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β-Arrestin recruitment and biased agonism at the M1 muscarinic receptor

Shayan Amiri[1,2], Paul Fernyhough[1,2]
[1]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada. [2]Division of Neurodegenerative Disorders, St Boniface Hospital Albrechtsen Research Centre, Winnipeg, Canada

Abstract

Background & Objectives. We recently showed that application of muscarinic acetylcholine type 1 receptor (M_1R) antagonists (pirenzepine (PZ) or muscarinic toxin 7 (MT7)) reverse nerve degeneration in different rodent models of peripheral neuropathy and β-arrestin played a role in mediating these effects. To understand the mechanism of action of PZ and MT7, we investigated whether these drugs possess β-arrestin-biased agonism at M1R.

Methods. HEK 293 cells and cultured adult rat dorsal root ganglia (DRG) sensory neurons were used. Inositol-phosphate one (IP1) measurement, NanoBRET and luminescence-based M_1R internalization assays were used. Phospho-specific immunoblotting for serine and threonine was performed on purified M_1R. Gαq inhibitor and β-Arrestin KO HEK293 cells were used to determine the role of Gαq-protein and β-arrestins. Western blot was used to measure ERK activation. Statistics was determined using one-way ANOVA followed by post hoc analysis (n=3, minimum 2 assays).

Results. M_1R agonists and antagonists induced Halo-tagged β-arrestin2 recruitment to M_1R-Nluc in a dose-dependent manner at 5 and 30 min, respectively. Unlike MT7 and PZ, muscarine increased IP1 level, while PZ and MT7 dose-dependently inhibited muscarine-induced IP1 generation. MT7 and PZ increased ERK phosphorylation in transfected HEK293 and DRG neurons. Results suggest PZ/MT7 possess β-arrestin-biased agonism. Unlike Gαq protein, β-arrestins are necessary for PZ/MT7-induced ERK phosphorylation. PZ/MT7 impacted serine/threonine phosphorylation status of M_1R. Surprisingly, unlike carbachol, PZ/MT7 not only did not induce M1R internalization but increased surface expression of the receptor.

Conclusion. Selective/specific muscarinic receptor antagonists act as biased agonists at M_1R.

Keywords: Biased agonism, M_1R, antagonists, DRG neurons, ß-Arrestin

A microfluidic brain-on-a-chip model for examining blood-brain barrier (BBB) response to the tumor microenvironment and approaches to enhance drug delivery to the brain

Stacey Line[1], Donald Miller[2], Magimairajan Vanan[2], Sajesh Babu[2], Teruna Siahann[3], Kelly Schwinghamer[3]
[1]University of Manitoba, Narol, Canada. [2]University of Manitoba, Winnipeg, Canada. [3]University of Kansas, Kansas, USA

Abstract

Key words: microfluidics, blood-brain barrier, drug delivery

Background: Microfluidic technology has been applied to in vitro BBB models, having advantages of fluid flow, cell-cell contact, and fluid sampling

Objectives: Establish a dynamic microfluidic (DMF) BBB-brain tumor co-culture model to investigate tumor induced changes in endothelial barriers and identify methods for improving drug delivery to brain tumors

Methods: Human brain microvessel endothelial cells (hCMEC/d3) were seeded (10,000 cells/µL) into the vascular channel and various human brain tumor cell lines (SF8628, U87, and U251) were seeded in the adjacent brain channel of the microfluidic unit. Tumor-induced alterations in barrier function were examined using paracellular and transcellularly transported dyes and correlated with changes of expression of selected BBB specific genes and secretome. Modulation of permeability using cadherin peptides was examined to determine potential for improving drug delivery across the BBB.

Results: Compared to monoculture (4.7x10-8 cm/s) , no significant changes in FDX70,000 permeability were observed with U251 co-culture model (5.4x10-8 cm/s). However, SF8628 enhanced barrier properties (2.0x10-8 cm/s) while U87 increased paracellular leakiness (4.6x10-7 cm/s). Examination of the barrier enhancing microenvironment observed with the SF8628 co-culture model indicated a Sonic Hedgehog dependent process. Cadherin peptide increased FDX70,000 permeability in all the tumor models. (No treatment: SF8628 8.6x10-9 cm/s, U251 1.4x10-8cm/s, U87 5.2x10-8cm/s. HAVN1 treatment: SF8628 1.19x10-6, U251 8.9x10-7cm/s, U87 9.9x10-7 cm/s. T-test statistics with Bonferroni correction for multiple comparison $p<0.05$*, $p<0.1$**, $p<0.001$***

Conclusion: The DMF model can be used to identify tumor-driven changes in brain endothelial cells.

Activation of free fatty acid receptor 3 (FFA3) stimulates glucagon-like peptide-1 (GLP-1) release from enteroendocrine L-cells by non-canonical signaling mechanisms

Karly E. Masse, Jackie Han, Paramveer Love, Van B. Lu
Western University, London, Canada

Abstract

Background: Bacterial fermentation of complex carbohydrates yields high levels of short-chain fatty acids (SCFAs, >100mM) in the gastrointestinal tract. Previous reports indicate SCFAs stimulate enteroendocrine L-cells to secrete the hormone glucagon-like peptide-1 (GLP-1). However, canonical signals downstream of a SCFA receptor expressed in these cells, the free fatty acid receptor 3 (FFA3), involve inhibitory $G_{\alpha i}$-coupled signaling pathways.

Objective: To reconcile this discrepancy, our goal in this study was to elucidate the signaling pathways recruited following FFA3 activation and determine its contribution to GLP-1 secretion.

Methods and Results: The selective FFA3 ligand, AR420626 (10μM), was used to activate FFA3 in a murine cell line model of L-cells, the GLUTag cells. AR420626 significantly increased GLP-1 release when co-administered with the secretagogue, forskolin (one-way ANOVA, N=9 from 4 independent experiments, *p<0.05*). Two methods were used to investigate downstream FFA3 signaling pathways: i) Fura2 fluorescence Ca^{2+} imaging to measure intracellular Ca^{2+} levels and ii) a luminescence-based cAMP biosensor to measure intracellular cAMP levels. Of 19 GLUTag cells tested, AR420626 increased intracellular Ca^{2+} levels in 11 cells (mean increase in Fura2 ratio=22% of max response). In GLUTag cells stably expressing the cAMP biosensor GloSensor, AR420626 significantly increased the luminescence signal when pre-stimulated with forskolin (as a % before treatment: control=73.0±2.0%; AR420626=101.2±1.4%, *p<0.0001*), an effect that was unaffected in the presence of the G_i-uncoupler, pertussis toxin.

Conclusions: We have demonstrated that FFA3 activation is stimulatory in GLP-1 releasing L-cells and does not involve $G_{\alpha i}$ recruitment.

Keywords: short-chain fatty acids, enteroendocrine cells, free fatty acid receptor

Alpha-lipoic acid protects against gliclazide-induced hepatotoxicity in high glucose-exposed HepG2 cells and type 2 diabetic rats

George Dugbartey[1,2], Karl Alornyo[1], Stephen Atule[1], Richard Obeng-Kyeremeh[1], Daniel Amoah[1], Samuel Adjei[1]
[1]University of Ghana, Accra, Ghana. [2]University of Western Ontario, London, Canada

Abstract

Background: Sulfonylurea-induced hepatotoxicity contributes significantly to progressive worsening of liver health in type 2 diabetes mellitus (T2DM) patients.

Objectives: To determine whether alpha-lipoic acid (ALA) exhibits hepatoprotective effect against sulfonylurea-induced hepatoxicity.

Methods: HepG2 cells were incubated with high-glucose DMEM in the presence or absence of gliclazide and ALA for 72 hours, and cell viability and death were measured. Next, Sprague-Dawley rats underwent 12 hours of fasting, and fasting blood glucose was measured. The rats were randomized into four groups: HC (healthy control rats), T2DM (untreated diabetic rats), GLC (diabetic rats that received 15mg/kg/day gliclazide) and GLC+ALA (diabetic rats that received gliclazide + 60mg/kg ALA daily). T2DM was induced by a bolus injection of 110mg/kg nicotinamide and 55mg/kg streptozotocin intraperitoneally. The experimental protocol lasted for 6 weeks after which rats were sacrificed, and blood and liver samples were collected for analysis.

Results: Exposure of HepG2 cells to high glucose induced significant cell death, which was exacerbated with gliclazide treatment but markedly reduced following ALA treatment ($p<0.01$). In GLC-treated rats, severe histopathological changes were observed in the liver compared to T2DM rats ($p<0.01$). Also, severe hypertransaminasemia and increased expression of pro-inflammatory (ED-1, iNOS, IL-1β, IL-6 and TNF-α) and pro-apoptotic (caspases-3, Bax and Bid) markers were also found in GLC group compared to T2DM rats ($p<0.001$). Interestingly, ALA administration prevented these pathological changes and protected the diabetic liver to levels comparable to HC rats.

Conclusions: ALA protected against gliclazide-induced hepatotoxicity, suggesting its clinical application in T2DM patients under gliclazide or another sulfonylurea therapy.

Characterization of N,N,N-Trimethyl-L-Alanyl-L-Proline betaine (TMAP) as a Candidate Biomarker of Kidney Function

Nicole Sidor[1], Sophie Nie[1], Thomas Velenosi[2], Gilles Lajoie[3], Rommel Tirona[1], Bradley Urquhart[1]
[1]Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada. [2]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada. [3]Department of Biochemistry, University of Western Ontario, London, ON, Canada

Abstract

Background: N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP) is a candidate biomarker of kidney function, whereby plasma concentration increases as kidney function declines. Kinetic properties and influence of liver function on TMAP concentrations are unknown.

Objectives: To evaluate extent of plasma protein binding and to quantify plasma TMAP concentration in Non-Alcoholic Fatty Liver Disease (NAFLD) patients. We hypothesize TMAP exhibits low plasma protein binding and is not impacted by NAFLD.

Methods: Plasma samples from healthy control, chronic kidney disease (CKD), hemodialysis (HD) (n=3 for each) and peritoneal dialysis (PD) patients (n=1) were spun through centrifugal filters with a 10kDa molecular weight cut off. The filtrate was analyzed by LCMS. Concentration of TMAP in filtered and unfiltered samples was compared. Three time points (fasted baseline, postprandial 6 hours and 12 hours later) of plasma samples from healthy controls (n= 12) and NAFLD patients (n= 22) were analyzed by LCMS.

Results: TMAP plasma protein binding was 29% in controls, 27% in CKD, 43% in HD and 48% in PD patients. A two-way ANOVA yielded no significant difference in mean concentrations of plasma TMAP across the three time points between control and NAFLD patients. There was a 28 ng/mL (14%) and a 17ng/mL (9%) decrease in mean plasma TMAP concentration from baseline to 6 hours later within control ($p < 0.001$) and NAFLD ($p < 0.01$) groups in a one-way ANOVA, respectively.

Conclusions: Results suggest TMAP exhibits moderate protein binding. Liver dysfunction does not alter plasma TMAP concentrations. TMAP appears to have diurnal variation.

Characterizing a Metabolic Phenotype Susceptible to Polyamine Pathway Targeted Treatment in Triple Negative Breast Cancer

Chloe White, Thomas Velenosi
University of British Columbia, Vancouver, Canada

Abstract

Background

Triple negative breast cancer (TNBC) has the highest mortality rate of all breast cancer subtypes. Polyamines are small cationic molecules important for tumour growth. Spermidine/spermine N1-acetlytransferase (SAT1) is the rate-limiting polyamine catabolic enzyme.

