St. Michael's
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Research Training Centre

Trainee Research Day

Abstract Book

About the Research Training Centre (RTC)

The Research Training Centre (RTC) is part of the larger Research Institute at St. Michael's Hospital, which is comprised of the Keenan Research Centre for Basic Science (KRCBS) and the Li Ka Shing Knowledge Institute (LKSKI). The RTC seeks to provide a stimulating research training program and to create a nationally and internationally recognized training environment for future scientists. By bringing together expertise in translational scientific and applied health services research, the RTC works with trainees and the broader research community to support and advance their research training experiences.

What kind of trainees is the RTC supporting?

We provide support for individuals conducting research at St Michael's Hospital as a graduate student (at either the Master's or Doctoral level) or as a Postdoctoral Fellow, and under the supervision of a St. Michael's Hospital scientist; as well we also provide support to supervisors who participate in research training.

A graduate student is an individual enrolled in a graduate program pursuing a Masters or PhD degree which is focused on research.

A postdoctoral fellow (PDF) is an individual with a PhD degree who is pursuing post-doctoral research training.

Our Mission Statement

Creating an outstanding, internationally-recognized training environment for tomorrow's biomedical and health researchers to flourish.

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http://stmichaelshospitalresearch.ca/research-training-centre/

Research Training Centre Trainee Research Day Agenda

Date: Monday, November 5th, 2018
Time: 9:15am - 6:00pm
Location: The Allan Waters Family Auditorium / The Bernie and Mildred Syron Exhibition

Registration and Breakfast *Students and honoured guests will sign in. Poster presenters to set up in Bernie and Mildred Syron Exhibition Hall. Tea, coffee and a light breakfast will be available.*	**8:30am to 9:15am**
Executive Welcome *Dr. Janet Parsons & Dr. Katalin Szaszi, Co-Directors of the RTC* *Dr. Ori Rotstein, Director, Keenan Research Centre for Biomedical Science*	**9:15am to 9:25am**
First keynote: What is Science? Dr. Andras Kapus, Platform Director, Keenan Research Centre for Biomedical Science	**9:30am to 10:15am**
Break	**10:15am to 10:30am**
Oral Presentations	**10:30am to 1:00pm**
Lunch Break	**1:00pm to 2:00pm**
Poster Competition: Open Viewing *Posters will be open for viewing.*	**1:00pm to 3:00pm**
Poster Judging *Judging committee will evaluate posters. Presenters are given five minutes to present and five minutes for questions.*	**1:30pm to 2:30pm**
Second keynote: 8 Facts About A Career in Research That'll Make Your Hair Stand on End *Dr. Patricia O'Campo, Interim Executive Director, Li Ka Shing Knowledge Institute*	**3:15pm to 4:00 pm**
Award Ceremony *Awards ceremony in the Bernie and Mildred Syron Exhibition Hall*	**4:00pm to 4:15 pm**
Networking *Wine and cheese networking event in the Bernie and Mildred Syron Exhibition Hall / The Tony and Anne Arrell Classrooms.*	**4:15pm to 6:00pm**

Oral Competition Agenda

Date: Monday, November 5th, 2018
Time: 10:30am – 1:00pm
Location: The Allan Waters Family Auditorium

Preclinical development of a small molecule identified from a zebrafish screen as a novel candidate drug for sepsis targeting vascular leakage *Presented by Dr. Anju Philip*	**10:30am – 10:45am**
Profibrotic stimuli upregulate the RhoA exchange factor GEF-H1 through a cytoskeleton-dependent positive feedback involving MRTF and SRF *Presented by Dr. Shruthi Venugopal*	**10:45am – 11:00am**
Salvianolic Acids Inhibits Blood Coagulation and Platelet Aggregation: A Potential New Class of Thrombin Antagonists *Presented by Tiffany Ni*	**11:00am – 11:15am**
Ultrasound and Microbubbles to Deliver Therapy to the Injured Lung in ARDS *Presented by Victoria Mintsopoulos*	**11:15am – 11:30am**
Ultrasound-targeted microbubble destruction-mediated miRNA-495 delivery improves cardiac function post myocardial ischemia/reperfusion injury through inhibiting apoptosis *Presented by Zaman Afrasiabi*	**11:30am – 11:45am**
Food sources of fructose-containing sugars and body weight: a systematic review and meta-analysis of controlled feeding trials *Presented by Annette Cheung*	**11:45am – 12:00pm**
The Three I's of diabetes integration in emerging adulthood: developing a framework to individualize diabetes care *Presented by Benjamin Markowitz*	**12:00pm – 12:15pm**
Trends in Elective and Ruptured Abdominal Aortic Aneurysm Repair by Practice Setting in Ontario, Canada from 2003 to 2016 *Presented by Konrad Salata*	**12:15pm – 12:30pm**
Functional MRI of the clock-drawing test in mild cognitive impairment *Presented by Natasha Talwar*	**12:30pm – 12:45pm**
Systematic Review and Meta-Analysis of Non-Caloric Sweetened Beverages versus Water and Glycemic Control *Presented by Néma McGlynn*	**12:45pm – 1:00pm**

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Oral Presentation Abstracts

Preclinical development of a small molecule identified from a zebrafish screen as a novel candidate drug for sepsis targeting vascular leakage

Presenter: Dr. Anju Philip

Supervisor: Dr. Xiao-Yan Wen

Abstract

Loss of endothelial barrier function leading to the leakage of plasma-borne proteins, tissue edema, and tissue/organ damage is a major pathological feature of sepsis. Sepsis is one of the leading causes of hospital deaths (50-80%) worldwide with an economic burden of $16 billion/year in North America. Despite intense research into the pathogenesis of sepsis, over 100 clinical trials failed and current therapies remain largely supportive. Currently there is no therapy to effectively limit or reverse the loss of capillary membrane permeability. Part of the difficulty in identifying novel therapies is the inability to replicate the complexities of human sepsis in fast, cheap and informative animal models to fast track drug candidates to preclinical models. Hypothesis: Developing a zebrafish model for sepsis will allow high-throughput screening of hundreds of small molecules in vivo, helping to fast-track drug candidates to preclinical models. Methodology: 3dpf zebrafish larvae treated with lipopolysaccharide (LPS) develop critical features of sepsis including vascular leakage, reactive oxygen species (ROS) production, and mortality. Using a robotic system, hundreds of "septic" fish in microplates can be treated with drug compounds that are tested for their ability to limit or reverse 3 primary features of the disease: mortality, vascular leak and ROS production. Top leads identified are validated in human cell models of vascular leakage using FITC dextran assays and TEER measurements as well as mice CLP-sepsis models. Results and Conclusion: Drug U, a lead compound identified through this approach rescued mortality, LPS induced vascular leakage and increased ROS production in the zebrafish. Drug U also protected human pulmonary microvascular endothelial cells (HPMEC) from LPS induced FITC dextran leakage, and further protected mice from CLP induced protein leakage and mortality. In conclusion, using a fast, cheap and efficient drug screening approach, we have identified a potential drug candidate for sepsis.

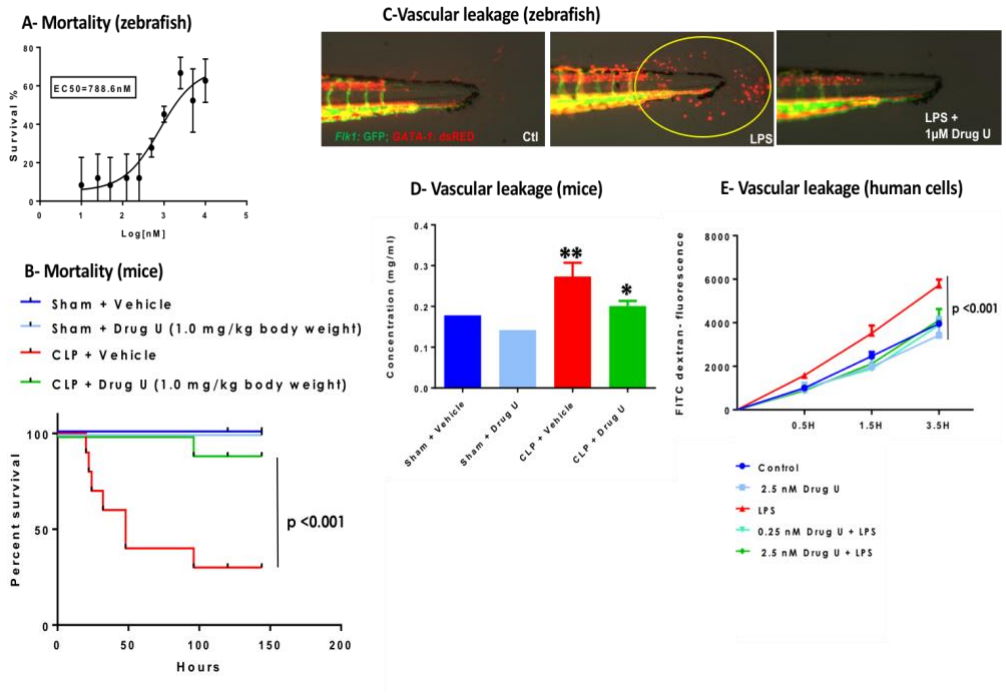


Figure 1: Drug U rescues sepsis associated phenotypes in zebrafish, mice and human cell models of the disease

Drug U dose dependently improved survival in LPS treated zebrafish larvae with an EC50 of 789 nM (A). It also improved survival in CLP-septic mice (B). Drug U protected against vascular leakage in septic zebrafish (reduced the leakage of RBCs at the tail fin region; C) and significantly lowered the exudation of protein into the alveolar space in CLP-septic mice (D). Drug U also significantly lowered LPS induced FITC dextran leakage in human pulmonary microvascular endothelial cells (E).

Profibrotic stimuli upregulate the RhoA exchange factor GEF-H1 through a cytoskeleton-dependent positive feedback involving MRTF and SRF

Presenter: Dr. Shruthi Venugopal

Supervisor: Dr. Katalin Szaszi

Abstract

Kidney fibrosis is the final pathway towards kidney failure in chronic kidney disease, a common complication of diabetes and hypertension. The role of the tubular epithelium in kidney fibrosis is now well recognized. Injury and inflammatory stimuli induce cytoskeletal remodeling and genetic reprogramming in tubular cells, leading to secretion of pro-fibrotic cytokines. Tumor Necrosis Factorα (TNF) and Transforming Growth Factorβ1 (TGF) are crucial inflammatory and fibrogenic cytokines. Our group has previously shown that in tubular cells these cytokines activate the small GTPase RhoA, a master regulator of cytoskeleton remodeling and epithelial reprogramming, through the guanine nucleotide exchange factor GEF-H1. The goal of the current project was to gain mechanistic insights into the profibrotic pathways controlling this molecule. Using unilateral ureteral obstruction, a mouse kidney fibrosis model, we showed that GEF-H1 protein and mRNA were upregulated in tubular cells. Prolonged exposure of cultured tubular cells (LLC-PK1) to pro-fibrotic cytokines or mechanical stimuli (cell stretch) also led to GEF-H1 mRNA and protein upregulation. Interestingly, this was attenuated by RhoA silencing or inhibition. Similarly, silencing the RhoA effector transcriptional coactivator/transcription factor pair Myocardin Related Transcription Factor (MRTF) and Serum Response Factor (SRF) also prevented GEF-H1 upregulation. Conversely, overexpression of MRTF or its activation by the actin-polymerizing drug Jasplakinolide, caused GEF-H1 upregulation. Since GEF-H1 is upstream from the RhoA/MRTF axis, these findings suggest a positive feed-back cycle. Indeed, silencing endogenous GEF-H1 attenuated cytokine-induced activation of a transfected luciferase-coupled GEF-H1 promoter. Finally, activation of the GEF-H1 promoter induced by cytokines or MRTF overexpression were mitigated when the transcription factor Sp1 was inhibited or silenced, suggesting a collaboration between MRTF and Sp1 in GEF-H1 control. In summary, we identified a self-augmenting pathway involving RhoA and MRTF that mediate cytokine-induced GEF-H1 upregulation. This positive feedback may be crucial for progression of kidney fibrosis.

Salvianolic Acids Inhibits Blood Coagulation and Platelet Aggregation: A Potential New Class of Thrombin Antagonists

Presenter: Tiffany Ni
Supervisor: Dr. Heyu Ni

Abstract

Salvia miltiorrhiza root (danshen) extracts have been used to control cardiovascular disease for centuries. In 2005, China approved intravenous administration of the depside salt of danshen for treatment of chronic angina. Salvianolic acids are thought to be the active component of danshen extracts. Salvianolic acid B (SAB), the most abundant salvianolic acid, has been previously shown to inhibit platelet aggregation and thrombosis. However, the mechanism of its action has not yet been adequately explored. A better understanding of SABs will improve their use and prevent adverse drug interactions. Through a series of in vitro coagulation assays, we found that SAB significantly reduced clot weight in whole blood, and delayed the initiation of coagulation in blood plasma using thromboelastography. Confocal microscopy revealed that SAB significantly reduced the fibrin network density in murine platelet poor plasma. These data suggest that SAB targets coagulation factors. Previous network pharmacology analyses suggested SAB interacts with coagulation factor XIII (FXIII) or thrombin. We observed that SAB dose-dependently reduced generation of FXIIIa from FXIII (a thrombin-dependent process) rather than FXIIIa activity, indicating direct inhibition of thrombin activity. Through structural analysis we found that SAB contains similarities to dabigatran, a known oral thrombin inhibitor. In silico molecular modeling predicts that SAB binds within the thrombin active site – interacting with similar residues as dabigatran. Using isothermal titration calorimetry and kinetic thrombin inhibition assays we corroborate these findings and reveal SAB as a direct inhibitor of thrombin activity. Consistently, SAB abrogated thrombin induced human gel-filtered platelet aggregation. Furthermore, using our intravital microscopy laser injury thrombosis model, we demonstrated that SAB significantly decreased murine thrombus growth in vivo. These data demonstrate a novel role for SAB in the inhibition of blood coagulation and platelet aggregation, likely through direct thrombin inhibition, as a new class of herb-derived anticoagulants and low-cost dabigatran analogs.

Ultrasound and Microbubbles to Deliver Therapy to the Injured Lung in ARDS

Presenter: Victoria Mintsopoulos

Supervisor: Dr. Warren Lee

Abstract

Introduction & Objectives: Acute respiratory distress syndrome (ARDS) is characterized by leakage of the alveolar-capillary membrane, leading to pulmonary edema and a loss of lung compliance. Patients show heterogeneous injury on CT scans, with normal-appearing regions interspersed among consolidated regions, making treatment extremely challenging. We aim to use ultrasound-destruction of microbubbles (USMB) to deliver drugs specifically to the injured lung regions in ARDS.

Methods: C57BL/6 mice were infected intratracheally with 5X107 CFU of E. coli. Six hpi mice were administered gentamicin (1.5mg/kg), microbubbles (1X109), and thoracic ultrasound for 5 minutes. Lung gentamicin levels and bacterial CFUs were measured following ultrasound.

In our large animal model, pigs (25-35kg) were administered 1X1011 CFU of E. coli into the right middle lobe (RML) using a bronchoscope. Twenty-four hpi, pigs were given Evan's blue dye (EBD) with microbubbles (7.5X109) IV simultaneously with thoracic ultrasound for 10 min over the RML. Lung EBD deposition was measured following ultrasound.

Results: In our mice model, bacterial growth was reduced almost 10-fold in mice that received gentamicin and USMB compared to controls ($p=0.001$ by ANOVA, $p<0.05$ or 0.01 by post-hoc t-tests). USMB caused a 2-fold increase in gentamicin levels in the BALF and lung homogenates ($p<0.05$) compared to controls that received only gentamicin. We observed a similar increase in EBD in the lung tissue of the RML of infected pigs compared to the same animal's uninjured left upper lobe ($p=0.03$). We demonstrated that this technique does not work on uninjured lung, and that ultrasound or microbubbles alone does not show the same effect.

Conclusion: Our mice model permitted a ten-fold increase in bacterial killing at a low systemic dose of gentamicin. Feasibility in humans is suggested by the porcine model. USMB may therefore allow for targeted delivery of therapies – antibiotics, antivirals, genes – to the injured lung in ARDS.

