Table of Contents

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

Abstract A.1.a

Development of nano-carriers for tumor-targeted delivery of novel inhibitors of polynucleotide kinase phosphatase (PNKP) in colorectal cancer

Tongtong Xu[a], Sams M. A. Sadat[a], Igor Paiva[a], Dennis Hall[c], Michael Weinfeld[bd], Afsaneh Lavasanifar[ae]
[a] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[b] Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada
[c] Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada
[d] Division of Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada
[e] Department of Chemical and Material Engineering, University of Alberta, Edmonton, AB, Canada

Purpose: Human polynucleotide kinase/phosphatase (PNKP) is a key DNA-repairing enzyme that phosphorylates DNA 5'-termini and dephosphorylates DNA 3'-termini, allowing DNA ligases to rejoin the strands. Cancer cells lacking a tumor suppressor protein, phosphatase and tensin homolog (PTEN) are shown to be effectively killed upon downregulation of PNKP. Our research team has recently developed a novel inhibitor of PNKP, namely A83B4C63, and its nanoparticle formulation. The long term objective of this research is to develop a tumor-targeted nano-formulation for PNKP inhibitors, so that the therapeutic index of these potential new anticancer drugs can be enhanced. In this study, *in vitro* anticancer activity of A83B4C63 against HCT116/PTEN$^{+/+}$ and HCT116/PTEN$^{-/-}$ colorectal cancer (CRC) cells as well as its bio-distribution in HCT116/PTEN$^{+/+}$ and HCT116/PTEN$^{-/-}$ xenografts in mice were compared to that of nano-encapsulated A83B4C63. Methods: Poly(ethylene oxide)-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-*b*-PBCL) copolymers were synthesized and used to prepare A83B4C63-encapsulated nanocarriers. Free drug and its nano- formulation were treated with HCT116/PTEN$^{+/+}$ and HCT116/PTEN$^{-/-}$ cells at different incubation times, then subjected to MTT assay. Tissue and plasma concentrations of A83B4C63 as part of cremophor EL formulation and its nano-formulation were determined using LC/MS in HCT116/PTEN$^{-/-}$ and HCT116/PTEN$^{+/+}$ xenografts in NIH-III mice (n=3) 24h after the last IV injection at a dose of 25 mg/kg, three times, every other day. Results: At a concentration 10 μM, A83B4C63 treatment of HCT116/PTEN$^{-/-}$ cells led to < 50% cell viability after 72h incubation. Under the same conditions, wild-type HCT116 cells showed > 80% viability. A significantly higher concentration of A83B4C63 was measured in blood plasma and tumor when delivered by nano-formulations in the HCT116/PTEN$^{-/-}$ xenografts. A similar trend was observed in animals with HCT116/PTEN$^{+/+}$ xenografts which did not approached statistical significance. Analysis of other tissues is currently underway. Conclusion: PEO-*b*-PBCL micellar delivery of A83B4C63 demonstrates a promising new monotherapeutic option in CRC with PTEN-loss. Support: Alberta Cancer Foundation, Canada.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.1.b

Use of an antioxidant adjuvant to attenuate clozapine (Clozaril®) toxicity in vitro

Thom Le*[1], Dinesh Babu*[1], Béla Reiz[2], Arno Siraki[1]

*These authors contributed equally to this work

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

[2]Department of Chemistry, Faculty of Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Clozapine (Clozaril®) is an atypical antipsychotic drug that is being used as the first-line treatment in refractory schizophrenia. Approximately 1% of patients taking clozapine experience agranulocytosis resulting in risk of infections, therefore hindering its clinical use. As such, costly blood monitoring is required for clozapine use. Thus, identifying adjuvants that can prevent the harmful effects of clozapine without compromising its pharmacological efficacy would be advantageous. Edaravone (Radicava®) is the only drug which is believed to function through its antioxidant activity, and is presently approved in the US to treat amyotrophic lateral sclerosis. The aim of the present study was to investigate whether edaravone can potentially attenuate the toxicity of clozapine through a foundational in vitro study. We studied the effect of edaravone on neutrophil myeloperoxidase (MPO), an enzyme capable of drug metabolism, and evaluate how edaravone could influence clozapine metabolite formation through MPO.Methods: The effect of edaravone on MPO activity and the metabolism of clozapine were determined using UV-visible spectrophotometry and LC/MS. The protective effect of edaravone on the cytotoxicity of clozapine in HL-60 cells was determined by Cell Counting Kit-8 (CCK-8, Dojindo) assay.Results: Treatment of H_2O_2 with live HL-60 cells significantly increased MPO activity and addition of edaravone to this system attenuated the MPO activity comparable to an MPO inhibitor, 4-ABAH. Kinetic spectra after addition of clozapine to purified MPO and H_2O_2 resulted in a characteristic peak indicative of a new product, which was not apparent after the addition of edaravone and 4-ABAH. Edaravone also inhibited clozapine metabolism as observed with the changes in the peak area from LC/MS analysis. Moreover, edaravone also attenuated clozapine-induced cytotoxicity of HL-60 cells. Conclusion: Our findings suggest that the antioxidant potential of edaravone might play a role to prevent clozapine-mediated toxicity in vitro based on its modulation of MPO activity.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.1.c

Inflammasome activation and characterization of H9c2 myoblast cells

Tim YT Lee[1], Kevin Khey[2], John M Seubert[2]
[1]Department of Pharmacology, Faculty of Science, Edmonton, AB, Canada
[2]Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton, AB, Canada

Purpose: Exposure to environmental toxins is well known to cause damage to mitochondria but much of the mechanism how this fully impacts cardiac cells remains unknown. Lipopolysaccharide (LPS) is an integral component of the cell wall in gram-negative bacteria, which can directly modulate cell death and cell survival pathways through inflammatory and oxidative injury pathways. The multiple and complex biological effects following chronic exposure to low concentrations of LPS induces extensive metabolic complications attributable to mitochondrial damage. New evidence suggests that impaired mitochondrial function leads to the activation an NLRP3 inflammasome cascade that has a significant role in adverse outcomes. In order to protect against inflammasome activation, a better understanding of the novel mechanism is required. H9c2 myoblast cells are a common rat cell used in cardiovascular research. The current project aims to characterize LPS induced inflammasome activation in undifferentiated and differentiated H9c2 cells. Methods: H9c2 cells were plated at a density of $2x10^5$ cells, incubated at 37ºC with 5% CO_2 for 2d, cultured in 5.5 or 25mM glucose DMEM with 10% FBS. Differentiating cells toward a cardiomyocyte phenotype was achieved by using 2% FBS in DMEM and 10nM retinoic acid. H9c2 cells were treated with LPS (10µg/mL) for 6 hrs then with 3µM nigericin for 1 hr for inflammasome activation. Microscopy images were used to assess cell morphology. Cell viability was determined using CCK-8 assay and immunoblots were run to assess alterations in inflammasome protein expression. Results: Differentiated H9c2 cells exhibited cardiac phenotypic characteristics which included a fused and striated morphological appearance with multi-nucleation and expression profiles of cardiomyocyte proteins such as troponin T. Significant differences in cell viability and inflammasome activation were observed in undifferentiated and differentiated H9c2 cells following treatments. Conclusion: H9c2 cells are an acceptable model for studying inflammasome activation. Support: Research supported by NSERC.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.1.d

Genetic deletion and pharmacological inhibition of soluble epoxide hydrolase attenuates ventricular tissue injury in aged mice following myocardial infarction.

Deanna K. Sosnowski[1], K. Lockhart Jamieson[1], Wang Wang[2], Pavel Zhabyeyev[2], Matthew Edin[5], Darryl Zeldin[5], Zamaneh Kassiri[2,4], Gavin Y. Oudit[2,4], John M. Seubert[1,3,4]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, [2]Department of Physiology, [3]Department of Pharmacology, [4]Mazankowski Alberta Heart Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada, [5]National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, USA

Purpose: Myocardial infarction (MI) contributes to significant morbidity and mortality amongst the ageing human population. Epoxyeicosatrienoic acids (EETs) are CYP450 metabolites of arachidonic acid and possess cardioprotective properties following myocardial ischemia. An endogenous enzyme, soluble epoxide hydroxylase (sEH), further metabolises EETs to less potent dihydroxyeicosatrienoic acids, attenuating cardioprotection and augmenting cell death, detrimental remodeling, and dysfunction. This study investigated whether pharmacological inhibition of sEH with *t*AUCB or genetic deletion of sEH protected hearts from MI injury in aged animals. Methods: Aged (16-18 months) WT and sEH knockout C57/Bl6 mice underwent ligation of the left anterior descending coronary artery and were treated with *t*AUCB (10mg/ml) or vehicle (0.1% DMSO) in drinking water. Groups were sacrificed after 7 or 28 days. Infarct size was determined by 2,3,5-triphenyltetrazolium chloride assay and analyzed using ImageJ Software. Ventricular tissue was stained using Masson's trichrome and hematoxylin and eosin (H&E). Fibrosis, myofibril arrangement, and neutrophil infiltration were visualized with light microscopy and ZEN-ZEISS Software. LCMS was used to obtain ventricular tissue metabolite profiles. Caspase-3 and aconitase activities were measured using microplate assay kits and spectrophotometry. Results: Infarct size was significantly reduced in sEH knockout and *t*AUCB treated mice compared to WT counterparts. sEH null and *t*AUCB treated ventricular tissue displayed reduced neutrophil infiltration, fibrosis, and improved myofibril integrity. No significant changes were observed in aconitase activity. Caspase-3 activity was significantly lower in sEH knockout mice 7 days post-MI. However, levels were comparable between treatment groups 28 days post-MI indicating possible apoptotic resolution. Conclusions: Pharmacological sEH inhibition and genetic deletion of sEH expression may attenuate apoptotic response post-MI and reduce myocardial infarct size and remodeling. Inhibition of sEH serves as a potential pharmacological target to reduce post-ischemic myocardial injury. Support: This research is supported by grants from HSFC and CIHR.

ABSTRACT A.1.e

Elucidating Potential Alterations in Skeletal Muscle Ketone Body Metabolism in Mice Subjected to Experimental Obesity

Jadin J. Chahade[1,2,3], Rami Al Batran[1,2,3], Keshav Gopal[1,2,3], Amanda A. Greenwell[1,2,3], Kim L. Ho[1,2,3], Farah Eaton[1,2,3], John R. Ussher[1,2,3]

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

[2]Alberta Diabetes Institute, University of Alberta, Edmonton, AB Canada

[3]Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada

Purpose: Type 2 Diabetes (T2D) is a rapidly growing health epidemic in our world, with intense investigation on whether perturbations in skeletal muscle carbohydrate and fatty acid metabolism contribute to obesity-induced T2D. Conversely, whether perturbations in skeletal muscle ketone body metabolism also contribute to obesity-induced T2D has been comparatively understudied. However, this is becoming more relevant due to the increased willingness of individuals to consume a ketogenic diet as a non-pharmacological strategy for weight loss and improved blood sugar control. Therefore, our goal was to investigate potential alterations in ketone body metabolism during the pathology of obesity-induced T2D. We hypothesized that succinyl CoA:3-ketoacid CoA transferase (SCOT), the rate limiting enzyme of ketone body oxidation, would be elevated in skeletal muscles from obese mice. Methods: We placed C57BL/6J mice on a low-fat (lean) or high-fat (obese) diet for 12 weeks, following which animals were euthanized and gastrocnemius muscles were extracted for assessment of SCOT mRNA/protein expression via real-time PCR (qPCR)/western blotting methods. Furthermore, we cultured C2C12 myotubes and utilized siRNA or plasmid-mediated transfection approaches to knockdown or overexpress SCOT, respectively, following which we assessed changes in mRNA/protein expression for key enzymes of glucose/fatty acid metabolism via both qPCR and western blotting. Results: Obese mice demonstrated a marked increase in both SCOT mRNA and protein expression within gastrocnemius muscles, which was associated with an increase in SCOT enzymatic activity. Furthermore, SCOT knockdown in C2C12 myotubes resulted in increased AMPK (regulator of fatty acid metabolism) and decreased PDH (regulator of glucose metabolism) phosphorylation. Conversely, PDH phosphorylation was increased in C2C12 myotubes following overexpression of SCOT. Conclusions: Our results suggest that ketone body metabolism is augmented in skeletal muscle during the pathology of obesity, which may contribute to perturbations in skeletal muscle glucose and fatty acid metabolism in obese individuals. Support: AIHS Summer Studentship.

