#1

mGluR5 regulates autophagy in Alzheimer's disease in a sex-specific manner

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Background: Alzheimer's disease is characterized by neurotoxicity due to accumulation of beta amyloid (Aβ) oligomers, causing progressive cognitive decline. We have previously shown that genetic deletion or chronic pharmacological inhibition of mGluR5 by the negative allosteric modulator CTEP, rescued cognitive function and reduced Aβ aggregation in male APPswe/PS1ΔE9 (APPswe) mice via the activation of novel ZBTB16-medaited autophagic mechanism (1, 2).
Objectives: To show that CTEP mitigates AD pathology and regulates ZBTB16 autophagic pathways in females APPswe similar to male mice
Methods: We tested brain samples from male and female APPswe mice treated for 12 weeks with either vehicle or CTEP (2mg/kg) for changes in the autophagic clearance of Aβ oligomers via ZBTB16 pathway.
Results: Both male and female APPswe mice exhibited higher Aβ oligomers levels at 6 months of age. To our surprise, CTEP significantly reduced Aβ burden and improved cognitive function in male mice yet, it exacerbated Aβ oligomers accumulation and did not improve cognitive function in female APPswe mice. mGluR5 blockade activated ZBTB16 autophagic pathway to reduce Aβ burden in male but not female APPswe mice. Interestingly, a significant reduction in the surface expression of mGluR5 was detected in female APPswe compared to male mice.
Conclusions: We also provide evidence, for the first time, that mGluR5 signaling is not conserved between males and females and there are sex-specific differences that must be considered when embracing mGluR5 as a drug target for neurodegenerative disease.

1. Abd-Elrahman et al., 2017. *Sci. Signal.*
2. Abd-Elrahman et al., 2018. *Mol. Brain.*

Keywords: GPCR, autophagy, Alzheimer's disease, mGluR5, sex

#2

Evaluating the Use of the Lymphocyte Toxicity Assay for Diagnosis of Delayed-Type Allergy to Amoxicillin.

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Background: Drug allergy especially to beta-lactam antibiotics (BLAs) is a major health problem. The prevalence of self-reported allergy to BLAs in the general population is very high (~10%), however, true, immune-mediated reactions are over 10 times less. The most popular course of action in such cases is drug avoidance, which result in exposing the patient to less effective, more expensive alternatives with inferior safety profiles. This is mainly due to lack of any reliable and safe diagnostic tool for this type of reactions.
Objectives: The objective of this study was to evaluate the performance of an *in vitro* test (the lymphocyte toxicity assay, LTA) as a diagnostic tool for BLAs-induced delayed type allergic reactions.
Methods: We recruited 8 patients (5 females and 3 males), age range 18 months to 16 years, who developed symptoms (skin rash with or without other systemic symptoms) highly suggestive of delayed allergy to amoxicillin after 6 to 10 days of exposure to the drug. Blood samples were obtained from the patients as well as another 8 healthy control volunteers. Peripheral blood monocytes (PBMCs) were isolated and the LTA test was performed on all subjects.
Results: Cells from the 8 patients exhibited significantly more toxicity ($p<0.05$) as compared to cells from the healthy controls.
Conclusions: This is a very small sample size; however, this preliminary data highlights the potential of using the in vitro toxicity test as safe diagnostic and research tool in patients suspected of having delayed allergic reactions to BLAs.
Keywords: in vitro toxicity assay, beta-lactam antibiotics, amoxicillin, drug allergy, hypersensitivity reactions

#3

Xenobiotic metabolism and tobacco-associated cancers – A case of CYP1A1, ALDH3A1 and GSTM1 gene signature?

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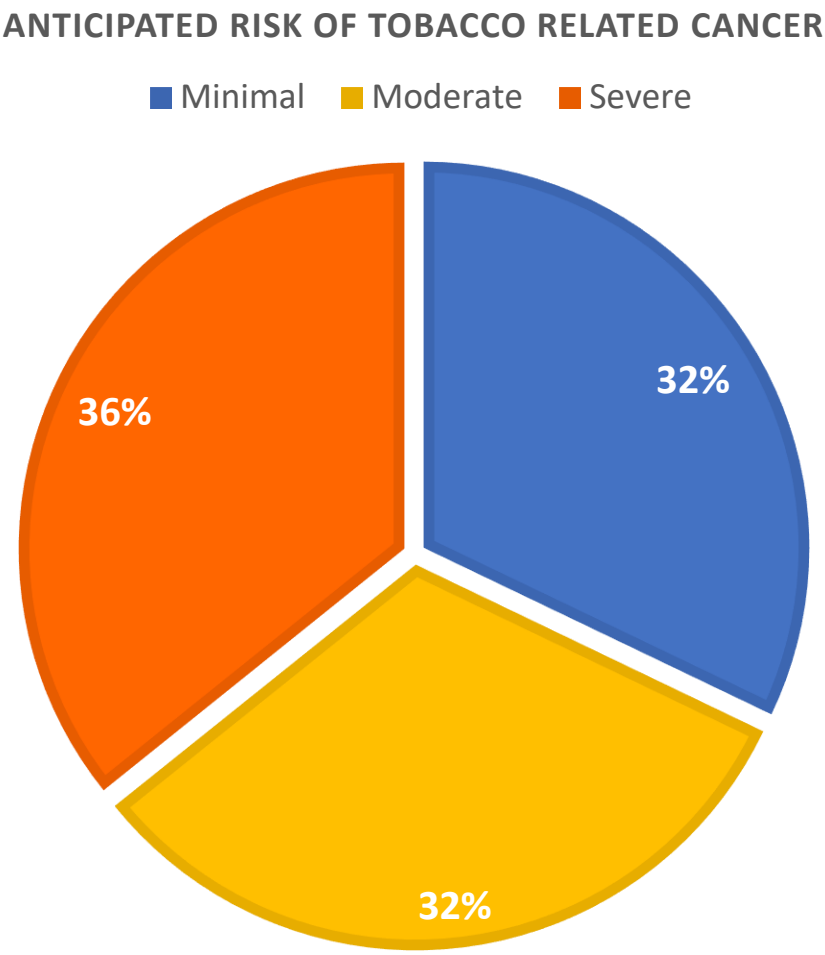
Background: Tobacco smoke contains numerous pro-inflammatory and carcinogenic substances, such as polycyclic aromatic hydrocarbons (PAHs) which are activated by CYP1A1 and detoxified by GSTM1 and ALDH3A1 within respiratory tract, from lips to lungs. Genetic polymorphism of these enzymes may influence development of conditions like chronic obstructive pulmonary disease as well as oropharyngeal and lung cancer. Indeed, Pakistan has one of the highest incidence of such cancers in the world. Although the role of CYP1A1 and GSTM1 is known with varying severity, new evidence regarding ALDH3A1 has renewed the interest in this regard in global perspective.
Objectives: To estimate the prevalence pattern of SNVs of CYP1A1, GSTM1 and ALDH3A1 in Pakistani population and evaluate its role as a genetic signature predisposing to the increased incidence of tobacco-associated cancers.
Method: In this study, 155 healthy adults (99 females) were included from all districts of Karachi. DNA was extracted from saliva and genotyped for SNVs either through PCR (GSTM1), RFLP (CYP1A1) or pyrosequencing (ALDH3A1).
Results: About 64% of the participants were born to parents who were unrelated to each other. There was generally a higher prevalence ($p<0.05$) of variant alleles of CYP1A1*2B, ALDH3A1, and GSTM1 in this study cohort than in other ethnicities reported in the HapMap database (Table 1). When viewed as a gene signature (Figure 1), 68% population had high risk to develop tobacco related COPD and cancers.
Conclusions: Karachiites have a significantly different prevalence of xenobiotic metabolizing gene signature, which could have putative clinical consequences on gene-environment interaction and carcinogenesis.
Key Words: Lung cancer, Oro-pharyngeal cancer, PAH, Tobacco, Environmental toxicity

FIGURE 1: Distribution of CYP1A1, ALDH3A1 and GSTM1 gene signature in healthy Pakistanis. The definition of gene signature in terms of estimated risk is given as the data table in this figure.