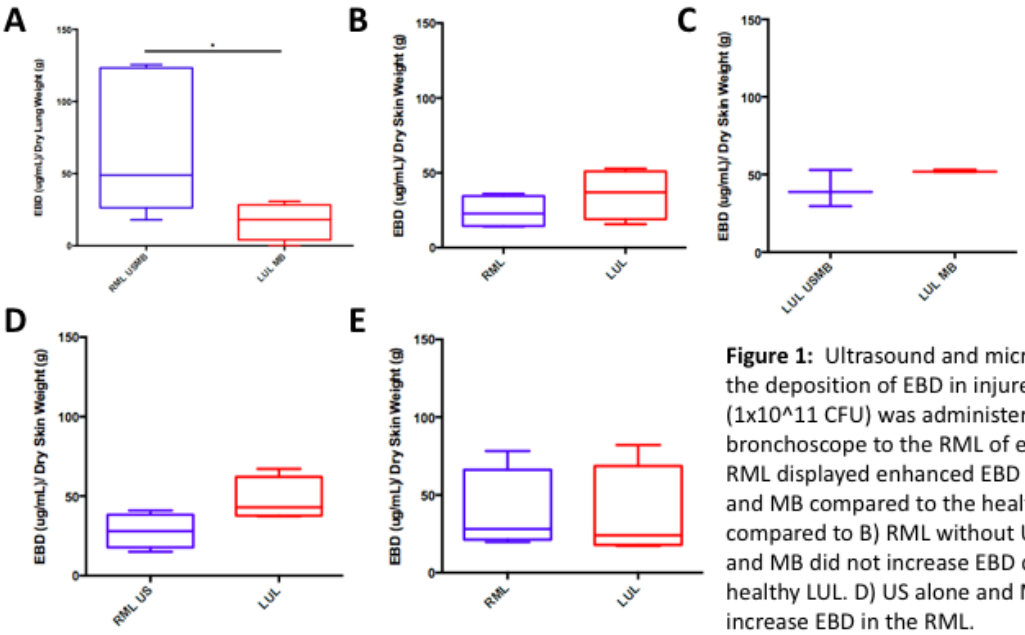


Figure 1: Ultrasound and microbubbles enhance the deposition of EBD in injured lung. *E. coli* (1x10^11 CFU) was administered via bronchoscope to the RML of each pig. A) The RML displayed enhanced EBD deposition with US and MB compared to the healthy LUL and compared to B) RML without US and MB. C) US and MB did not increase EBD deposition in the healthy LUL. D) US alone and MB alone did not increase EBD in the RML.

Ultrasound-targeted microbubble destruction-mediated miRNA-495 delivery improves cardiac function post myocardial ischemia/reperfusion injury through inhibiting apoptosis

Presenter: Zaman Afrasiabi

Supervisor: Dr. Howard Leong-Poi

Abstract

Introduction: MiRNAs play crucial regulatory roles in multiple pathways involved in cardiovascular diseases. RUNX3, a post-transcriptional factor, induces apoptosis in various cells.
Hypothesis: We hypothesized that miR-495 attenuates myocardial ischemia/reperfusion (I/R) injury by targeting RUNX3 and apoptosis.
Methods: In vivo, miR-495 was delivered using ultrasound-targeted microbubble destruction (UTMD) post myocardial I/R in 120 male Sprague Dawley rats. Cardiac function was evaluated by echocardiography. MiR-495, RUNX3 expressions (qPCR) and infarct size (triphenyl tetrazolium chloride (TTC) assay) were measured at day 1 post I/R. In vitro, neonatal rat ventricular myocytes (NRVMs) were transfected with miR-495, scrambled or non-transfected and exposed to hypoxia/reoxygenation (H/R). MiR-495 (qPCR) and RUNX3 (qPCR/western blotting) expressions were quantified. Apoptosis was assessed using western blotting and fluorescence-activated cell sorting.
Results: In vivo, miR-495 expression was decreased and RUNX3 expression was increased at day 1 post I/R vs controls without I/R. UTMD of miR-495 delivery resulted in overexpression of miR-495 and downregulation of RUNX3 (1.25±0.20 vs 2.5±0.54 fold-change, $p<0.05$) at day 1 post I/R. In miR-495-treated rats, LVEF and fractional area change were significantly improved at day 28 post I/R injury. TTC assay indicated smaller infarct size (12.5±1.9 vs 22.4±1.4 %, $p<0.05$) at day 1 in miR-495-treated rats vs controls. In vitro, non-treated NRVMs showed downregulation of miR-495 expression and upregulation of RUNX3 mRNA and protein level post H/R vs normoxia. MiR-495-transfected cells demonstrated decline in RUNX3 mRNA (0.6±0.1 vs 1.6±0.2 fold-change, $p<0.05$) and protein level (1.4±0.1 vs 2.6±0.1 fold-change, $p<0.05$) vs non-treated NRVMs post H/R. MiR-495-treated cells indicated upregulation of Bcl-2 and downregulation of Bim, Bax, and cleaved caspase-3 (0.9±0.02 vs 1.2±0.03 fold-change, $p<0.05$) protein level compared to non-treated NRVMs. FACS showed early and late apoptosis was declined in the miR-495-treated NRVMs.
Conclusions: Our data demonstrate that miR-495 decreases RUNX3 expression and apoptosis to improve myocardial remodeling and LV function post myocardial I/R injury.

Food Sources of Fructose-Containing Sugars and Body Weight: A Systematic Review and Meta-Analysis of Controlled Feeding Trials

Presenter: Annette Cheung
Supervisor: Dr. John Sievenpiper

Abstract

Objective: Excess intake of fructose-containing sugars is associated with increased weight gain. It remains unclear if various food sources of sugars contribute to weight gain. A systematic review and meta-analysis of controlled feeding trials was conducted to assess the effect of different food sources of fructose-containing sugars on body weight using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Methods: MEDLINE, EMBASE, and The Cochrane library were searched through September 2017. Randomized and non-randomized controlled trials of ≥2-weeks duration were included and assessed at four levels of energy control: substitution (energy matched comparisons); addition (energy from sugars added to diet); subtraction (energy from sugars subtracted from diet); or ad libitum (energy from sugars freely replaced). Two independent reviewers extracted relevant data and assessed risk of bias (Cochrane Risk of Bias tool). Data was pooled using random effects inverse variance method and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q) and quantified (I2 statistic).

Results: 134 trials with 9,193 subjects were included (13 subtraction, 44 addition, 78 isocaloric and 4 ad libitum trials). Foods identified included fruit, fruit juice, sugar-sweetened beverages, candy, honey, cake, complex carbohydrates, and mixed sources. In addition studies, total sugars increased body weight (MD=0.47 [95% CI=0.20, 0.74] with substantial heterogeneity across individual food sources ($p<0.001$). Body weight increased with fruit juice, sugar-sweetened beverages (SSBs) and mixed sources, but was reduced with fruit intake. There was no effect of total sugars on weight in substitution, subtraction and ad libitum studies.

Conclusions: The effects of fructose-containing sugars on body weight are both energy and food source dependent. Higher quality trials are required to improve strength of evidence.

Protocol registration: ClinicalTrials.gov Identifier, NCT02558920

Funding: American Society for Nutrition, The Physicians' Services Incorporated Foundation, Canadian Diabetes Association

The Three I's of diabetes integration in emerging adulthood: developing a framework to individualize diabetes care

Presenter: Ben Markowitz
Supervisors: Dr. Andrew Advani & Dr. Janet Parsons

Abstract

Early adulthood is widely recognized as being a challenging time in the life of an individual with Type 1 diabetes, being associated with an increased risk of acute complications, loss to follow-up and the emergence of long-term complications. In an effort to smooth the transition process, guidelines refer to the need to individualize diabetes care and provide "developmentally appropriate support". To help providers better understand the lived experiences of emerging adults with Type 1 diabetes so that they can individualize their care, we undertook a qualitative study employing a narrative approach. In-depth interviews were performed with 33 emerging adults (18 to 24 years) with Type 1 diabetes (51% female, age 20.6±0.3 years, diabetes duration 12±1 years). Narrative analysis defined typology and themes across first-hand accounts. Participants narrated life with diabetes through one of three 'lenses': ingrained (n=14), intrusive (n=12) and inconspicuous (n=7). Participants conveying an ingrained lens described actively integrating diabetes within their lives. Through an intrusive lens, participants described struggles trying to accept diabetes and striving for control. Participants conveying an inconspicuous lens expressed a desire to minimize attention towards diabetes to protect their normalcy. HbA1c levels were lower for participants conveying an ingrained lens (HbA1c (%), ingrained 7.4±0.2, intrusive 9.3±0.5 ($p<0.01$ vs. ingrained), inconspicuous 8.6±0.6). Along with stories about HbA1c, participant accounts about relationships with health care providers, family and peers demonstrated that transitions in diabetes care were experienced differently according to each lens. In intrusive and inconspicuous narratives, participants described being under scrutiny from parents, health care providers, the public and technology (e.g. blood glucose monitors). In contrast, participants conveying an ingrained lens believed they were exemplary self-managers who earned the trust of others. Armed with an understanding of these lenses, adult care providers can look beyond HbA1c towards a more holistic approach to diabetes care.

Trends in Elective and Ruptured Abdominal Aortic Aneurysm Repair by Practice Setting in Ontario, Canada from 2003 to 2016

Presenter: Dr. Konrad Salata
Supervisor: Dr. Mohammed Al-Omran

Abstract

Introduction: Recent years have seen centralization of vascular surgery services in Ontario. We sought to examine the trends in overall and approach specific elective (eAAA) and ruptured abdominal aortic aneurysm (rAAA) repair by hospital type (teaching vs. community).

Methodology: We conducted a population-based time-series analysis of eAAA and rAAA repairs in Ontario, Canada from 2003 to 2016. Quarterly rates of repairs per 100,000 Ontarians > 40 years old were calculated. We fit exponential smoothing models to the approach and hospital type stratified data to examine repair trends.

Results: We identified 19,219 eAAA and 2,722 repairs from 2003 to 2016. Open surgical repairs (OSR) were almost equally split between teaching and community institutions, with 56% (6,724/11,985) of elective and 54% (1,336/2,458) of ruptured OSRs conducted at teaching institutions. However, most endovascular (EVAR) repairs were conducted at teaching institutions [83% (5,969/7,234) of elective and 86% (226/264) of ruptured EVARs]. The rates of eAAA repair and elective open surgical repair (OSR) in teaching and community hospitals decreased by 1.15% ($p=0.0077$), 67% ($p<0.0001$), 23% ($p<0.0001$), and 60% ($p=0.0002$), respectively (Figure). The rate of elective endovascular repair (EVAR) increased 667% in teaching hospitals, ($p<0.0001$). Elective EVAR began in community centres after 2010 and increased to 0.98/100,000 ($p<0.0001$), resulting in a rebound in overall eAAA repair rates in the community. Overall rAAA repairs and ruptured OSR decreased by 84% ($p=0.0007$) and 88% ($p=0.0017$) at community centres. Ruptured EVAR at community centres increased from no procedures prior to 2006, to 0.03/100,000 in 2016 ($p=0.0048$).

Conclusions: Endovascular aortic repair has seen substantial uptake in teaching and community hospitals in Ontario. Furthermore, community hospital uptake of EVAR has begun decentralization of AAA repair. Increased experience and training in EVAR, and reduced specialized care requirements will likely lead to continued decentralization.

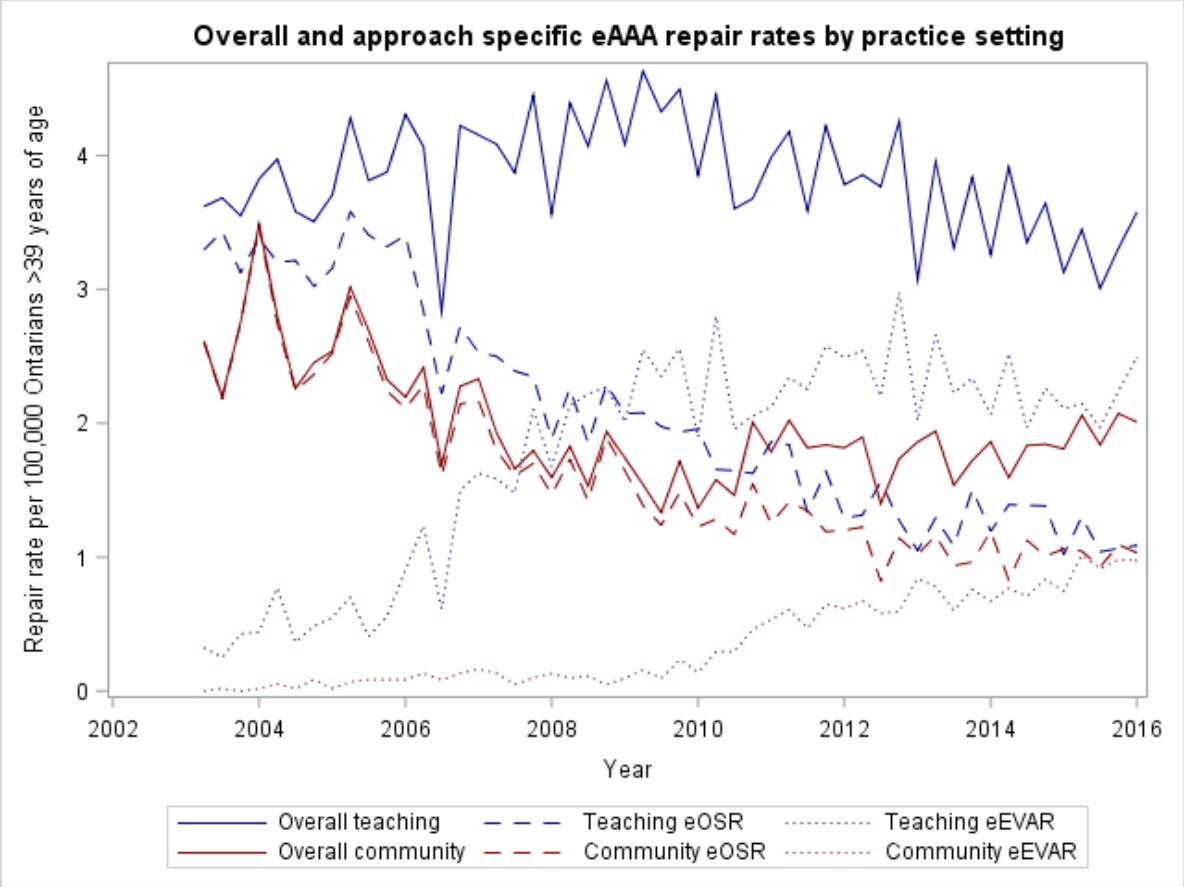


Figure 1: Overall and approach specific eAAA repair rates by practice setting in Ontario from 2003 to 2016

eAAA=elective abdominal aortic aneurysm; eEVAR=elective endovascular aortic repair;

eOSR=elective open surgical repair.

Functional MRI of the clock-drawing test in mild cognitive impairment

Presenter: Natasha Talwar
Supervisor: Dr. Tom Schweizer

Abstract

The clock-drawing test (CDT) is a cognitive assessment tool used to screen for numerous neurocognitive disorders, including mild cognitive impairment (MCI). However, there is limited information on the effect of MCI on brain activity during the test. Until now, the CDT had not been realistically replicated in an MRI environment due to technological limitations. This is the first study to create a naturalistic version of the CDT using functional magnetic resonance imaging (fMRI) and a novel MRI-compatible tablet to identify brain regions activated during the test in healthy and MCI groups. Results may help pinpoint neuropathological changes in brain function associated with impaired CDT performance in MCI.

This study combined fMRI, an MRI-compatible tablet with stylus and an augmented reality system with visual hand feedback, to measure brain activity during completion of the CDT in 17 patients with MCI and 17 matched control participants. Statistical activation maps were calculated using a general linear model (cluster-size thresholded at $p<0.005$, cluster size=20).

This is the first study to show patterns of brain activation in MCI and control groups during the CDT. Both groups showed task-related activation in the frontal, occipital and parietal lobes and the supplementary motor area and precentral gyrus (Figure 1). Compared to controls, patients with MCI exhibited reduced task-related activity, specifically in the frontal and parietal lobes, which are involved in critical cognitive functions for the CDT. Individuals with MCI also scored significantly lower on the CDT as determined by a Wilcoxon test ($p=0.03$). These results demonstrate that the CDT is sensitive to detecting early neuroimaging biomarkers of cognitive decline, however current methods for measuring performance are not sensitive enough to consistently reveal this impairment. This suggests that better performance metrics need to be developed in order to use the CDT as a clinical screening tool for MCI.

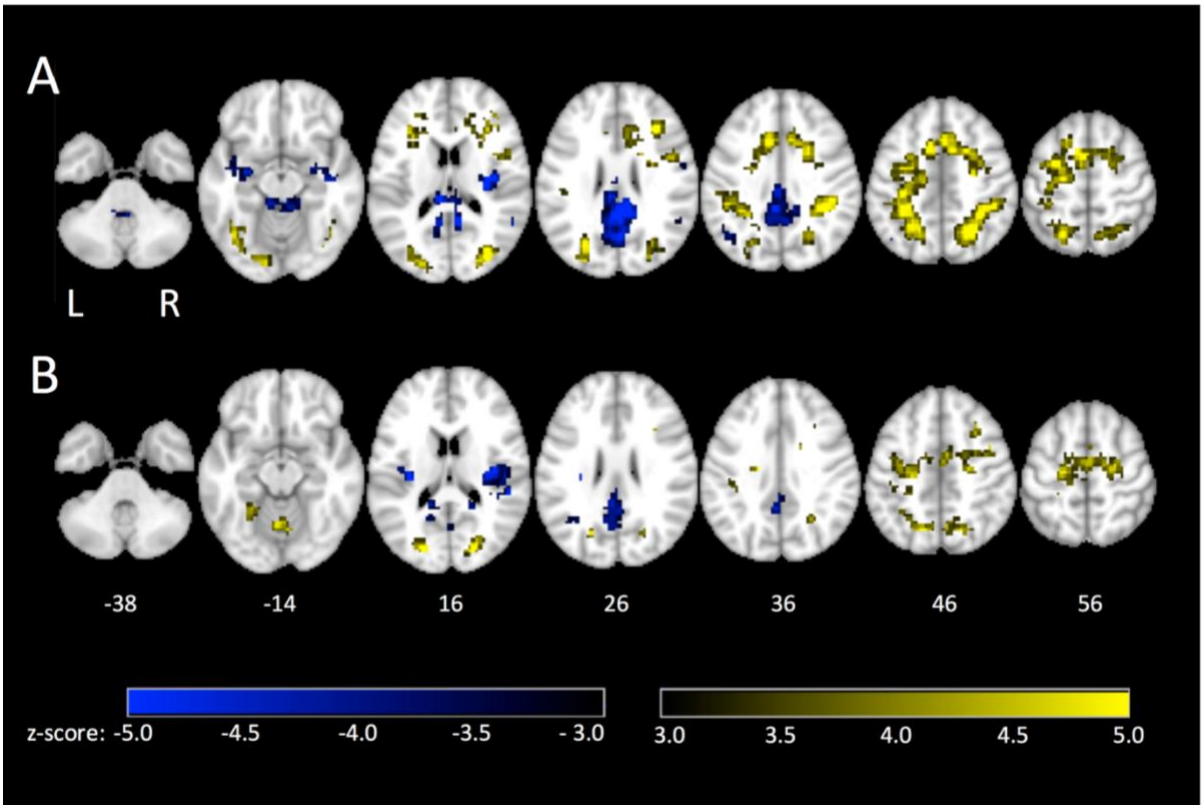


Figure 1. Brain activation associated with completion of the clock-drawing test in (A) healthy controls and (B) patients with MCI. Yellow represents regions with increased brain activity during the test, while blue represents regions with decreased brain activity during the test.