ABSTRACT A.1.f

The Use of Computational, Biophysical, and Molecular Biological Techniques to Screen and Identify Compounds for Juvenile Myelomonocytic Leukemia

Anna Jutla[1], Jitendra Kumar[2], Marawan Ahmed[1], Michael Overduin[2], Khaled Barakat[1]
[1]Faculty of Pharmacy and Pharmaceutical Sciences, [2]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

Purpose: The Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) protein plays an important role in signal transduction from the cell surface to the nucleus. SHP2 is a cancer driver, and its overstimulation is a key player in Juvenile Myelomonocytic Leukemia and Triple Negative Breast Cancer. The aim of this project is to identify a drug-like molecule to inhibit the activity of SHP2. Methods: Structure-based computational database screens have been used to filter libraries of millions of chemicals for their ability to interact with the allosteric regulatory sites or the lipid binding sites of SHP2. Thirty-eight short-listed ligands were ordered from Molport. Recombinant SHP2 protein was expressed in E. coli and purified for use in interaction studies. Due to instability of the protein and time restrains, we chose five compounds to characterize by phosphatase activity assays, Protein Thermal Shift (PTS), NMR spectroscopy and Surface Plasmon Resonance (SPR), and to establish the ligand-protein interaction experimental conditions. Results: We have determined that two of the selected compounds bind to SHP2 as shown by TS assays. These compounds demonstrated a consistent shift in the melting temperature, i.e stability of SHP2. One compound was chosen for further analysis by NMR Spectroscopy and SPR ($KD = 1.175*10^{-13}$M, in comparison to NSC-87877, a known inhibitor of SHP2 with $KD=1.723*10^{-8}$M) and confirmed binding with SHP2. Conclusions: This project demonstrates the importance of integrating structure-based drug design screening by computational methods with biochemical and biophysical techniques. We have established protocols for this screening, putting us well on the way for identifying potential drug fragments/molecules which can inhibit the activity of SHP2. We have characterized one compound that binds to SHP2 but does not inhibit activity. Identified molecules will be chemically modified to produce a molecule with enhanced affinity and inhibitory action.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.a

What characteristics are associated with success in healthcare practitioners? A scoping review

Eddie Huang, Danielle Nagy, Madellyn Heth, Katina Woo, Janice Kung, Theresa L. Charrois, Ken Cor, Jill Hall

Faculty of Pharmacy and Pharmaceutical Sciences, John W. Scott Health Sciences Library, University of Alberta, Edmonton, Alberta, Canada

Purpose: The primary objective of this study was to conduct a scoping review of the literature regarding characteristics that contribute to professional success. Methods: A comprehensive search was conducted in Ovid MEDLINE (1946 to May 2018) to retrieve publications that could demonstrate an association between specific characteristics or traits with professional success in healthcare practice. Characteristics of interest were determined through a previous study that identified themes extracted from pharmacist interviews that are thought to potentially lead to success, including: motivation, critical thinking, emotional intelligence, core competencies and work-life balance. Two students independently screened titles and abstracts based on predefined inclusion criteria, then independently reviewed each full-text article. Two additional research students then extracted data and independently coded each article for recurring themes. Results: Of the 1118 articles identified, 10 articles were relevant and included. The content analysis revealed six broad themes: personal mastery, commitment, collaborator, problem solver/critical thinker, inspirer/influencer/mentor, and excellence. Each theme was further broken down into sub themes. Conclusions: Six themes were identified as characteristics that contribute to healthcare professional success.

ABSTRACT A.2.b

Save Your Breath! Piloting a Tobacco Cessation and COPD Screening Service in Community Pharmacies

LeAnna Kalvi[1], Doug Kremp[2], Darrel Melvin[3], Tatiana Makhinova[1]
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
Alberta Health Services, Onoway, AB, Canada
Alberta Health Services Tobacco Reduction Program, Red Deer, AB, Canada

Purpose: Chronic Obstructive Pulmonary Disease (COPD) is a progressive pulmonary condition that is largely associated with smoking. It is estimated that two thirds of patients with COPD are unaware of their diagnosis. Pharmacists play an important role in providing tobacco cessation (TC) services and are well situated to identify patients at risk for COPD by screening current smokers. The aim of this study is to develop (Phase I) and pilot a COPD screening and TC program in community pharmacy settings (Phase II). The objectives are to (1) demonstrate the utility of proactively screening current smokers for airflow limitations and (2) determine the success rates of TC services. Methods: Phase I included analysing current evidence of TC provision and determining the main patient outcomes to be measured. This was followed by identification of tools that could be used by pharmacists during TC counseling and development of recruitment, training and workflow processes. Results: As a result of Phase I, we were able to develop data collection tools and a workflow and recruitment strategy, involving collaborative opportunities with a respiratory therapist, peer-support pharmacist and tobacco reduction counsellor. We launched the program in two pharmacies in Athabasca, Alberta, where participating pharmacists were trained to identify current smokers, measure their lung function with a portable spirometer and refer for full spirometry testing if airflow obstruction was identified. In Phase II pharmacists will offer TC services to smokers and will follow up with them at one and three months. Data from the patients will be collected via surveys during the three sessions. Conclusions: We were able to develop and initiate a pilot project in which pharmacists provide TC and COPD screening services. This study will help to identify the prevalence of undiagnosed COPD in Athabasca and demonstrate a model of interdisciplinary collaboration for providing TC services.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.c

Evaluating a patient decision aid (PDA) for managing early surgical menopause

Alisha Shivji[1], Tasneem Siyam[1], Dr. Sue Ross[2], Dr. Nese Yuksel[1]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta Edmonton, AB

[2]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB

Purpose: An evidence-based patient decision aid (PDA) was developed to help women who have had an early surgical menopause decide on hormone therapy to help manage symptoms and long term health consequences. The purpose of this study was to evaluate the PDA, "SheEmpowers" with patient stakeholders to ensure their priorities and decisional needs had been met.

Methods: A convenience sample of women who had participated in the initial focus group conducted during the development phase of the decision aid were contacted for participation. Women who agreed to participate attended a one-on-one semi-structured interview between May 31 and July 19, 2018 to review the tool and answer questions on its acceptability using a validated 9-item acceptability questionnaire. Women also completed a questionnaire to capture demographics, education and employment status. All interviews were digitally recorded and transcribed. Quantitative measures were reported as descriptive statistics. Two researchers independently completed content analysis of the qualitative data to identify common themes.

Results: Of the 31 women approached for participation, twelve participants were interviewed. Participants were white (100%), half were between ages 40 to 49 and most were currently employed (83%). Overall, participants rated the acceptability of different sections of the PDA as good or excellent (83%). The length of the tool and amount of information were rated as just right (92% and 83% respectively) and women did not feel the tool was biased (83%). Themes related to the value of the tool, context of use, dissemination and other expectations of support were identified when women were asked to share views on strengths of the PDA and areas for improvement.

Conclusions: The interviews provided invaluable insight into patient perspectives on the tool and helped inform the refinement of the PDA to meet the needs of women who have undergone a surgical menopause with their decision making.

ABSTRACT A.2.d

Assessment of IV Antibiotic Use at Fort Saskatchewan Community Hospital Outpatient Infusion Clinic

Erica McGinn, Marg Hadley, Micheal Guirguis, Ingo Gottstein, Kirstie McLelland, Lynora Saxinger
Alberta Health Services, Edmonton, Alberta, Canada

Purpose: To evaluate current prescribing patterns and identification of potential areas for antimicrobial stewardship improvement at the Fort Saskatchewan Outpatient Antibiotic Therapy Clinic, and to evaluate the impact of a form intended to facilitate infectious disease (ID) care. Methods: A retrospective chart review of antimicrobial therapy offered at the Fort Saskatchewan Community Hospital IV Outpatient Clinic was examined before introduction of ID physician support in Phase 1 (2015 Fiscal Year) and in Phase 2 (2017 Fiscal Year).Next, a form to facilitate care of patients in the same outpatient clinic was introduced in June 2017, Phase 3 of the retrospective chart review was then completed (2018 Fiscal Year). Antimicrobial utilization, duration of therapy by indication, the proportion of charts with a complete care plan, overall IV Outpatient Clinic Antibiotic Utilization in Defined Daily Doses (DDD)/100 patient and antibiotic expenditures calculated in dollars/100 patient visits and Dollars/DDD were captured. Results: A total of 171 charts were reviewed across all three Phases of the project. Virtually all records reviewed had a stated indication of therapy, although detailed rationale was often absent. Mean duration of antibiotic therapy in 2015 was 7.4 (± 9.8) days (Median of 5.7 days) while in 2017 it was 7.1 (± 10.4) days (Median 4.2 days), and final phase it was 4.5 (±4.5) days (Median 3 days), demonstrating high levels of variability in all audit periods. Total expenditure for antibiotics used in the outpatient setting was $56,152 in the 2017 fiscal year and approximately $43,300 in 2018 fiscal year, a dramatic drop from the 2014 where outpatient antibiotic expenditure was $90,000. Conclusions: The introduction of the form appears to have reduced the length of antibiotic therapy, although the high level of variability precluded any definitive conclusions.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.e

Development and Evaluation of a Practice Tool for Management of Combined Hormonal Contraceptives (CHC)

Camille Yearwood, Lapinda Chitnuyanondh, Nese Yuksel

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

Purpose: With the current scope of practice in Alberta including prescribing, pharmacists are ideally positioned to manage the hormonal contraceptive needs of women. The purpose of this study was to update and evaluate a practice tool to guide pharmacists in the management of CHCs.

Methods: A previously developed practice tool for combined oral contraceptives was updated to incorporate the most recent guidelines and content for all CHCs. A convenience sample of pharmacists from a variety of settings (hospital, community, PCN) were contacted for evaluation of the tool. A prototype of the tool was sent to each pharmacist agreeing to participate for review. A questionnaire was created to help guide feedback and one on one interviews were conducted either in person or over the phone. Respondents also had the option to send feedback through email. Questions included perspectives on content, clarity and usefulness of the tool. Responses were documented directly in the questionnaire and reviewed by the research team. Results: Overall 16 pharmacists were contacted and 13 agreed to participate, of whom 3 were women's health experts and 10 were front-line pharmacists (7 community, 3 hospital). All 13 pharmacists thought that the tool was comprehensive, easy to follow, non-biased, and information was clear. Majority agreed that it was accurate (77%). Comments on the amount of information in the tool were equally divided, with most commenting it was an appropriate amount (54%), and some thinking it was too much. Barriers to the practice tool included that it may be too long. Areas for tool improvement included providing a table of CHCs in Canada, listing signs and symptoms of VTE, and including questions for sexual history assessment. Conclusions: The pharmacists provided valuable feedback to the content and applicability of the CHC practice tool. The tool will be revised further based on this feedback.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.f

Development of a Practice Tool to Screen, Assess and Manage Drug Induced Osteoporosis

Anupreet Dhillon, Megan Elliott, Teri Charrois, Nese Yuksel

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

Purpose: Many drug classes can negatively impact bone; however, there is a lack of clear recommendations surrounding assessment and management of risk associated with drug induced osteoporosis. The purpose of this study was to develop a practice tool to screen, assess and manage drug induced osteoporosis. Methods: We completed a literature search to identify drug categories associated with osteoporosis, as well as to provide further insight into the relationship between the medication and osteoporosis risk. From November 2017-May 2018 the following databases were searched: TRIP, ACCESSSS, PubMed, UpToDate, and Cochrane Library. A practice tool was created for clinicians summarizing the mechanism of action, screening, and management recommendations for classes of medications known to impact bone. Results: The practice tool includes 4 sections: effect of drug on bone, nature of association, screening required and management/follow-up. Drugs were categorized into 4 categories based on actionability as follows a) Category 1 medications warrant initiation of osteoporotic medication when appropriate, b) Category 2 medications should be accompanied by close monitoring and follow-up, with initiation of osteoporotic pharmacotherapy when indicated, c) Category 3 medications require monitoring but typically osteoporosis medication is not initiated, d) Category 4 medications do not warrant additional action beyond what is recommended for the general population. Conclusions: The practice tool is intended to serve as a tool to assist clinicians in assessing, managing, and mitigating the risk of drug-induced osteoporosis. Further plans are to have the tool evaluated by experts in the field, as well as evaluation of the feasibility and applicability of the tool in a clinical setting.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.g

International students and their accessibility to on-campus healthcare services

Dillon H. Lee, Ken M. Cor, Lisa M. Guirguis

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

Purpose: The well-being of international student holds significant implications for all students, health services professionals on campus, and university policymakers. There is limited information pertaining to specific healthcare needs of international students and how comfortable they feel accessing healthcare services at the University of Alberta. The aim of this study was to characterize the international student patient experience and factors that influence it, including knowledge, attitudes, and perceived barriers. Method: This exploratory descriptive study employed a mixed-method approach to produce qualitative and quantitative data. Through snowball sampling starting with targeted contacts from the International Student Centre (ISC), interviews were held using a semi-structured interview guide. Interview data were explored using thematic analysis. A 44 item survey was developed to measure the University of Alberta specific experiences including, help-seeking preferences, perceived cultural barriers, and attitudes towards using on-campus health resources. The survey was distributed using the ISC and Faculty Student Services mailing lists. Descriptive analysis was used to characterize data. Results: Results draw from nine interviews with international students, on-campus healthcare providers, and ISC advisors and 59 survey responses from international students. The study determined three themes associated with international students: the issue of knowledge translation, insurance imperatives, and the unique challenges with medications. Quantitative findings support the themes. For example, more than 50% of students were not knowledgeable about the Canadian healthcare system. Conclusions: Explaining the values and the structure of Canada's free healthcare is a crucial step in reconciling the healthcare expectations and realities for international students. The University of Alberta should support student initiatives and quality improvement projects that better help international students to explore the Canadian healthcare system: these could include peer-mentoring by pharmacy students and the development of a targeted healthcare guide for international students. Support: Undergraduate Research Initiative (URI) Social Sustainability Research Award

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT A.2.i

Implementation of Pharmacogenomics in Community Pharmacies in Alberta: Perceptions and Challenges

Amy McMurdo[1], Riad Abou Alwan[1], Patrick R. Mayo[1] and Dalia A. Hamdy[1,2]
[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2] AbEx Health Services LTD, Edmonton, Alberta, Canada

Purpose: To study the perspectives of patients, pharmacy students and pharmacists towards pharmacogenomics (PGx) testing in Alberta community pharmacies. Methods: Three self-administered, anonymous, online surveys were developed and validated. Surveys were composed of 9, 12 and 18 questions for patients, pharmacy students and pharmacists, respectively. Participants were invited via social media and/or email to a one-time link to SurveyMonkey.com. Data were collected over 20 days during June 2018. Descriptive and quantitative analysis were performed. Results: A total of 112 patients, 105 pharmacy students, and 70 pharmacists completed the surveys. 78%, 52% and 21% of pharmacy students, pharmacists and patients were previously introduced to pharmacogenomics, respectively. 75% of patients showed interest in knowing more about the topic, 73% of them agreed on sharing their pharmacogenetic data with their healthcare providers whereas only 40% agreed to pay for the test. 44% of participants thought that the best age to perform this test was at the birth, 33% suggested taking it between the age of 16 and 30 years old and 10% disapproved testing. Both pharmacy students and pharmacists agreed that introducing pharmacogenetic testing, identifying patient candidates and educating them about the test and interpreting their results and sharing with other healthcare providers is an important add on to their daily practice, however, only 25% of the practicing pharmacists were comfortable/confident interpreting the test results based on their current knowledge and experience. Conclusions: Patient education and pharmacist training are essential for the introduction of pharmacogenetic testing and interpretation to pharmacy practice in Alberta. Practicing community pharmacists and pharmacy students are willing to take steps towards the proper addition of PGx to the pharmacist's scope of practice. More patient awareness of the topic is required, hands on experience through dedicated CE units for practicing pharmacists is advisable.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT B.1.a

Does the IgC-domain of B7-1 (CD80) impact receptor binding – a key process in T-cell immunomodulation?