Objective

To characterize a TNBC metabolic phenotype for targeting the polyamine pathway using a SAT1 agonist, diethylnorspermine (DENSpm), alone or in combination with doxorubicin treatment.

Methods

Patient-derived xenograft models of three different TNBC tumour phenotypes, with high (TM99), moderate (TM98), and low (TM96) baseline expression of SAT1 respectively, were engrafted into NSG mice. Tumours were excised, sliced, and treated ex vivo with DENSpm, and/or doxorubicin. SAT1 mRNA expression and levels were measured by qPCR. Polyamine levels were measured using LC-MS.

Results

Treatment of TM98-moderate SAT1 tumours with 1µM doxorubicin resulted in a significant 2-fold induction of SAT1 expression ($p < 0.05$). Interestingly, combining doxorubicin and DENSpm treatment resulted in a 2.7-fold increase in SAT1-generated diacetylspermine and a 61% depletion of spermidine and spermine at sub-therapeutic doxorubicin concentrations. In TM99-high SAT1 tumours, SAT1 was induced up to 6-fold with DENSpm treatment, while the addition of doxorubicin did not significantly affect SAT1 expression.

Conclusions

The results indicate that SAT1 can be induced by DENSpm or combination DENSpm-doxorubicin in tumours with high and moderate baseline SAT1 expression, respectively. SAT1 depletes polyamine levels and therefore, the addition of DENSpm to doxorubicin treatment may contribute to treatment effectiveness in TNBC with moderate and high SAT1 baseline expression.

Keywords: triple-negative breast cancer, patient-derived xenograft, metabolic biomarker, polyamine

Characterizing Opioid Overdose Deaths in Children (COODC Study)

Katrina Assen[1], Michael Wilson[2], Elizabeth Urbantke[3], Michael Rieder[1]
[1]Western University, London, Canada. [2]Office of the Chief Coroner, Thunder Bay, Canada. [3]Office of the Chief Coroner, London, Canada

Abstract

Introduction

With the opioid epidemic there are increasing numbers of fentanyl related adult deaths with limited knowledge of this effect on pediatric deaths. Our aim is to describe these cases and their characteristics to help guide public policy and health care system response.

Methods

Patient information was taken from the opioid investigative aid (OIA) database, which collects all suspected opiate-related deaths in Ontario. A chart review was preformed on patients less than 10 years of age between October 1st, 2017, to October 31st, 2021. Patient characteristics were calculated as percentages and descripted analysis was conducted.

Results

10 childhood deaths occurred, the average age was 1.9 with the oldest patient being 4 years and 9 months. The causative opioid was fentanyl alone in 40% of cases, fentanyl in combination in 40% and hydromorphone and methadone with 10% each. Most cases involved improperly stored medication/illicit substances with drug paraphernalia present in 80%. All had previous child protection service involvement and 70% had previous police involvement.

Conclusion

In patients less than 10 years of age there were ten opioid related deaths over four years all in those younger than 5 years. Fentanyl was the primary drug involved in 80% of cases. This demonstrates a proportional rise in fentanyl related opioid deaths in children and elucidates areas for change including education on the pediatric threat of fentanyl, proper storage of illicit substances and implications for how the child protection system works in homes where substance use is reported to occur.

Opioids, Mortality, Pediatric

Characterizing the mechanism of doxorubicin-mediated SAT1 induction in triple-negative breast cancer

Caroline Liang, Chloe White, Thomas Velenosi
The University of British Columbia, Vancouver, Canada

Abstract

BACKGROUND

The effective treatment of triple-negative breast cancer (TNBC) with chemotherapy drugs such as doxorubicin is limited by drug resistance. Consequently, there is a need to detect effective drug response early during chemotherapy. In TNBC, polyamine pathway upregulation can accelerate tumour growth, demonstrating the utility of polyamines as metabolic biomarkers. A previous study showed that doxorubicin increases tumour spermidine/spermine N1-acetyltransferase (SAT1) gene expression, resulting in increased urine levels of the polyamine pathway metabolite, diacetylspermine; however, the underlying mechanism is unknown.

OBJECTIVES

Identify the mechanism of doxorubicin-mediated SAT1 induction using TNBC cell lines and a TNBC patient-derived xenograft (PDX) ex vivo tumour model.

METHODS

BT549 cells and TM98-PDX tumour ex vivo slices were treated with vehicle, 1 µM doxorubicin, or 10 µM diethylnorspermine (DENSPM), a polyamine analog that induces SAT1. Quantitative PCR was performed to measure relative pre-mRNA, mature mRNA, and the SAT1-X splice variant mRNA expression.

RESULTS

Doxorubicin-treated BT549 cells demonstrated a significant increase in SAT1 pre-mRNA, mature mRNA, and SAT1-X mRNA transcript expression compared to controls ($P<0.05$). DENSPM-treated cells trended towards increased SAT1 pre-mRNA and mature mRNA, but reduced SAT1-X mRNA expression. Similarly, preliminary data suggest that pre-mRNA and mature mRNA expression are elevated in TM98-PDX tumours following doxorubicin and DENSPM treatment; however, SAT1-X mRNA transcript expression was unaffected.

CONCLUSIONS

The increase in SAT1 pre-mRNA and mature mRNA in cellular and ex vivo tumour TNBC models suggest that the mechanism of SAT1 induction may be mediated through transcriptional activation.

Keywords: pharmacometabolomics, TNBC, polyamines, biomarker

Cytoprotection by nanobioparticle drug delivery

Benny Habiyambere[1,2,3], Anirban Ghosh[4], Kyle Wells[1,2,3], Ashley Eadie[1,2,3], Keith Brunt[1,2,3]
[1]Dalhousie Medicine New Brunswick, Saint John, Canada. [2]Dalhousie University, Halifax, Canada.
[3]IMPART Investigator Team Canada, Saint John, Canada. [4]Pividl Bioscience, Moncton, Canada

Abstract

Background-Plants naturally synthesize nanobioparticles to transport cellular materials that contain potential active pharmaceutical ingredient (API). Extracting nanobioparticles from plants containing the APIs is an alternative to harmful synthetic nanoparticles. Curcuminoids (e.g. curcumin) are the most abundant APIs in turmeric spice (from the *Curcuma Longa* plant), a traditional medicine with anti-oxidative/anti-inflammatory effects. Numerous chronic diseases largely feature oxidative stress and excess inflammation at the level of the macrophage. Curcumin is GRAS by the FDA but has poor stability, solubility and bioavailability. Nano-encapsulation of curcumin can improve its stability, solubility and bioavailability to elicit meaningful pharmacological effects.

Objectives-We aim to show that turmeric nanobioparticles (i.e., encapsulates curcumin) will be superior at eliciting anti-oxidative cytoprotective effects in human macrophages compared to a pure curcumin molar equivalent.

Methods-Transmission electron microscopy and dynamic light scattering was used to assess the biophysical properties of turmeric nanobioparticles (PVDL-005). Heme-oxygenase-1 expression (i.e., regulates anti-oxidation/anti-inflammation and cytoprotection) was evaluated by Western Blot. Fluorescent microscopy was used to observe PVDL-005 cellular localization.

Results-Biophysical properties of PVDL-005 are preserved after extraction, where the nanobioparticles are spherical in shape, with a size and surface charge of 172.3 nm and -0.349 mV, respectively. $[5\mu M\ curcumin]_{eq}$ in PVDL-005 induced 2.5-fold more heme-oxygenase-1 than 5µM pure curcumin. PVDL-005 localizes in the cytoplasm and nucleus of human macrophages at 6hr.

Conclusions-These early results show the potential to harness the intrinsic properties of an active natural ingredient (e.g. curcumin) by nano-encapsulation and exploit its cytoprotective effects *in vitro*.

Keywords-Curcumin, turmeric nanobioparticles, cytoprotection, macrophages, heme-oxygenase-1

Developing a Mouse Model to Study the Mechanisms of Peg-asparaginase-induced Pancreatitis

Esther Bonitto[1], Zara Forbrigger[1,2], Ketan Kulkarni[1,2], Kerry Goralski[1,2]
[1]Dalhousie University, Halifax, Canada. [2]IWK Health Centre, Halifax, Canada

Abstract

Keywords: Pancreatitis, peg-asparaginase, cancer, mouse model

Background: Pancreatitis is a severe adverse drug response (ADR) occurring in 2-16% of pediatric acute lymphoblastic leukemia patients treated with peg-asparaginase. The causative proponents of pancreatitis development are unknown, but it is suspected that genetic factors may contribute. There is presently no reliable mouse model that fully incapsulates peg-asparaginase-induced pancreatitis, and one must be generated to study the genetic risk factors and mechanisms leading to this ADR.

Objectives: To establish a mouse model that accurately reflects peg-asparaginase-induced pancreatitis observed in pediatric patients.

Methods: Balb/cByJ, A/J, and C57 mice were intraperitoneally injected with either ~1.5 IU/g of peg-asparaginase (n=5 per strain) in PBS, or an equivalent volume of PBS (n=4 or 5 per strain) alone. Body mass was recorded daily. Mice were euthanized on day 4 or 5 for terminal blood sample collections and pancreas dissections. Pancreatitis will be assessed by measurement of pancreatic enzymes lipase and amylase in serum, and H&E staining of pancreatic sections.

Results: Peg-asparaginase A/J mice lost a significant amount of weight (14.8% +/- 1.4 over 4 days, $P<0.05$), and were euthanized humanely on day 4. C57 lost a smaller amount (6.7% +/- 1.2 over 5 days, $P<0.05$), and Balb/cByJ experienced no significant weight loss.

Conclusions: The response to peg-asparaginase treatment is mice-strain dependent based on observed body weight changes. Completion of the pancreatic enzyme analyses and histology evaluation of pancreatic sections will reveal which mouse model is best for investigating genetic predictors of peg-asparaginase-induced pancreatitis.

Development of a polygenic score to predict cisplatin-induced ototoxicity

Deanne Nixie Miao[1], Mackenzie Wilke[1], Feryal Ladha[1], Mary McAuley[1], Urim Iyasere[1], Britt Drogemoller[1,2,3]
[1]Department of Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [2]CancerCare Manitoba Research Institute, Winnipeg, Canada. [3]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada

Abstract

Background: Hearing loss is a common adverse drug reaction (ADR) associated with cisplatin treatment. Previous research has shown that 38-47% of the variability in this ADR can be attributed to genetics, and this susceptibility can be traced to multiple loci.

Objective: To create a polygenic score (PGS) to predict cisplatin-induced ototoxicity (CIO).

Methods: We developed a PGS using self-reported hearing loss data from the UKBiobank (*n*=353,983) and SBayesR. To improve the score's predictive capabilities, we also developed an audiogram-based PGS using data from the Canadian Longitudinal Study on Aging (CLSA) (*n*=18,955). The relevance of these scores to CIO were tested in a pediatric CIO cohort (*n*=390) using ReAct. Enrichment analyses were also conducted on murine inner ear single-cell RNA-sequencing data to assess variant associations with hearing loss in specific inner ear cell types.

Results: Examination of the self-reported hearing loss PGS revealed that this score was significantly associated with CIO ($P=3.8\times10^{-3}$, $R^2=0.02$). However, use of the audiogram-based PGS significantly improved the predictive capacities of this score ($P=5.52\times10^{-10}$, $R^2=0.09$). Enrichment analyses revealed that variants mapping to genes expressed in all cell types in the epithelial cochlea, and basal, intermediate, and spindle/root cells in the stria vascularis were more likely to be associated with hearing loss ($P<2.0\times10^{-16}$).