Tobacco related cancer risk	**CYP1A1*2B (m1+m2)** 2454+3800 0=AT/AT, 1=AT/GC, 2=GC/GC	ALDH3A1 985 C>G 0=CC, 1=CG, 2=GG	GSTM1 0=Null
Minimal	0	0	1
	0	1 or 2	1
	1 or 2	0	1
Moderate	0 or 1	0	0
	1 or 2	1 or 2	1
Severe	1 or 2	1 or 2	0
	2	0	0
	0	1 or 2	0

TABLE 1: Comparison of variant allele frequency with other ethnic groups. The Chi square value was computed with df=1.

Genotype	KHI Sample	HapMap Ethnicities	Variant Allele %	χ^2 Value	p-value
*CYP1A1*2*	11.07	CHIN	25.6	7.05	0.008
		CAUC	3.1	4.83	0.028
		GUJ	10.2	0.04	0.84
		AFR	0	6.18	0.013
*CYP1A1*2A*	32.64	CHIN	37.5	0.52	0.47
		CAUC	10	15.28	<0.001
		GUJ	-	-	
		AFR	14.4	9.25	0.002
ALDH3A1 985C>G	66.89	CHIN	44.4	10.25	0.001
		CAUC	29.2	28.46	<0.001
		GUJ	-	-	
		AFR	44.1	10.5	0.001
GSTM1	30.13	CHIN	-	-	
		CAUC	0	20.4	<0.001
		GUJ	-	-	
		AFR	0	20.4	<0.001

KHI, Karachi sample; ***CHIN***, Chinese of Han ancestry; ***CAUC***, Caucasian of Northern and Western European ancestry, ***GUJ***, Gujrati Indians in Houston Texas; ***AFR***, African of Yoruba Nigerian ancestry.

#4

Physiologically-based pharmacokinetic modeling of amantadine and acetylamantadine metabolites for potential applications as cancer biomarker.

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Background: Cancer is the second leading cause of death globally. Despite advances in treatment, there is a need for faster and economical screening tests for early diagnosis of cancer. Spermidine/spermine N^1-acetyltransferase (SSAT-1) is overexpressed in many cancers. Recent studies suggest that that SSAT-1 based acetylation of amantadine could serve as a biomarker for lung cancer. However the potential use of amantadine for detection of tumor in other tissues is unclear.
Objectives: Use physiologically-based pharmacokinetic modeling to determine whether appropriate amounts of amantadine and acetylamantadine could be achieved in other tissue/organs.
Methods: Physiologically-based pharmacokinetic modeling was performed using Gastroplus™. Plasma and tissue compartment kinetics for amantadine (200 mg PO) was simulated and prediction of acetylamantadine metabolite in urine under normal conditions and following increases in SSAT-1 enzyme expression was performed.
Results: The simulated plasma C_{max} of 750 ng/ml and half-life of 12 hours was similar to reported values in healthy subjects. Modeling of the acetylamantadine metabolite concentrations in urine under low SATT-1 expression were consistent with levels observed in healthy controls (approximately 3 ng/ml). When the expression of SATT-1 was increased 3-fold simulating increased expression in lung tumor, the levels of acetylamantadine in urine increased to levels comparable to patients with lung cancer (around 10 ng/ml).
Conclusions: Physiologically-based pharmacokinetic modeling of amantadine indicates significant accumulation of the drug in tissue including the brain. Acetylamantadine metabolite formation may be useful for cancer detection and therapeutic monitoring in various solid organ tumors.
Keywords: Amantadine, Acetylamantadine, Lung cancer, PBPK modeling

#5

Paracrine effects of perivascular adipose tissue on atherogenesis: Role of exosomal intercellular communications

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Background: Development of atherosclerosis depends on the interaction between various factors. Type 2 diabetes accelerates these interactions and predisposes to rapid progression of atherosclerosis. Diminution of vasoprotective effects of perivascular adipose tissue (PVAT) in metabolic disorders suggests a molecular link between diabetes and atherosclerosis.
Objectives: To assess the paracrine role of the PVAT on the progression of diabetic atherosclerosis, via intercellular communications between PVAT and the underlying vasculature.
Methods: Periaortic adipose tissue from Type 2 diabetic (db/db) mice were transplanted around the right common carotid arteries of $ApoE^{-/-}$ mice, followed by 16 weeks of atherogenic diet. Carotid arteries and adipose tissues were assessed for lesion formation and inflammatory markers, respectively. Adipose stem cells (ASCs) from PVAT were treated with lipopolysaccharide (1µg/ml), palmitate (200µM), and high-glucose (42 mM) for 24 hrs (denoted as P-ASCs) to mimic type 2 diabetes-associated metabolic alterations. Exosomes were isolated from the conditioned media. Aortic vascular smooth muscle cells (SMCs) were incubated with ASC-derived exosomes (25µg/ml). The migratory potential of SMCs was evaluated by wound healing assay.
Results: Histological analysis displayed atherogenic plaque formation with transplantation of type 2 diabetic PVAT around the carotid arteries of $ApoE^{-/-}$ mice, that is otherwise resistant to plaque formation.. Pro-inflammatory markers were significantly increased in periaortic adipose tissue from db/db compared to WT. Exosomes secreted from P-ASCs greatly enhanced the SMC migration when compared with the control ASCs.
Conclusions: Our data shows type 2 diabetes-accelerated progression of atherosclerosis is mediated by PVAT-derived exosomes**.**
Keywords: Exosomes, Diabetes, perivascular adipose tissue, Atherosclerosis

#6

Management of Marfan aortic root remodelimg with ARBs: blood pressure or endothelial function?

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Background: Marfan syndrome (MFS) causes accelerated aortic root widening and aneurysm. The anti-hypertensive angiotensin II (AngII) receptor type 1 (ATR1) blocker (ARB) Losartan has shown underwhelming efficacy in MFS patients, along with the other guideline approve beta blocker atenolol. We have reported that Losartan's anti-aortic root remodeling effects are likely BP-independent, but rather endothelial function and nitric oxide (NO)-dependent. Whether other ARBs can provide greater protection against MFS aortic root widening is unknown.
Objectives: To study the anti-aortic root remodeling properties of other ARBs, some at doses that do not reduce BP.
Methods: Aortic root dilation, aortic vessel remodeling, and vessel contractility were compared following treatment with Losartan, Telmisartan and Valsartan between MFS and wild-type (WT) mice with normal and blunted ATR1 expression.
Results: Loss of ATR1 expression does not interfere with the activity of ARBs in MFS aortic widening. Although Valsartan had no effect on BP, all ARBs reduced MFS aortic root widening, medial thickening, and elastic fiber fragmentation with Telmisartan reaching >95% inhibition efficacy. All ARBs decreased vascular contractility ex vivo between 60-80% in both MFS and WT aortas in a NO-sensitive fashion.
Conclusions: ARBs likely mediate their effect in an ATR1 independent fashion. Telmisartan was the most effective ARB at blocking MFS aortic root widening and activating endothelial function, whereas, Valsartan showed a high degree of efficacy with little to no BP-lowering effects. Aortic root protection could be achieved with lesser side effects for MFS patients, addressing key compliance issues. Future clinical trials should focus on other ARBs than Losartan for the management of MFS-associated aortopathies.
Keywords: aorta, nitric oxide, angiotensin, Marfan.