Rui Chen[1], Elena Arutyunova[2], Tae Chul Moon[1], Aravindhan Ganesan[1], M. Joanne Lemieux[2], Khaled Barakat[1,3]

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

[2]Department of Biochemistry, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada

[3]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB, Canada

Purpose: B7-1 (CD80) is a primary modulator of T-cell mediated immune responses that remains an attractive target for immunotherapeutic intervention against autoimmune diseases and cancers. B7-1 is expressed on the surface of antigen presenting cells and interacts with two homologous surface receptors of T-cells, namely CD28 and CTLA-4 (CD152). The B7-1-CD28 interactions co-stimulate T-cells activation; whereas, its binding to CTLA-4 elicits an inhibitory signal. B7-1 is a member of the Ig superfamily and includes two extracellular domains namely Ig variable-like (IgV) and Ig constant-like (IgC). While the X-ray crystallographic studies have demonstrated that with B7-1 the IgV domain is involved in binding to its receptor CTLA-4, other studies have reported that IgC domain also has crucial roles in the binding affinities, possibly though an indirect effect. In this work, we seek to further understand the effect of the presence and absence of the IgC domain of B7-1 on the binding to two different receptors (CD28 and CTLA-4) through biochemical experiments. Methods: We expressed recombinant human B7-1 proteins, with only IgV domain (B7-1^{IgV}) and with both IgC and IgV domains (B7-$1^{IgV/C}$), in BL21 *E. coli*. The expressed proteins were purified using affinity chromatography followed by refolding slowly with dialysis. The quality of refolding was tested using size exclusion chromatography. Subsequently, binding of B7-$1^{IgV/C}$ proteins against the natural receptors were conducted by ELISA. Results: Preliminary results show recombinant B7-$1^{IgV/C}$ had lower binding to human CTLA-4 compared to the commercial B7-$1^{IgV/C}$. Conclusions: Preparation of highly purified B7-1 is the premise of disclosing its IgC domain significance in receptor binding. Optimization of refolding and purification are needed to improve the binding activity of purified B7-1 to CD28 and CTLA-4. More understanding of B7-1 in its binding to CD28 and CTLA-4 will help developing drugs for autoimmune disease and cancers.

ABSTRACT B.1.b

Evidence of myeloperoxidase-mediated oxidation of a novel anti-oxidant drug edaravone

Yeon Kyoung Suh[1], Arno G. Siraki[1]
[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Excessive neutrophil ROS production at the sight of inflammation can cause mitochondrial dysfunction, endothelial dysfunction and tissue injury. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a highly potent antioxidant drug known to scavenge radical species and prevent free-radial induced lipid peroxidation. In activated neutrophils, edaravone has shown to reduce oxidative stress by scavenging singlet oxygen and hydroxyl radical. In this study, we investigated the impact of edaravone on the activity of myeloperoxidase (MPO), an enzyme responsible for the production of an array of neutrophil-derived oxidants, as well as the MPO-mediated metabolism of edaravone. Method: The effect of edaravone on the MPO peroxidation cycle and the halogenation cycle was examined by kinetic measurements of UV-Vis spectral changes in the heme active site (430 – 460 nm) and in the monochlorodimedone (MCD, 291 nm), an indicator used for HOCl generation, respectively. Then UV-Vis spectrum of edaravone was measured to investigate whether edaravone is a substrate of MPO. Hypothetical mechanism of MPO-mediated metabolism of edaravone was established using liquid chromatography–mass spectrometry (LC-MS) and electron paramagnetic resonance (EPR) spectroscopy. Result: Addition of Edaravone to the reaction of MPO and H_2O_2 significantly enhanced the cycling of MPO compound II back to native MPO. In addition, MPO-medicated chlorination of MCD dose-dependently increased with edaravone concentration. MPO-catalyzed product of edaravone was identified at 350 nm by kinetic analysis of UV-Vis spectroscopy. A number of MPO-catalyzed metabolites of edaravone were suggested from the LC-MS, including oxidized dimers from edaravone radicals. EPR spectroscopy confirmed MPO-derived edaravone radical formation by spin trapping with 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), and further confirmed that edaravone radical is able to co-oxidize GSH into glutathionyl radical. Conclusion: This study, for the first time, demonstrates evidence of edaravone oxidation by MPO. Implications of edaravone metabolism in the cellular redox balance and inflammatory response of activated neutrophils will be further investigated.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT B.1.c

Modeling the near-open conformation of human CaV1.2 using external electric field and ion pulling simulations

Tianhua Feng[1], Subha Kalyaanamoorthy[1], Aravindhan Ganesan[1], Khaled Barakat[1,2,3]

[1]Faculty Of Pharmacy And Pharmaceutical Sciences, University Of Alberta, Edmonton, Ab, Canada.

[2]Li Ka Shing Institute Of Virology, University Of Alberta, Edmonton, Alberta, Canada.

[3]Li Ka Shing Applied Virology Institute, University Of Alberta, Edmonton, Alberta, Canada.

Abstract:

Purpose: Voltage-gated calcium channels (CaVs) are widely distributed in the human body and remain essential for normal cardiac and neuronal functions. Activation of CaVs leads to the influx of calcium ions and promotes different cellular events. Similar to voltage-gated ion channels, CaVs undergo a voltage-dependent gating mechanism and transforms into different conduction/conformational states, such as open, close, and inactivated to modulate the flow of ions through the channel. Identifying the amino acids that are significant for this gating process is vital to understand the calcium influx mechanisms and the structure-function relationships of the channel. However, the three-dimensional (3D) structure of the Cav1.2 channel is yet to be resolved. Therefore, we have used computational molecular modeling and molecular dynamics (MD) simulations to model the 3D structure and dynamics of the CaV1.2 channel. Methods: Homology modeling and threading-based methods were applied to model the 3D structure of the closed human CaV1.2 channel from the recently resolved cryo-EM structure of rabbit Cav1.1 channel. The POPC lipid-embedded Cav1.2 model was equilibrated in the presence of water molecules and 150 mM concentration of ions using the classical MD approach. An external electric field of -40mV, corresponding to the activation potential of the channel, was applied to enable extended sampling of the conformational states. Finally, the calcium influx mechanism was studied using the steered molecular dynamics approach. Results: We identified several key residues that are important for the calcium ion influx mechanism. Our simulation also reveals the gating mechanism for transition from a closed conformational state to a near-open conformation (NOC) of the Cav1.2 channel. Conclusions: Our model and simulations provided novel insights into the solvation structure, key barriers, and mechanisms by which the internal gate would be opened during the gating process.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT B.1.d

DDDPLUS APPLICATION IN EARLY DRUG DEVELOPMENT

Juliet Obianuju Njoku1 , Gregory K. Webster2 , Raimar Loebenberg1 .

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada1 , Global Research and Development, AbbVie Inc., North Chicago, IL USA2 .

Purpose: In early drug development, the selection of a formulation platform and decisions on formulation strategies have to be made within a short timeline and with minimal use of API. There is limited information available about the physicochemical and biopharmaceutical properties of a new drug candidate. The current work investigated the adoption of in silico and in vitro tools with improved prediction accuracy on the dissolution profile of immediate release tablets.

Method: DDDPlus software was used to simulate dissolution test profiles of immediate release tablets of ritonavir. Minimum data points required to make predictions were assessed. Data input from ADMET predictor module and Chemicalize were compared to ascertain which input parameters gave better prediction accuracy. Solubility of ritonavir was determined experimentally. A surfactant model was developed and a transfer model to mimic in vivo conditions was simulated. All simulations were compared with experimental dissolution test results.

Results: Solubility versus pH profile was obtained through a combination of in silico and in vitro tools to give better predictions. Data input from ADMET predictor alone showed a lower solubility at pH 1 as opposed to data from Chemicalize showing a higher solubility at pH 1. Three minimum data points was shown to be enough to make predictive simulations. However, predictions at pH 2.0 showed an overestimation of drug release while pH 1.0 and 6.8 were close to the measured values. The two phase dissolution transfer model simulates surfactant solubility in a single vessel and is at this point not suitable to predict the in vivo environments separately, the predictions for the stomach were too high.

Conclusion: For weak bases like ritonavir a minimum of three solubility data points is required for in silico predictions. A surfactant solubility model results in good predictions as long as surfactant solubility was determined experimentally. In silico predictions need some real solubility data to be predictive. A combination of experimental data and simulations can support the dissolution development. Further studies are needed to include excipient effects.

ABSTRACT B.1.e

Assessment of drug metabolizing enzyme expression and activity after high fat feedings, and after a switch back to a normal diet in female rats

Hamdah Al Nebaihi, Dion R. Brocks
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: To investigate the functional changes in enzyme hydroxylation and protein expression of drug metabolizing enzymes triggered by a high fat diet, and whether diet normalization can overturn these changes, in female rats. We hypothesized reductions in enzyme expression and activity after a high fat diet, with rebound increases after an additional 4 weeks of normal diet. Methods: Female Sprague Dawley rats (n=8 total) were started on a 14 week course of 45% high fat rat chow with water starting at 1 month of age. At the end of the 14 week period, half of the animals were euthanized under isoflurane. In the remaining rats, the diet was switched to normal rat chow (fat content 4.5%). Four weeks after, those rats were euthanized. For each of the 14 and 18 week groups, control rats (4 each) were included where the diet was 4.5% fat content. Tissues were collected and frozen at -80°C. Liver microsomes were harvested and lidocaine was incubated with the microsomal proteins with cofactors at 37°C with pH 7.4. HPLC was used to assay the formation rate of lidocaine metabolite, monoethylglycinexylidide (MEGX). Western blot was used to determine protein expression of hepatic enzymes. Those proteins measured were those involved in hydroxylation of lidocaine and drugs (CYP2E1, CYP3A1, CYP1A2, CYP2C12 and CYP2D1). Results: After 14 weeks on high fat diet, MEGX maximal formation rate was reduced significantly ($p< 0.05$). Expressions of CYP3A1, CYP1A2 and CYP2C12 were also significantly lower (≥40% reduction compared to control group using unpaired student t-test). Interestingly, the expressions were elevated back to normal levels when the normal diet was implemented from weeks 14 to 18. Conclusions: Diet-induced obesity was associated with reduction in the hepatic microsomal rate of hydroxylation and some metabolizing enzyme expressions. The changes were reversible by a switch to normal diet.