Conclusion: This is the first PGS developed to predict the risk of CIO using large-scale hearing loss cohorts. Future analyses will focus on single-nuclei RNA-sequencing of cisplatin-treated murine inner ear samples to identify relevant cell types for CIO.

Keywords: cisplatin-induced ototoxicity, pharmacogenomics, polygenic

Dissecting the role of branched-chain amino acids and branched-chain keto acids in modulating cardiac adverse remodelling in heart failure

Qutuba Karwi[1], Liyan Zhang[1], Cory Wagg[1], Keshav Gopal[2], Golam Mezbah Uddin[1], Kim Ho[1], Qiuyu Sun[1], Sai Panidarapu[1], Kaya Persad[1], Betul Altunay[1], Shaden Deman[1], Jody Levasseur[1], John Ussher[2], Jason Dyck[1], Gary Lopaschuk[1]
[1]Cardiovascular Research Centre, University of Alberta, Edmonton, Gabon. [2]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Abstract

Background: Alterations in branched-chain amino acid (BCAA) oxidation has been linked to aggravating adverse remodelling in the failing heart. Interestingly, these alterations are associated with the accumulation of BCAAs and their metabolites, namely branched-chain keto acids (BCKAs), making it challenging to ascertain whether BCAAs or BCKAs are exacerbating adverse remodelling. We have recently demonstrated that cardiac-specific deletion of mitochondrial branched-chain aminotransferase ($BCATm^{Cardiac-/-}$), the enzyme that converts BCAAs into BCKAs and vice versa, increases cardiac BCAA levels and left ventricular (LV) mass.

Objective: To characterize the impact of selective augmentation of BCAA levels on cardiac energy metabolism and adverse remodelling in heart failure.

Methods: $Wild^{Cre+/+}$ and $BCATm^{Cardiac-/-}$ male mice underwent a sham or transverse aortic constriction (TAC) surgery to induce heart failure. Cardiac function and structure were monitored pre-and post-TAC using echocardiography, and cardiac energy metabolism was accessed using an isolated working heart model.

Results: Five weeks post-TAC, BCATm deletion exacerbated adverse hypertrophy, as evidenced by increased LV mass, compared to the WT^{Cre+} failing hearts. The mTOR/P70S6K/4E-BP1 signalling pathway was also triggered in $BCATm^{Cardiac-/-}$ mice hearts. BCATm deletion did increase insulin-stimulated glucose oxidation rates and cardiac efficiency in the failing hearts, which was associated with enhanced mitochondrial Akt activity. This was probably due to a lowering of BCKAs, which impair cardiac insulin signalling.

Conclusion: Augmented BCAA levels worsen adverse remodelling and offset any potential beneficial effects of lowering BCKA by triggering the mTOR/P70S6K/4E-BP1 signalling pathway in the failing heart.

Keywords: branched-chain amino acids, branched-chain keto acids, heart failure, adverse remodelling

Early endothelial function activation by losartan prevents aortic stiffness in a model of type 1 diabetes.

Chris Yuen[1], Zoe White[1], Angela Devlin[1,2], Pascal Bernatchez[1]
[1]University of British Columbia, Vancouver, Canada. [2]BC Children's Hospital Research Centre, Vancouver, Canada

Abstract

Background/Objective: Type 1 diabetes (T1D) is an autoimmune disease that causes insulin-producing β-cell destruction and ultimately cardiovascular diseases. In addition to antihypertensive properties, angiotensin II receptor blockers (ARBs) are effective in activating protective, nitric-oxide (NO)-dependent endothelial function. It is unknown if ARBs can reverse early changes in vascular homeostasis caused by T1D to improve cardiovascular outcomes.

Methods: Ins2+/Akita (Akita) mice were assessed as a model of diabetes and compared to age/sex-matched Ins2+/+ controls at diabetes onset (blood glucose ≥ 16.6mmol/L); 4 weeks post-diabetes onset; 12-weeks post-diabetes onset. Treatment with ARB losartan (0.6g/L drinking water) was initiated at 4 weeks post-diabetes onset. Mean arterial blood pressure (MABP) and aortic pulse wave velocity (PWV), an indicator of aortic stiffness, were measured. Ex vivo wire myography was conducted to study vascular reactivity and basal NO production.

Results: At 4-weeks post-onset, Akita mice showed 70.2% greater phenylephrine-induced constriction ($p<0.04$), a 24.5% reduction in Ach-induced NO-dependent vasorelaxation ($p<0.01$) and 51.7% greater PWV compared to control. At 12-weeks post-onset, Akita mice had 16.2% greater MABP($p<0.01$), 241.1% greater PWV ($p<0.01$), 16.2% greater phenylephrine, and 20.2% reduction ($p<0.01$) in Ach reactivity. In addition to lowering of MABP, losartan reduced phenylephrine in Akita (46.1%) and control (71.1%) mice ($p<0.01$). Losartan lowered PWV in male Akita mice to control levels and normalized impaired Ach-induced vasodilation.

Conclusion: Early intervention with losartan prevents aortic stiffness and rescues endothelial protective properties, which may be an effective treatment to reduce T1D-associated cardiovascular complications.

Keywords: endothelium, diabetes, angiotensin

Effect of nucleocapsid protein of SARS-COV-2 on endothelial inflammation

Mehreen Tufail, Qingping Feng, Xiangru Lu, Brent Tschirhart, Stephen Barr
Western University, London, Canada

Abstract

Background: COVID-19 is caused by SARS-CoV-2 and can lead to severe complications like ARDS and sepsis. The nucleocapsid protein (N-protein) is a structural protein of SARS-CoV-2 and current literature suggests that it induces endothelial inflammation. However, the mechanism by which N-protein exerts its effects remains unclear.

Objectives: To assess if N-protein induces an inflammatory response in mouse and human endothelial cells, and to determine its mechanism of action.

Methods: Mouse and human endothelial cells were treated with vehicle, lipopolysaccharide (LPS), or recombinant N-protein in the presence or absence of LPS-neutralizing agent polymyxin B, for 4 hours. mRNA levels of inflammatory markers (TNFα, IL-6, E-selectin and ICAM-1) were measured through qPCR. Monocyte adhesion was measured to detect endothelial activation. The endotoxin levels in N-proteins were assessed.

Results: Our data show that treatment with either LPS or N-protein resulted in at least a 15-fold increase in inflammatory marker levels, which were inhibited over 95% by polymyxin B. Similarly, LPS or N-protein alone resulted in 3 times increase in monocyte adhesion to vascular endothelial cells. In the presence of polymyxin B, monocyte adhesion to vascular endothelial cells was reduced to control levels.

Conclusion: Our data suggest that N-protein may not induce endothelial inflammation, contrary to the current literature. The inflammatory effects of N-protein that have been observed thus far may be due to endotoxin contamination, implying that it may not play a role in SARS-CoV-2 induced endothelial inflammation.

Keywords: N-protein, inflammation, SARS-CoV-2, endothelial cells, endotoxins

Erythropoietin single nucleotide polymorphic rs507293 correlates with dyslipidemia

Victoria Northrup[1,2], Jermey A. Simpson[3,2], Keith R. Brunt[4,2]
[1]Dalhousie Univerisity, Halifax, Canada. [2]IMPART investigator team Canada, Saint John, Canada.
[3]University of Guelph, Guelph, Canada. [4]Dalhousie University, Halifax, Canada

Abstract

Background/Objective: Dyslipidemia is an abnormal amount of lipids (triglycerides, cholesterol and/or lipoproteins) and is a risk factor for cardiovascular disease. Erythropoietin (EPO) is an erythropoietic cytokine that may have a role in regulating cellular metabolism. Recombinant human EPO (rhEPO) has been used to treat anemia but has also been shown to decrease cholesterol and low-density lipoprotein (LDL). Single nucleotide polymorphisms (SNPs) in EPO have been linked to anemia and diabetic microvascular complications but have not been studied in association with dyslipidemia. We investigated the association between EPO SNPs and dyslipidemia.

Methods: DNA was isolated from blood samples obtained as part of the OPOS study. Sanger sequencing was used to genotype three EPO SNPs (rs507392, rs1617640 and rs551238). Genotypes were compared to clinical outcomes using SNPstat (SNPstat.net) and lipid profiles using student t-test. EPO concentrations were measured in plasma by ELISA.

Results: The SNP rs507392 genotype of GG was associated with dyslipidemia in a recessive model. This was associated with lower EPO in plasma and elevated total cholesterol and LDL. The SNP rs1617640 genotype of CC was associated with reduced EPO and Hb and with slightly higher total cholesterol and LDL but did not reach significance for dyslipidemia. The SNP rs551238 genotype of GG did not change EPO concentrations or associate with dyslipidemia.

Conclusion: The G allele of rs507392 and C allele of rs1617640 results in lower EPO and the latter is associated with lower Hb, while the former shows an elevated risk of dyslipidemia.

Keywords: Erythropoietin, Dyslipidemia, Anemia

Exploring the molecular promiscuity of L-phenylalanine (Phe) activation of G protein-coupled receptors

O. Peter Oni, Madeline E. Power, Jillian L. Rourke
Mount Allison University, Sackville, Canada

Abstract

Background/Objective: Recent advances in nutrient-sensing mechanisms revealed that amino acids (AA) act as ligands for class C G protein-coupled receptors (GPCRs). The AA phenylalanine (Phe) is proposed to activate the adhesion GPCRs GPR56 and GPR97 and class A GPCRs GPR142 and GPR139. This study aimed to determine the extent to which Phe activates class A GPCRs.

Methods: We quantified the activation of 277 GPCRs treated with a L-Phe using a high-throughput b-arrestin2 recruitment assay (PRESTO-Tango). GPCRs were classified as a candidate Phe receptor if the activation magnitude exceeded 2-fold and was statistically significant (GraphPad Prism, two-way ANOVA with multiple comparisons, $p<0.05$).

Results: The treatment of 277 class A GPCRs with Phe revealed that it significantly activates ~53% of the tested receptors and 34 receptors (12.3%) had greater than 5-fold change. The top 5 receptor candidates with the greatest average Z Score fold change were GPR88 (8.36), DRD4 (8.40), NPBW1 (9.23), SSTR4 (9.82), and MTNR1B (11.54). Nine candidate orphan and lipid receptors were treated with increasing concentrations of Phe, revealing individual receptor efficacies and potencies to Phe with Emax ranging from 13.61 - 57.82-fold change. Using in vitro signalling luciferase reporters, significant downstream signalling profiles were identified for some receptors, while others did not impact tested pathways.

Conclusions: These data suggest that Phe is a promiscuous endogenous ligand for numerous GPCRs. This contributes to the growing evidence supporting AAs as signaling meesnegers in nutrient status sensing.