#7

A tri-peptide IRW (Ile-Arg-Trp) from egg white protein ovotransferrin improves mitochondrial status in metabolic disease models

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Background: Obesity and hypertension are critical unfavorable health metrics that have a disastrous impact on health and contribute to other metabolic diseases such as type 2 diabetes mellitus, dyslipidemia, and certain cancers. However, appropriate nutritional and pharmacological interventions with feasible potential to delay such diseases and promote healthspan remain rare.
Objectives: The objectives of the current study were to evaluate the ability of a tri-peptide IRW (Ile-Arg-Trp) to improve mitochondrial status towards mitigation of metabolic disorders.
Methods: The ability to tri-peptide IRW was evaluated in different mammalian cell lines followed by measurement of mitochondrial mass and activity. Animal models of diet-induced obesity (C57BL/6J) and hypertension (SHRs) were used to examine the impact of IRW at different dosage (15 and 45 mg/kg BW). Further, the target identification of peptide was completed using MS/MS studies.
Results: Administration of IRW at a moderate dose (15 and 45 mg/kg BW) improves mitochondrial status in C57BL/6J and SHRs *in vivo*. Further, it enhances mitochondrial mass in mammalian cells and induces mitochondrial biogenesis as indicated by elevated expression of TFAM and COXIV. Also, the target identification indicated the interaction of IRW with FAM120B, a transcriptional co-activator of PPARγ and a newly established regulator of DNA repair. The cas9 guided KO of FAM120B, lowered the efficacy of IRW in 293T cells.
Conclusions: To the best of our knowledge, IRW is the first peptide which improves mitochondrial status in both *in vitro* and *in vivo* models.
Keywords: Obesity, Hypertension, mitochondria, biogenesis, peptide

#8

The Pharmacogenomics of Clozapine-Induced Myocarditis (PROCLAIM) Consortium

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Background: Clozapine has proven efficacy in treatment-resistant schizophrenia (TRS). However, the occurrence of clozapine-induced myocarditis, an unpredictable and often fatal adverse drug reaction, has substantially limited its use. Markers for and the mechanism by which clozapine induces myocardial inflammation and damage is unknown.
Objectives: (1) To identify clinical and genomic factors associated with clozapine-induced myocarditis, and (2) develop a novel *in vitro* model system using patient-derived induced pluripotent stem cells (iPSCs) to explore how clozapine induces myocardial inflammation and damage and ultimately identify a mechanism.
Methods: Recruitment of adult TRS patients aged 18-65 with and without a history of clozapine-induced myocarditis is on-going at 11 participating sites. Patients provide DNA samples, which are used for genomic analysis. In a subset of participants, blood is used to generate iPSCs that are differentiated into cardiomyocytes for *in vitro* functional studies to identify abnormal cellular pathways and responses to clozapine.
Results: To date, 42 treatment-resistant schizophrenia individuals with a history of clozapine-induced myocarditis and 68 without a history have been recruited. Details related to recruitment rates at participating sites and inclusion/exclusion criteria as well as preliminary results of genomic and patient iPSCs analyses will be presented.
Conclusions: The PROCLAIM consortium is an innovative initiative that if successful will lead to safer and expanded use of clozapine. In addition, success will provide a novel and clinically-relevant *in vitro* model for elucidating the etiology of clozapine-induced myocarditis and for the study of other drug-induced cardiotoxicities.
Keywords: pharmacogenomics, adverse drug reaction, precision medicine

#9

Characterizing T-cell phenotype in patients with hypersensitivity reactions to sulfonamides and beta-lactam antibiotics

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Background: Delayed drug hypersensitivity reactions (DHRs) are idiosyncratic and can present days after exposure to the culprit drug. There is currently no consensus on how T-cell activation occurs. Previous research groups different resulting skin reactions, often without accounting for rash type or severity, although some may have different cytokine profiles and T-cell subset involvement. We hypothesize that differences in activated T cell subsets lead to the different clinical presentations observed in DHRs to sulfamethoxazole and beta-lactam antibiotics.
Objectives: This project will address this issue and determine DHR mechanisms in the context of both drug type and clinical presentation.
Methods: Peripheral blood mononuclear cells (PBMCs) are isolated from participants with DHRs to sulfamethoxazole or beta-lactam antibiotics. PBMCs are stimulated in vitro with the culprit drug or anti-CD3. T-cell subset proliferation is assessed by T-cell specific surface markers using flow cytometery and 3H-thymidine incorporation, and secreted effector cytokines are measured by Luminex.
Results: Proliferation and surface staining of isolated T-cells has been optimized. 3H-thymidine for measuring T-cell proliferation and flow cytometry for T-cell CD69 expression/activation using control blood samples have provided satisfactory results. Participants with DHRs are being recruited to participate. Preliminary results have suggested that different clinical presentations occur due to response of different T-cell subsets and effector cytokines.
Conclusions: When completed, this study will provide evidence into the underlying pathophysiology of DHRs. Identifying differences in cytokine profiles between skin rashes in DHRs to sulfamethoxazole and beta-lactam antibiotics can help with developing reliable, minimally-invasive, diagnostic and predictive tests.
Keywords: Hypersensitivity, beta-lactam, sulfamethoxazole, pathomechanism, T-cell

#10

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis Caused by Antibiotics- an International cohort study

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Background: Antibiotics have saved millions of lives; however, antibiotics are also one of the most common causes to induce potentially fatal adverse events, such as severe cutaneous adverse reaction (SCAR)- Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).
Objectives: To investigate the epidemiological characteristics of antibiotic-associated SCAR and to identify the genetic susceptibility to specific antibiotic-SCAR
Methods: We retrospectively reviewed the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) database and Taiwan Chang Gung Memorial Hospital system database over a 14-year period (2004-2018).
Results: From a review of 6,450 individuals in CPNDS database, 3,828 (59.3%) participants were prescribed at least one antibiotic treatments. Among them, twenty-three percent of patients experienced antibiotic-related cutaneous adverse reactions, ranging from common rash to serious systemic reactions (i.e. SCARs). Thirty severe patients met the criteria of the SJS/TEN definitions. Among all antibiotics, β-lactams, particularly amoxicillin, is the leading cause in the Canadian cohort and is responsible for 41% of all SJS/TEN, following by sulfonamides (i.e. sulfamethoxazole). Consistently, β-lactam accounts for half (50.1%) of all SCAR cases in the Taiwanese cohort. Our next step is to identify genetic variants associated with SJS/TEN induced by β-lactams and sulfonamides in different ancestries.
Conclusions: β-lactams are the most common culprit antibiotics to induce SJS/TEN in the both Canadian and Taiwanese cohorts. Given the widespread use of antibiotics, understanding the pathogenesis of antibiotic-associated SCAR can develop interventions to prevent its onset, resulting in improving the safety of antibiotics use.
Keywords: antibiotics, ADR, SCAR, SJS/TEN

#11

Severity of Celiac disease and oral felodipine pharmacokinetics: Comparison to the interaction with grapefruit juice.