ABSTRACT B.1.f

Cardiac Pyruvate Dehydrogenase Phosphorylation is Elevated Prior to the Development of Heart Failure in a Mouse Model of Barth Syndrome

Amanda A. Greenwell[1,2,3], Keshav Gopal[1,2,3], Malak Almutairi[1,2,3], Farah Eaton[1,2,3], Jennifer Kruger[4], Rami Al Batran[1,2,3], John R. Ussher[1,2,3]
[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2]Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada
[3]Women and Children's Health Research Institute, University of Alberta, Edmonton, AB, Canada
[4]Health Sciences Laboratory Animal Services, University of Alberta, Edmonton, AB, Canada

Purpose: Heart failure (HF) presents as the leading cause of infant mortality in individuals with Barth syndrome (BTHS), a rare genetic disorder due to mutations in the tafazzin (*TAZ)* gene, which encodes for a phospholipid transacylase critical in the remodelling of the mitochondrial phospholipid, cardiolipin. Despite well-characterized mitochondrial/electron transport chain dysfunction, information regarding perturbations of cardiac energy metabolism in individuals with BTHS remains limited. Hence, our objective was to identify these potential metabolic perturbations and determine whether optimization of cardiac energetics may be a novel approach to attenuate cardiomyopathy development in BTHS children. Methods: Cardiac function in a mouse model of BTHS (tetracycline-inducible *Taz* knockdown (TAZKD) mice) was assessed via ultrasound echocardiography and compared to their wild-type (WT) littermates at ~2 months of age. Hearts were subsequently extracted from ~2.5-month-old TAZKD and WT mice for mRNA/protein expression profiling via semi-quantitative real-time PCR/western blotting techniques. Results: TAZKD mice exhibited early development of a hypertrophic cardiomyopathy as evidenced by increased left ventricular (LV) anterior (0.95±0.04 vs. 0.82±0.03 (mm)) and posterior (0.85±0.05 vs. 0.79±0.09 (mm)) wall thickness during diastole, and impaired LV volumes during both systole and diastole. Conversely, no signs of systolic dysfunction or HF were apparent. Of interest, inhibitory phosphorylation of pyruvate dehydrogenase (PDH), the rate-limiting enzyme for glucose oxidation, was increased in hearts from TAZKD mice. This change coincided with increased protein expression of PDH kinase 4 (PDHK4, gene name *Pdk4*), the primary PDHK isoform in the heart inhibiting PDH activity, but did not coincide with an increase in *Pdk4* mRNA expression. Conclusions: Our results suggest a potential reduction in myocardial glucose metabolism prior to the development of overt HF in TAZKD mice. Furthermore, our future studies will determine whether correcting BTHS-related alterations in cardiac PDH activity may be a possible mechanism to reduce HF development/progression in BTHS.

ABSTRACT B.2.a

Evaluation of Electronic Medication Administration Records in Supporting Safe Medication Administration Practices within Long-term Care Facilities

Andrew E.C. Fuller, BScPharm, Lisa M. Guirguis, BScPharm, MSc, PhD, Cheryl A. Sadowski, B.Sc.(Pharm), PharmD, Mark J. Makowsky, BSP, PharmD

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

Purpose: To evaluate the frequency, type, and severity of reported medication errors within a long-term care facility (LTCF) that utilizes electronic medication administration records and barcode medication administration (eMAR-BCMA) technology to support medication administration. Methods: Retrospective review of paper-based, medication incident reports voluntarily submitted by nursing staff from June 2015 to October 2017 at a 239-bed designated assisted living facility, in Edmonton, AB, Canada that implemented eMAR-BCMA in 2013. Using a standardized template, a single researcher reviewed each medication incident report, classifying errors according to medication-use phase, error type, and severity based on established definitions. Results: A total of 268 incident reports were completed by nursing staff and reviewed. A mean of 9.24±3.58 medication incidents/month were submitted. Six reports were non-resident specific: Incorrect narcotic counts (n=4) and eMAR-BCMA software issues (n=2). Overall, 155 residents were involved in at least one of the 262 resident specific medication incident reports. The majority of medication incidents occurred during medication administration (63.4%), where 45.3% involved missed/omitted medications, incorrect time (23.5%), incorrect dose (11.8%), incorrect medication (11.8%), incorrect resident (4.7%) and incorrect route (0.6%). The majority of medication incidents reached the resident, but caused no harm (56.5%), while 1.9% reached the resident and caused temporary harm. Conclusion: In our study, medication incident reports demonstrated that medication administration errors (MAE) still occur with eMAR-BCMA. Further research is required to assess how preventable MAEs still occur in LTCF that utilize eMAR-BCMA.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT B.2.c

Healthcare student competence and confidence with prescribing: the development of a mixed methods study

Cassandra Woit, Theresa L. Charrois, Nese Yuksel

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

Purpose: Previous research has shown that prescribing competence is poorly correlated with prescribing confidence, and has questioned whether undergraduate programs adequately prepare interns and junior practitioners for safe and rational prescribing. The goal of this project is to investigate whether prescribing competence and perceived prescribing confidence of fourth year pharmacy and medicine students at the University of Alberta are correlated. Methods: A mixed method design will be used to quantitatively measure prescribing competence using five prescribing case scenarios, and qualitatively explore prescribing confidence using a survey. Answers to the cases will be graded based on therapeutic appropriateness and inclusion of all legal requirements. The confidence survey will assess confidence of both assessment and prescribing skills, and consists of a four-point confidence scale. The cases and survey were pilot tested by practicing pharmacists and a physician. Results: Recruitment is currently underway, and results are expected by the end of November 2018. To assess the internal consistency of competence scores and self-perceived confidence ratings, cronbach's alpha will be used. The Spearman correlation coefficient (r) will be used to determine the correlation between prescribing competence and confidence for both cohorts independently. Significance will be defined as $P < 0.05$. The overall level of prescribing competence and self-perceived confidence will be calculated and compared between the two cohorts of students. Conclusion: This project will gauge the level of competence and confidence of future prescribers graduating from the University of Alberta, which may guide curriculum changes and/or improvements.

ABSTRACT C.1.a

Induction of the antioxidant enzyme NAD(P)H:quinone oxidoreductase-1 (NQO1) by clozapine

Md Harunur Rashid[1, 2], Dinesh Babu[1], Arno G. Siraki[1]
[1]Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada
[2]Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh

Background: Clozapine (Clozaril®) is an important antipsychotic drug that is metabolized in neutrophils to a free radical, which then rapidly forms an electrophilic species that is thought to be the clozapine nitrenium ion (CNI). The latter may generate oxidative stress and covalently bind to critical targets in neutrophils, which may be involved in its toxicity. The mechanism(s) for this toxicity is currently unknown but may involve modulation of phase II detoxifying enzymes. Certain electrophilic species are known to induce expression of NAD(P)H:quinone oxidoreductase-1 (NQO1) via nuclear factor erythroid 2-related factor-2 (Nrf2) signaling utilizing the antioxidant response element (ARE). Since clozapine supposedly forms CNI (an electrophilic species), we hypothesize that clozapine (CNI) induces NQO1 activity and expression. Research design and methods: We designed in vitro studies to test this hypothesis. Clozapine's effect on cell viability was determined using a water-soluble tetrazolium salt, WST-8, as a metabolic indicator in HL-60 (human promyelocytic leukemia cell). We investigated the effect of clozapine on expression and enzymatic activity of NQO1 in HL-60 using dichlorophenolindophenol (DCPIP) as a probe substrate. Results: A concentration-dependent cytotoxicity was seen with clozapine treatment for 24 hours ($IC_{50} \approx 35$ µM). Also, a dose-dependent induction of NQO1 activity was found by clozapine. Treatment with 10 µM clozapine caused an approximately two-fold increase of NQO1 activity in comparison to dimethyl sulfoxide (DMSO, vehicle control) and treatment with 25 µM clozapine produced an approximately three-fold increase. Conclusions: The increase of NQO1 activity in response to clozapine demonstrates that clozapine can stimulate transcription of NQO1, potentially through Nrf2 signaling pathway. Induction of the human NQO1 enzyme by clozapine could afford protection against clozapine toxicity reactions via molecular pathways utilizing the ARE. More studies are required to determine the consequences of NQO1 activity and expression. Support: Bangladesh Atomic Energy Commission and University of Alberta.

ABSTRACT C.1.b

Encapsulation of a novel PNKP inhibitor into GE11 peptide-decorated micellar nanoparticles

Igor Paiva[1], Zahra Shrine[2], Sams Sadat[1], Amir Soleimani[1], Mohammad Vakili[1], Gary Martin[3], Frank Jirik[3], Michael Weinfeld[2], Afsaneh Lavasanifar[1].
[1]: Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.
[2]: Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.
[3]: Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada.

Purpose: Surface-modified drug nanocarriers with peptides specific to transmembrane receptors overexpressed on cancer cells is hypothesized to enhance the drug homing in tumors. The objective of this study was to test this hypothesis in an orthotopic mice model of colorectal cancer (CC) using traceable polymeric micelles modified on their surface with GE11 peptide that targets epidermal growth factor receptor (EGFR) on CC cells. Methods: Poly(ethylene oxide)-poly(-caprolactone) (PEO-PCL) or poly(ethylene oxide)-poly(-benzyl carboxylate- -caprolactone) (PEO-PBCL) were developed with and without peptide and Cy5.5 attachment (Garg et al., 2017). The micellar cell uptake, using HCT116 and SW620 cells, was measured using flow cytometry and confocal microscopy. An orthotopic colorectal mice model was used to assess the biodistribution of plain versus GE11 modified-micelles following intravenous administration and imaging through IVIS equipment. The PNKP inhibitor A83B4C63 was loaded in all the tested micelles (Shire et al., 2018). Results: GE11-micelles had a higher *in vitro* uptake than plain-micelles in HCT-116 cells. At early time points, Cy5.5-tagged micelles showed higher signal in the mice tumor site when the core was PCL, whereas micelles with PBCL core accumulated more into the tumor at later time points (i.e. ~24h). The results also showed faster clearance of PEO-PCL micelles, mostly through kidneys, leading to lower accumulation in non-target tissues as well as tumor. A trend was found for a higher tumor accumulation among micelles with GE11 peptide. The A83B4C63 loading was only slightly higher among PBCL based micelles, but their kinetic stability as well as their drug release were significant better compared with PCL based micelles. The GE11 surface decoration had no influence on the drug encapsulation behavior. Conclusion: Peptide decoration positively impacted CC cell uptake and the presence of benzyl in the micellar core significantly contributed to a stronger encapsulation of the novel PNKP inhibitor, A83B4C63.

ABSTRACT C.1.c

Empagliflozin Increases Cardiac Energy Production In Diabetes

Kim L. Ho[1,2], Sonia Rawat[1], Cory S. Wagg[1], Liyan Zhang[1], Hwee Teoh[3], John E. Dyck[1], Golam M. Uddin[1], John R. Ussher[2], Gary D. Lopaschuk[3]
[1]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
[2]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada
[3]St. Michael's Hospital, Toronto, Ontario, Canada

Purpose: Empagliflozin treatment of diabetic patients at risk for cardiovascular disease results in a significant decrease in the risk of heart failure development and mortality. However, the way in which empagliflozin confers its cardiovascular benefits remains poorly understood. Therefore, we wanted to investigate whether empagliflozin's cardiovascular benefits are associated with an improvement in cardiac energy production. Methods: 18 week-old diabetic (*db/db*) male mice were treated with empagliflozin or vehicle for 4 weeks (10/mg/kg/day via their food). The rates of myocardial glucose, fatty acid and ketone oxidation were measured in isolated working hearts perfused with 0.8mM palmitate, 5 mM glucose, 0.5 mM ß-hydroxybutyrate (ketone), and 500 µU/ml insulin. Results: Empagliflozin treatment prevented the development of *ex vivo* cardiac dysfunction in *db/db* mouse hearts. Furthermore, while vehicle-treated *db/db* mouse hearts had decreased overall cardiac energy production compared to C57BL/6J mouse hearts, empagliflozin-treated *db/db* mouse hearts had a 31% increase in energy production compared to the vehicle-treated *db/db* mouse group. Empagliflozin-induced increases in energy production was attributed to a 61% increase in glucose oxidation, not ketone oxidation. Moreover, despite an improvement in energy production, cardiac efficiency was not improved with empagliflozin treatment in the *db/db* mouse hearts. Lastly, since *db/db* mouse hearts presented depressed myocardial ketone oxidation alongside impaired cardiac efficiency, we next determined whether the addition of ketones to *db/db* mouse hearts would improve cardiac efficiency. The addition of ketones, while not affecting glucose or fatty acid oxidation rates, resulted in increased ketone oxidation rates but no improvement in cardiac efficiency. Conclusions: In a diabetic mouse model, empagliflozin's cardiovascular benefits may be due to improvements in myocardial energy production via an increase in glucose oxidation without changes in ketone oxidation or cardiac efficiency. Support: Grant from Boehringer Ingelheim International.