Keywords: GPCR, Phenylalanine, Signal Transduction

Genomic Risk Factors of Vincristine-Induced Peripheral Neuropathy

Kheireddin Mufti[1,2], Miguel Cordova[2,3], Erika Scott[1,2], Jessica Trueman[2,3], Jessica Lovnicki[2,3], Catrina Loucks[2,3,4], Shahrad Rassekh[2,5], Colin Ross[1,2,6], Bruce Carleton[1,2,3,7]
[1]Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, Canada. [2]British Columbia Children's Hospital Research Institute, Vancouver, Canada. [3]Division of Translational Therapeutics, Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada. [4]Department of Anesthesiology, Pharmacology & Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, Canada. [5]Division of Hematology, Oncology & Bone Marrow Transplant, Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada. [6]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada. [7]Pharmaceutical Outcomes Programme, BC Children's Hospital, Vancouver, Canada

Abstract

Background: Vincristine-induced peripheral neuropathy (VIPN) is a debilitating toxicity of treatment that can reduce both quality of life and survival. Previous studies have identified genetic variations associated with VIPN risk; however, their clinical relevance has been limited due to small sample sizes and unclear clinical phenotypes.

Objectives: To identify pharmacogenomic risk factors of VIPN.

Methods: A nested case-control study of 1,100 pediatric cancer patients from ten Canadian academic health centres. VIPN cases (CTCAE Grade ≥ 2; n=550) and controls (CTCAE Grade 0; n=550) were matched by vincristine cumulative exposure and genetic ancestry. Patients with mild VIPN (CTCAE Grade 1; n=155) were excluded to ensure case/control phenotypic discrimination. A genome-wide association study (GWAS) was performed using genotype data generated with custom Illumina Infinium® Global Screening Array (GSA v2.0) and supplemented with genotype imputation.

Results: The GWAS revealed significant associations ($p<5.0x10^{-8}$) between VIPN risk and genetic variations in several genes, including *MCM3AP* that strongly increases VIPN risk by 6-fold (OR=6.3 [95%CI:2.9-12.5], $p=3.1x10^{-8}$), *NRG3* (OR=0.15 [95%CI:0.07-0.33] $p=3.2x10^{-8}$), *FBN2* (OR=0.36 [95%CI:0.25-0.50], $p=4.3x10^{-8}$) and *LRRTM3* (OR=0.14 [95%CI:0.06-0.33], $p=4.8x10^{-8}$) significantly decrease VIPN risk by 3-7 fold. *MCM3AP* gene causes recessive forms of Charcot-Marie-Tooth disease, a common disorder that results in peripheral nerve demyelination, while *NRG3* and *FBN2* contribute to peripheral nerve myelination, and *LRRTM3* regulates excitatory synapse development, which is crucial for normal neuronal transmission.

Conclusions: Genetic variations related to heritable peripheral neuropathy, nerve myelination and synaptic assembly and transmission play an essential role in VIPN susceptibility.

Keywords: Vincristine; Neuropathy; Adverse Drug Reactions; Pharmacogenomics

Gestational Diabetes Mellitus Induces Cardiac Dysfunction and Altered Mitochondrial Protein Acetylation in the Offspring Heart

Caitlin Menzies[1,2], Mateusz Tomczyk[1,2], Bo Xiang[1,2], Stephanie Kereliuk[1,2], Vernon Dolinsky[1,2]
[1]Department of Pharmacology and Therapeutics,University of Manitoba, Winnipeg, Canada. [2]Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme, Winnipeg, Canada

Abstract

Background: Exposure to gestational diabetes mellitus (GDM) increases risk of cardiovascular disease in offspring later in life. Our lab has found that mitochondrial dysfunction is a major factor that contributes to the development of cardiomyopathy in GDM offspring. Protein lysine acetylation is a post-translational modification that regulates activity of metabolic enzymes within the mitochondria.

Objectives: To determine how GDM exposure affects lysine acetylation of mitochondrial enzymes and mitochondrial function in the offspring heart.

Methods: GDM was induced by feeding female mice a high fat and sucrose (HFS; 45% fat) diet for 6 weeks prior to mating and throughout pregnancy. Control lean dams were fed a low fat (LF; 10% fat) diet. Offspring from Lean and GDM dams were fed LF and HFS diets. Echocardiography was performed in 15-week-old offspring. Mitochondria were isolated from offspring hearts and acetylated peptides were extracted via immunoprecipitation and quantified by mass spectrometry.

Results: GDM induced cardiac hypertrophy (Lean-LF vs. GDM-HF $p=0.015$) and diastolic dysfunction (Lean-HF vs GDM-HF $p=0.006$) in offspring. GDM exposure differentially altered the acetylation of mitochondrial peptides which was exacerbated by a postnatal HFS diet (GDM-HF vs Lean-LF 88 peptides, $p<0.05$). Functional classification revealed prominent representation of acetylated proteins in fatty acid oxidation, respiratory electron transport, and mitochondrial biogenesis pathways in hearts of GDM offspring. We examine mitochondrial metabolism protein expression to investigate mechanisms of mitochondrial dysfunction in GDM offspring.

Conclusion: Cardiac enzyme acetylation contributes to GDM-induced mitochondrial dysfunction and cardiomyopathy in offspring.

Keywords: Cardiomyopathy, Gestational diabetes, Protein acetylation, Mitochondria

Infant Poisoning in the First Year of Life: A Cohort Study of an International Toxicology Surveillance Registry

Justin Williamson[1,2], Asaph Rolnitsky[3,4], Yaron Finkelstein[1,2]
[1]Department of Paediatrics, The Hospital for Sick Children, Toronto, Canada. [2]Departments of Paediatrics and Pharmacology and Toxicology, University of Toronto, Toronto, Canada. [3]Neonatal-Perinatal Medicine, Sunnybrook Health Sciences Centre, Toronto, Canada. [4]Department of Paediatrics, University of Toronto, Toronto, Canada

Abstract

Background/Objectives: Infants are highly vulnerable to poisoning due to their size, immature pharmacological pathways, inability to report exposure circumstances or protect themselves from harm. However, infant poisoning has not been systematically studied.

Methods: We conducted a cohort study of infant (≤12 months) poisonings recorded in the American College of Medical Toxicology's Toxicology Investigators Consortium (ToxIC) database between 3/2010 to 3/2022. This prospective registry captures all emergency department visits for poisoning, which received a bedside medical toxicology consultation from 47 participating hospitals globally. We collected demographic and clinical data, including age, sex, exposure circumstances, intoxicating substances, clinical presentation, management, and outcome.

Results: 758 infants were identified. Toxic exposure to a pharmacological and non-pharmacological agent accounted for 385 (50.8%) and 268 (35.4%) of cases, respectively, while the rest included exposures such as envenomation. Of 901 agents identified, 207 (23.0%) were analgesics, with opioids accounting for 72.0%, followed by 128 (14.2%) sympathomimetics, 84 (9.3%) cardiovascular drugs, 65 (7.2%) psychoactive drugs, and 47 (5.2%) household substances. The most common presenting symptoms were central nervous system (CNS) depression/coma (n=229, 30.2%) and tachycardia (n=138, 18.2%). Overall, 307 (40.5%) infants were admitted to a critical care unit, including 48 (6.3% of total) who were mechanically ventilated. Six (0.9%) infants died.

Conclusion: Infants presenting to ToxIC sites were exposed to highly toxic substances. 1 in 3 experienced CNS depression/coma, almost half required critical care, and 1 in 100 died. Prevention strategies to mitigate infant poisoning and dire outcomes are needed.

Key Words: Infants, Poisoning, Toxicology

Investigating the Anticancer Properties of Anthocyanin-Rich Extracts from Aponogeton madagascariensis in Triple Negative Breast Cancer Cells

Shanukie Embuldeniya, Kerry Goralski, Arunika Gunawardena
Dalhousie University, Halifax, Canada

Abstract

Background: Triple Negative Breast Cancer (TNBC) is a human breast cancer subtype accounting for 10 – 20 percent of all breast cancers. Natural Products (NPs) from plant extracts contain diverse bioactive compounds that can reduce cancer cell viability, proliferation, and metastasis. However, the exact pharmacological mechanisms responsible for these anticancer properties remain unclear.

Objective: To identify the anti-cancer properties and pharmacological mechanisms of young and mature leaf extracts from the aquatic plant Aponogeton madagascariensis (Lace Plant) in MDA-MB-231 triple negative, human breast cancer cells.

Methods: MDA-MB-231 and control breast epithelial MCF-10A cells were treated for 24-48 h with crude anthocyanin-rich extracts, prepared from young and mature leaves of lace plants grown in axenic cultures. Cell viability was measured using the MTT cell viability assay. Cell proliferation was measured using a fluorescent-based CyQuant® proliferation assay. Intracellular reactive oxygen species (ROS) were measured using a CM-H2DCF-DA fluorescent-based assay.

Results: Young and mature anthocyanin-rich extracts (25–250 μg/mL) reduced MDA-MB-231 cell viability by 20–30 percent in a dose-dependent manner ($P<0.05$) while having no effect on MCF-10A cell viability after 48 h. MDA-MB-231 cell proliferation was reduced by 20-30 percent ($P<0.05$), 24-48 h post-anthocyanin extract treatments compared to vehicle control. Intracellular ROS levels increased by 1.5 – 2-fold in 24h anthocyanin extract-treated MDA-MB-231 cells compared to vehicle controls.

Conclusion: Anthocyanin-rich (crude) extracts from young and mature leaves of the lace plant display cytotoxic and anti-proliferative effects in MDA-MB-231 in TNBC cells which could be due to elevated intracellular ROS.

Keywords: Breast Cancer, Natural-Products, Anthocyanin

In Vitro Anti-Diabetic Activity and Mechanism of Action of Root Extract of Sansevieria liberica Gerome & Labroy (Dracaenaceae) and its Different Solvent Extracts.

Omowunmi Amao[1], Margaret Sofidiya[2], Adedunni Olusanya[1], Oluwamuyiwa Fasina[1]
[1]College of Medicine, University of Lagos, Idiaraba, Nigeria. [2]University of Lagos, Idiaraba, Nigeria

Abstract

Background

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent chronic hyperglycaemia with impaired insulin secretion, resistance to peripheral actions of insulin or both. One of the anti-diabetic therapeutic approaches is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase.

Objectives

The aim of this study was to investigate the possible mechanism of action of anti-diabetic activity of Sanseviera liberica (SL) roots extract and its fractions.

Methods

The different concentrations (50, 125, 250, 500, and 1000 µg/mL) of extract, its fractions and the standard (acarbose) were evaluated for α-amylase, and α-glucosidase enzyme inhibition activities. The in-vitro method used for this procedure involved the use of synthetically manufactured alpha amylase enzyme and crude alpha glucosidase which was extracted by the method of Lowry et al., 1951. The percentage of α- amylase, and α- glucosidase inhibitory activity and IC50 values were calculated (Telagari and Hullatti, 2015).

Results

The assay results showed that Sanseviera liberica and its fractions exhibited significant inhibitory activity on α-amylase and α- glucosidase enzymes. The percentage inhibition varied from 8.697 ± 3.513 at 50 µg/ml to 106.38 ± 13.306 at 1000 µg/ml with IC50 of 221.32 µg/ml for alpha amylase and 19.363 ± 0.982 at 50 µg/ml to 125.86 ± 10.166 with IC50 of 381.60 µg/ml for alpha glucosidase.

Conclusions

The study gave an insight into the mechanistic actions of the extract by inhibiting carbohydrate enzymes.

Keywords: Diabetes; Sanseviera liberica; alpha amylase; alpha glucosidase

Jadomycin B Causes Human Breast Cancer Cell Death Through Inhibition of Cyclooxygenase-2 Signalling

Brendan T. McKeown[1,2], Kerry B. Goralski[1,2,3]
[1]Dalhousie University, Halifax, Canada. [2]Beatrice Hunter Cancer Research Institute, Halifax, Canada. [3]IWK Health Centre, Halifax, Canada

Abstract

Background:

Cyclooxygenase-2 (COX2), the rate limiting enzyme in converting arachidonic acid (AA) to prostaglandin E2 (PGE2), is an important pro-tumorigenic pathway. Previous experiments show that jadomycin B (JadB) acts synergistically with COX2 inhibitors to kill human breast cancer cells *in vitro*. These data suggest JadB may act as an inhibitor of COX2 signalling.