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Background: Celiac disease is a hypersensitivity reaction to gluten-containing foods in genetically susceptible individuals. It is characterized by damage to the small intestinal mucosa that ranges from inflammation to villous atrophy. CYP3A4 is constitutively expressed in human small intestinal villi and accounts for first-pass prehepatic metabolism of drugs. Celiac patients with severe disease have low duodenal CYP3A4 expression.
Objectives: To determine whether oral felodipine bioavailability would be dependent on celiac disease severity and be caused by a grapefruit-like mechanism.
Methods: Celiac patients were histologically stratified into three categories: Group A (n=15, normal), B+C (n=16, intraepithelial cell invasion with/without mild villous blunting) and D (n=16, moderate/severe villous blunting). Single dose oral pharmacokinetics of felodipine 10 mg were assessed. Healthy volunteers (n = 68) undergoing similar testing in prior felodipine–grapefruit juice interaction crossover studies served as negative and positive controls.
Results: Groups A, B+C and D had linear trends of increasing felodipine AUC0-8 (mean $\pm$ SEM, 14.4$\pm$ 2.1, 17.6$\pm$2.8, 25.7$\pm$5.0; $p<0.05$) and Cmax (3.5$\pm$0.5, 4.0$\pm$0.6, 6.4$\pm$1.1; $p<0.02$), respectively. Healthy subjects receiving water had lower felodipine AUC0-8 (11.9$\pm$0.9 vs 26.9$\pm$0.9, $p=0.0001$) and Cmax (2.9$\pm$0.2 vs 7.7$\pm$0.2, $p=0.0001$) versus those receiving grapefruit juice. Group A and D had similar felodipine pharmacokinetics for healthy subjects with water and grapefruit juice, respectively.
Conclusions: Patients with severe celiac disease had increased oral felodipine bioavailability like that with grapefruit juice from low small intestinal CYP3A4 protein expression. They could be at risk of serious overdose toxicities with numerous grapefruit-affected drugs and should be considered for altered pharmacotherapy.
Keywords: Celiac disease, prehepatic metabolism, CYP3A4, felodipine, pharmacokinetics

#12

Genomic analyses of L-asparaginase-induced Pancreatitis in Pediatric Cancer Patients

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Background: L-asparaginase is highly effective in the treatment of pediatric acute lymphoblastic leukemia. Unfortunately, the use of this treatment is limited by the occurrence of pancreatitis, a severe and potentially lethal adverse drug reaction, which occurs in 2-18% of patients. As previous studies have been unable to identify strong associations between clinical variables and susceptibility to L-asparaginase-induced pancreatitis, genetic factors are expected to play an important role this adverse drug reaction.

Objectives: We sought to explore the role of these genetic susceptibility factors to L-asparaginase-induced pancreatitis in pediatric cancer patients.

Methods: Patients who were treated with L-asparaginase were recruited from 13 pediatric oncology units across Canada (*n*=284) and extensive clinical data were collected for all patients. Genotyping was performed using the Illumina HumanOmniExpress and Global Screening Arrays and pancreatic gene expression profiles were imputed in these individuals using GTEx v7 and S-PrediXcan. Genome- and transcriptome-wide associations (GWAS and TWAS) were performed to identify associations with L-asparaginase-induced pancreatitis.

Results: GWAS analyses identified significant associations between genetic variants in *HLA-DQA1* and *–DRB1* and pancreatitis, while TWAS revealed that individuals experiencing L-asparaginase-induced pancreatitis exhibited lower expression levels of *HLA-DRB5*. Further interrogation of the TWAS data revealed an enrichment in genes involved in the somatic diversification of immune receptors.

Conclusions: These analyses uncovered an association between genetic variation in immune-related genes and the development of L-asparaginase-induced pancreatitis. These associations mirror previous associations with the *HLA* region and (i) pancreatitis induced by other drugs and (ii) L-asparaginase-induced hypersensitivity.

Keywords: Genome-wide association study, L-asparaginase, Pancreatitis, Pharmacogenomics

#13

Sex Differences in the Role of Pannexin-1 in Neuropathic Pain

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Background: Neuropathic pain is among the most debilitating types of chronic pain conditions and its clinical management is difficult because the underlying causes are poorly understood. Pannexin-1 (Panx1) channels have recently been implicated in the modulation of neuropathic pain. Our preliminary findings indicate that Panx1 channels expressed in the central nervous system differentially modulate neuropathic pain in male and female rats.
Objectives: To investigate the role of Panx1 in the expression of neuropathic pain in male and female rats.
Methods: Nerve injury was induced in male and female Sprague Dawley rats using the spared nerve injury (SNI) model. Mechanical threshold was assessed using the von Frey filament test and in a subset of animals, thermal threshold was tested using the Tail Flick test. We investigated the importance of Panx1 in the expression of neuropathic pain by intrathecally administering 10panx, a Panx1 blocking mimetic peptide, into male and female rats with SNI.
Results: Peripheral nerve injury produced robust mechanical allodynia in male and female rats. A single intrathecal injection of 10panx transiently reversed mechanical allodynia in male rats on day 7 after SNI. In contrast, 10panx produced a partial reversal of the established mechanical allodynia in females. We confirmed that in sham control rats, intrathecal administration of 10panx did not alter baseline thermal threshold.
Conclusions: Our results suggest that Panx1 expressed in the central nervous system is necessary for the ongoing expression of neuropathic pain, and that there may be sex differences in the contribution of spinal Panx1 in the expression of neuropathic pain.
Keywords: Pannexin-1, neuropathic pain, chronic pain, sex difference

#14

Bend Beauty, Inc.'s oral skincare supplement, *Anti-Aging Formula,* protects skin cells from ultraviolet light and reactive oxygen species

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Background: Ultraviolet (UV) light overexposure is associated with multiple health risks. The ingestion of natural product photoprotectors can increase skin's UV-resistance. Bend Beauty, Inc.'s skincare supplement, marketed as "*Anti-Aging Formula*" (AAF), contains daily doses of eicosapentaenoic acid (1050 mg), docosahexaenoic acid (350 mg), gamma-linolenic acid (120 mg), zeaxanthin (2.5 mg), lutein (5 mg), and vitamin D3 (1,000 IU), and reduces UV-induced skin erythema (sunburn) clinically.
Objective: To determine AAF's photoprotective and antioxidant properties in dermal fibroblasts.
Methods: Primary human dermal fibroblasts were treated with 0.005% AAF or vehicle for up to 14 days. (i) Photoprotection assays: AAF-treated fibroblasts were exposed to UVA (365 nm; 100-216 J/cm^2) or UVB (312 nm; 15.6-8,000 mJ/cm^2); cell viability quantified *via* methyltetrazolium (MTT) assays or confocal microscopy (dyed with phalloidin, propidium iodide, and Hoechst 32258). (ii) Reactive oxygen species (ROS): AAF-treated fibroblasts were exposed to H_2O_2 (11.1-2,700 μM); cell viability and ROS activity quantified *via* MTT assays and ROS-probe (CM-DCFH$_2$-DA) fluorescence, respectively.
Results: (i) The viability (MTTs) of UVA- or UVB-irradiated fibroblasts was increased by 4.2- and 3.2-fold, respectively, by AAF treatment vs. control (P<0.05). AAF-treated fibroblasts were protected against structural damage as visualized with confocal microscopy (Figure 1). (ii) The viability of H_2O_2-treated fibroblasts was increased up to 2.5-fold, and H_2O_2-induced ROS activity reduced by 38%, by AAF treatment vs. control (P<0.05).
Conclusions: AAF protects human dermal fibroblasts from the damaging effects of UVA, UVB, and H_2O_2, demonstrating its cellular photoprotective and antioxidant properties.
Keywords: Photoprotection, lipids, essential fatty acids, skin health, academic-industry collaboration