ABSTRACT C.1.d

Development and Validation of a Sensitive Liquid-Chromatography Tandem Mass-Spectrometry Assay for Mycophenolic Acid, Mycophenolic Acid Glucuronide, and Mycophenolic Acid Acyl-Glucuronide in Cell Culture Medium

Yan Rong, Tony KL Kiang

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

Purpose: Mycophenolic acid (MPA) is an immunosuppressant frequently used to prevent graft rejection after solid organ transplantation. MPA can be subjected to clinically relevant drug-drug interactions, which are best characterized *in vitro* using liver cells (e.g. HepaRG cells). The purpose of this study was to develop and validate a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantification of MPA and its metabolites (MPA glucuronide [MPAG] and MPA acyl-glucuronide [AcMPAG]) in the culture medium of HepaRG cells. Methods: Deuterated-MPA and MPAG (MPA-d_3 and MPAG-d_3) were utilized as internal standards. Cell culture medium proteins were precipitated with a mixture of acetonitrile and methanol (0.4% acetic acid). Chromatographic separation was achieved with a C18 column (4.6×250 mm, 5 µm) using a gradient elution with two mobile phase solutions (A) water and (B) methanol, each containing 0.1% formic acid and 2 mM ammonium acetate. Dual ion source, a combination of electrospray ionization and atmospheric pressure chemical ionization, along with positive multiple reaction monitoring (MRM) mode were utilized. Results: MRM mass transitions (m/z) were: MPA (320.95→207.05), MPAG (514.10→303.20), and AcMPAG (514.10→207.05). The calibration curves were linear over concentrations ranging from 0.00467-3.2 µg/mL for MPA/MPAG and 0.00467-0.1µg/mL for AcMPAG. Complete assay validation indicated that the inter- and intra-day accuracy/precision were < 15% (<20% for lower limit of quantitation) for quality control concentrations. The assay was selective, with no matrix interference or carryover effects. The workup conditions were stable with respect to long (30 day)/short-term (1 day) storage, benchtop stability (6 hour), and repeat freeze/thaw (1-3 cycles). Conclusions: We have successfully developed a sensitive and high throughput assay to determine the concentrations of MPA and its metabolites in HepaRG cell culture medium. This is the first assay, to our knowledge, capable of detecting all three analytes in this biological matrix.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT C.1.e

Inhibitory Effects of P-cresol on Mycophenolic Acid Glucuronidation in HepaRG Cells

Yan Rong, Tony KL Kiang

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

Purpose: Mycophenolic acid (MPA) is commonly used for preventing graft rejection in kidney transplant recipients. Uremic toxins such as p-cresol, known to accumulate in these patients, have been shown to affect the catalytic activities of UDP-glucuronosyltransferase (UGT) enzymes. We hypothesized that p-cresol can inhibit the glucuronidation of MPA (primarily metabolized by UGT1A9) in humans and this may be a cause for the large variability in the pharmacokinetics of MPA. Methods: Metabolically competent HepaRG cells were differentiated at passage 16 for all experiments. Cells (0.4 million/well; cultured in high density) were initially exposed to MPA (0.4 µg/mL) for different amounts of time to determine the linear enzymatic conditions. Subsequently, cells were treated with MPA (0.2, 0.4, 0.6, 1, and 2 µg/mL, physiological free concentration range) with/without p-cresol (5-200 µM, concentrations likely observed in patients). Niflumic acid (10 µM, a potent and specific inhibitor of UGT1A9) was utilized as the positive control. Concentrations of MPA and its major metabolite, MPA glucuronide (MPAG), in the culture medium were determined using a newly validated LC-MS/MS assay. Results: Concentrations of MPA and MPAG formed in HepaRG cells were comparable to that found in humans.

Rates of MPA depletion or MPAG formation were linear from 0 – 12 hours (6 hour being the optimal incubation time). Linear increases in MPAG formation (or MPA depletion) were evident in cells exposed to escalating physiological concentrations of MPA. Likewise, p-cresol reduced MPAG formation in a concentration–dependent manner. The Maximum inhibition by p-cresol was observed with the 200 µM concentration (53.6±6.3%, N=3). Niflumic acid, the positive control, significantly reduced MPAG formation. Conclusions: We have developed a novel *in vitro* HepaRG model that has relevant *in vivo* physiological characteristics. We have also characterized, for the first time, the inhibitory effects of p-cresol toward MPA metabolism. These findings are potentially clinically relevant.

ABSTRACT C.1.f

Pharmacokinetics and biodistribution of traceable poly(ethylene oxide)-*block*-poly(ester) based micellar formulations of diclofenac: the effect of poly(ester) structure

Hanan Al Lawati, Mohammad R Vakili, Afsaneh Lavasanifar, Fakhreddin Jamali
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: Nonsteroidal anti-inflammatory drugs are associated with elevated cardiovascular (CV) risk, depending on the extent of their accumulation in the heart and kidneys. We have developed poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles encapsulating diclofenac ethyl ester (DFEE) which favourably altered the pharmacokinetics and disposition of diclofenac in rats. Herein, we investigate whether modifying the PCL structure to poly(α-benzyl-carboxylate-ε-caprolactone) (PBCL) in micelles can induce further changes to the disposition of delivered diclofenac. Methods: DFEE was encapsulated in traceable (Cyanine-5.5 attached) PEO-*b*-PBCL (PBCL-TM) or PEO-*b*-PCL micelles (PCL-TM). The micelles were characterized for their size distribution, DFEE encapsulation, and *in vitro* release. Diclofenac pharmacokinetics and tissue distribution was studied at 24 h following intravenous administration of micellar formulations or free diclofenac (n=3). Excised organs *were* fluorescent imaged. Results: An average diameter of 37.2±0.06 nm was observed for PBCL-TM which was significantly smaller than that for PCL-TM (45.1±0.06 nm). The diclofenac concentration was comparable for both PBCL-TM and PCL-TM in blood and kidneys, significantly higher than free diclofenac in blood (2.3±1.4 and 1.9±0.6 μg/mL for micelles, respectively, vs below detection), and significantly lower than free drug in the kidneys (0.4±0.3, 0.5±0.5, vs 1.5±0.3 μg/g). In heart, PBCL-TM showed significantly lower diclofenac levels compared to PCL-TM and free diclofenac (0.3±0.03 vs. 0.5±0.1, 0.8±0.1 μg/g). In liver and spleen, treatments showed comparable diclofenac concentrations. Both micellar formulations similarly reduced diclofenac partition in the heart and kidneys (heart:blood ratios of 0.4±0.1, 0.7±0.2, and 4.4±0.7 and kidney:blood ratios of 0.8±0.06, 1.2±0.4, and 5.5±2.1 for PBCL-TM, PCL-TM, and free diclofenac, respectively). Near-infrared fluorescence images showed micellar carrier tissue accumulations in-line with those achieved for diclofenac. Conclusions: PBCL based micelles further improved the biodistribution of diclofenac compared to PCL based micelles evidenced by reduced drug accumulation in the cardiac tissue. Both micelles show strong potential for a cardiac-safe delivery of diclofenac.

ABSTRACT C.2.a

Resveratrol Protects Against Angiotensin II-Induced Cellular Hypertrophy through Inhibition of CYP1B1/Mid-Chain Hydroxyeicosatetraenoic Acid Mechanism

Sherif M. Shoieb, Ayman O.S. El-Kadi.

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

Purpose: Cytochrome P450 1B1 (CYP1B1) and its associated cardiotoxic mid-chain hydroxyeicosatetraenoic acid (HETEs) metabolites have been reported to directly contribute to the development of cardiac hypertrophy. Resveratrol (RESV) is naturally occurring and commercially available polyphenol that possess beneficial effects in wide array of cardiovascular diseases. Since RESV is a well-known CYP1B1 inhibitor, the purpose of this study is to investigate whether RESV protects against angiotensin II (Ang II)-induced cellular hypertrophy through inhibition of CYP1B1/mid-chain HETEs mechanism. Methods: Human ventricular cardiomyocytes RL-14 and rat H9c2 cells were treated with vehicle or 10 μM Ang II in the absence and presence of 2, 10 or 50 μM RESV for 24 h. Thereafter, the level of mid-chain HETEs was determined using liquid chromatography–mass spectrometry (LC/MS). Gene expression was measured using real-time PCR and Western blot analysis was performed to assess protein level of CYP1B1. Results: Our results demonstrated that RESV, at concentrations 10 and 50 μM, was able to protect against Ang-II- induced cellular hypertrophy as evidenced by a substantial inhibition of hypertrophic markers, β-myosin heavy chain (MHC)/α-MHC and ANP. Ang II significantly induced the protein expression of CYP1B1 and increased the metabolite formation rate of its associated mid-chain HETEs namely 5-, 8-, 9-, 12- and 15-HETE in both cell lines. Interestingly, the protective effect of RESV, at concentrations 10 and 50 μM, was associated with significant decrease of CYP1B1 protein expression and mid-chain HETEs to nearly control levels. Conclusions: Our results provide the first evidence that RESV protects against Ang II-induced cellular hypertrophy at least in part through CYP1B1/mid-chain HETEs-dependent mechanism. Support: This work was supported by a grant from the Canadian Institutes of Health Research [Grant 106665] to A.O.S.E. S.M.S. is the recipient of Antoine Noujaim Graduate Scholarship in Pharmaceutical Sciences.

ABSTRACT C.2.b

Nano-delivery of PNKP inhibitor exhibits synthetic lethality in PTEN-deficient colorectal cancer xenograft mice.

Sams M. A. Sadat[a], Zahra Shire[b], Igor Paiva[a], Timothy D. R. Morgan[c], Dennis Hall[c], Michael Weinfeld[bd], Afsaneh Lavasanifar[ae]

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

[b] Department of Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

[c] Department of Chemistry, Faculty of Science, University of Alberta, Edmonton, AB, Canada

[d] Division of Experimental Oncology, Cross Cancer Institute, Edmonton, AB, Canada

[e] Department of Chemical and Material Engineering, University of Alberta, Edmonton, AB, Canada

Purpose: Phosphatase and tensin homolog (PTEN) is a tumor-suppressor protein that is lost in up to 75% of aggressive colorectal cancers (CRC). Our research group has found that the co-depletion of PTEN and a DNA-repair protein; polynucleotide kinase phosphatase (PNKP) leads to significant death in many cancer cell lines including CRC cells followed by synthetic lethality. This finding inspired the development of novel PNKP inhibitors as potential new drugs for treating PTEN-negative CRC. Our objective was to investigate the anticancer activity of lead PNKP inhibitor (A83B4C63), developed by our team, delivered with either cremophor EL or methoxy poly(ethylene oxide)-b-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-*b*-PBCL) nano-formulation in wild-type $PTEN^{+/+}$ and $PTEN^{-/-}$ CRC xenograft in mice. Methods: A83B4C63 was either encapsulated in PEO-*b*-PBCL nanoparticles or solubilized with the aid of cremophor EL: ethanol. The anticancer activity of both formulations was determined in HCT116/$PTEN^{-/-}$ and HCT116/$PTEN^{+/+}$ xenograft in NIH-III nude mice (n=5) after three IV injections of A83B4C63 at two different doses of 10 and 25 mg/kg. Control animals received either equivalent doses of cremophor EL or PEO-*b*-PBCL without drug, or 5% dextrose. Histopathological evaluation for different organs of healthy CD-1 mice was performed to identify the toxicity of A83B4C63 as free drug and its nanoparticle formulation following three IV injections at a dose of 50 mg/kg (n=4). Results: The nanocarriers of A83B4C63 reduced the rate of HCT116/$PTEN^{-/-}$ xenograft growth more efficiently than free drug. This was in contrast to wild-type HCT116/$PTEN^{+/+}$ xenografts which showed similar growth rates following administration of A83B4C63 (in either formulation), formulation excipients (without drug) or dextrose. Moreover, no toxicity was observed in histology samples of different organs of the treated mice that received A83B4C63 injection at a dose of 50mg/kg. Conclusion: The results at this point to A83B4C63, particularly as nanoparticle formulation, demonstrate a potential new monotherapy for PTEN-deficient colorectal cancer. Support: Alberta Cancer Foundation, Canada.

ABSTRACT C.2.c

Cardioprotective Effects of CYP-Derived Epoxy Metabolites of Docosahexaenoic Acid Involve Limiting NLRP3 Inflammasome Activation

Ahmed M. Darwesh[1], K. Lockhart Jamieson[1], Hedieh Keshavarzbahaghighat[1], John M. Seubert[1,2]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2]Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

Purpose: Recent evidence suggests that impaired mitochondrial function and activation of the NLRP3 inflammasome cascade has a role in the adverse outcomes following myocardial ischemia-reperfusion (IR) injury. Ecosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the n-3 polyunsaturated fatty acids (PUFAs) derived from food sources. The role of n3-PUFAs and their CYP epoxygenase metabolites in protecting the heart is controversial. The current study investigated whether EPA and DHA and their corresponding CYP metabolites, 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) and 19,20-epoxydocosapentaenoic acids (19,20-EDP), limit IR injury. Methods: Isolated mouse hearts were perfused in the Langendorff mode with vehicle, DHA, 19,20-EDP, EPA or 7,18 EEQ for 40 min of baseline, 30 min of global no flow ischemia and followed by 40 min of reperfusion. Cytosolic and mitochondrial heart fractions were used to assess NLRP3 inflammasome complex components as well as proteins regulating mitochondrial dynamics. Moreover, mitochondrial respiration was assessed in permeabilized cardiac fibers. Results: In contrast to EPA and 17,18-EEQ, DHA and 19,20-EDP exerted protection against IR, shown by a significant improvement in postischemic functional recovery. The activation of the NLRP3 inflammasome complex was induced by IR and attenuated by DHA or 19,20-EDP pretreatment. Intriguingly, pretreatment with either DHA or 19,20-EDP protected against both the degradation of the mitochondrial fusion protein Opa-1 as well as the excessive localization of mitochondrial fission protein Drp-1 induced by IR injury. Furthermore, DHA and 19,20-EDP were able to maintain the antioxidants activities of both the cytosolic TRX-1 and the mitochondrial TRX-2 under IR conditions. Notably, DHA cardioprotective effect was partly attenuated by the specific CYP epoxygenase inhibitor MSPPOH. Conclusions: Our data indicate a differential cardioprotective response between DHA, EPA and their epoxy metabolites toward IR injury. Where 19,20-EDP protected hearts against IR injury by maintaining mitochondrial function and reducing a detrimental inflammatory response. Support: Research supported by CIHR.