Objective:

To investigate the role of JadB inhibition of COX2-mediated signalling in MDA-MB-231 human breast cancer cells.

Methods / Results:

PGE2 concentrations increased in vehicle-treated cells after 24h (201 ± 56 pg/mL), 48h (271 ± 42 pg/mL) or 72h (353 ± 36 pg/mL) compared to 6h concentrations (114 ± 70 pg/mL) as measured in medium by ELISA assay ($P \leq 0.05$). JadB treatment (2.5 μM, 5 μM) prevented any significant change from 6h PGE2 concentrations at 24h, 48h, and 72h. Consistent with these results, JadB treatment (5 μM) increased the cellular concentration of the COX2 substrate AA by 13 ± 5% and 34 ± 18%, while dihomo-γ-linolenic acid (an AA precursor) was increased 16 ± 8% and 50 ± 24% (24h and 48h, respectively) compared to control, as measured by gas chromatography ($P \leq 0.05$). Interestingly, in purified enzyme assays JadB (1-20 μM) alone had no effect on COX2 enzyme activity, but synergistically enhanced the inhibitory effect of celecoxib (0.15 μM) by 5-36% ($P \leq 0.05$).

Conclusions:

Our results suggest JadB is a novel, allosteric inhibitor of COX2. Future studies will explore if JadB acts synergistically with endogenous competitive inhibitors of COX2 (docosahexaenoic/docosapentaenoic acid) leading to its anticancer effect.

Keywords:

cyclooxygenase-2, cancer, jadomycins, prostaglandins

Maternal RESV Supplementation and the Effects on Mitochondrial Metabolism and Cardiac hypertrophy in Fetal Cardiomyocytes.

Marcelo Ninalaya[1,2], Mateusz Tomczyk[1,2], Bo Xiang[1,2], Gabriel Brawerman[1,2], Stephanie Kereliuk[1,2], Vernon Dolinsky[1,2]
[1]Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme of the Children's Hospital Research Institute of Manitoba, Winnipeg, Canada. [2]Department of Pharmacology and Therapeutics, Rady Faculty of Health Science, College of Medicine, University of Manitoba, Winnipeg, Canada

Abstract

Background:

Gestational Diabetes Mellitus (GDM) is a metabolic condition observed during pregnancy. GDM interventions include diet and insulin therapy. Other treatments have shown effectiveness but there is risk of adverse pregnancy outcomes and long-term effects on offspring are unknown. Our previous studies found GDM offspring experience mitochondrial dysfunction and induced cardiac hypertrophy.

Objectives:

We hypothesize that administration of Resveratrol (RESV) to GDM female rats will mitigate mitochondrial dysfunction and cardiac hypertrophy in offspring exposed to GDM.

Methods:

Female Sprague-Dawley rats were fed a low-fat (Lean) (10% kcal fat) or high-fat and sucrose (GDM)(45% kcal fat) diet six weeks before mating to induce GDM. A subgroup of GDM dams were switched to a diet containing RESV (GDM+RESV)(45% kcal + 4g/kg RESV). At embryonic day 18.5 fetal echocardiography was performed to assess cardiac structure. E.20 pups were sacrificed for cardiomyocyte isolation and measurement of mitochondrial respiration.

Results:

Echocardiography revealed maternal RESV attenuated GDM-induced cardiac hypertrophy. GDM-exposed Offspring showed 1.4 times thickness in the intraventricular septum and left ventricular posterior wall when compared to both Lean and GDM+RESV offsprings (Lean vs. GDM, $p<0.05$) (Lean vs. GDM+RESV, $p<0.05$). Cardiomyocytes isolated from GDM-offspring had approximately 20% lower levels of ATP-production and maximal respiratory capacity compared to lean and GDM+RESV offspring (Lean vs. GDM, $p<0.05$)(Lean vs. GDM+RESV, $p<0.05$).

Conclusion:

Our findings suggest that maternal RESV supplementation improves mitochondrial efficiency and capacity and attenuated GDM-induced cardiac hypertrophy in rat offspring.

Keywords (3-5): Gestation diabetes; Resveratrol; Mitochondrial metabolism; Cardiac hypertrophy

Mechanisms of Mitochondrial Calcium Uniporter (MCU) Inhibition by the Dominant-Negative Beta Subunit (MCUb)

Danielle M. Colussi, Ryan Grainger, Murray Junop, Peter B. Stathopulos
Western University, London, Canada

Abstract

Background: Mitochondrial Ca^{2+} uptake into the matrix is regulated by the mitochondrial Ca^{2+} uniporter complex (mtCU). Mitochondrial Ca^{2+} uniporter (MCU), the pore-forming subunit, has high conservation to the dominant-negative inhibitory paralogue, MCUb. Phosphorylation of Ser92 in the MCU amino (N)-terminal domain (NTD) disrupts channel dimerization, and divalent cation binding to an acidic patch of the NTD critically regulates mtCU assembly and Ca^{2+} uptake.

Objectives: My work aims to characterize structural and biophysical consequences of MCUb-NTD Ser77 phosphorylation (MCU Ser92 exists as MCUb Ser77), as well as mechanisms underlying MCUb-mediated inhibition of mtCU function in the presence and absence of divalent cations.

Methods: The Ser77Asp phosphomimetic was introduced into a pET-28a-MCUb-NTD vector for recombinant protein generation, and an MCU-MCUb-NTD fusion construct was created to stabilize the heterocomplex. Optical spectroscopies, light scattering and NMR spectroscopy analyses of wildtype, mutant, and fusion proteins in the presence of Ca^{2+} and Mg^{2+} will be applied. Homology modeling of MCUb integrated into human mtCU structures was performed.

Results: The heterodimeric MCU-MCUb-NTD fusion (24.7 ± 0.2k Da) is markedly destabilized by Ca^{2+} and Mg^{2+} (ΔT_m=-10.2˚C and -10.0˚C, respectively). Solution NMR spectra of both MCUb-NTD and MCU-MCUb-NTD are well-dispersed with most backbone amides visible. Homology modeling suggests reduced interactions with the gating regulator upon MCUb integration into human mtCU structures.

Conclusions: Divalent cations regulate MCUb:MCU-NTD assembly akin to MCU-NTD homomerization, and perturbed interactions involved in gating may underlie MCUb-mediated mtCU inhibition, which can be leveraged to control mitochondrial Ca^{2+}-overload-related cellular dysfunction.

Keywords: mitochondrial Ca^{2+}, MCUb, phosphorylation

Metabolic Shock-Induced Yap Translocation in Cardiomyotubes as a Model of Adaptation to Cardiac Injury

Victoria Nelson[1,2], Lester Perez[1], Michael Connolly[1], Malav Madhu[1], Mathew Platt[3], Keith Brunt[1,2], Jeremy Simpson[3,2]
[1]Dalhousie University, Saint John, Canada. [2]IMPART, Saint John, Canada. [3]University of Guelph, Guelph, Canada

Abstract

Background: Due to myocardial infarction (MI) a loss of viable cardiomyocytes significantly reduces cardiac function and patients' quality of life. This loss is caused in part by defined stressors of ischemia. The Yap signalling pathway is a regulator of fetal heart development—its role in adult cardiomyocytes after MI remains to be defined.

Objectives: We seek to better understand the role of the Yap pathway in cardiac ischemia in hopes of promoting regeneration or positive cardiac remodelling of adult cardiomyocytes after MI.

Methods: We used differentiated rat cardiomyotubules (H9C2) to screen independent stressors expected within an MI. We found Yap signalling was sensitive to nutrient deprivation. We then created a metabolic shock model, media deprived of nutrient substrates: glucose, fatty acids, and amino acids. Yap and phospho-Yap (S127 & S397) were measured by western immunoblotting. Yap-associated genes were measured using qPCR.

Results: Metabolic shock elicited a significant increase in whole-cell phospho-Yap S397&S127 ($P=0.0065$ & $P=0.0221$). Protein fractions resulted in increases in phospho-Yap S397&S127 in both the nucleus ($P=0.0106$ & $P=0.0008$) and phospho-Yap S397 in the cytoplasm ($P=0.0321$) 1hr after metabolic shock compared to controls. The re-addition of isoleucine [0.80mM] decreased phospho-Yap S397 in the cytoplasm and increased connective tissue growth factor gene expression 1000x compared to metabolic shock alone ($P<0.0001$).

Conclusion: Our data suggest translocation of phosphorylated Yap is affected under metabolic stress. Additionally, isoleucine may play an important role in cytoplasmic retention and contributes to the upregulation of structural integrity-related genes.

Cardiac-regeneration, Myocardial Infarction, Heart Failure

Opioids Safety in Pediatric Procedural Sedation with Ketamine and Opioids

Neta Cohen[1], Gidon Test[1], Yehonatan Pasternak[1], dana Singer Harel[1], Suzan Schneeweiss[1], savithiri ratnapalan[1], Suzanne Schuh[1], YARON Finkelstein[2]
[1]Hospital for Sick Children, Toronto, Canada. [2]Toronto, Toronto, Canada

Abstract

Background: Pediatric painful procedures frequently require administration of procedural sedation and analgesia (PSA) in emergency departments (ED). Ketamine is the most popular medication used, however, opioids are frequently added. The safety of their combination has been questioned.

Objectives: To evaluate the effects of pre- and intra-procedural opioids on adverse events in children undergoing procedural sedation with ketamine in the emergency department (ED).

Methods: We conducted a retrospective cohort study of all children 0-17 years old who underwent procedural sedation with intravenous ketamine alone, or in combination with an opioid, at a tertiary-care pediatric emergency department between June 1st, 2018 and August 31st, 2020. We explored predictors of serious adverse events (SAEs), desaturation, respiratory intervention, and vomiting.

Results: Of 1,164 included children (694 males, 59.6%; median age 5.0 years [IQR 2.0-8.0]), 80 (6.8%) vomited, 63 (5.4%) had a desaturation episode or required respiratory interventions and six (0.5%) had SAEs. Pre- and intra-procedural opioids were not independent predictors of sedation-related adverse events. A concurrent respiratory illness (aOR 3.73 [95% CI 1.31-10.60], P=0.01), sedation for dental procedures (aOR 3.05 [1.25-7.21], P=0.01), and a higher total ketamine dose (aOR 1.75 [1.21-2.54], P=0.003) were independent predictors of desaturation or respiratory interventions. A higher total ketamine dose (aOR 1.86 [1.16-2.98], P=0.01) and older age (aOR 1.15 [1.07-1.24], P<0.001), were independent predictors of vomiting.

Conclusions: Pre- and intra-procedural opioids do not increase the likelihood of sedation-related adverse events. SAEs are rare during pediatric procedural sedation with ketamine in the ED.

Key words: sedation, children, ketamine, adverse events

Parameterization of Rat Liver and Kidney Scaling Factors for Improved Pharmacokinetic and Toxicokinetic Prediction

Michael Doerksen, Abby Collier
The University of British Columbia, Vancouver, Canada

Abstract

Background

Pharmacokinetic modelling can be used to characterize drug disposition and clinical trial dose selection. The metabolism data informing these models can be generated in vitro and extrapolated to predict in vivo kinetics using scaling factors. Using scaled data, pharmacokinetic models can reduce the use of animals in drug development. The objective is to investigate sex differences in rat scalars and examine effects of buffer condition on derived scalars.