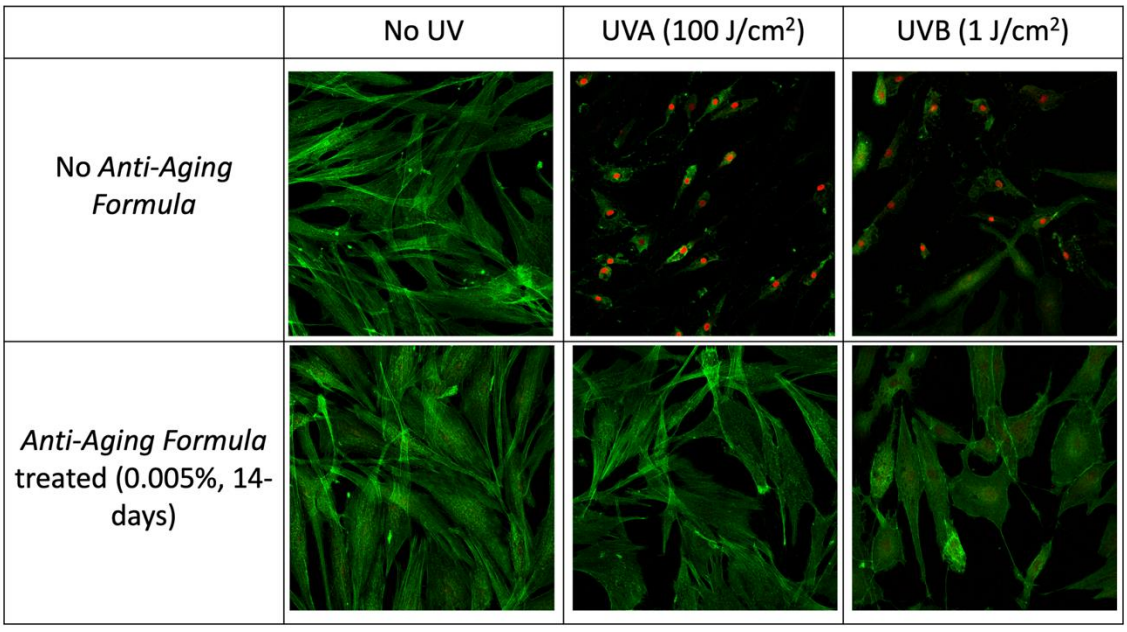

Figure 1

#15

Abstract title: Contribution of Cyclic AMP and β-arrestin-2-dependent Mechanisms to β2-adrenoceptor-mediated Gene Expression Changes in Human Airway Epithelial Cells

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Background: β_2-adrenoceptor (β_2-AR) agonists are used routinely in asthma management. However, chronic use as a monotherapy is associated with adverse events (AEs). Previous studies have reported that β_2-AR agonists promote a significant transcriptional response in BEAS-2B airway epithelial cells, which could contribute to their AEs. However, the signalling mechanism(s) responsible is unclear.
Objectives: To investigate the extent to which canonical (G-protein/cAMP/protein kinase A [PKA]) and non-canonical (β-arrestin/extracellular signal-regulated kinase [ERK]) signalling regulate gene expression in human primary bronchial epithelial cells (HBECs).
Methods: The effect of different β_2-AR agonists on ERK phosphorylation quantified by using western blotting was used as an index of β-arrestin-dependent signalling. Adenovirus-mediated over-expression of an inhibitor of PKA (PKIα) and silencing of dual specificity phosphatase-1 (DUSP1) were used to interrogate the role of canonical signalling. To evaluate the genomic effect of β_2-AR agonists, HBECs from five normal subjects were treated with vehicle, formoterol, forskolin and tumor necrosis factor-α (TNFα) alone and in their various combinations. Total RNA was extracted, quantified, and sequenced.
Results: In BEAS-2B cells and HBECs, β_2-AR agonists dephosphorylated basal ERK expression. This effect was time-dependent and inhibited in cells treated with a β_2-AR antagonist (ICI 118,551), PKIα and siRNAs that target DUSP1. In HBECs, formoterol promoted a significant number of gene expression changes in absence and presence of TNFα which were replicated by forskolin highlighting the cAMP dependency of this genomic effect.
Conclusions: These data indicate that cAMP/PKA signalling plays a dominant role in regulating LABA-induced gene expression changes in HBECs and BEAS-2B cells.
Keywords: β_2-receptor, β-arrestin, Cyclic AMP.

#16

TRPV1 channels modulate the activity of Opioid receptor via β-Arrestin2 nuclear shuttling.

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Background: Inflammation enhances the analgesic properties of opioids, and there is much interest in determining the mechanisms by which inflammatory mediators prime opioid receptor signaling in afferent nociceptors. Here, we report that the Transient Receptor Potential Vanilloid type 1 (TRPV1) channel, a key transducer of inflammatory signals, stimulates a mitogen-activated protein kinase (MAPK) signaling pathway that was accompanied by the shuttling of the scaffold protein β-arrestin2 to the nucleus. We investigated whether the nuclear translocation of β-arrestin2 could prevent its recruitment to the agonist-bound μ opioid receptor (MOR), the subsequent internalization of MOR, and the suppression of its activity that occurs upon receptor desensitization.
Objectives: To assess the role of TRPV1 activation in 1. ERK 1/2 phosphorylation, 2. β-arrestin2 cellular localization and 3. the regulation on opioid receptor activity.
Methods: 1. western blotting to assess ERK1/2 Phosphorylation
2. Confocal microscopy to identify the MOR1 and β-arrestin2 cellular localization
3. BRET assay to assess MOR-β-arrestin2 interaction
4. complete Freund's adjuvant (CFA) inflammatory pain model to examine the role of TRPV1 in regulating endogenous opioid analgesia
Results: 1. The activation of TRPV1 channels induced the shuttling of β-arrestin2 to the nucleus.
2. Resiniferatoxin activation of TRPV1 induced ERK1/2 phosphorylation
3. TRPV1 activation disrupts MOR-β-arrestin2 interaction and subsequent receptor internalization
4. TRPV1 is essential to endogenous opioid mediated regulation of inflammatory pain.
Conclusions: Activation of TRPV1 mediates β-arrestin2 nuclear translocation. thus preventing β-arrestin2 -mediated MOR internalization and desensitization and enhancing endogenous opioid analgesia during inflammation.
Keywords: TRPV1, Beta-Arrestin2, μ-opioid receptors, inflammatory Pain.

#17

The Effect of Vitamin C on the Vasodilator Response to Nitroglycerin in those with and without ALHD-2 polymorphism

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Background: Humans with the ALDH-2 polymorphism who have little ALDH-2 activity have been reported to have blunted responses to nitroglycerin (GTN). We hypothesized that lack of ALDH-2 activity leads to accumulation of reactive aldehydes, which impair the bioactivation of GTN.
Objectives: To test the hypothesis that supplemental Vitamin C would increase vascular responses to GTN in humans with the ALDH-2 polymorphism.
Methods: East Asian subjects with and without the ALDH-2 polymorphism received 2, sequential intra-arterial infusions of GTN at 5, 11 and 22 nmol/min, separated by a 30-minute recontrol. Both infusions were carried out in the presence and absence of vitamin C using a randomized crossover design. Venous occlusion plethysmography was used to measure forearm blood flow responses to GTN.
Results: During the first GTN infusion there was no difference in the blood flow response to GTN between subjects with and without functional ALDH-2. During the first infusion, vitamin C did not modify GTN responses in either group (RM-ANOVA, P=NS). In subjects with the ALDH-2 polymorphism, the response to GTN was significantly blunted during the second GTN infusion while the response of the wildtype group was unchanged (RM-ANOVA, effect of Genotype P=0.03). The co-administration of vitamin C restored blood flow responses to GTN during the second GTN infusion.
Conclusions: Subjects with the ALDH-2 polymorphism developed acute tolerance to GTN, possibly due to lack of ROS defense mechanisms, since the antioxidant vitamin C, prevented loss of GTN responses during the second exposure to GTN (P=NS, vs first infusion).
Keywords: Nitroglycerin, Aldehyde Dehydrogenase 2, Vitamin C, Oxidative stress, Tolerance

#18

Design, Synthesis, and Antifungal Activity of New Triazole Derivatives

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Background: Fungal diseases are a menace to human life. They are a major problem, not only for individuals suffering from primary infection, but also for individuals suffering from fungal infection as a secondary infection and for immunocompromised patients suffering from other disorders. Among the antifungal agents, azoles were used widely in treatment of fungal infections.
Objectives: This study describes the design, synthesis and evaluation of a novel series of fluconazole derivatives bearing nitrotriazole (series A) or piperazine ethanol (series B) side chain.
Methods: It docked in the active site of lanosterol 14α-demethylase enzyme (1EA1) using the Autodock 4.2 program (The scripps research institute, La Jolla, CA, USA). The structures of synthesized compound were confirmed by various methods including elemental and spectral (NMR, CHN, and Mass) analyses. Then antifungal activities of the synthesized compound were tested against several natural and clinical strains of fungi using a broth microdilution assay against several standard and clinical fungi.
Results: Nitrotriazole derivatives showed excellent and desirable antifungal activity against most of the tested fungi. Among the synthesized compounds, **5a**–**d** and **5g**, possessing nitrotriazole moiety, showed maximum antifungal activity, in particular against several fluconazole-resistant fungi.
Conclusions: Here two series of novel fluconazole-derivatives containing nitrotriazole or 2-(piperazin-1-yl) ethanol moieties were synthesized. All the synthesized derivatives except **5i**, **5j** and **5h** exhibited moderate to high in vitro antifungal activities. Compound **5b** was the most active antifungal agents.
Keywords: Fluconazole, Lanosterol 14α-demethylase, Docking, Nitrotriazole