Systematic Review and Meta-Analysis of Non-Caloric Sweetened Beverages versus Water and Glycemic Control

Presenter: Néma McGlynn
Supervisor: Dr. John Sievenpiper

Abstract

Introduction: International health authorities recommend a reduction in free sugars, especially sugar sweetened beverages (SSBs). Water is the preferred strategy for replacing SSBs as there are concerns that non-caloric sweetened beverages (NSBs) may contribute to increased diabetes risk. To address this concern, we conducted a systematic review and meta-analysis (SRMA) of randomized controlled trials using GRADE to assess the evidence of the effect of NSBs versus water on glycemic control.

Methods: We searched Medline, EMBASE and Cochrane Library through March 6th, 2018. We included randomized controlled trials ≥ 7 days duration that compared the effect of NSBs (intervention) vs water (control) on HbA1c, fasting plasma glucose (FPG), fasting plasma insulin (FPI), the homeostasis modal assessment of insulin resistance (HOMA-IR) and two-hour post prandial glucose (2hpp). Two independent reviewers extracted relevant data and assessed risk of bias (Cochrane Risk of Bias tool). Data were pooled using generic inverse variance method with random effects models and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q) and quantified (I2 statistic). The overall certainty of the evidence was assessed using GRADE.

Results: Eligibility criteria were met by 7 randomized controlled trials in 959 predominantly overweight/obese participants. Compared with water, NSBs did not show an effect on HbA1c (0.24 [-0.01, 0.48]), FPG (0.05 [-0.04, 0.13]), FPI (10.36 [-0.83, 21.54]), HOMA-IR (0.23 [-0.21, 0.67]), or 2hpp (0.15 [-0.16, 0.45] with evidence of substantial heterogeneity across all outcomes (I2>50%, p<0.0001). The certainty of the evidence was graded as "high" for FPG, "moderate" for FPI, HOMA-IR and 2hpp and "very low" for HbA1c.

Conclusions: Current evidence does not allow us to conclude that the consumption of NSBs is any worse than water in its effect on glycemic control. More high quality randomized controlled trials are needed to improve our estimates.

Poster Presentation Abstracts

Regulation of the long non-coding RNA transcriptome in endothelial cells in response to shear stress

Presenter: Aravin Sukumar
Supervisor: Dr. Philip Marsden

Abstract

Endothelial cells (EC) play a crucial role in regulating blood flow by adapting to changes in shear stress and modifying the expression of important EC genes, such as endothelial nitric oxide synthase (eNOS). In the absence of healthy (laminar) flow, EC gene expression becomes dysregulated which can predispose the vessel to plaque development. A recent paradigm in gene regulation encompasses a novel class of RNA molecules that do not code for proteins called long non-coding RNAs (lncRNAs). The influence of flow on the lncRNA transcriptome in ECs and their roles in regulating EC phenotype is not well studied. To evaluate if the lncRNA transcriptome is responsive to flow, human umbilical vein ECs (HUVEC) were exposed to static or laminar flow (10 dynes/cm2, 48 hrs) using a parallel-plate flow chamber. Genome-wide profiling of lncRNAs was performed using a custom microarray which revealed 395 (1.3%) upregulated and 323 (1.05%) downregulated lncRNAs (≥2 fold change, uncorrected p-value<0.05) out of 30,586 lncRNAs screened. The top three highly responsive lncRNAs were validated using qRT-PCR which revealed a robust upregulation with flow (60-1500 fold) primarily due to very low expression levels in static conditions. We identified a novel lncRNA that is induced by flow and is preferentially expressed under laminar vs. disturbed flow conditions. Knockdown of this lncRNA using siRNA resulted in dysregulation of several genes involved in vascular homeostasis, such as c-type natriuretic peptide (CNP). We conclude the lncRNA transcriptome is regulated by flow but is more responsive compared to protein-coding genes. Furthermore, we identified an unannotated lncRNA which responds differentially to flow patterns observed in healthy vs. diseased vessels and regulates key EC genes. This work warrants further exploration of the role of lncRNAs in mediating the EC response to shear stress which will expand our understanding of vascular homeostasis and cardiovascular diseases.

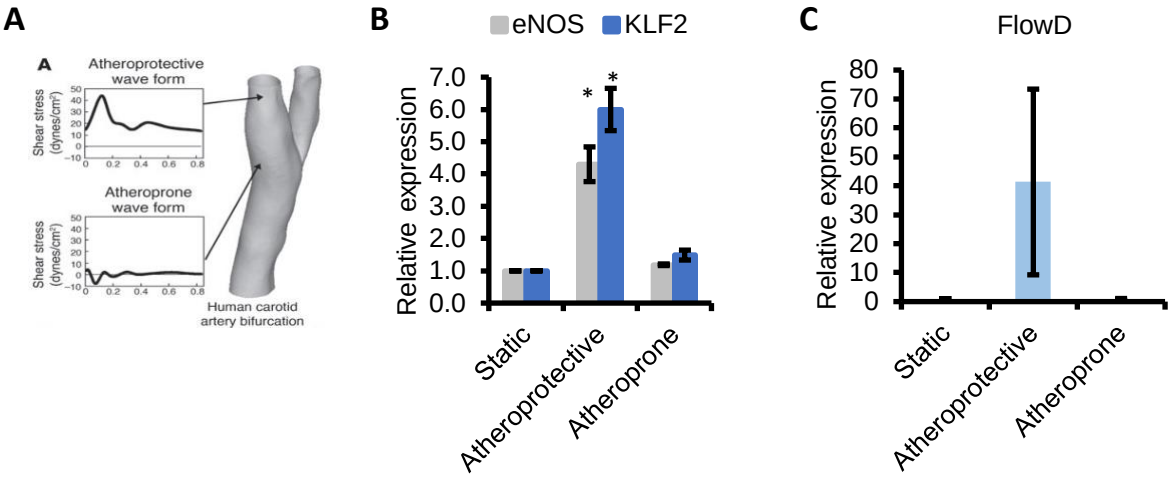


Figure 1: The flow-regulated lncRNA, FlowD, is differentially regulated by atheroprone vs. atheroprotective waveforms. A) Atheroprotective and atheroprone flow waveforms were modeled using a cone-and-plate apparatus to mimic waveforms derived from a human carotid artery bifurcation (Dai et al., *PNAS* 2004). HUVECs were subjected to static, atheroprotective, and atheroprone flow for 24 hours and isolated RNA was analyzed using qRT-PCR. B) eNOS and KLF2 were measured using qRT-PCR to confirm the endothelial response to distinct biomechanical stimuli. Up-regulation of eNOS and KLF2 is associated with an atheroprotective EC phenotype (n=3, mean±SEM, *p<0.05) C) qRT-PCR measurements of flow-regulated lncRNA that is preferentially up-regulated with atheroprotective flow (FlowD). qRT-PCR data normalized to cyclophilin (n=2, mean±SEM).

Needs and experiences of primary care providers when managing incidental findings from genomic sequencing

Presenter: Agnes Sebastian
Supervisor: Dr. Yvonne Bombard

Abstract

Genomic medicine is a way to personalize healthcare by using an individual's genetic variation to diagnose, predict, and prevent health conditions. Genome sequencing (GS) yields more information than standard genetic tests, including findings incidental to the primary reason for testing. With a limited number of medical geneticists, it is critical to support nongeneticists such as primary care providers (PCPs), who will increasingly be tasked with managing many types of incidental findings. Previous studies show that in a hypothetical context, PCPs anticipate major obstacles to their capacity to manage GS incidental findings. However, it is not known what PCPs' needs and experiences will be when managing these findings from their actual patients, in the context of their real practice. I will investigate this by conducting semi-structured telephone interviews of 15-20 PCPs who have a patient receiving GS incidental findings as part of a larger randomized controlled trial. I will purposively sample until thematic saturation is achieved. My interview guide will explore barriers and facilitators such as the PCPs' genetics knowledge, prior experience with genetic tests, counseling/communication of results, impact on management, and educational needs (e.g., online education, point-of-care tools). The interviews will be audio-recorded and transcribed. I will use qualitative descriptive methodology and analyze using conventional content analysis and counting responses. I will begin with first-level coding, then create categories with pattern coding. I will ultimately develop a descriptive summary of how PCPs manage GS incidental findings and whether their anticipated needs and experiences hold true. My results will show whether new concerns were identified and whether anticipated concerns were relevant during real-life management. This provides novel evidence on the current capacity of PCPs to manage genomic medicine. Critical insights from this study will inform the design of targeted educational interventions for providers to optimize delivery of GS in clinical care.

Effects and Mechanisms of Increased Lung Tension in Pulmonary Repair and Regeneration

Presenter: Alice Luo
Supervisor: Dr. Haibo Zhang

Abstract

Introduction & Objective: Cellular turnover is a homeostatic process whereby dying cells in an organ is replaced by newly divided cells. Adult lung normally has low turnover rates, however, in response to injury, the lung exhibits enhanced cell division whereby endogenous lung stem and progenitor cells proliferate to repair and regenerate the damaged tissue. Recent studies have shown that alveolar type II epithelial cells (AECII) cells are endogenous progenitors and can differentiate into alveolar type I epithelial cells (AECI). In a mouse model of left pneumonectomy, the remaining right healthy lung showed regenerative properties upon compensation of increased workload of breathing. Unfortunately, in extensive lung injuries, such as acute respiratory distress syndrome (ARDS), whereby alveolar epithelial damage is diffused, the regeneration potential is disrupted and it may lead to fibrosis. To recapitulate the repair response, we aim to use the non-invasive continuous positive airway pressure (CPAP) to induce appropriate mechanical tension that can stimulate AECII-proliferation and differentiation for lung repair and regeneration in ARDS.

Methods: Male C57BL/6 mice were instilled oro-intratracheally with hydrochloric acid to induce acute lung injury (mouse model of ARDS). Mouse model of ARDS was established and confirmed by 1) histological assessment of the lung, 2) PaO2/FiO2 ratio, and 3) cytospin of bronchioalveolar lavage fluids to determine the inflammatory cell infiltration. In a subsequent pilot study mice were randomized, 48 h after HCl instillation, to receiving CPAP at 10 - 20 cmH2O, or maintaining spontaneous breathing 5 h/day for 4 consecutive days to determine the dose-dependent tolerance in response to the CPAP treatment. The mice were monitored for additional 48 h after the final CPAP treatment and lungs were excised for assessment of alveolar repair and regeneration by histological and immunological examination.

Results: Alveolar collapse and hypercellularity was persistently observed on day 3, 7 and 10 post-HCl injury. PaO2/FiO2 remained low 24 h after HCl instillation (304.84 vs 470.63 in NS control), and neutrophil infiltration was presented at day 3. Mice receiving 20 cmH2O CPAP for 5h/day exhibited faster weight gain post-injury, diminished hypercellularity and less lung collapse as compared to those receiving 10 cmH2O CPAP or under spontaneous breathing. Recovery of alveolar cellular linage was evidenced by increased expression of the AECI marker, Aquaporin-5 (AQN5), in mice treated with 20 cmH2O CPAP. The increased AECI proliferation was associated with an increase in AECII renewal (SPC+Ki67+ cells). In fact, in red-fluorescent protein (RFP)-tagged AECII mice with comparable lung injury as the C57/BL6 as assessed by histological staining, lineage tracing revealed co-staining of AQN5 and RFP post CPAP treatment – suggesting an increase in AECII-derived AECI population.

Conclusion: CPAP-mediated lung tension induced AECII proliferation and differentiation to AECI, thereby promoting lung repair and regeneration in mouse ARDS model. These results may lead to promising use of CPAP in treating ARDS patients. Mechanisms of action are yet to be elucidated.

Detection of Immunoregulatory microRNAs in Cardiac Tissue of Septic Mice Treated with Mesenchymal Stromal/Stem Cells

Presenter: Amin Ektesabi
Supervisor: Dr. Claudia Dos Santos

Abstract

Although bone marrow–derived mesenchymal stromal/stem cell (MSC) systemic administration mitigates sepsis-induced myocardial dysfunction, organ injury, and mortality in clinically relevant models of polymicrobial sepsis, the mechanisms of action remain incompletely understood. Here we hypothesize that MSC administration regulates the differential expression of host-derived miRNAs that in turn determine the transcriptional response profile in the heart, one of the major target organs in sepsis. To experimentally model polymicrobial sepsis, cecum ligation and puncture (CLP) was performed in mice, and the miRNA expression profile was compared between sham, CLP, and MSC-treated CLP hearts. Bioinformatics analysis identified a total of five miRNAs as significantly changed in MSC- vs. placebo-treated septic hearts (false discovery rate <0.05?). To establish the biological relevance of our in silico findings, we determined differential expression of target mRNAs for all five miRNAs using the Illumina microarray expression array. Putative mRNA-miRNA interactions were elucidated. The five miRNAs and 318 putative targets were identified as significantly regulated following MSC administration in septic hearts. Functional enrichment highlighted roles in suppression of inflammation and apoptosis, as well as upregulation of cardiac-specific structural proteins. Hub-gene analysis identified a central role for miR-187 and its target genes Itpkc, Lrrc59, Tbl1xr, all of which are known to play fundamental roles in cardiac inflammation and cardiomyocyte apoptosis. Quantitative real-time PCR validated differential expression of these in silico targets in vivo, as well as markers of apoptosis, in murine septic hearts treated with placebo or MSCs. MSC administration results in the upregulation of host-derived miRNAs involved in protecting cardiomyocytes from sepsis-induced inflammation and apoptosis.

Supported by: Canadian Institutes of Health Research (MOP-137002 to CCDS), Early Research Award from the Ministry of Research and Innovation of Ontario (ERA/MRI 2011) and SCORR ORF: Ontario Research Fund (to DJS, CCDS)

Vegetarian Diets with Cardiovascular Disease Outcomes: A Systematic Review and Meta-Analysis of Prospective Cohort Studies

Presenter: Andrea Glenn
Supervisor: Dr. John Sievenpiper

Abstract

Introduction: Vegetarian diets are recommended for cardiovascular disease (CVD) prevention; however, the role of vegetarian diets and incident CVD outcomes remains unclear.
Objective: To update the European Association for the Study of Diabetes (EASD) clinical practice guidelines for nutrition therapy, we undertook a systematic review and meta-analysis of the association of vegetarian diets compared to non-vegetarian diets with incident CVD in prospective cohorts.
Methodology: MEDLINE, EMBASE, and Cochrane databases were searched through September 6th, 2017. Prospective cohorts ≥1 year, inclusive of diabetes participants, were included. Two independent reviewers extracted data and assessed study quality (Newcastle-Ottawa Scale). Risk ratios for associations were pooled using generic inverse-variance method and expressed as risk ratios (RRs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q-statistic) and quantified (I2-statistic). The overall certainty of the evidence was assessed using Grading of Recommendations, Assessment, Development, and Evaluation (GRADE).
Results: Eight prospective cohort comparisons (197,737 participants, 7,407 events) were included. A vegetarian diet was associated with reduced coronary heart disease (CHD) mortality (RR, 0.78 [CI, 0.69, 0.88]) and incidence (0.72 [0.61, 0.85]). The association with CVD mortality (0.92 [0.84, 1.02]) and stroke mortality (0.92 [0.77, 1.10]) were not significant. The overall certainty of the evidence was graded as "very low" for all outcomes.
Conclusions: Current evidence indicates that vegetarian diets are associated with reduced CHD incidence and mortality but showed non-significant associations with CVD and stroke mortality.

