyme for glucose oxidation, our objective was to generate a cardiac-specific PDH deficient (PDHCardiac-/-) mouse model to study the importance of glucose oxidation in the setting of experimental diabetic cardiomyopathy.
Methods: We generated PDHCardiac-/- mice via using the tamoxifen inducible Cre-loxP system, which involved crossing mice expressing a floxed PDH allele (Pdha1) with mice expressing Cre recombinase under control of the myosin heavy chain-□(□MHC) promoter. Cardiac-specific PDH deletion was induced via 6 treatments of tamoxifen spread over 8 days (50 mg/kg) and assessed by western blot analysis in various tissues after 6 weeks post-tamoxifen. In vivo cardiac function was assessed via ultrasound echocardiography under isoflurane anaesthesia. In addition, myocardial glucose oxidation rates were assessed using isolated working heart perfusion.
Results: Protein levels of PDH were significantly reduced exclusively in the heart following tamoxifen treatment only in PDHCardiac-/- mice, but not in their control littermates. Furthermore, basal in vivo cardiac function was unaltered in PDHCardiac-/- mice when compared to their control littermates. Although cardiac function was similar, left ventricular mass was significantly elevated in PDHCardiac-/- mice. Moreover, glucose oxidation rates

were markedly depressed and consistent with negligible PDH expression/activity in PDHCardiac-/- mice. Conclusions: As our results indicate that PDHCardiac-/- mice have normal baseline cardiac function despite a marked reduction in glucose oxidation rates, we anticipate that these mice will be a valuable tool to study the role of PDH/glucose oxidation in diabetic cardiomyopathy and other diabetes-related cardiovascular diseases.

E8 Victor Samokhvalov

Mitochondrial dysfunction and cytotoxicity caused by endogenously produced linoleic acid following LPS treatment in cardiac cells

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Purpose: Linoleic acid (18:2n6, LA) is the primary source of the essential n-6 PUFA, which is converted to arachidonic acid (20:4n6, AA) by desaturation and elongation via enzyme systems within the body. Biological transformation of LA may occur through CYP-mediated hydroxylation, epoxidation and allylic oxidation. The primary CYP-derived epoxy metabolites are two regioisomeric epoxides, 9,10- epoxyoctadecamonoenic acid (EpOME) and 12,13-EpOME. Both of these metabolites are rapidly hydrolyzed by sEH to the corresponding vicinal diols, 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME) and 12,13-DiHOME. In the current study, we investigated whether LPS-induced cardiotoxicity is mediated through endogenously produced metabolites of linoleic acid.
Methods: HL-1 cardiac cells were treated with either LPS (1μG/ml), 9,10-DiHOME (0.01, 0.1 or 1□M) or 9,10-EpOME (0.01, 0.1 or 1□M) for 12-24 h with or without the sEHi (tAUCB). Cell viability and mitochondrial function were assessed.
Results: We observed that treatment with LPS induced a sharp decrease in cell viability associated with pronounced mitochondrial dysfunction. Importantly, mitochondrial respiratory control ratio was significantly lowered when both complex I and II respiratory substrates were used. This finding correlated with the declined intracellular ADP/ATP and NAD+/NADH ratios demonstrating profound metabolic collapse of cardiac mitochondria. Treatment with 9,10-DiHOME produced spectrum of cytotoxic and metabolic adverse effects strongly resembling what was observed in LPS-exposed cardiac cells. Furthermore, we found that 9,10-DiHOME triggered pronounced mitochondrial structural abnormalities, which also contributed in the development of extensive mitochondrial dysfunction in cardiac cells.
Conclusions: We provide novel evidence that LPS-induced cardiotoxicity can be mediated through DiHOME-instigated mitochondrial dysfunction. Support: This study was supported by CIHR grant.