#19

Pharmacological inhibition of mitochondrial fission prevents development of abdominal aortic aneurysm in mouse model

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Background: Abdominal Aortic Aneurysm (AAA) is progressive dilatation of aorta due to abnormal alterations in structural integrity of aorta. Complex pathophysiology and limited therapeutic interventions warrant identification of novel mechanisms of pathogenesis or therapeutic agents.
Objective: To study the role of mitochondrial dynamics in the development of AAA.
Methods: Mitochondrial division inhibitor 1 (mDivi-1) (1.2 mg/kg/day) was administered to 8-10 weeks old male ApoEKO mice model of angiotensin II (Ang II)-induced AAA. The structural alterations in abdominal aorta and mitochondria were assessed by histology staining and transmission electron microscopy (TEM). In-vitro protective effects of mdivi-1 (50 μM) on staurosporine (1 μM) treated vascular smooth muscle cells (VSMCs) were evaluated by mitochondria labelling and flow cytometric analysis for apoptosis and mitochondrial permeability transition pore (mPTP) opening. Mitochondrial metabolism in treated VSMCs was assessed using Seahorse analyser. The data was compared using one-way ANOVA and P value <0.05 was considered statistically significant.
Results: Using TEM, increased mitochondrial fission was observed in abdominal aorta of AAA mouse model. Treatment of VSMCs with mDivi-1 showed reduced apoptosis, mPTP opening, and elongated mitochondrial structure. Metabolic profile of VSMCs showed higher oxygen consumption rate, extracellular acidification rate, and metabolic potential in response to mDivi-1. mDivi-1 significantly attenuated the dilatation of abdominal aorta in AAA mice model. Immunostaining and histological assessment showed reduced matrix remodeling and VSMCs apoptosis along with normal mitochondrial morphology.
Conclusions: The data indicates inhibiting mitochondrial fission protects against development of AAA, suggesting a novel pharmacological strategy.
Keywords: Abdominal aortic aneurysm, mitochondrial fission, mDivi-1

#20

Human Red Blood Cell Transport of Selenite in the Presence of Arsenite

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Background: Over 200 million people worldwide are exposed to the proven human carcinogen arsenic, due to contaminated drinking water. Animal studies have shown that arsenic and the essential trace element selenium can undergo mutual detoxification through the formation of the seleno-bis(S-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ which undergoes biliary excretion, resulting in fecal elimination of both metalloids. $[(GS)_2AsSe]^-$ has been shown to form in animal red blood cells (RBCs), resulting in the sequestration of arsenic and selenium. In rat RBCs, selenite (Se^{IV}) uptake is inhibited by 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS), suggesting uptake is mediated by the erythrocyte anion-exchanger 1 (eAE1, or Band 3).
Objectives: In human RBCs (hRBCs), the influence of arsenic on selenium accumulation is largely unknown. We hypothesized that the presence of arsenite (As^{III}) would increase radioactive $^{75}Se^{IV}$ accumulation in hRBCs by means of Band 3.
Methods: Uptake was quantified using $^{75}Se^{IV}$ transport assays ± As^{III} ± DIDS ± bovine serum albumin (BSA).
Results: Se^{IV} uptake by hRBCs was inhibited by ~90% in the presence of DIDS (50 µM). As^{III} was able to increase Se^{IV} accumulation by approximately two-fold after 10 minutes in the presence of BSA (36 mg/mL), and pre-loaded RBCs effluxed $^{75}Se^{IV}$ in the presence of BSA.
Conclusions: Under physiological conditions As^{III} is able to increase Se^{IV} accumulation in hRBCs, with Se^{IV} uptake mediated by Band 3. Consistent with animal RBCs, human RBCs can sequester arsenic in the presence of selenium and this likely has a protective function.
Keywords: Arsenic, Selenium, Band 3, Toxicity

#21

Comparative analysis of the transcriptomic changes produced by a long acting β2 adrenoceptor agonist and a prostanoid EP4 receptor agonist in airway epithelial cells

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Background: Patients with severe, chronic obstructive pulmonary disease (COPD) often respond suboptimally to mainstay therapies including long-acting β_2-adrenoceptor agonists (LABAs) and inhaled corticosteroids (ICS). It has been proposed that selective agonists of the EP_4-receptor, which typically elevate cAMP, could be alternative therapeutic candidates as they exhibit bronchodilator and anti-inflammatory activity. We have reported previously that LABAs promote significant gene expression changes in human airway epithelial cells by activating a canonical Gsα-cAMP-PKA pathway and that this could contribute to their beneficial effects in COPD. However, there is literature precedent for compartmentalized cAMP signalling whereby the cAMP generated by different GPCRs leads to different functional outcomes. Accordingly, we hypothesized that the transcriptomic signatures of a LABA and an EP_4-receptor agonist could be fundamentally different and that this could be exploited to therapeutic advantage.
Objectives: We compared the transcriptomic changes produced by the LABA, vilanterol and the EP_4-receptor agonist, ONO-AE1-329 in BEAS-2B airway epithelial cells.
Methods: Changes in global gene expression was determining by RNA-sequencing.
Results: Gene expression changes produced by vilanterol and ONO-AE1-329 were highly correlated suggesting that the β_2-adrenoceptor and the EP_4-receptor shared a common mechanism of action. However, whereas vilanterol was a full agonist on all gene expression changes, ONO-AE1-329 was a partial agonist with intrinsic activity values that varied from 0.1 to 0.9.
Conclusions: Contrary to our hypothesis, LABAs and EP_4-receptor agonists generated similar genomic signatures in BEAS-2B cells. Thus, if genomic mechanisms are important, targeting the EP_4-receptor might not offer an advantage over the β_2-adrenoceptor.
Keywords: Prostanoid EP_4 receptor, β_2-adrenoceptor, RNA-seq, Transcriptome, Airway epithelium

#22

Development and Characterization of Magnetic Nanoparticles for Treating Glioblastomas

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Background: Glioblastoma multiforme (GB) is the most aggressive brain cancer with a median survival time of 12 months. One of the main impediments for early stage GB therapy is the highly restrictive BBB. Nanoparticles are widely investigated as drug carriers for cancer applications. Among nanoparticles, non-spherical shapes are of interest as they have been shown to exhibit higher cell internalization than spherical nanoparticles. Here we investigate a novel non-spherical brick shaped iron oxide nanoparticle (IONB_EDT) as a potential drug carrier for GB.
Objectives: To investigate the usefulness of brick-shaped magnetic iron oxide nanoparticles for GB therapy and identify nanoparticle formulations for further *in vivo* assessment.
Methods: Two human GB cell lines, U-87 MG and U-251 MG were cultured in Dulbecco's Modified Eagles' F12 medium. The cellular uptake of IONB_EDT in these cell lines was estimated with and without magnetic field at 4ºC and 37ºC. The cytotoxicity of IONB_EDT and doxorubicin loaded IONB_EDT in GB cells were determined using MTT assay.
Results: IONB_EDT were not toxic at 50µg/mL to bEnd3 and N9 cells over 24h exposure. The cellular uptake of IONB_EDT was higher at 37ºC, indicating an energy-dependent pathway, predominantly caveolae-mediated endocytosis. The IONB_EDT_Dox produced over 50% cell death in both U87 and U251 cells, which was further enhanced to over 80% in the presence of lysosome modulator desloratadine
Conclusions: IONB_EDT_DOX was promising as a carrier for GB cells. Studies using BBB and animal models are warranted to further establish its compatibility and usefulness for brain drug delivery.
Keywords: (iron oxide nanoparticles, glioblastoma, caveolae)