Development of patient "profiles" to tailor counseling for incidental genomic sequencing results

Presenter: Chloe Mighton
Supervisor: Dr. Yvonne Bombard

Abstract

Background: Genomic sequencing (GS) is increasingly used to guide diagnosis and management of hereditary conditions, including hereditary cancer syndromes. A challenge to its delivery is the generation of a large volume of disease risks unrelated to the reason for testing. Guidelines recommend that providers engage patients in shared decision-making about receiving these secondary and incidental results (IR), but this can be time consuming given the myriad of IR and variation in preferences. Genetics resources are limited; there is a need to identify efficiencies in delivering GS and IR. We aimed to develop patient profiles to streamline pre-test counseling for IR.
Methods: We conducted semi-structured interviews with adult cancer patients as part of a randomized controlled trial of the Genomics ADvISER.com, a decision aid for selecting IR. Interviews explored factors participants considered when deliberating over learning IR and were analyzed by thematic analysis.
Results: Participants were mostly female (28/31) about half were over age 50 (16/31). We identified five patient profiles that reflect common contextual factors, attitudes, concerns and perceived utility of IR. Information Enthusiasts self-identified as "planners" and valued learning most/all IR to enable planning and disease prevention because "knowledge is power". Concerned Individuals defined themselves as "anxious," were reluctant to learn IR, anticipating negative psychological impacts from IR. Contemplators were discerning about the value and limitations of IR, weighing health benefits with the impacts of not being able to "un-know" information. Individuals of Advanced Life Stage did not consider IR relevant for themselves; primarily considered the implications for family members. Reassurance Seekers were reassured by previous negative genetic test results which shaped their expectations for receiving no IR: "hopefully [GS will] be negative, too. And then I can rest easy".
Conclusions: These profiles could streamline counseling for IR by providing a framework to address common values, concerns and misconceptions.

Table 1. Summary of profile attributes

	Information Enthusiasts	Concerned Individuals	Contemplators	Individuals of Advanced Life Stage	Reassurance Seekers
Attitude toward IR	Enthusiastic Sense of agency related to learning IR Confident in their decisions	Hesitant Reluctant to learn IR or pursue GS	Recognize potential value Discerning about the limitations Engage in extensive deliberation	Primarily consider impacts for family members Consider how their decisions would have differed at an earlier life stage	Expect not to carry IR Hope for reassurance Possible misunderstanding of IR
Concerns about IR	Limited concerns	Emotional and psychological impacts Limitations of the technology	Uncertainty related to IR	Passing disease risks to family members How their future illness may impact family	Receiving multiple IR
Self-Definition	A "planner"	"Anxious"	Not stated	Altruistic "Experienced"	Not stated
Perceived utility of IR	Medical actions, lifestyle changes Planning for self and family Sharing results with family Seeking further information "Knowledge is power"	Medical actions Weigh utility against potential negative impacts Low perceived utility overall	Medical actions Planning	Planning, particularly for family members Planning to maintain quality of life	Reassurance about disease risk if they do not carry IR Medical actions
Sample category selection	✓Category 1: Medically Actionable ✓Category 2: Common Disease Risks ✓Category 3: Rare Genetic Diseases ✓Category 4: Brain Diseases ✓Category 5: Carrier Status	✗Category 1: Medically Actionable ✗Category 2: Common Disease Risks ✗Category 3: Rare Genetic Diseases ✗Category 4: Brain Diseases ✗Category 5: Carrier Status	✓Category 1: Medically Actionable ✓Category 2: Common Disease Risks ✓Category 3: Rare Genetic Diseases ? Category 4: Brain Diseases ✓Category 5: Carrier Status	✓Category 1: Medically Actionable ✓Category 2: Common Disease Risks ✓Category 3: Rare Genetic Diseases ✓Category 4: Brain Diseases ? Category 5: Carrier Status	✓Category 1: Medically Actionable ✓Category 2: Common Disease Risks ✓Category 3: Rare Genetic Diseases ✓Category 4: Brain Diseases ✗Category 5: Carrier Status
Contextual factors	Typically middle-aged Typically have children	Past distressing experience with genetic testing, or profound health experience	Tend to be younger than other participants	Lived experience with disease Typically have adult children	Expectations for IR are shaped by their previous negative genetic test result

Illustrative quote	*"I'm very much for planning and being prepared. And, also if there are things I'm doing that I should be doing differently that could make a difference in the end result kind of thing, then I would want to know what those things were more specifically."* – SB03	*"I really feel like the emotional component is the one that I was struggling with the most. Because, I do not believe I really fully understand what I can handle and what I can't handle."* – SB27	*"It's a tricky thing […] I absolutely want to be informed […] Then at the same time, once you know something, you can't really un-know it."* – MK42	*"I don't want to be a burden on my kids […] I would want to do everything ahead of time […] I would want them to see me in a good light. I don't want them to remember me in a sick state." – SB31*	*"I want to participate in any learning possible and because I already had a negative one, let's just do it and get the rest of it, and it'll hopefully be negative, too. And then I can rest easy and sleep well."* – MK40

Reporting Bias in Randomized Controlled Trials from Two Network Meta-Analyses: Comparison of Clinical Trial Registrations and Their Respective Publications

Presenter: Dr. Chantelle Lachance
Supervisor: Dr. Andrea Tricco

Abstract

Background: Registration of clinical trials facilitates transparency in study conduct and reporting. Public reporting of serious adverse events (SAEs) in clinical trial registries allows independent review of harms associated with an intervention. Discrepancy between registry data and publications may indicate concealment of results, leading to reporting bias. No study has compared SAE reporting between trial registries and the primary publications included in network meta-analyses (NMAs). Potential reporting bias should be explored as the validity of NMAs depends on the quality and accuracy of the published studies.

Objectives: To determine if there is a difference in the frequency of overall SAEs reported in clinical trial registrations and their respective primary publications.

Methods: We conducted a retrospective review of the published randomized controlled trials (RCTs) included in two recent NMAs investigating cognitive enhancers for Alzheimer's disease and long acting inhaled agents for chronic obstructive pulmonary disease. We included RCTs published in 2005 or later (English only), since the International Committee of Medical Journal Editors (ICMJE) mandated the registration of clinical trials for results to be eligible for publication in its member journals in 2005. Two reviewers independently abstracted study and SAE details from the included study publications and trial registrations.

Results: Of the 203 RCTs included from the two NMAs, 140 (69.0%) were registered with a clinical trial registry and 72 (35.5%) posted results in the registry. Of the publications with results posted in a clinical trial registry, 14 (19.4%) had inconsistent reporting of overall SAEs (publication vs. registry); 7 studies did not report SAEs in the publication, but did in the registry.

Conclusions: We identified inconsistent reporting of SAEs in RCTs from two NMAs. Findings highlight the importance of including trial registries as part of the grey literature search and verifying safety data within these registries before incorporating it into meta-analyses and NMAs.

The Impact of Psychological Factors on the Outcome of Surgical Repair for Rotator Cuff Tears: A Prospective Cohort (IMPROVE)

Presenter: Christine Schemitsch
Supervisor: Dr. Aaron Nauth

Abstract

Background: Rotator cuff tears are a significant cause of pain and reduced function in the shoulder. Despite the advanced surgical techniques that are available to repair these tears, poor results are sometimes seen in a subgroup of patients. To date, studies that have examined predictors of a successful outcome following rotator cuff repair surgery have been limited to components of physical health or surgical technique. An increasing body of evidence has demonstrated a strong correlation between pre-operative psychological factors and functional outcome following several orthopaedic procedures. This association, however, has not been fully demonstrated or effectively investigated in the context of rotator cuff surgery.

Methodology: The IMPROVE study is a multi-centre, prospective, observational cohort of 223 patients. The main objective is to determine the impact of several psychosocial factors on post-operative outcomes following rotator cuff repair surgery. Patients will complete several psychosocial questionnaires prior to surgery (measuring symptoms of depression, anxiety, catastrophic thinking, pre-operative expectations, and social support), in addition to standardized assessments of their surgical outcome. The primary outcome of interest is the change in the Western Ontario Rotator Cuff Index (WORC) measured at baseline and one year post-operatively.

A multiple linear regression analysis will be performed to determine the relationship between the pre-operative psychosocial factors and the WORC score assessed at one year, taking into account baseline characteristics of the patients.

Results: Patient recruitment is ongoing, and 90 patients are currently enrolled.

Discussion: The information provided by this study will allow us to better understand the complex interplay between surgical outcomes following rotator cuff repair surgery and specific psychosocial factors. This information may allow for better patient screening in the future and/or the design of psychosocial interventions to improve upon patient outcomes following rotator cuff repair surgery.

The role of focal adhesion kinase and yes-associated protein signaling in adipose tissue fibrosis and insulin resistance

Presenter: Daniel Han
Supervisor: Dr. Cynthia Luk

Abstract

Fibrosis is the excessive deposition of extracellular matrix (ECM) components and a leading cause of chronic disease in vital organs such as the heart, liver, lung, and kidney. Observational studies suggest that fibrosis increases in adipose tissue of obese humans and correlates with insulin resistance and type 2 diabetes. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase important in integrin signaling, a fundamental pathway that responds to ECM stimuli. Another important fibrosis signaling pathway involves yes-associated protein (YAP), which is a transcriptional co-activator of Hippo signaling that can also retain transcription factors Smad2/3 in the nucleus and promote profibrotic TGF-□ signaling. The precise role of these pathways in adipose tissue and fibrosis remain unclear. Therefore, the aim of this study is to identify the role of FAK and YAP in adipose tissue fibrosis in the setting of type 2 diabetes. Our preliminary data show that the expression of YAP increased in inguinal, perigonadal, and interscapular fats of mice fed a high-fat diet and mice that are genetically obese compared to the control group. Furthermore, knocking down adipocyte FAK in both control and high-fat diet-fed groups increased YAP expression. This suggests that FAK integrin signaling regulates YAP, which plays a major role in the development of fibrotic disorders. Future studies may examine the expression of phosphorylated Smad2/3 when FAK or YAP is inhibited in adipocytes. Additionally, adipose tissue-specific knockouts of FAK and YAP can be generated to determine their in vivo role in adipose tissue fibrosis and insulin resistance. Mechanistically, inflammatory mediators associated with adipose tissue fibrosis, such as TNF-□, could inhibit insulin signaling pathways and result in insulin resistance. Therefore, this work could identify an important role of FAK and YAP signaling in adipose tissue, which may form the foundation for new therapeutic approaches to obesity and diabetes.

Characterizing and developing new program models for diabetes control and self-management among those experiencing homelessness

Presenter: Dr. David Campbell
Supervisor: Dr. Gillian Booth

Abstract
Introduction: Due to various health and social challenges, patients with diabetes who are experiencing homelessness often struggle with self-management. As a result, glycemic control tends to be quite poor. More frequent hospitalizations and more severe complications result from poor ambulatory care. Unfortunately, diabetes self-management education programs are only attended by a fraction of newly diagnosed patients; attendance among the urban-dwelling poor, as well as those with mental illness is even lower. Our objective is to obtain a deeper understanding of the current care offered and the challenges faced by this population.
Methodology: This is a multi-faceted qualitative program of research that has three distinct components:
(1) Scoping Review: We will search published and grey literature for any reports of novel interventions for enhanced diabetes care or education for individuals who are experiencing homelessness; (2) Environmental Scan: We will conduct interviews with stakeholders and care providers in five major Canadian cities to understand what diabetes care is being provided in academic centres, hospitals and community-based facilities which is often absent from published and grey literature; (3) Participatory-Action Research: We will recruit a group of 10-15 individuals who are now or have recently experienced homelessness to join as research collaborators. Together we will explore the challenges to diabetes self-management and work towards developing ideas that might address these problems.
Results: Results for this work are forthcoming, as it is currently work in progress.
Conclusions: It is our hope that through coming to a more thorough understanding of the challenges to optimal diabetes self-management faced by individuals who are experiencing homelessness, we will be able to collectively develop interventions and new models of care that address the common barriers. This would hopefully lead to a reduced burden of acute and chronic diabetes-related complications among this population.

Functional Deficiency of Dicer in Atherosclerosis: Role for Hemodynamics Shear Stress Regulation of the miRNA Biogenesis Pathway

Presenter: Eileen Tran
Supervisor: Dr. Philip A. Marsden

Abstract

Endogenous microRNA (miRNA) acts as cytoplasmic post-transcriptional gene regulators. Targeting specific miRNAs can modulate atherosclerosis development. Particularly, miR-145 is significantly downregulated in atherosclerotic lesions and we have shown that the re-expression of miR-145 to basal levels in ApoE-/- (atherosclerosis murine model) was associated with 60% size reduction of plaque lesions. Dicer is an essential RNA interference (RNAi) enzyme which cleaves pre-miRNA into its functional mature miRNA. The regulation of Dicer and its effects on miRNAs implicated in atherosclerosis remains undefined. Dicer expression is frequently shown to be downregulated in diseased conditions. Investigating the mechanism that contributes to functional deficiency of Dicer is important in understanding atherosclerosis development and can provide novel insight for therapeutic targets using RNAi. Aortic arches of ApoE-/- mice were harvested to determine the expression of miRNA biogenesis factors in-vivo. Dicer expression was significantly reduced in these murine models. Global profiling of miRNA expression in ApoE-/- aorta via a microarray shows 77% reduction of miRNA levels. Endothelial cells (EC) from distinct regions of the aorta in C57Bl/6 (wild-type) mice were isolated to examine hemodynamics' effects on miRNA biogenesis. Dicer expression was downregulated in athero-susceptible regions that experience atheroprone (disturbed/non-laminar) flow patterns. In-vitro hemodynamics studies via a parallel plate flow apparatus were conducted; where human ECs were exposed to atheroprotective (laminar) flow complemented our in-vivo results. ECs were treated with/without Dicer ASOs and/or TNF to investigate differential inflammatory gene expression due to Dicer deficiency and/or TNF induction. Several proinflammatory genes were found to be regulated by Dicer. In conclusion, Dicer expression is regulated by hemodynamics; upregulated in atheroprotective and downregulated in atheroprone flow. Dicer deficiency is associated with dysregulated miRNAs in atherosclerosis. The presence or absence of Dicer affects differential expression of inflammatory genes in ECs. Dicer deficiency can potentially prime athero-susceptible regions by de-repressing specific proinflammatory genes.

Important Food Sources of Fructose-Containing Sugars and Cardiovascular Outcomes: A Systematic Review and Meta-Analysis of Prospective Cohort Studies

Presenter: Fei (Rodney) Au-Yeung
Supervisor: Dr. John Sievenpiper

Abstract

Introduction: Sugar-sweetened beverages (SSBs) are associated wth cardiovascular disease. Whether this association is mediated by blood lipids and holds for other important sources of fructose-containing sugars is unclear. To address this question, we conducted a systematic review and meta-analysis of trials on food sources of fructose-containing sugars on LDL-cholesterol using GRADE.

Methods: MEDLINE, EMBASE, and Cochrane Library were searched through March 9, 2018. We included controlled feeding trials ≥7-days in people with diabetes assessing the effect of different food sources of fructose-containing sugars on fasting blood lipids at 4 levels of energy control: substitution (energy matched comparisons); addition (energy from sugars added to diet); subtraction (energy from sugars subtracted from diet); or ad libitum (energy from sugars freely replaced). Three independent reviewers extracted data and assessed risk of bias. Data were pooled using generic inverse variance and expressed as mean differences (MDs) with 95% confidence intervals (CIs). The overall certainty of evidence was assessed using GRADE.

Results: 20 substitution (n=441) and 7 addition (n=175) trials met eligibility criteria. No subtraction or ad libitum trials were identified. There was no effect on LDL-cholesterol (mmol/L) of total fructose-containing sugars (MD, 0.01 [95% CI, -0.09,0.10])) or individual food sources including fruit (-0.21 [-0.58,0.17]), SSBs (0.03 [-0.43,0.48]), sweets (-0.10 [-0.49,0.29]), or mixed sources (0.03 [-0.07,0.14]) in substitution trials. Similarly, there was no effect of total fructose-containing sugars (-0.14 [-0.40,0.12]), fruit (-0.10 [-0.52,0.31]), SSBs (0.14 [-0.57,0.86]), or mixed sources (-0.18 [-0.51,0.15]) in addition trials. The overall certainty of evidence was "moderate" for mixed sources in substitution and addition trials and "low" for all other comparisons.

Conclusions: Fructose-containing sugars do not have an adverse effect on LDL-cholesterol in people with diabetes irrespective of energy control or food source. Further research is needed to improve our estimates. Protocol Registration: ClinicalTrials.gov Identifier, NCT02716870

The Significance of the Fc region of CD44 Antibodies in the Amelioration of Immune Thrombocytopenia (ITP), In-vivo

Presenter: Gurleen Kaur
Supervisor: Dr. Alan Lazarus

Abstract

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by an isolated decrease in platelet counts. The underlying pathology is thought to be the production of autoantibodies directed against platelets, followed by Fcγ receptor-mediated phagocytosis of platelets. Currently, a major treatment for ITP is administration of intravenous immunoglobulin (IVIg). However, replacements for IVIg are imperative as it is expensive[1], not available in sufficient quantity[1], can result in hemolysis[2] and coagulation[3], and runs the theoretical risk of pathogenic contamination[4]. A proposed replacement is recombinant monoclonal antibodies directed against the transmembrane glycoprotein, CD44. Comparisons of activity between CD44 antibodies and IVIg show a 100 percent concordance when tested in various autoimmune animal models[5]. Furthermore, previous work in our lab has shown the ability of certain CD44 antibodies to prevent thrombocytopenia, some at a 3-log fold lower dose than IVIg[6]. The purpose of this study is to identify the mechanisms used by CD44 antibodies in ITP amelioration. To initiate the study, the ability of KM-114 (a CD44 antibody) in ameliorating thrombocytopenia was compared between two mouse strains, as well as mice housed under specific-pathogen free versus standard conditions. Moreover, changes in the expression levels of cell-surface CD44 upon administration of KM-114 was also examined in inbred versus outbred mice, in an attempt to understand the antibodies' mechanism of action. Lastly, activity of intact KM-114 was compared to an Fc inactivated version (deglycosylated KM-114) in order to understand whether the anti-inflammatory activity of the antibody is Fc region dependent. By understanding the conditions and requirements contributing to the anti-inflammatory activity of CD44 antibodies, it should help us to determine whether this therapeutic can be used to replace IVIg. The outcome of this study could result in a therapeutic which is less expensive, theoretically unlimited in supply, and free from risks associated with IVIg utilization.