Methods

Organs (n = 3 per strain and sex) were dissected and separated into two buffers (Sucrose-HEPES or Tris-HCl). Kidney scalars were generated from one whole kidney; liver scalars were generated from a liver section. Following homogenization, microsomal and cytosolic subcellular fractions were generated, and scalars parameterized, including intactness of microsomes using the mannose-6-phosphatase assay.

Results

There were no sex differences in hepatic scalars or intactness in Sprague Dawley and Wistar rats. However, hepatic microsome intactness was significantly greater in the Sucrose-HEPES microsomes compared to Tris-HCl microsomes (84.98 ± 4.35% vs. 74.11 ± 5.66%, $p < 0.05$) for Wistar rats, but not Sprague Dawley rats. For kidney scalars and intactness, there were no sex differences or differences between buffer conditions.

Conclusions

Differences in microsomal intactness were observed between Sucrose-HEPES and Tris-HCl buffers, and between strains, suggesting that buffer condition and strain may impact scalar parameterization. Characterizing scaling factors, and the methods used to parameterize them, improves the predictive utility of pharmacokinetic models, which can reduce the use of animals in pre-clinical drug development.

Keywords

Scaling factors, In vitro-in vivo-extrapolation, buffer composition

Pharmacokinetic Characterization of a Novel Cannabidiol Analogue

Aaron Simkovich[1,2], Tamer Abdelghany[3,4], Aurelia Bihari[1,5], Doaa Mossaad[1], Pavan Mandapati[1], Eden Reid[2], Brad Urquhart[2], Mahmoud Moustafa[1]
[1]London Research and Pharmaceutical Corporation, London, Canada. [2]Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Canada. [3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. [4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Heliopolis University for Sustainable Development, Cairo, Egypt. [5]Department of Surgery, Division of Orthopaedic Surgery, Schulich School of Medicine & Dentistry, Western University, London, Canada

Abstract

Background

With purported analgesic, anti-epileptic, and other favourable properties, cannabidiol (CBD) use is increasing. Effective pharmacotherapy of CBD is hindered by low oral bioavailability, and variable pharmacokinetics. We synthesized a CBD analogue with favourable physicochemical properties to improve the bioavailability and pharmacokinetic profile.

Objective

Characterize the pharmacokinetic properties of the novel CBD analogue.

Methods

Permeability of a novel CBD analogue was determined by evaluating flux across Caco-2 monolayers. Rat liver microsomes and BaculosomesTM were used to assess metabolic stability and phase I metabolism. Plasma protein binding was determined using equilibrium dialysis. In-vivo pharmacokinetic studies were performed by oral administration of CBD or CBD analogue in Sprague−Dawley rats. LC-MS was used to quantify plasma concentration of CBD and CBD analogue.

Results

Without transport inhibitors, the CBD analogue had an efflux ratio of 5.23 across Caco-2 monolayers. The efflux ratio with valspodar (P-gp inhibitor) or fumitremorgin C were 2.29 and 7.92 respectively. The CBD analogue was metabolized by CYP2C9, CYP2C19 and CYP3A4 and was extensively protein bound. Greater area under the curve values for the CBD analogue (62965.40 h*ng/ml) compared to CBD (6012.09 h*ng/ml) suggests higher drug exposure compared to CBD following oral administration ($p=0.00029$).

Conclusion

Our study demonstrates this novel CBD analogue has improved oral bioavailability compared to CBD. This suggests the pharmacokinetic profile of the analogue may be improved compared to CBD. Future studies will evaluate the pharmacodynamic and toxicology profiles of the CBD analogue to determine its suitability as a candidate for clinical investigation.

Pharmacokinetics of Recombinant Human Annexin A5 (SY-005) in Severe COVID-19

Brent J. Tschirhart[1,2], Xiangru Lu[1], Aristide Kognou[1], Claudio M. Martin[3,2], Douglas D. Fraser[3,2], Aleksandra Leligdowicz[3,4,5], Bradley Urquhart[6,2], Qingping Feng[1,2]
[1]Department of Physiology and Pharmacology, Schulich School of Dentistry and Medicine, Western University, London, Canada. [2]Lawson Health Research Institute, London Health Sciences Centre, London, Canada. [3]Division of Critical Care, Department of Medicine, Schulich School of Dentistry and Medicine, Western University, London, Canada. [4]Robarts Research Institute, Schulich School of Dentistry and Medicine, Western University, London, Canada. [5]Department of Microbiology and Immunology, Schulich School of Dentistry and Medicine, Western University, London, Canada. [6]University of Western Ontario, Schulich School of Medicine and Dentistry, London, Canada

Abstract

Background/Objectives

Annexin A5 is a phosphatidylserine binding protein and inhibits pro-inflammatory responses in rodent models of sepsis. This clinical trial aimed to evaluate the pharmacokinetic (PK) properties of the recombinant human annexin A5 (SY-005) in severe COVID-19.

Methods

This was a pilot randomized, double-blind, placebo-controlled trial. Severe COVID-19 patients were randomly assigned to receive intravenous 50μg/kg (low dose, n=3), 100μg/kg (high dose, n=5) of SY-005 or placebo (n=5) every 12 hours for 7 days. Plasma SY-005 levels were assessed using enzyme-linked immunosorbent assay (ELISA) and the PK parameters were determined using non-compartmental analysis.

Results

All patients had a normal baseline estimated glomerular filtration rate. Both low and high doses of SY-005 were cleared within 6-hours after intravenous administration. Plasma maximum concentrations (Cmax), half-life, clearance, and volume distribution of low and high doses of SY-005 were 331.6 and 1715.6 ng/mL, 1.22 and 0.96 hours, 7.18 and 4.44 L/h, and 12.62 and 6.20 L, respectively. Daily pre-dose circulating annexin A5 levels were not significantly different when SY-005 was administered at the low or the high dose 12-hour intervals. There was no significant effect on activated partial thromboplastin time (aPTT) or INR (international normalized ratio of prothrombin time) during 7 days of SY-005 treatment.

Conclusion

SY-005 doses of 50 and 100 μg/kg were detectable and subsequently cleared from the plasma in severe COVID-19 patients with normal baseline renal function. There was no plasma SY-005 accumulation and coagulation was not altered during 7 days of treatment.

Keywords: COVID-19, Sepsis, Annexin A5,Pharmacokinetics

Polygenic Risk Score associated with increased risk of L-asparaginase induced hypersensitivity

Spencer Anderson[1,2], Erika Scott[1,2], Catrina Loucks[2,1], Rod Rassekh[1,2], Colin Ross[1,2], Bruce Carleton[1,2]
[1]University of British Columbia, Vancouver, Canada. [2]BC Children's Hospital, Vancouver, Canada

Abstract

Background: L-asparaginase is a critical component of a multi-modal treatment for acute lymphoblastic leukemia, the most common cancer in children. Hypersensitivity reactions occur in up to 70% of treated patients and can be severe, resulting in emergency care, hospitalization, or death.

Objectives: The goal of this study is to identify genes strongly associated with the pathogenesis of hypersensitivity.

Methods: 927 children who received l-asparaginase were assessed for the occurrence of hypersensitivity through the Canadian Pharmacogenomics Network for Drug Safety. Severity of hypersensitivity was graded based on the Common Terminology Criteria for Adverse Events v5. Cases were defined as patients who experienced ≥ grade 2 hypersensitivity and controls were those who did not experience hypersensitivity despite equivalent exposure to l-asparaginase. A genome-wide association study (GWAS) was conducted utilizing the Illumina Global Screening Array v2

Results: The GWAS identified significant associations for variants in two genes: CYP1B1 ($p = 1.7 \times 10\text{-}8$; OR = 6.1 [3.0-12.5]) and ANKLE2 ($p = 2.2 \times 10\text{-}8$; OR = 5.6 [2.9-11.0]). A polygenic risk score (PRS) of variants that passed the screening threshold revealed significant associations between increased PRS and the presence of hypersensitivity reactions.

Conclusion: L-asparaginase-induced hypersensitivity reactions are predominantly severe and require hospitalization for management. The GWAS results suggest that genes involved in immune cell regulation and development, as well as critical lipid metabolism pathways regulating immune responses, are associated with l-asparaginase-induced hypersensitivity.

Keywords: Pharmacogenomics, oncology, adverse drug reactions

Protein kinase A mediated regulation of equilibrative nucleoside transporter subtype 2

Nayiar Shahid[1], James Hammond[2]
[1]University of Alberta, Edmonton, Canada. [2]Edmonton, Edmonton, Canada

Abstract

Background: Equilibrative nucleoside transporters (ENT) 1 and 2 mediate the transmembrane flux of endogenous nucleosides and nucleoside analogs used as anti-viral and anti-cancer drugs. Evidence suggests heterodimerization of ENT1 and ENT2 influence their function. It is critical that the functional characteristics and modes of ENT2 regulation are better defined in its native cellular environment.

Objectives: In silico analysis has predicted serine-282 of ENT2 as consensus site for PKA (protein kinase A) mediated phosphorylation. To examine the PKA effect on ENT2 activity, we have developed a novel HEK293 mutant (using CRISPR/cas9) lacking ENT1 and expressing ENT2 (HEK293-ENT1KO). We now report on the impact of PKA activator-forskolin and PKA inhibitor-H89-dihydrochloride on ENT2.

Methods: ENT2 function was assessed by measuring the rate of [3H]2-chloroadenosine uptake (1–150 µM) ± forskolin (10 µM, 30 min, 37°C) and ± H89-dihydrochloride (10 µM, 15 min prior to forskolin treatment, 37°C) using a 15 s uptake time point in serum free media.

Results: Treatment of HEK293-ENT1KO cells with forskolin resulted in a significant decrease in Vmax of ENT2-mediated uptake from 1.9±1.2 to 0.8±0.5 pmol/µl/s and Km shift from 200±41 to 65±18 µM (n=10, $p<0.0001$, 2-way ANOVA).This effect was reversed by H89-dihydrochloride (n=5, $p<0.05$,two-way ANOVA with Tukey's multiple comparisons post-test).

Conclusions: Endogenous ENT2 is regulated by PKA in HEK293-ENT1KO cells. This may be part of an endogenous feedback mechanism whereby adenosine receptor-mediated changes in cAMP modulate ENT2 activity, via PKA, to impact extracellular adenosine levels.

Keywords: CRISPR-Cas9, ENT2, regulation, PKA

Psilocybin and Eugenol Ameliorate LPS-induced Liver Inflammation in Mice

Gregory Robinson
University of Lethbridge, Lethbridge, Canada. University of Illinois Urbana-Champaign, Champaign, Canada

Abstract

Background: Inflammation is one of the leading mechanisms in the pathogenesis of acute and chronic liver diseases. Recently, psilocybin and eugenol have been implicated as potential anti-inflammatories, however, their effects on inflammation within the liver has not been studied.

Objectives: We aim to test the efficacy of psilocybin and/or eugenol to ameliorate LPS-induced hepatic inflammation.