#23

Prevention of hyperglycaemia-induced endothelial dysfunction by metformin: A novel role for Orphan Nuclear Receptor, NR4A1/Nur77

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Background: Metformin is known to improve hyperglycaemia-impaired endothelial function by an as-yet-unknown mechanism. Since metformin and the nuclear-orphan-receptor-NR4A1, are both involved in AMPKinase activation, we hypothesised that metformin and NR4A1 are linked to metformin's endothelial hyperglycaemia-protective actions.
Objectives: We tested this hypothesis using both murine aorta-ring organ cultures and aorta-ring-tissue-derived endothelial cell (EC) cultures from wild-type(WT) and NR4A1-null mice.
Methods: Isolated aorta rings from WT and NR4A1-null mice were cultured [48h/37°C/48h in DMEM containing either 25 or 10mM glucose(G)], without or with 1-5 μM metformin. Acetylcholine and 2-fLI (PAR2 agonist)-mediated endothelium-induced vasodilation was then evaluated by bioassay with endothelium-intact aorta rings. Cultured ECs from the same rings, maintained under 10 or 25 mM glucose for 48h without or with metformin(1-5μM), were assessed for eNOS levels and mitochondrial function (Seahorse-XFe24-measured oxygen-consumption-rate(OCR).
Results: Under hyperglycaemic culture conditions (25 mM-G), metformin(0.5-5 μM) preserved the wild-type-tissue ACh- and 2fLI- vasodilator responses (95±6% and 58±7%, respectively. $P<0.05$ vs no metformin), compared with endothelium-impaired metformin-untreated-tissue vasodilator responses (22±4% and 18±3%, respectively). Metformin did not preserve hyperglycaemia-induced endothelial dysfunction in Nr4a1-null tissues (30±4% vasodilator responses to ACh/2fLI in all tissues/25 mM-G). Further, 25 mM-Glucose-cultured Nr4a1-WT ECs exhibited an increased mitochondrial oxygen-consumption-rate(OCR) that was diminished by metformin (5 μM) treatment, whilst hyperglycaemia-cultured-Nr4a1-null ECs showed no reduction in mitochondrial-OCR in the presence of metformin.
Conclusions: Metformin (0.5-to-5 μM) can directly preserve endothelial function by a process involving the essential participation of Nr4a1.
Keywords: Hyperglycaemia, organ culture, metformin, mitochondria.

#24

The role that G protein-coupled receptors play in mast cell functions

Background: Mast cells are tissue-resident immune cells that are involved in inflammation and fibrosis but also serve beneficial roles including tissue maintenance, angiogenesis, pathogen clearance, and immunoregulation. As a result, mast cells have become an important target for drug discovery and diagnostic research. Recent work has focused on applying novel nanotechnologies to explore mast cell biology.
Objectives: Our lab has developed and designed several nanomaterials to target specific receptors on mast cells and thereby manipulate their function.
Methods: Microfluidic platforms have been used to create lipid nanoparticles for DNA encapsulation. Self-assembled protein nanomaterials have been designed to specifically target G protein couple receptors expressed by mast cells.
Results: Nanomaterials can be used successfully to either activate or inhibit mast cell functions, either by specifically targeting surface receptors or by targeting gene expression.
Conclusions: Nanomaterials can be customized to manipulate mast cell functions by targeting specific receptors or by modulating gene expression through DNA delivery.
Keywords: Human mast cells, Nanomaterials, GPCR

#25

Varied mechanisms and sites of action of anti-epileptic potassium channel activator compounds

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Background: Kv7 (KCNQ) voltage-gated potassium channels are targeted by a variety of activating compounds that shift the voltage-dependence of activation. The underlying pharmacology of these activator compounds is of growing interest for the treatment of epilepsy, pain, and other diseases. Retigabine/flupirtine is the first Kv channel activator approved for human use, but this drug class remains poorly understood.
Objectives: The objective of our research program has been to accelerate the development of Kv7 activators by understanding the molecular mechanism(s) of action of these drugs.
Methods: We use electrophysiological recording of Kv7 channel mutants and concatenated tetrameric channels with known subunit stoichiometry to investigate the mechanism of action of Kv7 channel activators.
Results: Retigabine is the prototype member of a class of pore-targeted activators that influence all non-cardiac Kv7 channels (Kv7.2-7.5) isoforms with little specificity. A single binding site is required for maximal effectiveness of pore-targeted activators. A second sent of activators binds exclusively to the activated state of the voltage-sensing domain (VSD). These VSD-targeted drugs, including ICA-069673, discriminate between different Kv7 subtypes and require four drug-sensitive subunits for maximal effectiveness. Specific amino acids have been identified that selectively abolish sensitivity to either pore- or VSD-targeted activators, suggesting they act at separate binding sites.
Conclusions: Our findings provide a framework for the classification of Kv7 potassium channel activators. Kv7 activators can be clustered into at least two subtypes that differ in their binding site, stoichiometry, and mechanism of action.
Keywords: potassium channel, epilepsy, electrophysiology, M-current

#26

Injury-responsive dermal dermal fibroblasts acquire divergent fates dependent on their location within the wound

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Background: Few adult mammals can completely regrow a damaged tissue – a process known as tissue regeneration. Instead, humans and most other mammals repair injuries by producing scar tissue, which has different properties compared to the original tissue it replaces. In mice, an interesting model combines regeneration and scar; After large wounds, regeneration occurs in the center zone whereas scar is formed in the periphery. However, the mechanisms driving regeneration in the central zone is unclear.
Objectives: We hypothesize that the skin progenitor cells recruited are different in center and periphery.
Methods: To test this hypothesis, we used Hic1-tdTomato-lineage to trace the mesenchymal progenitors. We compared centre and peripheral cells using single-cell-RNA-sequencing 14 days after injury.
Results: Our data showed that fibroblasts from regenerative zone reacquired some development cells fate (CRABP1) and some specific transcription factor (RUNX1). Conversely, scar fibroblasts expressed some specific scar transcription factor (DlK1). However, center and periphery fibroblasts share a lot of common markers. Indeed, their inducibility to become regenerative or scar fibroblasts seems to be dependent on the micro-environment signals. We found that epithelial and immune compartment are very different in the two zones and could be responsible for the specific signal in the centre by switching the fibroblasts in regenerative fibroblasts. Neutrophils and ROS production are two times higher in the regenerative part than in the scar part.
Conclusions: Ongoing studies are examining the functional role of neutrophils by blocking its infiltration into healing wounds. Hence, our work identifies novel strategies to improve skin regeneration by modulating the microenvironment during wound healing.
Keywords: Skin regeneration**,** Neutrophils**,** ROS production**,** Micro-environment, single-cell-RNA-sequencing