Keywords: Heart, N-3 PUFA, Mitochondria, NLRP3 inflammasome.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT C.2.d

FOXM1 Inhibitors: Emergence of a neglected binding force

Seyed Amirhossein Tabatabaei Dakhili, David J. Perez, Keshav Gopal, John R. Ussher, Carlos A. Velazquez
Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, CANADA

Purpose: The Forkhead boX M1 (FOXM1) is a transcription factor essential for normal activation of the cell cycle and replication. However, increasing evidence suggests that overexpression of this protein correlates with cancer and poor patient prognosis, which makes FOXM1 a promising drug target in medicinal chemistry. Based on a computer-based molecular modelling protocol reported by our group, we hypothesized that FOXM1 inhibitors bind to the FOXM1 DNA binding domain (DBD) by (i) a pi-sulfur interaction with His287, and (ii) a halogen bonding with Arg297 within the FOXM1 DNA binding domain. Methods: To test this hypothesis, we used the structure of FDI-6, a commercial FOXM1 inhibitor, to synthesize and screen a new series of derivatives. In this regard, we removed or replaced critical groups at the 4-fluorophenyl position of FDI-6; essentially, we exchanged a sulfur atom with nitrogen or an oxygen; then we determined their inhibitory effect on the expression of nuclear FOXM1 using a triple negative breast cancer cell line and we measured their binding affinity to DNA by ElectroMobility Shift Assay (EMSA). Next, using a site-directed mutagenesis technique, we confirmed specific binding interactions exerted by these molecules. Results: The replacement of 4-fluorophenyl group with different halogen atoms resulted in equipotent compounds while the bioisosteric replacement of methyl group significantly reduced the potency; confirming the role of halogen in drug-protein binding. On the other hand, swapping the sulfur atom by other heteroatoms and also mutating the His287 residue to Phe287 and Ala287 verified the existence of an interaction between sulfur atom and pi electron cloud. Conclusion: These results validate the role of essential binding interactions (pisulfur and halogen) predicted by computer simulations, and provide preliminary evidence to postulate a mechanism of action exerted by “direct” FOXM1 inhibitors.

ABSTRACT C.2.e

Thioester Linked Cationic Lipopolymers for co-delivery of TRAIL plasmid and its complementary siRNA targets: One Stone Two Bird Approach for Cancer Therapy

Bindu Thapa[1], Remant KC[2], Hasan Uludag[2]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, [2]Department of Chemical and Material Engineering, University of Alberta, Edmonton, AB, Canada

Purpose: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in variety of cancer cells without affecting normal cells. However, TRAIL therapy tested so far failed to exert robust anticancer activity in patients due to resistance and its rapid clearance. Hence, we aim, (i) to identify novel small interfering RNA (siRNA) targets, which sensitize breast cancer cells against TRAIL, (ii) to use TRAIL plasmid as promising alternative since it produces TRAIL protein at the site of action in higher concentration and (iii) to co-deliver TRAIL plasmid and identified complementary siRNA. Co-delivery of DNA and siRNA requires a special delivery vehicle which must display enough loading and delivery capacity of DNA/siRNA cocktails. Methods: To identify novel siRNA targets that sensitize breast cancer cells against TRAIL, a siRNA library against 446 human apoptosis-related proteins were screened in breast cancer MDA-MB-231 cells in presence or absence of TRAIL. A library of cationic lipopolymers (PEI-L) was prepared by grafting aliphatic lipids (L) onto small molecular weight (0.6, 1.2 and 1.8 kDa) polyethyleneimine (PEI) with amide or thioester bond. Potential polymer to delivery DNA/siRNA cocktail was identified. Results: Sixteen siRNAs were found to sensitize TRAIL-induced cell death. The most promising novel targets BCL2L12 and SOD1 were further evaluated. Co-delivery of TRAIL plasmid and siRNAs targeting BCL2L12 and SOD1 using thioester linked polymer resulted higher cell death than the separate delivery in breast cancer cells. Co-delivery resulted higher TRAIL secretion and sensitization of both MDA-MB-231 and TRAIL-resistant MCF-7 breast cancer cells against TRAIL. Conclusion: The therapeutic benefit by dual delivery of TRAIL plasmid and its complementary siRNA targets with single carrier provided a more effective way to treat breast cancer. Support: Alberta Innovates Graduate Studentship.

ABSTRACT C.2.f

Pharmacologic inhibition and genetic deletion of soluble epoxide hydrolase improves survival following myocardial infarction in aged female mice

K. Lockhart Jamieson[1], Deanna K. Sosnowski[1], Wang Wang[2], Pavel Zhabyeyev[2], Matthew Edin[5], Darryl Zeldin[5], Zamaneh Kassiri[2,4], Gavin Y. Oudit[2,4], John M. Seubert[1,3,4]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, [2]Department of Physiology, [3]Department of Pharmacology, [4]Mazankowski Alberta Heart Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada, [5]National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, USA

Purpose: Myocardial infarction (MI) accounts for a significant proportion of death and disability in aged individuals. CYP450 metabolism of n-6 PUFA arachidonic acid results in formation of numerous metabolites, called eicosanoids, that exhibit a wide range of cellular effects. Previous studies demonstrate eicosanoids can induce alterations to the mitochondria resulting in preserved cardiac function following ischemic injury. These metabolites are further metabolised by the enzyme soluble epoxide hydrolase (sEH) reducing their endogenous activity. This study investigated post-ischemic cardiac function in aged mice with either genetic deletion or pharmacologic inhibition of sEH. A primary component of this study was to elucidate potential sex differences between male and female mice undergoing the experimental protocol. Methods: Male and female WT and sEH null mice averaging 15 months old underwent permanent occlusion of the left anterior descending coronary artery (LAD). On the day of surgery, WT mice were given either vehicle (0.1% DMSO) or sEH inhibitor *t*AUCB (10 mg/ml) in drinking water for 28 days. Cardiac function was assessed at baseline, 7 days and 28 days post-MI by echocardiography and electrocardiogram. Protein expression was determined by immunoblotting techniques while mitochondrial enzymatic activities were assessed by spectrophotometry. Mitochondrial respiration in cardiac fibres was measured using a Clark-type electrode. Results: Female sEH null and *t*AUCB-treated mice demonstrated significantly improved survival at 28 days post-MI compared to both WT females and their male counterparts. Male mice that survived to 28 days demonstrated a similar phenotype to the females in overall cardiac function and mitochondrial activity, indicating a potential survival bias. Conclusions: These data provide the first evidence demonstrating sex differences in the cardiac response to ischemic injury in mice with genetic ablation or pharmacologic inhibition of sEH. Support: This research is supported by grants from the Canadian Heart and Stroke Foundation and CIHR.

ABSTRACT C.3.a

Application of in silico tools in clinical practice using Ketoconazole as model drug

Daniela Amaral Silva[1,2], Marcelo Dutra Duque[3], Neal M. Davies[2], Raimar Löbenberg[2], Humberto Gomes Ferraz[1,].

[1]Department of Pharmacy, Faculty of Pharmaceutical Sciences, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, Cidade Universitária, 05508-000, Butantã, SP, Brazil
[2]Faculty of Pharmacy & Pharmaceutical Sciences, Katz Centre for Pharmacy & Health Research, University of Alberta, Canada
[3]Department of Pharmaceutical Sciences, Institute of Environmental, Chemical and Pharmaceutical Sciences, Universidade Federal de São Paulo - UNIFESP, Brazil

PURPOSE: Hypochlorhydria is a condition where the production of hydrochloric acid in the stomach is decreased. As a result, the intragastric pH is elevated. This condition can be due to a series of causes, such as disease (gastric mucosal infection caused by *Helicobacter pylori* and is prominent in AIDS patients), ethnicity, age and also the use of antisecretory agents. This may significantly impact the absorption of other drugs that have pH-dependent solubility, such as ketoconazole, a weak base. Within this context, the purpose of this study was to demonstrate how GastroPlus™ – a physiological based software program- can be used to predict clinical pharmacokinetics of ketoconazole in a normal physiological state vs. elevated gastric pH and how different clinical approaches can be evaluated beforehand. METHODS: A physiologically based pharmacokinetic (PBPK) model was built and validated. The developed PBPK model was used to investigate to the impact of different physiologic conditions (hypochlorhydria, drug administered with water and Coca Cola®) on ketoconazole's bioavailability. RESULTS: The developed model was able to accurately predict the impact of increased pH and beverage co-administration on dissolution and absorption of the drug, and confirmed that complete gastric dissolution is essential. Particle size only mattered in hypochlorhydric conditions due to the incomplete gastric dissolution, as its absorption would depend on intestinal dissolution, which corroborates with previous studies. CONCLUSION: In silico approaches are a potential tool to assess a pharmaceutical product's performance and efficacy under different physiological and pathophysiological states supporting the assessment of different dosing strategies in clinical practice.

Faculty of Pharmaceutical Sciences Research Day 2018, University of Alberta

ABSTRACT C.3.b

Are Excipients Inert? Phenytoin: a follow-up half a century later with new insights

Daniela Amaral Silva[1], Neal M. Davies[1], Raimar Löbenberg[1]

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, 11361 – 87 Avenue, Edmonton, AB T6G 2E1.

PURPOSE: The U.S. Pharmacopeia defines excipients as substances other than the active pharmaceutic ingredient (API) that are added in a drug delivery system to aid in the manufacturing process and enhance stability, bioavailability, safety, effectiveness and delivery of the drug. The 1968 phenytoin intoxication outbreak in Australia is a classic example of an API–excipient interaction. When administered with CaSO4 the absorption of phenytoin was reduced. When CaSO4 was replaced by lactose, the amount of drug absorbed was much higher, resulting in the observed intoxication. It was hypothesized that phenytoin was converted to a calcium salt prior to ingestion. The purpose of this study was to mechanistically investigate the interactions between excipients and phenytoin to confirm the hypothesis of the previous reports. METHODS: Titration experiments with phenytoin and calcium salt were performed. Isothermal micro calorimetry was used to determine incompatibilities between excipients, phenytoin and milk. NMR was used to characterize the compounds. Dissolution tests containing CaSO4, lactose or sorbitol as excipients were also performed. Both Canadian and United States of America commercially available capsules were tested with milk and water. RESULTS: The calorimeter results indicate that phenytoin sodium interacts with CaSO4 in aqueous media and the dissolution profile of CaSO4 containing capsules showed a reduced dissolution rate. In addition, phenytoin sodium also interacts with lactose through a Maillard reaction that can occur at body temperature. Likewise, commercial Phenytoin sodium products interacted with milk and the products containing lactose showed browning in water. The reference product contains lactose as an excipient in the formulation, whereas the Canadian generic formulations do not. Any clinical relevance of this difference has not been determined. CONCLUSION: A new incompatibility between phenytoin and lactose has been discovered and an incompatibility with calcium was confirmed, which may have implications in regard to excipients and food effects.

ABSTRACT C.3.c

Development of discriminative and *in vivo* predictive dissolution test: Biphasic dissolution conducted at low buffer capacity conditions.

Daniela Amaral Silva[1], Raimar Löbenberg[1].

[1] Faculty of Pharmacy and Pharmaceutical Sciences, Katz Centre for Pharmacy and Health Research, University of Alberta, Canada.

PURPOSE: There is a great need to optimize performance test methods of drug products, due to its lack to reflect the *in vivo* conditions. Additionally, there is still a lack in studies to discriminate between minor formulation and process changes within the same dosage forms. The purpose of this study was to develop a novel dissolution method to address the deficiencies of the traditional dissolution tests and to assess drug performance under physiologically relevant conditions. METHODS: Different ibuprofen formulations were prepared with minor differences in excipient composition and different manufacturing processes (wet granulation and direct compression). In vitro release of the formulations was investigated using a biphasic dissolution system (5mM phosphate buffer pH 6.5 and octanol) with either 900mL or 200mL of aqueous media. Octanol layer replicates the concurrent absorption that occurs *in vivo* while the drug is being dissolved, whereas the low buffer capacity system is more physiologically relevant than compendial buffers. The results were compared to the conventional USP II dissolution test and single-phase low buffer capacity conditions. RESULTS: Single phase USP II dissolution tests lacked discrimination. In contrast, the single-phase low buffer capacity (900mL) was able to differentiate between manufacturing processes. However, the pH dropped markedly. In the 900mL biphasic dissolution system, the octanol phase was similar between all formulations, which can be attributed to convection characteristics within the vessel. Nevertheless, the organic phase in the 200mL system showed great discriminatory power between minor formulation changes. The release differences for the granulate formulations were correlated with microclimate effect. Moreover, the additional sink assisted to maintain the medium pH. CONCLUSION: The biphasic method provides great potential to discriminate between minor formulation and process changes compared to conventional dissolution methods. Dissolution test with physiologically buffer strengths combined with drug removal from the aqueous media is more *in vivo* predictive.