Cerebrovascular changes and inflammation after experimental subarachnoid hemorrhage

Presenter: Hoyee Wan
Supervisor: Dr. Loch MacDonald

Abstract

Subarachnoid hemorrhage (SAH) is a type of hemorrhagic stroke that typically involves the rupture of a cerebral aneurysm. Recent findings have implicated changes to the cerebral microcirculation and inflammation to cause poor outcome after SAH. The aim of the current study was to investigate changes to the cerebral microcirculation and inflammation in an experimental rodent model of SAH. We employed a prechiasmatic blood injection model in mice with implanted chronic cranial windows, allowing direct observation of microvasculature before and after SAH using two-photon microscopy. Changes to mouse cortical microvasculature were assessed before, immediately, 6 hours, 48 hour and 5 days after SAH ictus. Blood flow was measured using laser speckle imaging. To assess inflammation within the imaging window, we assessed markers of macrophage activation and inflammation in mouse cortex. We identified global vasoconstriction immediately after SAH induction, with arterioles, venules and capillaries undergoing severe vasoconstriction. There was a sustained vasoconstriction in penetrating arterioles and capillaries that persisted through the 6 hour imaging window, whereas venules returned to their baseline size. In the delayed imaging timepoints (2 days and 5 days), vasodilation of arterioles and capillaries was observed, with no change in venular diameter. Development of inflammation mirrored vasodilation in the delayed phase – with increased macrophage activation and proliferation, and activation of parenchymal astrocytes. Our results suggest that injury after SAH is two-fold. In the early phase, global vasoconstriction causes hypoxia, and in the delayed phase, inflammation could cause direct cellular injury and loss of vascular reactivity.

Timing of cow milk introduction and childhood growth

Presenter: Izabela Soczynska
Supervisor: Dr. Jonathon Maguire

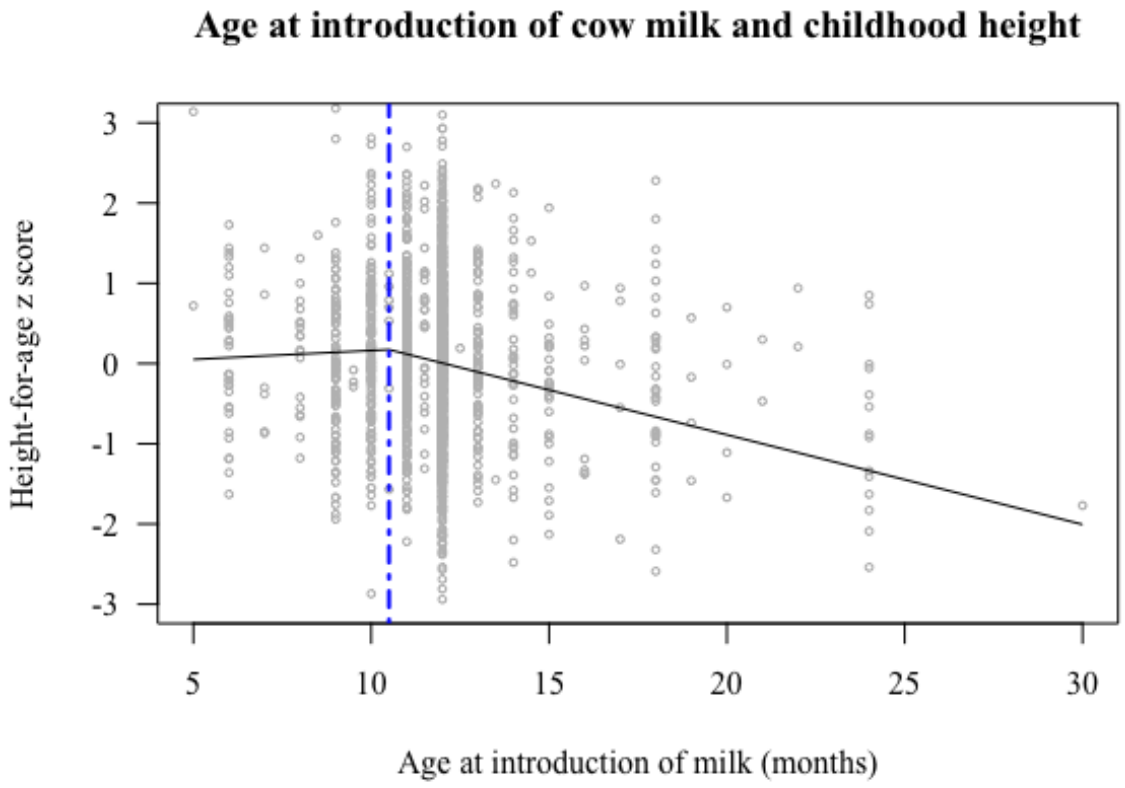
Abstract

Introduction: There has been considerable debate on what age to introduce cow milk into a child's diet. In the United States and the United Kingdom, it is recommended that cow milk not be started until one year of age. In Canada and Denmark, it is recommended to introduce cow milk at 9 to 12 months. Early cow milk exposure may positively influence child height, possibly through insulin-like growth factor-1 (IGF-1) found naturally in cow milk. However, early milk intake may also accelerate weight gain, which has been linked to child obesity.

Methods: This was a prospective study of healthy children (n = 1823) enrolled in the TARGet Kids! cohort. The primary exposure was the age of milk introduction measured at baseline (< 3 years) using a parent-completed questionnaire. Physical measurements were obtained at 3 to 5 years of age. The primary outcome was height-for-age z score. The secondary outcome was adiposity, measured as BMI z score. Multiple linear regression was used to determine the association between the age of milk introduction and growth.

Results: A significant association between younger age of milk introduction and taller children was identified; each month earlier that cow milk was introduced was associated with 0.04 higher height-for-age z score (95% CI: 0.02, 0.06; P < 0.01). Exploratory analysis revealed the association was apparent only after 10.5 months of age. For example, the height difference for a child aged 4 y introduced to cow milk at 12 months relative to 24 months was 2.0 cm (95% CI: 1.9; 2.3). No association between age of milk introduction and child adiposity was identified (P = 0.45).

Conclusions: After 10.5 months of age, earlier milk introduction was associated with taller stature. Future research is needed to understand the causal relationship between age of milk introduction and childhood height.


Age at introduction of cow milk and childhood height
Height-for-age z score
3
2
1
0
-1
-2
-3
5
10
15
20
25
30
Age at introduction of milk (months)

Maternal Anti-αIIB Antibodies Target Fetal HSCs and May Lead to Miscarriage in the Anti-αIIB Mediated FNAIT

Presenter: Jade Sullivan
Supervisor: Dr. Heyu Ni

Abstract

Background: Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is characterized by an aberrant maternal immune response against paternally inherited polymorphisms on fetal platelet antigens and can result in severe bleeding and miscarriage. Platelet surface receptor αIIbβ3 integrin is commonly targeted however there are far fewer reports of FNAIT against the αIIb subunit, possibly due to increased miscarriage. In addition to platelets, αIIb is also expressed on hematopoietic stem cells (HSCs) and early HSC progenitors.

Hypothesis: Miscarriage is prevalent in anti-αIIb mediated FNAIT and maternal anti-αIIb antibodies target HSCs resulting in decreased HSC and blood cell populations and miscarriage.

Methods: Using our established anti-αIIb mediated FNAIT mouse model, we collected fetal placenta, liver and blood and analyzed cell populations with flow cytometry. Miscarriage rates were measured in anti-αIIb and -β3 FNAIT models using ultrasound and weight-recording.

Results: Maternal anti-αIIb antibodies were able to bind HSCS *in vitro* and *in vivo*. Two types of HSC populations were measured: LPK34+ (HSCs and progenitors; $lin^-/CD62P^-/CD34^+/CD117^+$), and LSK+ (true HSC; $lin^-/CD49b^-/Sca\text{-}1^+/CD117^+$). FNAIT fetuses had reduced LPK34+, as well as αIIb^+ LPK34+ HSCs in the placenta, fetal liver and blood at E14.5 (14 days gestation) compared with healthy controls. The FNAIT fetuses had significantly reduced LSK+ HSCs in the placenta. Interestingly, the fetal livers had no difference in LSK+ HSCs, but significantly lower αIIb+ LSK HSCs. Preliminary blood analysis revealed FNAIT fetuses had lower RBC and WBC counts, compared with healthy controls. Our observed miscarriage rate in the anti-αIIb mediated FNAIT model appeared significantly higher than the anti-β3 model.

Conclusion: Anti-αIIb antibodies may bind fetal HSCs during embryonic development, contributing to fetal death. Destruction of early αIIb^+ HSC progenitors may explain the decrease in total HSCs and blood cell counts in FNAIT fetuses.

Does Timolol Affect Lymphatic Drainage from the Eye?

Presenter: Joseph Hanna
Supervisor: Dr. Yeni Yucel and Dr. Neeru Gupta

Abstract
Introduction: Lowering intraocular pressure (IOP) is the treatment goal in glaucoma, and timolol, a beta- blocker is known to reduce aqueous production. It is unknown whether timolol affects lymphatic drainage from the eye.
Purpose: To study the effect of timolol on ocular lymphatic drainage.
Methods: Wildtype CD1 mice were treated with topical timolol 0.5% (n=4; 10µL) or artificial tears (n=5; 10µL) to the right eye twice at 9pm and 9am the next morning. One hour after the last dose, QC1- quencher dye (1µM; Li-Cor Inc., USA) conjugated to Bovine Serum Albumin was injected intracamerally into the right eye. In vivo MSOT photoacoustic imaging of the head and neck region was performed before injection and immediately after at 20 minutes, 2 and 4 hours. IOP was measured by tonometry before injection and at 4 hours after injection. Both eye and cervical lymph node were outlined, and QC-1 signal intensity slopes and area under the curve (AUC) was measured using Native View MSOT software and compared between groups (*t*-tests).
Results: Right eye QC1 signal decay slopes decreased steadily and were steeper in controls compared to timolol-treated (-3.20E-4±7.35E-5 vs. -1.18E-4±1.81E-5; p=0.009, respectively). AUC showed no significant difference between both groups (1.73±0.11 vs. 2.21±0.22; p=0.075). In contrast, right cervical lymph node slopes showed a steady increase over 4-hour in both groups and were significantly steeper in controls compared to timolol-treated (1.82E-4±1.01E-4 vs. 2.36E-5±8.65E-6; p=0.033). Controls also showed significantly increased AUC compared to the timolol group (0.80±0.22 vs. 0.12±0.01; p=0.029). IOP was significantly decreased in timolol group compared with controls before and 4 hours after injection (10.0±3.0mmHg vs. 14.8±1.7mmHg; p=0.04) (11.5±1.2mmHg vs. 16.6 ± 3.0mmHg; p=0.019), respectively.
Conclusion: The novel finding that timolol reduces lymphatic drainage from the eye may be relevant to understanding adrenergic control of lymphatic drainage from the eye and IOP regulation.

Transcriptomic profiling of VHL-dependent long noncoding RNAs in clear cell renal cell carcinoma

Presenter: Joseph Samuel
Supervisor: Dr. Philip Marsden

Abstract

Introduction: Clear cell renal cell carcinoma (ccRCC), the most common form of kidney cancer, carries a median overall survival (OS) time of two years. About one third of patients present with metastases. Although inactivation of the *VHL* tumour suppressor is a well-characterized driver event in ccRCC, the exact molecular basis remains unclear. To address this gap, we performed a transcriptomic analysis, focusing on *VHL*-dependent long noncoding RNAs (lncRNAs). The role of lncRNAs in ccRCC oncogenesis has not been extensively examined. Accordingly, the aim of the present study is to characterize lncRNA transcripts that are differentially associated with *VHL* status.

Methods: Transcriptome-wide analysis was performed on the Arraystar Human LncRNA V4.0 array, using total RNA from 786-O ($VHL^{-/-}$) ccRCC cells, stably reconstituted with wild-type *VHL* (786-O-VHL) or mutant *VHL* (786-O-C162F). Agilent GeneSpring GX v12.1 software was used for statistical analyses. LncRNAs were cross-referenced to The Cancer Genome Atlas (TCGA) to identify those predictive of OS. Finally, RT-qPCR was used to validate the most differentially expressed lncRNAs in RCC4 cells and paired kidney tissue.

Results: 360 lncRNAs were differentially expressed fourfold or greater (FDR $p < 0.05$) in 786-O-C162F cells relative to 786-O-VHL cells. Of these, 269 were upregulated and 91 were downregulated. Cross-referencing to TCGA, 52 of the upregulated and 23 of the downregulated lncRNAs were predictive of OS in ccRCC patients (Cox and Log-rank $p < 0.05$). The top five up- and downregulated lncRNAs were validated with RT-qPCR.

Conclusion: To our knowledge, this is the first analysis assessing *VHL*-dependent lncRNA expression in ccRCC. We anticipate that these molecular and clinical findings will provide a framework for utilizing *VHL* status as a biomarker to stratify patients and to identify molecular targets. Future collaborations are needed to validate these findings in a large cohort of ccRCC samples.

Lymphatic Drainage from the Eye and In Situ Validation by Near-infrared Imaging

Presenter: Kirsten Cardinell
Supervisor: Dr. Yeni Yücel

Abstract

Introduction: Glaucoma is a leading cause of blindness and most treatments increase fluid drainage from the eye to lower intraocular pressure. We previously tracked and measured fluid drainage from the eye into lymph nodes using photoacoustic imaging. In situ validation of our in vivo results is an important step to further understanding of complex lymphatic drainage routes. The purpose of this study is to determine whether using near-infrared fluorescence imaging can confirm and shed new light on findings of lymphatic drainage from the eye.

Methodology: Albino mice (N= 7) were injected with near infrared fluorescent dye CF770 conjugated to albumin (1.4mM, 3µl) into the right anterior chamber. In situ fluorescence imaging (Spectralis, Heidelberg Engineering, Germany) was performed on 3 mice sacrificed 4 hours post-injection. Histopathological mapping was performed on 4 mice sacrificed at 2 hours (n=2) and 4 hours (n=2) after injection. Soft tissue blocks containing the cervical and the left inguinal lymph nodes were harvested and fixed with 2% paraformaldehyde. Specimens were cryoprotected, frozen, and serially sectioned. Sections were stained with collagen IV antibody (basement membrane), and Sytox green (nuclear staining), and imaged using a fluorescence microscope (BX51, Japan) equipped with a hyperspectral camera.

Results: In situ fluorescence images revealed the tracer within the right submandibular, accessory submandibular, and deep cervical nodes of all mice. No signal was detected in the left sided or superficial parotid nodes. Histopathological mapping showed presence of the fluorescence tracer in right submandibular nodes in all mice. No tracer was observed in the left submandibular or inguinal nodes.

Conclusion: Using a novel approach, we confirm that aqueous humour drains from the eye into the lymphatic system. Future studies to develop dual modality tracers capable of in vivo mapping and in situ validation will accelerate testing of novel glaucoma agents that stimulate the lymphatic pathway.

In vivo function of flow-responsive cis-DNA elements of the endothelial nitric oxide synthase gene: a role for chromatin-based mechanisms

Presenter: Kyung Ha (Kay) Ku
Supervisor: Dr. Philip Marsden

Abstract

Background: Endothelial nitric oxide synthase (eNOS) is predominantly expressed in medium- to large-sized arteries where endothelial cells (ECs) are exposed to high shear stress. Decreased shear stress due to Disturbed blood flow leads to decreased transcription of eNOS, which contributes to atherogenesis preferentially at bifurcations, branch points, and curvatures of major arteries. Two flow-responsive cis-regulatory DNA elements within the eNOS promoter, a Shear Stress Response Element (SSRE) and a Krüppel-Like Factor (KLF) binding element, are both argued to be functionally relevant to the positive effects of laminar shear on eNOS transcription in vitro. However, the functional role of these cis-elements in vivo remains unclear.

Methods: Transgenic mice with a mutation at each flow-responsive cis-element were generated using a murine eNOS promoter-beta-galactosidase reporter via linker-scanning mutagenesis. Murine ECs were isolated from the aortic arch (AA) and the descending thoracic aorta (DTA) to assess the levels of DNA methylation at the transgene and native eNOS proximal promoters by pyrosequencing.

Results: Wildtype mice with a functional murine eNOS promoter-reporter construct showed reduced endothelial staining in the lesser curvature of the AA. Surprisingly, mutation of the SSRE completely abolished the reporter expression in ECs. This was associated with aberrant hypermethylation at the transgene eNOS proximal promoter. Mutation of the KLF binding element also evidenced decreased expression, manifesting an integration site-specific decrease in eNOS transcription. The proximal promoter of native eNOS in wildtype murine ECs from the AA displayed hypermethylation relative to those from the DTA, suggesting a functional role for flow-dependent DNA methylation in transcriptional activation of eNOS in vivo.

Conclusion: The SSRE and the KLF binding element in the eNOS promoter are both required for flow-induced transcription of eNOS in arterial ECs in vivo. This study is the first in vivo study to report the functional importance of a flow-responsive DNA element.