Methods: C57BL/6 mice were injected with lipopolysaccharide (LPS, 0.83 mg/kg, IP) to induce systemic inflammation. Psilocybin (0.88 mg/kg), eugenol (17.57 mg/kg), or combinations of psilocybin and eugenol (1:10, 1:20, or 1:50) was administered daily (PO) twice daily prior to or once following LPS injection. Mice were euthanized 24 hours after the LPS injection. Livers were excised, weighed, cut, and frozen for qPCR/Western blots or histology. Data were analyzed with a one-way ANOVA and a Dunnett's posthoc test, or with multiple unpaired t-tests.

Results: LPS upregulated mRNA expression of *COX-2* ($P<0.0001$), *TNF-α* ($P<0.0001$), *IL-1β* ($P<0.05$), and *IL-6* ($P<0.01$) relative to *β-actin*. Protein expression for COX-2 and IL-1β relative to GAPDH was unaltered. LPS-induced increases in mRNA expression of *COX-2*, *TNF-α*, *IL-1β*, and *IL-6* relative to *β-actin* were ameliorated by most pre-treatments ($P<0.05$). In contrast, all post-treatments strongly downregulated mRNA expression of *COX-2* ($P<0.01$), *TNF-α* ($P<0.0001$), *IL-1β* ($P<0.0001$), and *IL-6* ($P<0.001$) compared to *β-actin*.

Conclusions: Pro-inflammatory pathways upregulated by LPS were ameliorated by psilocybin and eugenol as both pre-treatment and post-treatment. In particular, psilocybin and eugenol post-treatment are extremely effective anti-inflammatories within the liver.

Keywords: Liver Disease, Inflammation, Psilocybin

SIRT3 Deficiency in the Liver and Development of Gestational Diabetes

Khushali Trivedi[1,2], Bo Xiang[1,2], Vernon Dolinsky[1,2]
[1]Department of Pharmacology & Therapeutics, University of Manitoba, Winnipeg, Canada. [2]Diabetes Research Envisioned & Accomplished in Manitoba (DREAM) Theme of the Children's Hospital Research Institute of Manitoba, Winnipeg, Canada

Abstract

Background:

Gestational diabetes mellitus (GDM) is the most common transient pregnancy complication that puts mothers and their children at risk for developing type-2 diabetes later in life. GDM is characterized by hyperglycaemia and hyperinsulinemia during pregnancy and the mechanisms involved are poorly understood. Sirtuin 3 (SIRT3) is a protein deacetylase that regulates energy levels in the cell.

Objectives:

To determine whether deficiency of SIRT3, specifically in the liver is sufficient to induce diabetes during pregnancy.

Methods:

Mice with liver-specific-deletion of SIRT3 (SIRT3-LKO) were generated by crossing Sirt3tm1.1Auw mice from Jackson Labs that have loxP sites flanking exons 2-3 of the Sirt3 gene with Cre recombinase mice with an albumin promoter from Jackson Labs. SIRT3-LKO mice and wildtype (WT) controls were fed either a low-fat diet (LF;10% kcal fat) or a high-fat and sucrose diet (HFS;45% kcal fat) for 6 weeks prior to pregnancy and throughout the 3-week mouse pregnancy to induce GDM. Glucose homeostasis was assessed by performing glucose tolerance tests (GTTs) at e18.5 days of pregnancy and measuring insulin in the serum.

Results:

Body weight and gestational weight gain were similar across genotypes, though GTTs revealed glucose intolerance in pregnant SIRT3-LKO mice, compared to WT controls. Hyperinsulinemia trends in SIRT3-LKO mice was also observed (Two-way ANOVA, $p>0.05$), suggesting the presence of insulin resistance. Liver histology revealed hepatic steatosis, in SIRT3-LKO mice.

Conclusions:

SIRT3 deficiency promotes accumulation of lipids in the liver and glucose intolerance during pregnancy, a characteristic of GDM.

Keywords:

Diabetes, Metabolic Disorders, Mitochondria, Gestational Diabetes, Maternal Health

The effect of chronic metformin exposure on adult zebrafish stress physiology

Mobeen Ramzan[1], Maya Piasecki[1], Roshan Sivarajah[1], Shemar Williams[2], Jessica Qiu[2], Joanna Wilson[2], Oana Birceanu[1,2]
[1]The Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, ON, Canada
[2]Department of Biology, McMaster University, Hamilton, ON, Canada

Abstract

Metformin is a medication that increases the body's sensitivity to glucose by impacting energy producing pathways in the mitochondria. Recent studies in fish have shown that metformin impacts the steroidogenic pathways, although the mechanisms behind such effects remain unknown. This study aimed to understand the impact of chronic metformin on the stress physiology of adult zebrafish. We hypothesized that chronic metformin exposure would result in altered stress response in a sex-specific manner. The objectives of this study were to investigate the impact of chronic 30-day exposure on muscle, cortisol, lactate, and glucose profiles of zebrafish following an acute stressor and to determine whether these effects were male or female-specific. Healthy adult zebrafish (n=15 male and 15 females per tank) were exposed to 0, 4, and 40µg/L metformin for 30-days in controlled laboratory conditions, in triplicate. After the 30-day exposure, zebrafish were stressed by chasing with a net, following established protocols. Muscle was collected at 0h, 1h, and 6h post-stress. Metformin had no effect on muscle lactate or cortisol levels, irrespective of sex. Muscle glucose significantly decreased in control males from 5.20 ± 0.84 µmol/g ww (0-hours) to 2.31 ± 0.54 (6-hours, $p<0.05$), with no effect of Met on muscle glucose in females. Chronic metformin exposure appears to have sex-specific effects on male muscle glucose mobilization in zebrafish, with transient effects in females. Therefore, warranting future research focusing on sex-specific effects of metformin. Additionally, multigenerational studies exploring paternal and maternal effects should be conducted.

Keywords: Glucose, Cortisol, Lactate, Male, Female

The Effect of L-Phenylalanine at Glutamate Synapses in the Rat Dorsomedial Hypothalamus

Diwan Minocha, Karen Crosby, Jillian Rourke
Mount Allison University, Sackville, Canada

Abstract

Introduction: The dorsomedial hypothalamus (DMH) is a brain region containing neurons that stimulate hunger, but the control of these neurons is not fully understood. Dietary nutrients, including glucose, can affect the release of neurotransmitters, such as glutamate, in the brain, but the effect of proteins/amino acids are less well known. Phenylalanine (Phe) is an important dietary amino acid that binds to receptors expressed in the DMH, but nothing is known about how Phe affects glutamate transmission in the DMH.

Hypothesis: Phenylalanine alters glutamate transmission in the dorsomedial hypothalamus in young male Sprague-Dawley rats.

Methods: Whole cell patch clamp electrophysiology was performed on living neurons in the dorsomedial hypothalamus to assess changes in neuronal activity in the presence of 1 mM Phe and two experiments were performed (1) glutamate current amplitude was measured before and during Phe application and (2) Phe (or control solution) was continuously, and high frequency stimulation was delivered to incoming axons to assess whether Phe affects long lasting changes in strength of these synapses.

Results: We show that (1) Phe significantly decreases glutamate transmission compared to baseline (92.271 +/- 0.8021% baseline, $p < 0.0001$) and (2) glutamate synapses underwent a long-lasting decrease in synaptic strength in the presence of Phe compared to baseline (75.00 +/- 6.679 %baseline, $p = 0.0025$) with no effect observed under control conditions.

Conclusion: These results suggest that phenylalanine decreases glutamate release onto DMH neurons. This could decrease the excitability of these neurons, which could ultimately suppress appetite.

Key words: L-Phenylalanine, Glutamate Transmission, Dorsomedial Hypothalamus,

The effects of NETs on endothelial health and function

Chloé Landry[1,2], Lihua Zhu[1], Dylan Burger[1,2]
[1]Ottawa Hospital Research Institute, Ottawa, Canada. [2]University of Ottawa, Ottawa, Canada

Abstract

Background: Neutrophil extracellular traps (NETs) are webs of extracellular DNA, histones and granular protein that can contribute to endothelial injury in disease by inducing endothelial cell activation and apoptosis. The mechanisms by which NETs impair endothelial function and may contribute to microvascular injury in disease have yet to be fully elucidated.

Objective: This study examines the effect of NETs on endothelial cells in vitro and the mechanisms by which they mediate vascular injury.

Methods: HL-60 cells were differentiated to neutrophil-like cells by incubation in 1.25% DMSO for 5 days, and then stimulated with 500 nM PMA to induce NET formation. NETs were isolated by differential centrifugation and used to treat Human Umbilical Vein Endothelial Cells (HUVECs) at 0.5-500 ng/ml for 24 hours. Cell viability (XTT cell assay) and proliferation (BrdU assay) were then measured. Mouse mesenteric arteries were isolated and treated with NETs at 15 ng/mL for 30 minutes to assess the effect on endothelium-dependent vasorelaxation, as measured by wire myography.

Results: NETs decreased HUVEC viability ~2-fold at 5 ng/ml ($P=<0.0001$) and dose dependently decreased proliferation from 0.5-500 ng/ml, with proliferation being decreased by ~80% at 500 ng/ml ml ($P=<0.0001$). NETs significantly impaired endothelium-dependent vasorelaxation, as reflected in smaller pD2 values in NET-treated vessels ($p=0.0284$).

Conclusion: These results suggest that NETs impact endothelial cell health and function in vitro. Identifying the mechanisms of NET-induced endothelial injury will help further understand their contribution to target organ damage in disease.

Key words: Neutrophil extracellular traps, endothelial dysfunction

The expression and activities of alcohol dehydrogenase, aldehyde dehydrogenase, and aldehyde oxidase in minipig liver

Rei Sato[1,2], Austin Zimmer[1], Dickson Lai[1], Michael Doerksen[1], Miki Nakajima[2], Abby Collier[1]
[1]The University of British Columbia, Vancouver, Canada. [2]Kanazawa University, Kanazawa, Japan

Abstract

Background

Alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and aldehyde oxidase (AO) are enzymes that play important roles in metabolism of aldehyde and alcohol moieties. Although minipigs have been a popular pre-clinical model in industry for ~20 years, little is known about metabolism through these hepatic pathways.

Objectives

To determine sex, species and strain differences of ADH, ALDH, and AO expression and activities in minipig and human liver.

Methods

Pooled male and female minipig (Yucatan n=5 M, n=4 F, Gottingen n=4 M, n=1 F, and Sinclair n=3 M) and human (n=5 M, n=5 F) liver cytosols were prepared by differential centrifugation, or purchased from commercial sources. Activities of ADH and ALDH were evaluated using spectrophotometric assay kits and AO was measured by uHPLC/MS with phthalazine as the probe substrate.

Results

All ADH activities in minipig were similar to humans, and no sex or strain differences were observed. ALDH activity of all male minipigs was 2-fold higher than female minipigs, male and female humans ($P<0.001$). Minipig AO activities were similar to human, except female Gottingen minipig AO was 3-fold higher than all other male or female minipigs and humans ($P<0.001$).

Conclusions

There are significant differences in the activities of ALDH and AO enzymes in minipig liver compared to humans and these vary by sex, and strain. This study may lead to improved understanding of alcohol/aldehyde metabolism, and better understanding of minipigs as models for human drug and chemical metabolism.