ABSTRACT C.3.d

Myeloperoxidase-mediated bio-activation of NSAIDs in promyelocytic leukemia (HL-60) cells

Andrew Morgan[1], Dinesh Babu[1], Karim Michail[1,2], Arno Siraki[1]
[1]Faculty of Pharmacy and Pharmaceutical sciences, University of Alberta, Edmonton, Canada
[2]Faculty of Pharmacy, Alexandria University, Alexandria, Egypt

Purpose: The correlation between chronic inflammation and development of different forms of cancer and potential therapeutic benefit of NSAIDs in cancer therapy have been areas of investigation. In this study, we have investigated the acute bio-activation of NSAIDs and their metabolites via myeloperoxidase (MPO), the main peroxidase in acute myeloid leukemia (AML) cells. As bio-activation involves the formation of reactive metabolites, that could be correlated with leukemia cell toxicity. Methods: We tested the 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 peroxidase in UV–vis kinetic spectrophotometry, and electron paramagnetic resonance (EPR) experiments study the relative reactivity of the MPO-derived species towards oxidation of ascorbic acid and glutathione (GSH), respectively. For *in vitro* studies, HL-60 cells were used as a model of AML to perform trypan blue exclusion, cellular ATP analysis, mitochondrial membrane potential (MMP) and GSH assays. Results: Our results showed that diclofenac, 4'-OHD, 5-OHD, DMBI and DMI demonstrated significant cytotoxic effect in HL-60 cells through oxidation by intracellular MPO. Moreover, 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 contents; and only 4'-OHD also generated glutathionyl radical and caused a significant increase in ascorbate co-oxidation rate. Conclusions: MPO could bio-activate NSAIDs into reactive pro-oxidant metabolites, implying a possible correlation between acute cytotoxicity in promyelocytic leukemia cells (HL-60) and MPO-bioactivation of NSAIDs.
Support: Alberta innovates graduate studentship.

ABSTRACT C.3.e

Studying the molecular targets for a set of heterocyclic small molecules with immunostimulatory activity

Yasser Tabana[1], Isobel S Okoye[2], Shima Shahbaz[2], Marawan Ahmed[1], Dinesh Babu[1], Shokrollah Elahi[2], Arno Siraki[1], Khaled Barakat[1,*]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada

*Corresponding author: Dr. Khaled Barakat, kbarakat@ualberta.ca

Purpose: Cancer immunotherapy has emerged as the fourth pillar of cancer treatment, along with surgery, radiation, and chemotherapy. Stimulating the immune system to fight the tumor plays an important role in virtually all aspects of cancer immunotherapy. Although there are antibody-based therapeutics available for immunotherapy, they are associated with a high incidence of adverse drug reactions. The novelty of this project stems from its main objective, which is developing a small molecule immunotherapy drug. Methods: In our study, a set of two synthesized heterocyclic small molecules have been studied for their immunostimulatory effects. Peripheral Blood Mononuclear Cells (PBMCs) were collected from healthy volunteers, T cells proliferative capacity of the small molecules were tested by Carboxyfluorescein Succinimidyl Ester (CFSE) staining, and Interleukin-2 (IL-2) secretions were measured using Enzyme-Linked Immunosorbent Assay (ELISA) (1-2). PBMC cytotoxicity of the small molecules was determined using Cell Counting Kit-8 (CCK-8) assay (3). These effects were compared with pembrolizumab, a programmed cell death 1 (PD-1) immune checkpoint inhibitor. Results: The small molecules have shown significant immune booster activity by stimulating T-lymphocyte proliferation, as well as the release of IL-2. No cytotoxicity effect was observed against PBMCs in vitro. Conclusions: the current study identified the immunostimulatory activities of two small molecules which may be developed further into an effective anticancer agent. The future direction is designed to identify the molecular targets of the small molecules responsible for the immunological activities. Proteomic and transcriptomic profiling will be conducted to identify potential markers and targets that can be linked to their cellular responses, the key pathways will be identified using the Bioinformatic techniques. Support: This work was supported by grants from the Li Ka Shing Institute of Virology and the Li Ka Shing Applied Virology Institute (Project ID:RES0028141) at the University of Alberta and Alberta Cancer Foundation (ACF) (Project ID: RES0025662)

References

1- Schade, A. E., Schieven, G. L., Townsend, R., Jankowska, A. M., Susulic, V., Zhang, R., & Maciejewski, J. P. (2008). Dasatinib, a small-molecule protein tyrosine kinase inhibitor, inhibits T-cell activation and proliferation. *Blood*, *111*(3), 1366-1377.

2- Diao, Y., Wang, X., Wan, Y., Zhong, J., Gao, D., Liu, Y & Jin, Z. (2016). Antitumor activity of a novel small molecule TLR7 agonist via immune response induction and tumor microenvironment modulation. Oncology reports, 35(2), 793-800.

3- Lee, T. J., Kim, E. J., Kim, S., Jung, E. M., Park, J. W., Jeong, S. H.,& Kwon, T. K. (2006). Caspase-dependent and caspase-independent apoptosis induced by evodiamine in human leukemic U937 cells. *Molecular cancer therapeutics*, *5*(9), 2398-2407.

ABSTRACT C.4.a

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

Tasneem Siyam[1]; Sue Ross[2]; Alisha Shivji;[1] Nese Yuksel[1]
[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada;
[2]Department of Obstetrics and Gynecology, Faculty of Medicine and Dentistry, and Women and Children's Health Research Institute (WCHRI), University of Alberta, Edmonton, AB, Canada

Purpose: To systematically develop and evaluate an evidence-based patient decision aid (PDA) to help women decide on hormone therapy (HT) to manage early surgical menopause. Methods: The PDA development was guided by the Ottawa Decision Support Framework (ODSF) and the International Patient Decision Aid Standards (IPDAS). Development involved 3 phases: an exploratory phase to identify women's 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. 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 multidisciplinary group of researchers, clinicians and patient partners to ensure women priorities were met. Results: Informed by identified needs from the exploratory phase a prototype PDA was drafted and had 4 components: facts about surgical menopause and HT; HT outcome probabilities; values clarification; and guidance in decision-making. Clinician and patient experts from the steering committee provided positive feedback about the PDA and suggested improvements were implemented. Participants in the evaluation phase perceived the tool as acceptable and offered useful suggestions for targeted modifications. The tool met 39 of the 46 IPDAS quality criteria for content and development. Conclusion: Through our adopted, systematic, evidence-based and multidisciplinary approach the SheEmpowers PDA was developed that aims to help women overcome deterrents to decision-making related to lack of knowledge, decision-making skills and involvement in therapy decisions. The effectiveness of the tool in terms of achieving those outcomes is yet to be assessed. Support: WCHRI Seed Grant.

ABSTRACT C.4.b

Application of Qualitative reporting guidelines in Pharmacy practice research

Damilola Adesanoye[1], Matthew Witry[2], Lisa Guirguis[1]
[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2] College of Pharmacy, University of Iowa, Iowa City, IA, USA

Background: Qualitative approaches are valuable in understanding behaviours and processes that underpin medication use and patient care. Given their utility, it is not surprising that the number of pharmacy researchers publishing qualitative work has grown. Purpose: The study objective was to evaluate the quality of reporting in a collection of qualitative pharmacy research articles using current reporting criteria. Methods: A systematic literature search was conducted using Ovid MEDLINE to identify original peer-reviewed pharmacy articles employing qualitative research and published from January 2017 to December 2017 in English. We screened 81 titles and abstracts and excluded review papers or studies that used quantitative or mixed methods. Out of 36 relevant articles, we randomly selected 12 articles for full-text appraisal using two common reporting guidelines (SRQR and COREQ) and two additional criteria from literature - theoretical visibility and use of categories vs. themes. Results: Most studies provided sufficient information on the research questions and data collection methods. Over three-quarters of studies lacked information on the researchers' reflexivity, research paradigm, strategies for ensuring trustworthiness and visibility of relevant theory. There was also predominance of descriptive rather than interpretive data analysis. The results suggest the use of reporting standards embedded in broader principles of qualitative research could strengthen the rigour of qualitative studies. Conclusions: Reporting checklists serve as a starting point to improve the transparency of a study's rationale, assumptions and decisions though they are not quick fixes for conducting systematic qualitative research. In particular, the thoughtful application of both theory and thematic analysis may place qualitative research findings in the context of the broader literature and increase their meaningful use in practice.

ABSTRACT D1

PHARMACOLOGICAL INHIBITION OF FORKHEAD BOX O1 ATTENUATES DIABETIC CARDIOMYOPATHY

Keshav Gopal, Rami Al Batran, Malak Almutairi, Amanda A. Greenwell, Amina Eshreif, Farah Eaton, John R. Ussher
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Alberta Diabetes Institute.

Purpose: Cardiovascular disease represents the number 1 cause of death in people with type 2 diabetes (T2D). This includes diabetic cardiomyopathy, of which there are no approved therapies. Previous studies have shown that myocardial glucose oxidation rates are markedly impaired during T2D due to reduced pyruvate dehydrogenase (PDH) activity. Furthermore, activity of the transcription factor, forkhead Box O1 (FoxO1), is enhanced in T2D and has been shown to increase expression of PDH kinase 4 (PDHK4, gene name *Pdk4*), which phosphorylates and inhibits PDH activity. Our aim was to determine whether FoxO1 antagonism could mitigate experimental diabetic cardiomyopathy, and whether the potential mechanisms of benefit involve alterations in *Pdk4* transcription and subsequent PDH activity. Methods: 6-week old C57BL/6J male mice were fed with high-fat diet (HFD) for 10-weeks, injected with Streptozotocin (75 mg/kg) at 4-weeks post-HFD and then treated for 2-weeks with the FoxO1 antagonist AS1842856 (100 mg/kg twice daily) via oral-gavage starting at 8-weeks post-HFD. In vivo cardiac function was assessed via ultrasound echocardiography. At study completion, mice were euthanized, following which the heart and other peripheral tissues were extracted and evaluated for *Pdk1/2/4* mRNA/protein expression and PDH phosphorylation. Results: FoxO1 inhibition in mice with experimental T2D significantly alleviated diastolic dysfunction as assessed by the mitral E/A ratio (1.39±0.13 vs 2.02±0.12). Likewise, systolic function (ejection fraction; 57.4±2.4 vs 69.4±5.4, and fractional shortening; 29.9±1.7 vs 39.4±4.4) also showed signs of improvement following 2-weeks of treatment with AS1842856. FoxO1 inhibition significantly decreased *Pdk4* mRNA (~60%) and PDHK4 protein expression, which correlated with a decrease in PDH phosphorylation. Moreover, FoxO1 inhibition improved glucose homeostasis as determined by enhanced glucose clearance during glucose tolerance testing. Conclusions: Our results suggest that FoxO1 inhibition mitigates the progression of cardiomyopathy in mice with experimental T2D, which may be due to reduced *Pdk4* transcription in the heart and increased PDH activity/glucose oxidation.

ABSTRACT D2

In silico binding kinetics of hERG blockers

Subha Kalyaanamoorthy, Justin Co, Khaled H. Barakat
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: The rapidly activating delayed rectifier current (I_{Kr}) of the human *Ether-à-go-go*-Related Gene (hERG) channel is essential for restoring the resting phase of the cardiac action potential and is well known for their contributions to cardiotoxicity. Despite several efforts, the questions about specific ligand-binding interactions and the unusual affinity to bind variety of ligands is still open. Understanding the residence time of a drug to its target is an important measure for determining the efficacy of the drug. However, predicting the binding kinetics of a drug using *in silico* methods, such as classical molecular dynamics is a time- and resource- consuming process. Methods: We have employed an enhanced sampling molecular dynamics technique called the τ-random acceleration molecular dynamics (τRAMD) method to understand the unbinding mechanisms of few known hERG channel blockers and non-blockers. We also predict the residence time of these molecules using the bootstrapping technique. Results: Our results reveal the unbinding processes and key binding determinants of these small molecules. The predicted residence times shows a good agreement with the experimental binding affinities of these molecules. Conclusions: Predicting the occupancy time of a drug molecule in the target is important for understanding the pharmacological action of the drug.

ABSTRACT D3

hERGBinder: An integrated computational workflow for predicting the hERG liability and mode-of-binding of compounds

Subha Kalyaanamoorthy[1], Shawn M. Lamothe[2], Xiaoqing (Ervin) Hou[3,4], Tae Chul Moon[1], Harley T. Kurata[2], Michael Houghton[3,4], Khaled H. Barakat[1]

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

[2] Department of Pharmacology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada.

[3] Department of Medical Microbiology & Immunology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada.

[4] Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, AB, Canada.

Purpose: Cardiotoxicity is a severe adverse effect caused by the unwanted interactions of small molecule drugs with voltage-gated ion channels in the heart. The human ether-à-go-go-related gene (hERG) potassium channel plays a crucial role in repolarizing the heart by conducting rapid delayed rectifier current (I_{Kr}). The hERG potassium channel has an unusual ability to bind diverse small molecules and prolong the repolarization phase of the cardiac action potential, resulting in a condition called long QT syndrome (LQTS). In order to predict the ability of a compound to bind the hERG channel and to understand which interactions contribute to the stronger binding affinity, we have developed a computational workflow and validated the results using binding and electrophysiology experiments. Methods: A Python-based integrated workflow that performs flexible molecular docking, pose-clustering, membrane-embedded system preparation for molecular dynamics (MD) simulation, classical and ligand unbinding (enhanced-MD) simulation with the adaptive biasing force (ABF) method and binding free energy estimation using the molecular mechanics generalized Born and surface area (MMGBSA) calculation was developed. Small molecule analogs with known strong and weak hERG binding affinities were taken as test cases to validate our workflow. The effects of these molecules in the wild-type (WT)-hERG, and two mutants (Y652A-hERG, and F656A-hERG) were studied using the patch clamp technique on HEK293 cells. Results: Our computational workflow shows a clear differentiation of the known hERG blockers and non-blockers and also provides the critical interactions that lead to the stronger affinities of molecules. The results from modeling were validated using hERG predictor polarization assay and electrophysiology experiments. Conclusions: Our workflow can be used as a cost-effective and early-screening tool for predicting the hERG liability of a compound. The predicted mode of binding for the small molecules will be useful for medicinal chemistry optimization and salvaging of drugs with higher hERG blocking affinity.