MicroRNA-26a Attenuates the In-vitro Inflammatory State of an Abdominal Aortic Aneurysms Model

Presenter: Lina Elfaki
Supervisor: Dr. Howard Leong-Poi

Abstract

Introduction: Abdominal aortic aneurysms (AAAs) are highly fatal despite repair surgeries because there are no therapies that prevent unexpected rupture. Micro-RNA-26a (miR-26a) is a novel anti-angiogenic regulator of vascular smooth muscle cell (VSMC) function that is downregulated in AAA.
Hypothesis: We hypothesized that miR-26a can target AAA pathogenic pathways *in-vitro* to attenuate the inflammatory extracellular matrix breakdown and abnormal angiogenesis of AAA.
Methods: Rat aortic endothelial cells (RAECs) and VSMCs were stimulated with inflammatory interleukin-1β (IL-1β; 10 ng/ml) to simulate the AAA phenotype and then transfected with miR-26a, antimiR-26a or scrambled microRNA. AAA biomarkers, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs), and direct targets of miR-26a, phosphatase and tensin homolog (PTEN) and connective tissue growth factor (CTGF), were quantified (n=6 / group) via qRT-PCR and western blotting. Lastly, the angiogenic capacity of miR-26a-transfected RAECs was assessed using the Matrigel™ Assay (n=6 / group). P-values <0.05 were considered significant.
Results: IL-1β stimulation increased MMP2 (1.33±0.04) and MMP9 (1.55 ± 0.17) mRNA levels while miR-26a transfection reduced mRNA and protein levels to baseline. Conversely, upon IL-1β stimulation, miR-26a transfection upregulated TIMP1-4 levels (1.79±0.17) whereas antimiR-26a transfection showed reduction back to baseline. Also, IL-1β stimulation upregulated PTEN (2.17±0.12) and CTGF (1.62±0.13) mRNA and protein levels while miR-26a transfection downregulated them (PTEN: 0.74±0.01; CTGF: 0.34±0.15). Inflammatory VSMC TGFβ mRNA levels were downregulated after miR-26a transfection (0.84±0.03) but upregulated after antimiR-26a transfection (1.32±0.15). Lastly, overexpression of miR-26a reduced RAEC angiogenesis, with a lower number of tubes (4±1.51 vs. 20±3.68) and nodes (8±2.14 vs. 32±5.99) compared to the control.
Conclusion: In-vitro, miR-26a attenuates the AAA-associated inflammatory state of AAA VSMCs, inhibits RAEC angiogenesis and downregulates key regulators of proliferation and extracellular matrix synthesis in AAA - CTGF and PTEN. This suggests that miR-26a is a viable therapeutic option for AAA.

Effect of a SRT3025 Late Intervention on an experimental model of chronic kidney disease

Presenter: Matthew Veitch
Supervisor: Dr. Richard Gilbert

Abstract

Introduction: While patients with kidney disease present with established disease that progresses, most experimental studies examine prevention rather than treatment. Moreover, these studies frequently report reduction in albuminuria as the primary efficacy outcome, while in patients slowing glomerular filtration rate (GFR) decline is much more important. In a previous prevention study, our lab found that administering SIRT1-activating compound, SRT3025, to 5/6 nephrectomized (5/6NX) rats, one of the few models with GFR decline, reduced renal fibrosis, attenuated proteinuria, and prevented a decline in GFR. In this study, we sought to determine the efficacy of SRT3025 when given later in the course of disease.

Methodology: Nine-week old Sprague Dawley rats were randomized to 5/6NX or sham surgery. Six weeks later, animals were randomized again to receive either a SRT3025 diet or control diet. 12 weeks after surgery, the rats were euthanized and tissue samples were collected. GFR was measured at 6 and 12 weeks post-surgery, while 24-hour urine protein was measured at 6, 8, 10, and 12 weeks post-surgery.

Results: Late intervention SRT3025 attenuated proteinuria but had no effect on GFR or renal fibrosis. After 12 weeks, SRT3025-treated 5/6NX rats showed lower urine protein (84.3±13.9 mg/day) than untreated 5/6NX rats (199.8±33.8 mg/day). No difference in GFR was observed between SRT3025-treated and untreated 5/6NX rats (2.56±0.29 □L/min/g and 2.42±0.34 □L/min/g, respectively). Similarly, SRT3025 had no effect on the extent of glomerulosclerosis or collagen IV deposition in the glomeruli and interstitium of 5/6NX rats.

Conclusions: SRT3025 prevents proteinuria but does not attenuate GFR decline in the late intervention setting. Reduction in proteinuria does not signify improved histology or kidney function (GFR). We conclude that reduction in proteinuria is not a surrogate for improved GFR and further suggest that studies of new therapies should assess efficacy in delayed intervention as well as prevention.

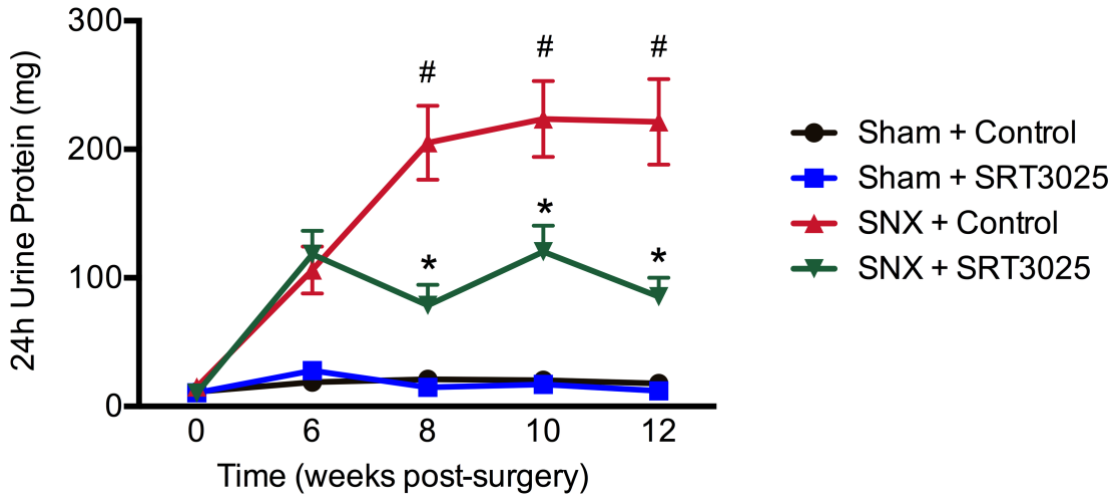


Figure 1: Time graph of 24-hour urine protein. Between-group comparisons within the same time points were analyzed using a one-way ANOVA with Fisher's least significant difference test. Same-group comparisons between time points were analyzed using a two-way ANOVA with Bonferroni's multiple comparison test. *$P < 0.05$ compared to respective control diet group, $^{\#}P < 0.05$ compared to same group at six weeks post-surgery.

Role of Hemodynamic-Mediated Endothelial Cell Cis-Acting DNA Element in Regulating Gene Expression Changes

Presenter: Michelle Dubinsky
Supervisor: Dr. Philip Marsden

Abstract

Endothelial nitric oxide synthase (eNOS) is a crucial combatant of vascular disease, as it produces the vasodilator nitric oxide. Reduced expression and transcriptional activity of eNOS is a hallmark of endothelial dysfunction, observed in the endothelium overlaying human atherosclerotic lesions. Arterial bifurcations and curvatures are susceptible to atherosclerosis and the endothelial cells (ECs) in these regions experience complex and disturbed (diseased) blood flow patterns. We have shown that these regions of the mouse aorta display reduced eNOS expression and transcriptional activity. Conversely, when laminar (healthy) flow is present in straight segments of the arterial network, eNOS is increased. Investigating the gene regulatory mechanisms that underlie vascular EC function in response to hemodynamic forces is crucial in understanding cardiovascular processes in health and disease.

EC gene expression patterns are regulated by blood flow through both cis-trans pathways and epigenetic mechanisms. Our objective is to elucidate the role of a major cis-acting DNA element, the shear stress responsive element (SSRE), influenced by hemodynamic forces. We hypothesize the SSRE cis-element and its corresponding transcription factors act as regulators of gene expression profiles in response to differential flow conditions.

Using our in vitro flow system, EC morphology is elongated. Our lab has mutated the SSRE in vivo and shown that eNOS gene expression is attenuated. Through sequence conservation analysis, we have noted the SSRE binding motif 'GAGACC' is only a small portion of the conserved residues likely to play a role in shear responsiveness of the eNOS promoter. Preliminary mass spectrometry analysis of the proteins that bind the SSRE reveals multiple signaling cascades influenced by flow. In conclusion, we predict proteins that bind to the SSRE contribute to the gene expression profiles of ECs to protect against atherosclerosis. This work will provide insights into transcriptional complexes that are involved in the etiology of atherosclerosis.

Calibration of a microsimulation model of bone loss among HIV-positive men

Presenter: Michelle Letchumanan
Supervisor: Dr. Ahmed Bayoumi

Abstract

Purpose: The optimal screening and treatment strategies to prevent fractures in HIV-positive individuals are unknown. We designed and calibrated a simulation model of the natural history of HIV-associated bone loss.

Method: We built this model in 50-year-old, HIV-positive men taking antiretroviral therapy with suppressed viral load levels. We incorporated a distribution of osteoporotic risk fractures to reflect the HIV-positive men in Ontario. We assumed men in the model were not taking tenofovir disoproxil fumarate. The model tracked bone loss over time as well as incident fractures at the hip, vertebral spine, and arm. We selected five calibration parameters. We used five calibration targets: cumulative fracture incidence (all types) at years 4 and 6, the proportion of all incident fractures up to 12 years that were at the arm and hip, and the proportion dying before age 70. Calibration targets were based on a literature review. To calibrate the model, we used simulated annealing and evaluated goodness of fit using the sum of Euclidian distances. We ran 1,000 first-order search iterations, each with a simulated sample size of 1,000.

Results: Among the 10 best-fitting parameter sets, cumulative fracture incidence was between 13 and 26 events per 1000 person-years (target 19.6) at 4 years and between 24 and 43 events per 100 person-years (target 22.4) at 6 years. Among the best-fitting parameter sets, between 25.5% and 37.2% of individuals were dead by age 70 (target 22.4%), between 37.7% and 48.4% of fractures were at the arm (target 45.4%), and between 35.4% and 40.8% of fractures were at the hip (target 35.7%).

Conclusion: Our model is reasonably well calibrated to predict fracture incidence and survival in 50-year-old HIV-positive men taking antiretroviral therapy. This model can be used to make predictions about the cost-effectiveness of osteoporosis screening and treatment strategies.

Actin remodeling during phagocytosis promotes ER-Plasma Membrane contact formation

Presenter: Minhyoung Lee
Supervisor: Dr. Greg Fairn

Abstract

Phagocytosis and efferocytosis are actin-dependent processes used to internalize particulate material (≥0.5 µm) that serves both antimicrobial and homeostatic functions. Impaired phagocytosis and efferocytosis occur in a variety of conditions. Many bacterial pathogens can prevent phagocytosis while failure to effectively degrade prey and reform lysosomes inhibits subsequent rounds of phagocytosis. Efferocytosis is responsible for clearing >200 billion apoptotic cells per day thereby fostering tissue renewal and remodeling. Impaired efferocytosis is described to occur in chronic inflammatory conditions such as chronic inflammatory lung disease, lupus, diabetes, and atherosclerosis, thus understanding biological process of phagocytosis is critical.

A role of the ER in phagocytosis has been a matter of much controversy. Electron micrographs of macrophages undergoing phagocytosis revealed the presence of ER in extensive, close contact with the forming phagosome, where ER potentially supply new membrane to the site of phagocytosis to support the engulfment process. However, follow-up proteomic studies and extensive microscopic analysis concluded that endosomes and lysosomes were the primary contributors of the membrane. We have found that during phagocytosis the disassembly of F-actin from the base of the phagocytic cup allows for the formation of ER-PM contact sites. These specialized regions are membrane-to-membrane interface typically 10-20 nm apart, thought to serve as a microdomain for numerous cellular processes. Using TIRF microscopy and synthetic probes for ER-PM junctions revealed that ER-PM contacts formed promptly after actin clearance. Furthermore, cortical actin clearance leads to the engagement of STIM1, a calcium sensor ER-PM junction protein, to the PM, thereby allowing SOCE mediated calcium replenishment. The formation of ER-PM contact sites allowed the ER-resident phosphatase, PTP1B, to localize and possibly dephosphorylate Fc Receptors. Together, we found that ER-PM contact site formation is spatially and temporally regulated by actin cytoskeleton during phagocytosis and that this may be critical to regulate phosphotyrosine and calcium signaling.

Investigating p300-mediated inhibition of p53 to protect against doxorubicin-induced cardiotoxicity

Presenter: Mohamed Adam
Supervisor: Dr. Kim Connelly

Abstract

Introduction: Doxorubicin is amongst the most widely prescribed chemotherapy drugs due to its effectiveness in cancer treatment, although, progressive treatment using doxorubicin severely increases the risk of congestive heart failure. Mechanistically, doxorubicin is known to induce reactive oxygen species production (ROS), then DNA damage, ultimately leading to p53-meadiated cardiomyocyte death. Currently, there are no clinically applicable preventative treatments for doxorubicin-induced cardiotoxicity and so, extensive research is being done in discovering a potential therapy. One such candidate is curcumin – a natural polyphenol compound non-toxic to humans. Interestingly, we have demonstrated that curcumin inhibits lysine acetyltransferase activity of p300, which acetylates/activates p53. Therefore, we hypothesize that curcumin protects against doxorubicin-induced cardiomyocyte death and cardiotoxicity via p300-mediated inactivation of p53.
Methodology: To investigate the effect of p300 inhibition in vitro, we induced toxicity using doxorubicin, then added curcumin in H9c2 rat cardiomyoblasts, and evaluated markers of p53 activation, DNA damage, and apoptosis. Additionally, in vivo, wild-type mice received a single dose of doxorubicin (20 mg/kg via intraperitoneal injection). Survival, heart function, and cardiac tissue morphology was assessed following doxorubicin treatment.
Results: Compared to control in vitro, doxorubicin-treated cells significantly experienced increase in p53 expression, DNA damage, and apoptosis. However, with the addition of curcumin, p53 expression, DNA damage, and apoptosis was significantly reduced. Experimentation in vivo is still ongoing, but preliminary results point towards increased mortality and reduced heart function for mice treated with doxorubicin.
Conclusion: Our data will provide the first evidence that curcumin protects cardiomyocyte cells against doxorubicin-induced apoptosis in vitro. In addition, our study may provide the first evidence of cardio-protection by p300 inhibition in the setting of doxorubicin treatment in vivo. Curcumin is a natural compound with little to no side-effects in humans, therefore our finding may provide a novel therapeutic target and treatment approach for doxorubicin-associated cardiotoxicity.

Patterns of Hepatitis C virus (HCV) Testing in a clinical HIV cohort in Ontario, 2000-2015

Presenter: Dr. Nasheed Moqueet
Supervisor: Dr. Ann Burchell

Abstract

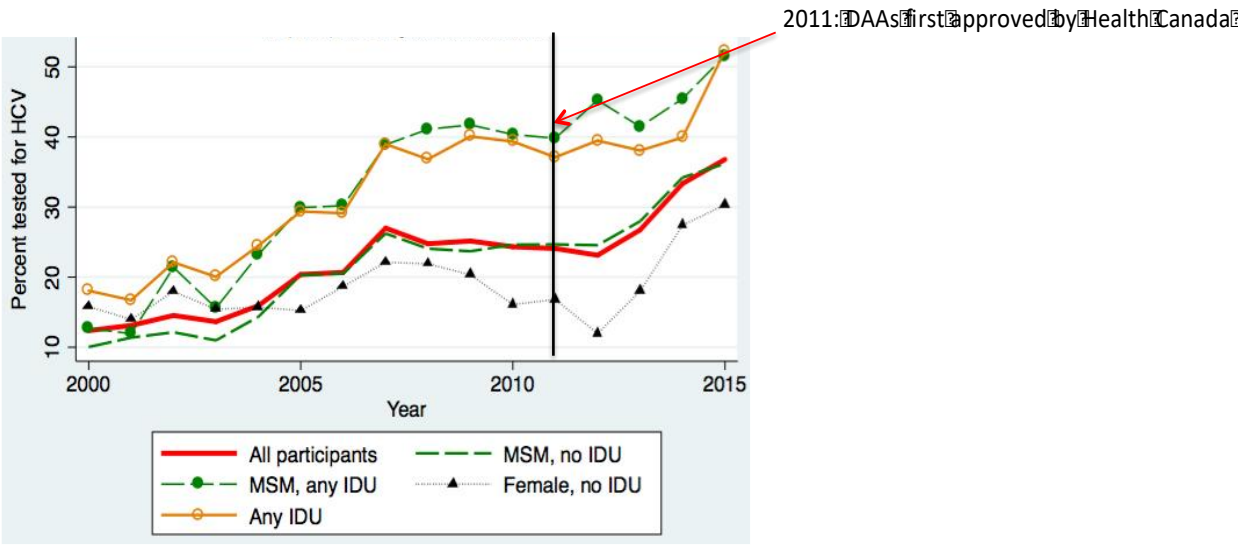
Background: Individuals with HIV are vulnerable to HCV acquisition via injection drug use (IDU), blood, and condomless anal sex. HIV care provides an opportunity for HCV screening and treatment with curative HCV therapies (direct-acting antiviral agents, DAAs). We sought to characterize temporal patterns of HCV testing in a cohort of HIV patients in Ontario, Canada.