Keywords

Alcohol dehydrogenase ; Aldehyde dehydrogenase; Aldehyde oxidase ; Minipig

The human MRS2 magnesium-binding domain is a regulatory feedback switch for channel activity

Sukanthathulse Uthayabalan
Ottawa, Ottawa, Canada

Abstract

Mitochondrial-RNA-splicing-protein-2 (MRS2) forms a magnesium (Mg2+) entry protein channel in mitochondria. Deletion of MRS2 has been reported to abolish Mg2+ influx into mitochondria, to induce functional defects in mitochondria, and to result in cell death. In the context of gastric cancer (GC), malignant cells over-express MRS2, and are less responsive to pharmacological insults. To reveal the fundamental basis for MRS2 action as the first step towards understanding its structural and functional mechanisms within GC, we employed several biophysical and structural characterization techniques. Here, we show that MRS2 NTD, consisting of ∼71% of the mature polypeptide chain, self-associates into a homodimer, with estimated molecular weights of 60.9 ± 1.8 kDa and 61.3 ± 0.50 kDa at 2.5 and 5.0 mg/mL, respectively, contrasting the pentameric assembly of CorA, an orthologous bacterial channel. Mutating pinpointed residues mediating Mg2+ binding to the NTD selectively decreases Mg2+-binding affinity. Apparent equilibrium dissociation constants of ∼0.14 ± 0.03, 1.01 ± 0.26 and 0.68 ± 0.30 mM were revealed for Mg2+, Ca2+ and Co2+ interactions, respectively, for MRS2 NTD, while, for Mutant MRS2, equilibrium dissociation constants of ∼0.98 ± 0.25, 0.74 ± 0.49 and 1.37 ± 0.51 mM suggested disruption of Mg2+ interactions, but not Ca2+ or Co2+. Disruption of NTD Mg2+ binding further abrogates Mg2+ binding-induced secondary, tertiary, and quaternary structure and strikingly potentiates mitochondrial Mg2+ uptake in WT and Mrs2 knockout cells. Our work exposes a mechanism for MRS2 autoregulation by negative feedback from the NTD and identifies a novel gain of function mutant with applicability to prospective pharmacological therapeutic development in GC.

The impact of glucocorticoid treatment on health-related quality of life in children with rheumatic diseases: A Scoping Review

Renee Pang, Barbara Murray, Erkan Demirkaya, Michael Rieder, Roberta Berard
Western University, London, Canada

Abstract

Background: Glucocorticoids (GCs) are used in the treatment for moderate to severe rheumatic diseases in children. Long-term treatment is associated with frequent adverse effects. The health-related quality of life (HRQOL) impact of long-term corticosteroid treatment has yet to be summarized for children with rheumatic disease (RD).

Methods: We conducted a scoping review of studies evaluating quality of life of children with RD treated with GCs at doses equal or greater than prednisone 30 mg per day, or 0.2 mg/k/day for at least 2 months. We searched MEDLINE, EMBASE, APA, CINAHL, Web of Science, and Cochrane Central of Controlled Trials from inception to May 2022. We used the PRISMA-ScR checklist to develop the protocol and the QUIPS tool to evaluate risk of bias.

Results: 362 records were retrieved, of which 9 studies were eligible for inclusion. Most studies were cross-sectional analytical studies (8) and 1 was a qualitative study. Three studies reported lower HRQOL for patients with childhood RD on long-term GCs due to physical appearance changes, and obesity. In addition, deficits in emotional functioning including symptoms such as anxiety were described while on systemic GCs. The frequency of side effects and disease damage was high for long-term GC use, and usually more noticeable after 5 years of treatment. Side effects related to treatments were an important priority for patients, and long-term disease and treatment-related damage had a negative effect on HRQOL scores.

Conclusions: GCs are associated with side effects and long-term damage which correlate with HRQOL scores.

The incorporation of single cell sequencing data to develop a polygenic risk score to predict cisplatin-induced tinnitus

Feryal Ladha[1], Urim Iyasere[1], Britt Drogemoller[1,2,3,4]
[1]Department of Biochemistry and Medical Genetics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada. [2]CancerCare Manitoba Research Institute, Winnipeg, Canada. [3]Children's Hospital Research Institute of Manitoba, Winnipeg, Canada. [4]Centre on Aging, Winnipeg, Canada

Abstract

Background: Cisplatin is an effective chemotherapeutic used to treat a variety of cancers. One of the limitations of cisplatin is that it causes tinnitus, which is a ringing or buzzing in one or both ears. Tinnitus is a constant and troublesome condition that lowers the quality of life for up to 36% of patients treated with cisplatin.

Objective: Develop a polygenic risk score (PRS) to predict cisplatin-induced tinnitus (CIT).

Methods: We performed a meta-analysis of tinnitus genome-wide association study data from the UK Biobank and FinnGen (88,950 cases, 309,202 controls). We then used SBayesR to develop a PRS for tinnitus and determined whether this score was predictive of CIT using ReAct and a cohort of cisplatin-treated patients (238 cases, 979 controls). Finally, we used murine inner ear single-cell RNA sequencing (scRNA-seq) data to filter our PRS for variants mapping to genes that are expressed in specific cell types within the inner ear.

Results: The tinnitus PRS was not significantly associated with CIT (P=0.67). The PRS that was filtered for variants mapping to genes that are expressed in the spindle/root cells of the stria vascularis was significantly associated with CIT (P=1.6x10^{-6}).

Conclusion: While a PRS developed for tinnitus was not predictive of CIT, the incorporation of scRNA-seq data increased the biological relevance of the PRS. This is the first study to create a PRS for CIT and represents the first step towards developing strategies to predict and prevent this adverse drug reaction.

Keywords: tinnitus, cisplatin, polygenic risk score

Training the next generation of pharmacist immunizers on the CARD (Comfort, Ask, Relax, Distract) vaccine delivery framework

Victoria Gudzak[1], Anna Taddio[1], Charlotte Logeman[2], Natalie Crown[1], Lucie M Bucci[3], Mike Folinas[1], Antonio N Ilersich[4], C Meghan McMurtry[5]
[1]University of Toronto, Toronto, Canada. [2]SickKids, Toronto, Canada. [3]Bucci-Hepworth Health Services, Pincourt, Canada. [4]University of Waterloo, Waterloo, Canada. [5]University of Guelph, Guelph, Canada

Abstract

Background: Concerns about needle fear and pain can lead to vaccination avoidance. CARD is a vaccine delivery framework that reduces immunization stress-related responses (ISRR), including fear and pain, and improves the vaccination experience. Mandatory injection training courses for pharmacy students currently do not include education about CARD.

Objective: To evaluate the perceptions of pharmacy students educated about CARD as part of their injections training course before and after using CARD in an influenza pop-up clinic.

Methods: Pharmacy students taking the injections course during the 2021-2022 academic year were educated about CARD. This included a self-paced CARD e-module, injection workshop discussion, and video assignment with relevant content. Eighteen students provided feedback in three separate focus groups. A sub-set of the class subsequently participated in influenza vaccination pop-up clinics in the fall of 2022, whereby they applied CARD. Fourteen provided feedback about their experiences.

Results: Students liked that CARD provided them with a "toolkit" of interventions they could offer to fearful patients, including communication strategies. However, some thought they should lead patient coping interventions rather than patients. Students recommended practice to help operationalize their learning. Students that implemented CARD reported very positive experiences. They stated that CARD was feasible and improved clinic efficiency. They thought that involving patients streamlined interactions and facilitated more positive and patient-centred interactions.

Conclusion: While pharmacy students appreciated learning about CARD in their injections training course, adding a practical component to CARD education improved student understanding, competence, and satisfaction.

Key words: vaccination, pain management, pharmacy education

Trick or Treat? Investigating the biological effects of sucralose signaling through GPR52 in the gut

SunMin Park[1], Nick Fernandez[2,3,1], Madeline Power[4,1], Grace Stapleton[1], Jill Rourke[1]
[1]Mount Allison University, Sackville, Canada. [2]University of Toronto, Toronto, Canada. [3]The Hospital for Sick Children, Toronto, Canada. [4]Dalhousie Medicine New Brunswick, Saint John, Canada

Abstract

Background/Objectives: Despite their widespread consumption, growing evidence raises concerns about the toxicity of non-nutritive sweeteners (NNSs) that may disrupt metabolic processes and gut physiology. We previously identified a novel mechanism that can distinguish between natural sweet molecules and selectively detect the NNS sucralose through the concentration-dependent activation of the orphan G protein-coupled receptor, GPR52. Here, we seek to assess whether and to what extent sucralose signaling through GPR52 can impact cell health and function in the human gut cell line, HCT116.

Methods: HCT116 cell health and function were quantitatively and qualitatively assessed following sucralose treatment using a panel of biochemical assays.

Results: Preliminary results from the MTT assay revealed that the sucralose treatment was more toxic to HCT116 cells with a maximal cell viability loss of 90-95% (IC_{50}=0.883 mM, p=0.0408) than the natural sugar sucrose (IC_{50}=3.296 mM, p=0.0008). Also at concentrations as low as 0.8 mM, HCT116 cells treated with sucralose resulted in a significant reduction in the number of colony growth, while qualitative microscopic investigation showed typical morphological features characteristic of apoptosis and necrosis. This cellular phenotyping will be repeated following TNFα-induced inflammation and *GPR52* gene inhibition to further characterize the pathogenic role of sucralose/GPR52 signaling.

Conclusions: These suggest that sucralose causes concentration-dependent toxicity in HCT116 cells, negatively impacting overall cell health and survival. Further investigation will enhance our understanding of the biological relevance of sucralose/GPR52 signaling in the gut and help inform pharmacological approaches to design NNSs with reduced metabolic side effects.

Keywords: Cell signaling, GPCRs, sucralose

Variation at the *TLR4* locus influences susceptibility to cisplatin-induced hearing loss in cancer patients

Asna Latif[1], Erika Scott[2,3], Abhinav Thakral[4], Jong Lee[4], Geoff Liu[4], Bruce Carleton[2], Colin Ross[2], Amit Bhavsar[1]
[1]University of Alberta, Edmonton, Canada. [2]University of British Columbia, Vancouver, Canada. [3]BC Children's Hospital Research Insititue, Vancouver, Canada. [4]University of Toronto, Toronto, Canada

Abstract

Background

Cisplatin is an versatile chemotherapeutic used in the treatment of a variety of cancers but is limited by its toxicity. In particular, irreversible hearing loss occurs in over 60% of pediatric patients. This has significant long-term repercussions on affected individuals and impacts cognitive development, particularly in children, and interferes with cancer therapy regimens. The incidence of cisplatin-induced ototoxicity (CIO) varies from person to person due to a genetic contribution to susceptibility. One potential therapeutic target, Toll-like Receptor 4 (TLR4), has recently been found to exacerbate cisplatin-induced inflammation and cell death; however, the relationship between TLR4 and genetic susceptibility to CIO is not defined.

Objectives

Determine the relationship between genetic differences at the TLR4 gene locus and susceptibility to CIO.

Methods

Candidate studies on the *TLR4* gene locus were conducted in pediatric and adult cisplatin-treated patient cohorts to investigate the relevance of single nucleotide polymorphisms (SNPs) in rendering protection from CIO. Functional analyses in a luciferase reporter system were used to investigate the relevance of these variants *in vitro*.

Results

SNPs at the TLR4 promoter in both adult (P= 1.68x10-4, OR=0.348 (0.201,0.603)) and pediatric cohorts (P= 0.0029, OR=0.316 (0.148,0.674)) were associated with protection from CIO. They were also shown to suppress TLR4 upregulation in functional assays.

Conclusions

TLR4 presents as an important therapeutic and prognostic target that can predict an individual's susceptibility to CIO during cancer treatment in order to optimize cancer treatment and minimize the risk of hearing loss in treated patients.

Keywords: ototoxicity, cancer, pharmacogenomics