ABSTRACT D4

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

Aravindhan Ganesan[1],Theinmozhi Arulraj[1], Khaled Barakat[2]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada.
[2]Li Ka Shing Institute of Virology, University of Alberta, Canada.

Abstract
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 present 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.

ABSTRACT D5

Zooming-into the molecular level differences in the costimulatory and co-inhibitory interactions mediated by B7-1 against its natural ligands for controlling T-cells.

Aravindhan Ganesan[1], Tae Chul Moon[1], Khaled Barakat[1,2]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada.
[2]Li Ka Shing Institute of Virology, University of Alberta, Canada.

Purpose: Activation of T-cells plays a central role in anti-cancer immune responses. B7-1, a surface protein on antigen presenting cells, interact with CD28 and CTLA-4, the homologous proteins on the T-cell surface, to mediate contradicting immunological signals such as co-stimulation and co-inhibition, respectively, to modulate the T-cells activity. Cancer cells upregulate the co-inhibitory pathways to disrupt this physiological counterbalance and tilting more towards the co-inhibitory signals for T-cell inactivation. This allows the cancer cells to grow without the T-cell immune defence. Therefore, developing inhibitors of the co-inhibitory CTLA-4/B7-1 interactions remains of significant interest in the field of cancer immunotherapy. In order to develop specific inhibitors, it is of paramount importance to understand the molecular level differences between the CD28:B7-1 and CTLA-4:B7-1 complexes to discern the specific hot-spots stabilizing them. The objective of this work is to develop the first molecular model of CD28:B7-1 complex and compare and contrast their interactions against the CTLA-4:B7-1 complex at molecular level. Methods: We performed advanced molecular modelling and extensive molecular dynamics (MD) simulations to characterize the interactions of CD28:B7-1 and CTLA-4:B7-1 complexes. Ensemble-based protein-protein docking and MD-based binding-free energy calculations were performed to build the first comprehensive model of CD-28:B7-1 complex. Subsequently, extended MD simulations of the two protein-protein complexes were carried out to unravel the similarities and differences in their interactions. Conclusions: Our results revealed the unique fingerprint hot-spot sites in CTLA-4:B7–1 and CD28:B7–1 complexes. The results presented in this work will, on a long-run, be useful to develop new generation of specific CD28 and CTLA-4 inhibitors for targeted immunotherapy. Support: The funding support from the Li Ka Shing Institute of Virology, the Li Ka Shing Applied Virology Institute, and Alberta Cancer Foundation, Canada.

ABSTRACT D6

Identification and biochemical validation of small-molecule inhibitors targeting the CTLA-4 pathway

Aravindhan Ganesan[1], Justin Shields[2], Dinesh Babu Nithyananda Babu[1], Elena Arutyunova[3], Isobel Okoye[4], Joydeb Kundu[5], Seyedeh Nargess Hosseini[6], Will Turnbull[6], Katharine Cheryl Agopsowicz[7], Tae Chul Moon[1], Arno Siraki[1], Joanne Lemieux[3], Shokrollah Elahi[4], Fredrick West[6], Mary Hitt[7], John Walker[7], Michael Smylie[7], Lorne Tyrrell[2,4,5], Michael Houghton[2,4,5] and Khaled Barakat[1,2,5]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

[2]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada.

[3]Department of Biochemistry, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada.

[4]Faculty of Medicine & Dentistry Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada.

[5]Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, AB, Canada.

[6]Department of Chemistry, University of Alberta, Edmonton, AB, Canada.

[7]Department of Oncology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada

Purpose: Blockade of immunosuppressive pathways to revive the T-lymphocyte (T-cell)-mediated antitumor immune response has emerged as a revolutionary approach for cancer immunotherapy. Immune checkpoints (or the negative immune regulators) transiently suppress the T-cell activation to regulate tolerance towards the normal cells and harmless antigens. Cancer cells often exploit this mechanism through upregulation of immunosuppressive pathways and escape from the immunological surveillance of T-cells. It has been clinically demonstrated that blocking the immune checkpoints such as the CTLA-4-B7 pathway using monoclonal antibodies (mAbs) can reactivate the T-cell immune responses to clear cancers. Given the high costs and severe side effects associated with mAbs, there is a need for developing small-molecule drugs that are safe, less expensive and controllable. Methods: In this work, we report the discovery of potent small-molecule inhibitors (named the N17-class) through high throughput virtual screening and validated using multiple biochemical experiments. Results: Results from Nuclear Magnetic Resonance (NMR) and thermal shift assay have confirmed that our compounds bind strongly to CTLA-4. The biochemical ELISA demonstrated dose-dependent activity of our compounds towards blocking the interactions of CTLA-4 with both of its ligands, B7-1 and B7-2. Conclusions: The N17 series represent a first-in-class drug-like small-molecule inhibitor for targeting the CTLA-4 pathway and our hope is to translate them for clinical development for the treatment of multiple cancer types. Support: Project ID: RES0028141, the Li Ka Shing Institute of Virology and the Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, Canada; Project ID: RES0025662, Alberta Cancer Foundation, Canada.

ABSTRACT D7

Discovery of novel small-molecule inhibitors targeting the CTLA-4-mediated immunosuppression.

Aravindhan Ganesan[1], Elena Arutyunova[2], Isobel Okoye[3], Lai Xu[3], James Nieman[4], Justin Shields[5], Dinesh Babu Nithyananda Babu[1], Joydeb Kundu[4], Seyedeh Nargess Hosseini[6], Katharine Cheryl Agopsowicz[7], Appan Srinivas Kandadai[4], Mostofa Hena[4], Alexandr Belovodskiy[4], Tae Chul Moon[1], Joanne Lemieux[2], Shokrollah Elahi[3], Arno Siraki[1], Fredrick West[6], Mary Hitt[7], John Walker[7], Michael Smylie[7], Lorne Tyrrell[3,4,5], Michael Houghton[3,4,]5, Khaled Barakat[1,4,5]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

[2]Department of Biochemistry, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada.

[3]Faculty of Medicine & Dentistry Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada.

[4]Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, AB, Canada.

[5]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada.

[6]Department of Chemistry, University of Alberta, Edmonton, AB, Canada.

[7]Department of Oncology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, AB, Canada

Purpose: Immune checkpoint blockade therapy –a powerful strategy of targeting negative immune regulators to revitalize antitumor immunity– has revolutionized the field of cancer immunotherapy. The therapeutic value of this approach has been recognized by the 2018 Nobel Prize in Physiology or Medicine. T-cells are modulated by constantly shifting immunological balance managed by co-stimulatory and co-inhibitory signals at the immune synapse, the former activates the T-cell mediated immune response and the latter inhibits the T-cell activation. Cancer cells often disrupt this physiological balance in favour of inhibitory signals by upregulating the inhibitory pathways such as the CTLA-4-B7-1 complex thereby allowing the survival and propagation of tumours. It has been clinically demonstrated that blocking the inhibitory pathways of CTLA-4-B7-1 interactions with monoclonal antibodies (mAbs) can revive the immune system and clear tumor cells. But, mAbs are expensive and have multiple toxic side effects. Methods: In this work, we report for the first-time discovery of novel small-molecule inhibitors through high-throughput virtual screening, and validated using rigorous biochemical and cell-based immunological assays. Results: Our inhibitors have been confirmed to bind CTLA-4 using Nuclear Magnetic Resonance (NMR), thermal shift and Isothermal Titration Calorimetry (ITC) assays. ELISA, AlphaLISA, and cell-based CTLA-4 blockade assays have confirmed that our inhibitors are able to block CTLA-4-B7-1 interactions. Conclusions: Although showing low activity, their soluble nature, low molecular weight, and favourable cytotoxic profiles make them a promising hit suitable for further lead development against CTLA-4 for more rationalized cancer immunotherapy. Support: Project ID: RES0028141, the Li Ka Shing Institute of Virology and the Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, Canada; Project ID: RES0025662, Alberta Cancer Foundation, Canada.

ABSTRACT D8

Genetic Impairment of Pyruvate Dehydrogenase Activity in Skeletal Muscle Promotes Lactic Acidosis and Exacerbates Exercise-Related Fatigue

Rami Al Batran[1,2,3], Keshav Gopal[1,2,3], Qutuba G. Karwi[2,3], Golam M. Uddin[2,3], Tariq R. Altamimi[2,3], Amanda A. Greenwell[1,2,3], Kim L. Ho[1,2,3], Jadin J. Chahade[1,2,3], Farah Eaton[1,2,3], Gary Lopaschuk[2,3], John R. Ussher[1,2,3†]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB Canada
[2]Alberta Diabetes Institute, University of Alberta, Edmonton, AB Canada
[3]Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada

Purpose: Fats and carbohydrates are the principal substrates that fuel aerobic ATP synthesis in skeletal muscle. However, the mechanisms that regulate the relative contribution of carbohydrates and fats for oxidative metabolism during exercise have not been fully elucidated. Therefore, our objective was to determine how skeletal muscle-deletion of pyruvate dehydrogenase (PDH), the rate limiting enzyme for glucose oxidation, would influence energy metabolism in response to exercise. Methods: 5-week-old PDH skeletal muscle knockout ($PDH^{SkM-/-}$) mice and their human α-skeletal actin (HSA)-Cre expressing littermates 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, 10% kcal from lard) or high-fat diet (HFD, 60% kcal from lard) for 12-weeks and subjected to various physiological assessments. Results: $PDH^{SkM-/-}$ lean mice exhibited significant mortality following 2 weeks on LFD, which was associated with decrease in body weight, length and lean mass compared with HSA-Cre mice. Furthermore, $PDH^{SkM-/-}$ lean mice showed no difference in circulating glucose levels, but exhibited significant increases in circulating lactate levels. Surprisingly, oxygen consumption and respiratory exchange ratios were augmented in $PDH^{SkM-/-}$ lean mice compared to their control. Conversely, obese $PDH^{SkM-/-}$ mice showed no differences in mortality, body weight, length and lean mass compared to HSA-Cre mice, but still demonstrated marked impairments in exercise capacity, which was associated with increases in circulating lactate levels post-exercise. Conclusions: Impaired skeletal muscle PDH activity worsens exercise tolerance, which is associated with excessive circulating lactate levels, and may therefore contribute to early life mortality during consumption of a diet enriched in carbohydrates. These observations illustrate the importance of glucose oxidation as an energy source for muscle during exercise, even in the context of increased muscle fatty acid supply during obesity.

ABSTRACT D9

Development of FOXM1 inhibitors as potential theranostic agents: initial steps in the validation of FOXM1 as a positron emission tomography (PET) probe for triple negative-breast cancer detection.

David J. Perez,[1,2] Seyed Amirhossein Tabatabaei Dakhili,[1] Cody Bergman,[2] Stephanie Mattingly,[2] Jennifer Dufour,[2] Frank Wuest,[2] Carlos A. Velazquez-Martinez.*[1]

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

[2] Department of Oncology, University of Alberta, Edmonton, Alberta, Canada.

Purpose: The FOXM1 transcription factor controls the expression of essential genes related to cell cycle progression and cell replication; under normal physiological conditions its expression is significantly decreased in terminally differentiated cells, but it is abnormally activated in most (if not all) malignant cells. During the last three years, our research group has worked on the development of novel (still experimental) FOXM1 inhibitors. We hypothesize that binding interactions exerted by FOXM1 inhibitors could not only inhibit its transcriptional activity but also serve as PET-based imaging probes, individually, ^{18}F-based imaging. Methods: We prepared derivatives from **FDI-6**, (a drug reported back in 2014 by Gormally et al.,) and evaluated them as FOXM1 inhibitors by using the electrophoretic mobility shift assay (EMSA) method. To determine their ability to exert *in vitro* FOXM1 downregulation and anti-proliferative activity, we used a triple negative breast cancer cell line (MDA-MB-231). We selected derivative **FDI-AF** and designed a suitable method to radiolabel it with a Fluorine-18 atom, and prepared **^{18}F-FDI-AF** (radioactive analogue). We measured its cellular uptake in MDA-MB-231 cells. Results: EMSA results showed that **FDI-AF** was able to dissociate the FOXM1-DNA complex with an IC_{50} = 46.4 ± 1.19 □M and Ki = 22.2 ± 0.56 □M, also exerted a time-dependent downregulation of FOXM1 and inhibited cell proliferation in MDA-MB-231 cells (MTT assay, IC_{50} = 41.91 ± 1.20 □M). We prepared **^{18}F-FDI-AF** in 60% radiochemical yield and measured its cellular uptake in MDA-MB-231 cells, reaching an overall 500% of radioactivity/mg of cell protein. Conclusions: We have set the initial steps toward establishing a FOXM1-based probe for PET imaging theranostic agents.