Methods: Data was collected at 9 specialty HIV clinics participating in the OHTN Cohort Study (2000-2015) using chart abstractions, annual interviews and record linkage with Public Health Ontario Laboratories. Among those not previously diagnosed with HCV, we estimated annual proportions of participants who tested for HCV (serological/RNA tests) per calendar year, overall and by HIV risk categories. We identified correlates of annual testing using generalized estimating equations.

Results: As of 2015, 91.8% of 5,400 under follow-up had tested for HCV atleast once, with 11.6% ever testing HCV positive. The proportion (95% CI) tested annually increased to 36.8% (35.2%, 38.4%) in 2015 ($p<0.0001$). Testing was highest in people who inject drugs in 2015: 55.1% (48.0%, 62.1%) and lowest among females with no IDU: 30.9% (27.0%, 35.0%) (Fig 1). HCV testing was more common among urban-dwellers [Proportion ratio, 95% CI= 1.13 (1.03,1.24)]; MSM [1.18 (1.09, 1.26); individuals ever testing positive for syphilis [1.16 (1.10, 1.23)] or with IDU history [1.45 (1.36,1.53)]; and post-DAA vs. pre-DAA [1.28 (1.24,1.33)]. Testing decreased per decade of age [0.91 (0.89, 0.94)] and HIV duration [0.95 (0.92, 0.99)].

Conclusion: Annual HCV testing increased over time, especially after DAA approval in 2011. Testing was higher among those with reported risk factors (sexual or IDU), reflecting pre-DAA testing practices which may miss HCV co-infections in those perceived to be at low risk. Future directions include examining patterns in repeat/frequent testers and HCV diagnoses.

Fig 1. Annual proportion tested for Hepatitis C virus (HCV) in the OHTN Cohort Study by calendar year and risk group, 2000-2015



MSM: men who have sex with men; IDU: injection drug use; HCV: Hepatitis C virus; DAA: direct-acting antiviral agents; OHTN: Ontario HIV Treatment Network

Molecular mechanisms of feedback inhibition of sphingolipid biosynthesis by Orm proteins

Presenter: Omar Mourad
Supervisor: Dr. Greg Fairn

Abstract

Sphingolipids are a class of membrane lipids that is ubiquitous in eukaryotes. They are essential structural components that influence membrane thickness and curvature. In addition to their biophysical properties, sphingolipids and their metabolites play critical roles in both intercellular and intracellular signaling. Serine palmitoyltransferase (SPT) catalyzes the first committed step in de novo sphingolipid biosynthesis. Like many rate-limiting steps in biological pathways, it is subject to feedback inhibition. However, this inhibition is not due to the direct interaction of a sphingolipid intermediate with SPT. Instead, it is thought to be mediated by Orm proteins, a highly conserved family of integral endoplasmic reticulum proteins. Overexpression of ORMDL3, a human Orm protein, is associated with the development of childhood asthma, although the disease mechanism is unknown. Co-immunoprecipitation studies have shown that Orm proteins physically associate with SPT in a multiprotein complex referred to as the SPOTS complex in yeast. Genetic studies and metabolic analyses have shown that Orm proteins are required for the negative feedback loop and attenuation of SPT activity. However, the precise molecular mechanisms involved in the metabolite sensing and enzymatic inhibition remain unclear. We speculate that Orm proteins function in a two-step process, first binding a downstream metabolite such as ceramide and then via protein-protein interactions inhibit the SPT complex. Using a combination of random and targeted mutagenesis we have generated several loss-of-function Orm1 proteins. While these proteins are stably expressed and localized to the ER, they can no longer act as a feedback inhibitor of the SPT complex. We are assessing the ability of these mutants to bind directly to ceramide and to interact with SPT. As aberrant regulation of the SPT is associated with asthma and other inflammatory diseases, a better understanding of the Orm proteins may help with the development of targeted therapeutic interventions.

Social and Economic Hardships Associated with Poor HIV Clinical Outcomes among HIV-Positive African, Caribbean, and Canadian Blacks Living in Ontario, Canada

Presenter: Dr. Pascal Djiadeu
Supervisor: Dr. LaRon E. Nelson

Abstract

Introduction: African, Caribbean and Canadian Blacks (ACB) communities represent 4.7% of the population of Ontario, but account for more than one-third of the HIV prevalence. Hardships are mechanisms by which social processes generate inequitable health outcomes. The purpose of the study was to test the hypothesis that timing of HIV infection and political, economic, social hardships were associated with poor HIV clinical outcomes in a sample of ACB adults living with HIV.

Methods: We used data on ACB adults (n=840) drawn from the Ontario Cohort Study (OCS) of patients from across the province of Ontario. Participants were classified according to the timing of their HIV infection, relative to arriving in Canada. We considered CD4 count, viral load and global self-rated physical health (dichotomized as poor/fair vs. all else) at time of first interview as indicators of health upon entering care. Descriptive statistics were analyzed using chi-squared tests and Fisher's exact test. Multivariate analyses employed logistic regression, using multiple imputation with chained equations to assuage problems with missing data.

Results: Compared to those for whom timing of infection could not be determined, those who acquired HIV post-immigration were less likely to have an elevated HIV viral load. Those with heterosexually-acquired infections were more likely than MSM to have a low CD4 count and an elevated HIV viral load. Compared to those who were employed full-time, those not in the labour force, or with an uncertain employment status were more likely to have poor/fair self-rated physical health. Among non-citizens, there were also significant differences in clinical indicators by timing of HIV infection for CD4 count and viral load.

Conclusion: Hardships are important factors that affect clinical outcomes of ACB people living with HIV. Structural level and policy interventions may be needed to mitigate social and economic hardships that undermine health status.

CD44 antibodies inhibit macrophage Fc gamma receptor-mediated phagocytosis of platelets in an IgG subtype- and Fc-dependent manner: a potential replacement for IVIg?

Presenter: Peter Norris
Supervisor: Dr. Alan Lazarus

Abstract

Immune thrombocytopenia (ITP) is an autoimmune disease characterized by immune-mediated reductions in platelet counts, leading to severe bleeding and potentially fatal intracranial hemorrhage. The majority of ITP patients possess anti-platelet autoantibodies that can mediate platelet destruction in the spleen through macrophage Fcγ receptor (FcγR)-mediated phagocytosis. Intravenous injection of pooled human serum immunoglobulin (IVIg) is used as a first-line treatment in ITP, but IVIg replacement therapies are of significant interest due to IVIg's high cost, recipient hemolysis, and demand on blood donors. We previously demonstrated that monoclonal antibodies to the hyaluronic acid receptor CD44 treats ITP in a murine antibody-mediated and FcγR-dependent ITP model by an unknown mechanism.

As CD44 is expressed on leukocytes including macrophages, we hypothesized that anti-CD44 increases platelet counts by interfering with macrophage phagocytosis. Our study aimed to determine whether CD44 antibodies inhibit macrophage FcγR-mediated phagocytosis. Two CD44 antibodies were tested for their ability to interfere with macrophage FcγR-mediated phagocytosis of antibody-opsonized platelets in an in vitro murine cell model. Both anti-CD44 antibodies inhibited phagocytosis of platelets in a dose-dependent manner, up to near complete (>90%) inhibition. However, using antibodies to opsonize murine platelets of mouse IgG2a, IgG2b, or IgG1 subtypes, we found anti-CD44 inhibited phagocytosis only when the opsonizing and therapeutic antibody subtypes were matched for putative FcγR binding. These results suggested to us that anti-CD44 inhibits FcγR receptor-mediated phagocytosis by blocking FcγRs in cis (same cell). Removal of anti-CD44 Fc-FcγR interactions by antibody deglycosylation or generation of F(ab')2 fragments led to complete loss of anti-CD44 activity. In conclusion, CD44 antibodies are potent inhibitors of macrophage FcγRs-mediated phagocytosis of platelets in a manner dependent upon anti-CD44 Fc and IgG subtype. These results suggest important implications of IgG subtypes in monoclonal antibody therapeutic efficacy and whether current therapeutic antibodies may incidentally block FcγRs remains to be explored.

Separating therapeutic activity from adverse events – Monoclonal anti-erythrocyte antibodies in a murine model of passive ITP

Presenter: Ramsha Khan
Supervisor: Dr. Alan Lazarus

Abstract

Introduction: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by low platelet counts and an increased risk of bleeding. Anti-D is an effective treatment but as a donor-derived product, it is associated with certain limitations. In addition, anti-D also carries an FDA-issued Black Box warning of serious complications that may occur in patients with ITP. To determine if some of the potential side effects of anti-RBC antibodies can be separated from therapeutic activity, a murine model of passive ITP was utilized with two different anti-RBC antibodies. Two major adverse events were evaluated: 1) Anemia, as defined by a significant decrease in the RBC counts and 2) Inflammatory activity, as defined by a significant change in the core body temperature.
Methods: Monoclonal antibodies TER-119 and M1/69 were evaluated for their ability to ameliorate ITP in two well characterized inbred mouse strains (C57BL/6 and BALB/c) and the outbred CD-1 strain of mice. Erythrocyte numbers were evaluated over 8 days and the body temperature of the mice was measured rectally for 30 minutes post anti-erythrocyte antibody injection.
Results and Conclusions: Both TER-119 and M1/69 ameliorated ITP in C57BL/6, BALB/c and CD-1 mice. TER-119 induced significant anemia in all three while M1/69 caused anemia only in C57BL/6 and BALB/c mice. We therefore conclude that anemia is not strictly required for ITP amelioration by anti-RBC antibodies in murine passive ITP. In addition, significant reductions in the body temperature were seen post TER-119 and M1/69 injections in CD-1 and BALB/c mice but not in C57BL/6 mice with either antibody. We therefore also conclude that inflammatory activity, as assessed by body temperature, is not required for ITP amelioration in murine passive ITP. Based on these results, it may be possible to develop a monoclonal anti-RBC antibody that ameliorates ITP without causing severe adverse events.

Cellular Mechanisms Involved in Mechanical Overventilation-promoted Pulmonary Fibrosis

Presenter: Razan Turki
Supervisor: Dr. Wolfgang Kuebler

Abstract

Mechanical ventilation is the current therapy for patients with severe cases of acute respiratory distress syndrome (ARDS). Despite its benefits, mechanical ventilation can cause ventilation-induced lung injury (VILI) that may ultimately lead to pulmonary fibrosis. In this study, we aim to uncover the molecular mechanisms implicated in pulmonary fibrosis induced by mechanical overventilation. The transcription factor TAZ, one of the key players of the Hippo-signaling pathway involved in cell proliferation, has been shown to be translocated in the nucleus of fibrotic lung tissue. To identify upstream regulators of TAZ nuclear localization, we looked at the Aurora kinases involved in mitotic processes and have been shown to modulate the TAZ inhibitor Lats2. We hypothesized that activation of Aurora kinases facilitate the nuclear translocation of TAZ through inhibiting Lats2 that results in increased profibrotic cytokine secretions such as TGF-β. To test this hypothesis, we assessed the expression and nuclear localization of TAZ in stretch-induced pulmonary endothelial cells. Our findings showed that TAZ nuclear localization was increased in human pulmonary microvascular endothelial cells (HPMEC) after 3h of stretch at 18% compared to the static control whereas stretching these cells at 5% increased TAZ nuclear translocation after 6h. These data suggest that TAZ translocates to the nucleus faster in HPMEC exposed to higher stretch percent than a lower one. Our data also showed that inhibiting Aurora kinases decreased TAZ nuclear localization in stretched HPMEC. These findings may uncover the link between mechanical ventilation and pulmonary fibrosis, which can provide insights into the key players that could be potentially targeted for mechanical overventilation induced pulmonary fibrosis.

Optimizing differentiation of the human induced pluripotent stem cells (iPSCs) into alveolar epithelial type II cells (AECIIs)

Presenter: Ryungrae (Benedict) Kim
Supervisor: Dr. Haibo Zhang

Abstract

Alveolar epithelial type II cells (AECIIs) are resident stem cells of the lung that can proliferate and differentiate into Alveolar epithelial type I cells to regenerate damaged lung tissue. Induced pluripotent stem cells (iPSCs) are directly derived from adult somatic tissues. Once reprogrammed to pluripotency, iPSCs can be differentiated into specific target cells. iPSCs have been shown to differentiate into AECIIs upon supplementation of culture media with various growth factors and morphogens, however little is known about their utility as a reliable cell source for lung regeneration. My project aims to optimize current differentiation protocols in order to achieve mature iPSC-derived AECIIs whose phenotype is stably maintained for *in vivo* application.

iPSC lines, HDF-SV and HDF-mRNA were differentiated into AECIIs according to established protocols. Cells were first cultured and differentiated into definitive endoderm, then specified into the anterior foregut endoderm fate, and finally differentiated to lung progenitor cells. Lung progenitors will then be matured using a combination of morphogens and growth factors.

After maturation, correct differentiation will be confirmed with immunocytochemistry and fluorescence microscopy for expression of the AECII-specific marker, pro-surfactant protein C (ProSPC). From this analysis, differentiation efficiency will then be calculated. The derived AECII cultures will then be purified using magnetic-activated cell sorting (MACS) and tested for consistent expression of ProSPC for up to 3 months. Detection of secreted surfactant protein C (SPC) in media will be conducted as a functional evaluation of matured AECIIs.

Approximately 70-75% of the cell population was positive for Pro-SPC following differentiation in our pilot study. Preliminary data showed that the cells expressed ProSPC at 105 days after maturation, suggesting that the AECII phenotype is being stably maintained.

Moving forward, MACS will be performed to purify the AECII population, which will then be continually evaluated for proper AECII phenotype.

Important food sources of fructose-containing sugars and serum uric acid: a systematic review and meta-analysis of controlled feeding trials

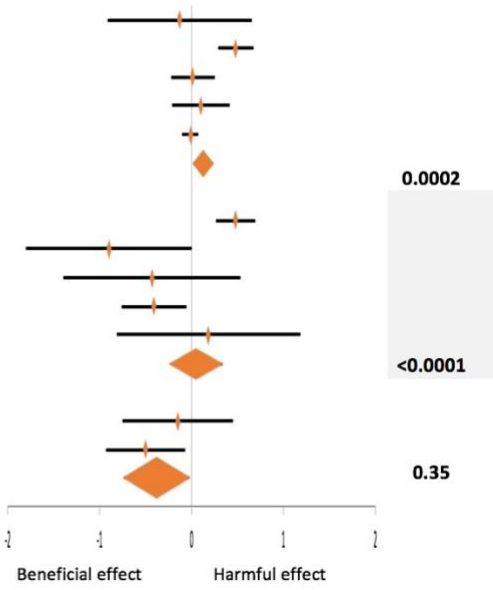
Presenter: Sabrina Ayoub-Charette
Supervisor: Dr. John Sievenpiper

Abstract

Background: Excess fructose intake is purported to increase blood uric acid (UA), yet the effect of important food sources of fructose-containing sugars on UA is unknown.
Methods: We conducted a systematic review and meta-analysis using GRADE. MEDLINE, EMBASE and Cochrane Library were searched (through September 9th, 2017). We included controlled feeding trials of ≥1-week of the effect of important food sources of fructose-containing sugars on UA at anyone of 4 levels of energy control: substitution (sugars in energy matched comparisons); addition (energy from sugars added to diet); subtraction (energy from sugars subtracted from diet); or ad libitum (energy from sugars freely replaced). Two independent reviewers extracted data and assessed study quality (Cochrane Collaboration Risk of Bias Tool). Data were pooled using the random effects model and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochrane Q statistic) and quantified (I2 statistic). The certainty of the evidence was assessed using GRADE.
Results: We identified 53 trial comparisons (n=1,401) involving 3 levels of energy control: substitution, addition, and subtraction. Total food sources of fructose-containing sugars increased UA in substitution comparisons (MD, 0.12 [95% CI, 0.02, 0.23]) and decreased UA in subtraction comparisons (MD, -0.38 (95% CI -0.73, -0.03]). No effect was seen in addition comparisons (MD, 0.05 [95% CI, -0.22, 0.33]). There was a significant interaction by food sources of fructose-containing sugars in subtraction and addition comparisons with only sugar-sweetened beverages showing an increasing effect (P<0.05). The certainty of the evidence was assessed as "moderate".
Conclusion: The effect of fructose-containing sugars on UA levels is dependent on both energy control and food source. Future research is needed to improve our estimates.

Protocol registration: NCT02716870.
Funding: PSI Foundation, Banting and Best Diabetes Centre, Canadian Institutes of Health Research and Diabetes Canada

Comparison	Trials	N	Mean difference (95%CI)	P-value	I^2	P-value interaction
SUBSTITUTION TRIALS						
Fruits	2	64	-0.13 (-0.91, 0.65)	0.75	79%*	
SSBs	6	154	0.48 (0.29, 0.67)	<0.001	23%	
Dairy Products	3	268	0.01 (-0.22, 0.25)	0.92	0%	
Baked goods, desserts and sweets	8	144	0.10 (-0.21, 0.41)	0.52	0%	
Mixed sources	16	563	-0.01 (-0.10, 0.07)	0.72	0%	
Total Food Sources	**35**	**1195**	**0.12 (0.02, 0.23)**	**0.02**	**40%***	**0.0002**
ADDITION TRIALS						
SSBs	6	164	0.48 (0.27, 0.69)	0.17	61%*	
Fruits	1	18	-0.90 (-1.80, 0.00)	0.05	-	
100% Fruit Juice	2	80	-0.43 (-1.39, 0.53)	0.38	77%*	
Fruit drink	3	68	-0.41 (-0.76, -0.06)	0.02	12%	
Mixed sources	2	44	0.18 (-0.81, 1.18)	0.72	12%	
Total Food Sources	**14**	**374**	**0.05 (-0.22, 0.33)**	**0.71**	**63%***	**<0.0001**
SUBTRACTION TRIALS						
SSBs	3	53	-0.15 (-0.75, 0.45)	0.62	0%	
Mixed sources	1	56	-0.50 (-0.93, -0.07)	0.02	-	
Total Food Sources	**4**	**109**	**-0.38 (-0.73, -0.03)**	**0.03**	**0%**	**0.35**



The Clinical Utility of Genomic Sequencing in Hereditary Cancer Syndrome Care: A Proposal for a Mixed-Methods Cohort Study

Presenter: Salma Shickh
Supervisor: Dr. Yvonne Bombard

Abstract

Background: Despite advances in detection and screening, cancer remains a major cause of morbidity and mortality in Canada. Although most cases of cancer are sporadic, up to 10% are caused by an inherited gene mutation. Individuals born with mutations have hereditary cancer syndromes (HCS) and are at increased risk to develop multiple early-onset cancers throughout their lifetime. Patients suspected to have a HCS undergo genetic testing to determine if they have a mutation. Those identified to have a mutation become eligible for enhanced screening programs and preventative surgeries that can lead to earlier cancer detection or prevention. However, standard testing for HCS only identifies a causative mutation in 10-15% of patients. Thus, most HCS patients are unable to benefit from current genetic tests. Genomic sequencing (GS), a novel genetic has the potential to reduce this diagnostic gap. Although there is literature indicating that GS has clinical benefits in some populations, its clinical utility in HCS populations is unknown. For my PhD study, I aim to evaluate the clinical utility of GS for HCS patients.

Methods: This will be a prospective, mixed-methods cohort study, embedded within a randomized control trial that will evaluate the health outcomes and costs of incidental GS results. My aim is to evaluate clinical utility by assessing (1) the diagnostic yield and (2) the impact of primary GS results on clinical management. Diagnostic yield will be determined by calculating the proportion of patients identified to have a disease-causing mutation in a cancer-related gene. The impact of results on management will be assessed through surveys and qualitative interviews with participants.

Results: Results of the study will help to inform the discussion on the clinical utility of GS for HCS.

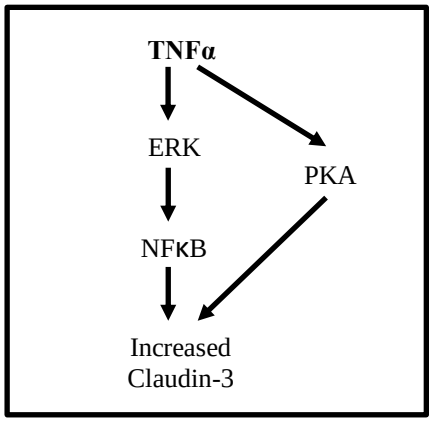
Conclusions: Establishing clinical utility is critical given its role as a fundamental determinant of health technology assessment and funding.

Tumor necrosis factor-α regulates Claudin-3 expression in kidney tubular epithelial cells

Presenter: Shaista Anwer
Supervisor: Dr. Katalin Szaszi

Abstract

Chronic kidney disease is a complication of common diseases such as diabetes and hypertension. Inflammation and injury affecting the tubular epithelium has emerged as a key factor in the development of kidney disease. The tight junctions are vital for the functional and structural integrity of the tubular epithelium, since they control permeability of the layer and are key signalling hubs. The inflammatory cytokine Tumor Necrosis Factor-α (TNFα) was found to be pathogenic in kidney disease and alters expression of tight junction proteins. Claudin-3 (Cldn-3) is a junction protein that reduces tubular permeability and affects cell migration. Interestingly, it is also overexpressed in many cancers. Our objective was to obtain a mechanistic understanding of the effects of TNFα on Cldn-3 and define functional outcomes. In LLC-PK1 tubular cells, we measured Cldn-3 mRNA expression using RT-PCR and protein levels using quantitative Western blotting. We found that TNFα increased Cldn-3 mRNA and protein levels. Using specific pharmacological inhibitors (PD98059, H-89 and IKK16) we showed that extracellular signal regulated kinase (ERK), protein kinase A (PKA) and the inflammatory transcription factor NFκB were crucial for Cldn-3 upregulation. Further, using these inhibitors, we determined that ERK was required for activation of NFκB, as measured by detecting phosphorylated p65 levels, suggesting a sequential activation of these pathways. Finally, we also found that in addition to expression changes, PKA also induced Cldn-3 phosphorylation. This was demonstrated by immunoprecipitation of endogenous Cldn-3 followed by western blotting with an antibody against phospho-PKA motifs. In summary, we uncovered the signalling pathway mediating TNFα-induced Cldn-3 upregulation. Our ongoing studies are aimed at defining functional relevance for permeability changes and repair of the injured epithelial layer. Overall, these mechanistic studies can inform the development of novel diagnostic and therapeutic tools for chronic kidney disease and other epithelial inflammatory conditions, as well as cancer.



Cow Milk Obesity pRevention Trial (CoMFORT) - Parent and Physician Contributions to Protocol Development

Presenter: Shelley Vanderhout
Supervisor: Dr. Jonathon Maguire

Abstract

Introduction: Clinical guidelines suggest children transition from whole (3.25% fat) milk to reduced (1% or 2%) fat milk at age 2 years; however, observational evidence supports a link between whole milk consumption and lower adiposity in children. The purpose of the CoMFORT randomized controlled trial is to determine which cow's milk fat minimizes excess adiposity (zBMI) and optimizes child nutrition and development. Children will be randomized to receive recommendations for either whole (3.25% fat) or 1% fat milk for 2 years.

Method: To understand how to design the trial protocol in a way that parents and practitioners will adhere, online questionnaires and structured in-person interviews were conducted with parents (n=65) and physicians (n=27). Descriptive statistics were used for questionnaire data, and thematic analysis was used to interpret interview transcripts.

Results: Questionnaire results showed that parents and physicians agreed on the benefits provided by cow's milk fat (38% of parents and 36% of physicians said healthier growth; 43% of parents and 36% of physicians said better nutrition). During primary healthcare, physicians recommend either 2% milk (n=4) or whole milk (n=5) at age 2 years and parents provide either reduced (1-2%) fat milk (n=27) or whole milk (n=18) to children over age 2 years. Interviewed physicians would recommend whole milk to all children over age 2 years, provided they were given evidence and guidelines for children around volume of milk consumption. Parent interviewees viewed milk as an important staple of childhood diets and a source of "healthy, essential fat." Parents trusted recommendations from their child's physician above other information sources (friends, internet).

Conclusion: These results suggest that the CoMFORT trial is feasible and the intervention will be agreeable to the majority of participants. Whole milk may be a simple, inexpensive and scalable cow's milk fat intervention to reduce childhood obesity.

Aspirin for Primary Prevention of Cardiovascular Events in Diabetics: A Systematic Review and Meta-Analysis

Presenter: Shuangbo Liu
Supervisor: Dr. Asim Cheema

Abstract

Background: Aspirin has an important role in secondary prevention of recurrent cardiovascular events, however its role in primary prevention remains controversial. Patients with diabetes mellitus (DM) are at high-risk of cardiovascular (CV) morbidity and mortality, and may therefore have increased benefit from aspirin for primary prevention. The objective of this systematic review is to assess the efficacy of aspirin for primary prevention of CV events in patients with DM.

Methods: A comprehensive literature search was performed. The primary outcome was the composite of CV death, non-fatal myocardial infarction (MI) and stroke at longest available follow-up (range 3.7–10.3 years). Outcomes were analyzed using a random effect Mantel-Haenszel model.

Results: The study cohort was comprised of 22958 patients (11425 aspirin, 11533 placebo) from 5 randomized controlled trials. The primary outcome did not differ between patients treated with aspirin vs placebo (9.02% vs 9.78%, RR 0.92 (0.85 – 1.00), p=0.06). Secondary outcomes of all-cause mortality (11.03% vs 11.70%, RR 0.94 (0.87 – 1.02), p=0.12), CV mortality (4.69% vs 5.12%, RR 0.91 (0.81 – 1.03), p=0.14), nonfatal MI (2.63% vs 2.63%, RR 1.00 (0.84 – 1.19), p=0.99), nonfatal stroke (2.84% vs 3.32%, RR 0.84 (0.65 – 1.09), p=0.19) and gastrointestinal bleed (1.93% vs 1.43%, RR 1.33 (0.80, 2.22), p=0.28) were also similar between the groups.

Conclusion: Aspirin as primary prevention in diabetic patients is not associated with a lower rate of cardiovascular events. Further research is needed to identify an appropriate patient population for aspirin as primary prevention.

Nut intake and measures of adiposity in metabolic syndrome: A systematic review and meta-analysis of controlled trials

Presenter: Stephanie Nishi
Supervisor: Dr. John Sievenpiper

Abstract

Introduction: Nuts have been shown to have health benefits, yet concern remains that nuts may contribute to weight gain due to their high energy density. Our aim was to conduct a systematic review and meta-analysis of the effect of nut intake in metabolic syndrome on markers of adiposity in controlled trials using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.

Methods: We searched MEDLINE, EMBASE, and Cochrane databases (through August 8, 2017). Controlled trials ≥ 3-weeks assessing the effect of nut intake on measures of adiposity in people with metabolic syndrome were included. Three independent reviewers extracted relevant data and assessed risk of bias of included trials. Data were pooled using the generic inverse variance method and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q statistic) and quantified (I2 statistic). The overall certainty of the evidence was assessed using GRADE.

Results: 6 randomized controlled trials in 477 people with metabolic syndrome met eligibility criteria. There was no adverse effect of nut consumption on global adiposity (BMI: MD 0.10 [95% CI: -0.03, 0.23]; body weight: MD 0.13 [95% CI: -0.19, 0.44], % body fat: MD -0.60 [95% CI: -1.78, 0.58]) or abdominal adiposity (waist circumference: MD -0.48 [95% CI: -0.94, -0.02]; visceral adipose tissue (VAT): MD -7.2 [95% CI: -47.4, 33.0]; subcutaneous adipose tissue (SAT): MD -11.8 [95% CI: -24.9, 1.3]). Overall certainty of the evidence was graded as "high" for body weight and waist circumference, "moderate" for body fat and SAT due to indirectness, and "low" for BMI and VAT owing to inconsistency and imprecision.

Conclusions: Pooled analyses show nut consumption does not have an adverse effect on measures of adiposity in metabolic syndrome. Concern that nuts may result in weight gain owing to their high energy density appears unwarranted.

Novel Mechanism of Thrombosis: Role of β3 Integrin PSI Domain in Blood Coagulation

Presenter: Tyler Stratton
Supervisor: Dr. Heyu Ni

Abstract

Integrin αIIbβ3 is a key receptor in platelet adhesion/aggregation. We recently showed the plexin-semaphorin-integrin (PSI) domain of β3 has thiol isomerase activity. We developed four monocloncal antibodies (mAbs) that bind PSI and inhibit thiol isomerase activity, platelet adhesion/aggregation and thrombosis. The inhibitory effect of the mAbs was much stronger in vivo than in vitro (anticoagulated), suggesting that anti-PSI mAbs decrease blood coagulation. The anti-PSI mAbs were shown to inhibit blood clot retraction. Anti-PSI mAb-treated platelet-rich plasma decreased fibrin network formation in laser scanning and spinning disk confocal microscopy. In thromboelastography, anti-PSI mAbs inhibited blood coagulation. Furthermore, anti-PSI B1 significantly inhibited blood coagulation more than other anti-β3 mAbs (JAN D1, M1 and Abciximab precursor 7E3). This supports a novel role of PSI in enhancing blood coagulation, particularly through intrinsic pathway factors. For the first time, αIIbβ3 has been targeted at an allosteric region (PSI). This novel anti-thrombotic inhibits platelet aggregation and blood coagulation without increasing bleeding or requiring combinatorial therapy.

Determining the relative contributions of the phosphatidylserine transfer proteins Osh6/7 and the secretory pathway in the development of cell polarity

Presenter: Yanbo Yang
Supervisor: Dr. Greg Fairn

Abstract

Cell polarity supports the formation of epithelial cell monolayers and the migration of neutrophils. In both cases the lipid PI3,4,5P_3 serves as a key spatial regulator. Cell polarity is also an important feature of fungal pathogens as it supports invasive growth and biofilm formation. Fungal organisms do not make PI3,4,5P3 and instead use phosphatidylserine (PtdSer) to support cell polarity. Two oxysterol-binding protein homologues, Osh6p and Osh7p, have been characterized as soluble PtdSer transfer proteins responsible for accumulation of PtdSer in the PM. However, PtdSer is also a constituent of secretory vesicles that deliver lipids and proteins to the PM. Currently, it is unclear to what extent non-vesicular and vesicular transport pathways control the cellular distribution of PtdSer and thus cell polarity. Our results indicated that Osh6p and Osh7p are dispensable for cell polarity. Accumulation of secretory vesicles via blockage in exocytosis revealed that secretory vesicles carry a considerable amount of PtdSer to the plasma membrane. Additionally, the accumulation of PtdSer in secretory vesicles is independent of Osh6/7p. Our results suggest that Osh6/7p may act as a fine-tuning mechanism rather than being the primary driver of PtdSer transport and are not required for fungal cell polarity unlike PtdSer which is pivotal.

The Presence of the IgG Fc Region is Essential for the Ability of Monoclonal Antibodies to Induce Antibody-Mediated Immune Suppression

Presenter: Dr. Yoelys Cruz Leal
Supervisor: Dr. Alan Lazarus

Abstract

Background: Hemolytic disease of the fetus and newborn (HDFN) is a disease provoked by erythrocyte antigenic incompatibility between mother and fetus. Polyclonal anti-D has been used to prevent HDFN and this mechanism has been referred as antibody-mediated immune suppression (AMIS). The major theory behind AMIS is based upon erythrocyte clearance. Recently, antigen loss has been proposed as a potential mechanism of AMIS; where Fcγ receptors and/or complement were required and we recently also demonstrated in a model system that immunoglobulin G Fc glycans are not essential for AMIS. However, the actual presence of the IgG Fc region on the ability of monoclonal antibodies (mAb) to induce AMIS has not been assessed. The aim of the present work was to determine the requirement for an IgG Fc region to mediate AMIS induction.

Methods: Erythrocytes from transgenic HOD mice, which express an antigen composed of hen egg lysozyme (HEL), ovalbumin (OVA) and the human Duffy transmembrane protein [HOD], were used as a source of foreign erythrocytes. HOD-RBCs were opsonized with antibodies against different regions of the molecule as well as their $F(ab')_2$ and F(ab) fragments of these antibodies and transfused in C57BL/6 mice. Transfusion of the HOD-RBC opsonized with $F(ab')_2$ and F(ab) fragments of HOD-specific mAb plus IgG or F(ab')2 fragments specific against the primary antibody were also performed. IgM and IgG antibody responses were measured by ELISA after HOD-RBC transfusion.

Results: Erythrocyte antigen-specific mAb avoided alloimmunization. However, F(ab) as well as $F(ab')_2$ fragments of these mAb did not inhibit the primary immune response. The inability of F(ab) or $F(ab')_2$ fragments of mAb to induce AMIS was restored when IgG but not $F(ab')_2$ fragments specific against the primary antibody were also administered.

Conclusions: The Fc region of IgG is essential for the successful induction of antibody-mediated suppression to foreign erythrocytes.

Development of Genetic Tool for Testing CFTR Gene Targeting in Pigs

Presenter: Zhichang (Peter) Zhou
Supervisor: Dr. Xiao-yan Wen

Abstract

Background: Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CF patients have defects in ion transport in epithelial cells. Persistent inflammation and infection in CF airway deteriorate lung function profoundly, leading to CF morbidity and mortality. Traditional medical therapies treat only symptoms but not the cause of the disease. Current CFTR-directed drugs, such as Ivacaftor, are effective for only small portion patients, and the combination of drugs, such as Ivacaftor and lumacaftor, have some efficacy for about half of the CF patients. Thus, novel therapeutic strategies for targeting all types of mutations are needed. CRISPR/Cas9-based gene targeting strategies have the potential for permanently correcting CF lung disease. We produced helper-dependent adenoviral (HD-Ad) vector which can deliver gene editing tools for integrating a functional CFTR gene into a genomic safe harbor of pig cells. The objective of this project is to test our CFTR gene targeting strategy in cultured CFTR deficient pig cell line and primary cells *in vitro*.

Methods: HD-Ad vector which contained CRISPR/Cas9, K18 promoter, and human CFTR cDNA was transduced into $CFTR^{-/-}$ cells. The function of hCFTR was assessed by forskolin-induced CFTR channel opening and inhibitor-induced channel closing through FLIPR and Ussing chamber assays.

Results/conclusions: Junctional PCR confirmed that the hCFTR transgene was successfully integrated at target site through CRISPR/Cas9 and homologous recombination. Transgene expressions were measurable at mRNA and protein levels at different cellular passages. Both FLIPR and Ussing chamber assays detected increased hCFTR channel-mediated conductance followed by forskolin stimulation. Meanwhile, the inhibition of hCFTR channel conduction was detected by CFTR inhibitor. Our study showed the potential of CRISPR/Cas9 mediated hCFTR gene transfer strategy to functionally rescue CFTR mutations.