#27

Metabolic Alterations in a Mouse Model of Cisplatin-Induced AKI

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Background: Cisplatin-induced acute kidney injury (AKI) occurs in 1/3 of cisplatin-treated patients. Cisplatin-AKI is diagnosed by elevated serum creatinine (SCr), but nephrotoxicity develops before measurable changes to SCr. Novel diagnostic/predictive markers of AKI may explain why only certain cisplatin-treated patients get AKI. FVB/N mice are more susceptible to cisplatin-AKI than C57BL/6. These two strains were used to model the interindividual variability of cisplatin nephrotoxicity.
Objectives: 1) Measure expression of renal transporters/enzymes involved in cisplatin disposition in FVB/N and C57BL/6 mice.
2) Investigate metabolic differences between FVB/N and C57BL/6 mice using metabolomics**.**
Methods: Mice were administered 15mg/kg cisplatin or saline by intraperitoneal injection and sacrificed 1,3, and 4 days post-treatment. AKI severity was assessed by plasma creatinine quantification and histological analysis. Gene expression was assessed using RT-PCR. LC-MS was used for untargeted metabolomics.
Results: Renal mRNA expression of transporters Oct2 and Oat1, and metabolizing enzyme Ggt1 were higher (+20%,+38%,+45%;$p<0.05$) in untreated FVB/N mice compared to C57BL/6. Principal component analysis (PCA) of untreated plasma samples showed separation based on strain. PCA of day 4 plasma samples separated cisplatin and saline groups for both strains. LysoPC(16:0), taurine, indoxyl sulfate, phenyl sulfate and p-cresyl sulfate were metabolites altered in cisplatin-AKI.
Conclusions: Compared to C75BL/6, FVB/N mice exhibited higher expression of various renal transporters/enzymes involved in cisplatin disposition. PCA clustering of plasma samples from untreated mice indicates baseline metabolic differences between strains; separation by treatment suggests that cisplatin alters the metabolic profiles of the mice. Future work will further characterize metabolic changes associated with cisplatin-AKI.
Keywords: Metabolomics, acute kidney injury, cisplatin, biomarkers

#28

Optimizing chronic Hepatitis C treatment in a Canadian cohort using precision medicine based methods

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Background: Chronic Hepatitis C virus (HCV) infection is a major cause of cirrhosis and liver disease, making it a leading indicator for liver transplantation. In the era of direct acting antiviral therapy for HCV, ribavirin is still used to improve outcomes and shorten therapy length. However, ribavirin can cause serious anemia. This compromises patient health, and treatment success, resulting in longer treatment periods and higher costs.

Objectives: We aim to validate previously identified genomic variants, discover novel variants associated with ribavirin-induced anemia in Canadian patients and develop a genomic prediction model identifying patients most likely to develop anemia due to ribavirin-containing treatments.

Methods: We have recruited patients treated with ribavirin-containing regimens from five sites across Canada. A genome-wide association study will then be conducted to validate the role that previously associated variants play in current ribavirin treatment regimens and uncover novel genomic predictors of ribavirin-induced anemia.

Results: We have recruited 191 patients treated with ribavirin-containing regimens. Of these, 98 cases were identified. Cases had a median hemoglobin decline of 38g/L during treatment and are comprised of a higher proportion of viral genotype 1 infected patients and lower proportion of viral genotype 3. We are currently assessing patients for ~700,000 variants genome-wide for genomic analyses which will be completed by May 2019.

Conclusions: Knowing in whom serious anemia to ribavirin is likely to occur, via precision medicine, will allow for tailoring of therapy to individual patients to increase the probability of treatment success and minimize the likelihood of developing serious anemia.

Keywords: Hepatitis C, ribavirin, pharmacogenomics

#29

Delay in Venous Thromboprophylaxis Initiation: Evaluation of the main causes

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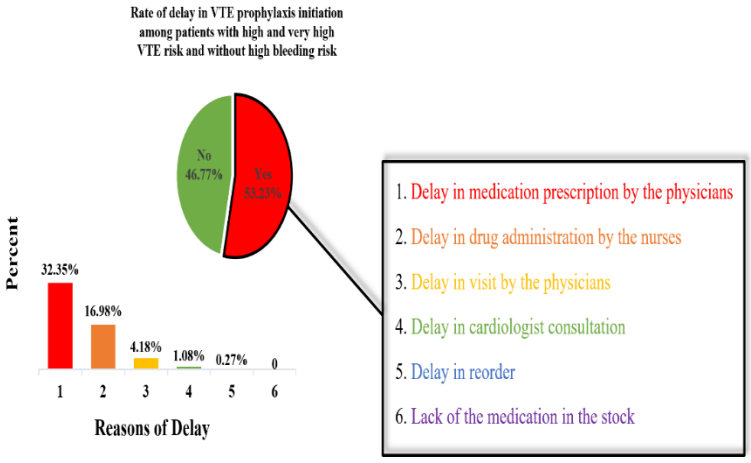
Background: VTE[1] includes DVT[2] and PE[3] which causes morbidity and mortality. Most hospitalized patients are at risk of VTE. An accurate prevention of VTE is vital to avoid acute complications and even mortality. It is also important to avoid unjustified anticoagulation therapy due to the risk of bleeding.
Objectives: This research was done to determine the rate of delay in VTE prophylaxis initiation and evaluation its causes.
Methods: In this study which was done in 2017, medical records of 742 patients, who were admitted to internal medicine and CCU wards of Masih Daneshvari hospital, were gathered through a valid checklist; it was developed based on the expert's opinion which was consisted of some sections such as demographic data, past medical history, Caprini score table, bleeding risk factors and main reasons of delay in the VTE prophylaxis initiation. According to guidelines, first six hours after admission is the golden time for thromboprophylaxis initiation. The medical records were reviewed by investigators and were analysed by SPSS software version 23.
Results: The patients had the same distribution pattern in term of demographic parameters ($P > 0.05$). %34.3 and %50.1 of patients had high and very high VTE risk[4] respectively. Also, %4.9 of patients had high bleeding risk[5], so were excluded from the study. More details about results:



Conclusions:
This study revealed that delay's main reason was the delay in prescribing medication by the physicians. Hence, by implementing an up to date hospital guideline, involving clinical pharmacist's consultation and making sure all the doctors are aware of and have access to the guidelines, VTE and its complications will decrease significantly.
Keywords: VTE, DVT, PE, Thromboprophylaxis

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[1] Venous thromboembolism
[2] Deep Vein Thrombosis
[3] Pulmonary Embolism
[4] Based on Caprini score
[5] Based on HAS-BLED score

#30

Individualized Amiodarone Maintenance Doses Prediction Using Response to a Loading Dose and a Two-Compartment Pharmacokinetics-Based Decision Rule

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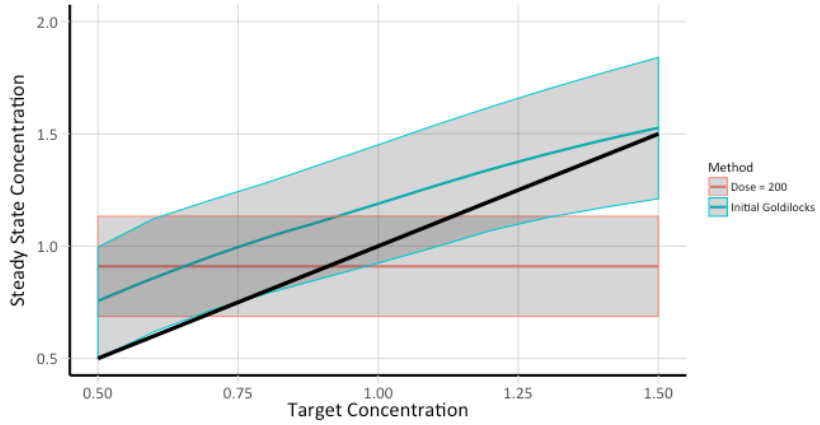
2. Stanford University, Palo Alto, CA, USA.

Background: One-dose-fits-all is not Personalized Medicine, but adjusting amiodarone (AM) dose is challenging (55d half-life). Stable AM personal-PK, makes maintenance dose proportional to accumulated [AM] at first point that good clinical effect is observed during loading. Since measuring [AM] is not always available, we examined a model to pharmacokinetically predict optimum individual AM maintenance dose from loading dose history prior to good effect (e.g. sinus rhythm).
Objectives: Develop a pop-PK decision rule using information from a 77 pt population.
Methods: Rule-predicted dosing to achieve therapeutic AM [steady-state] (range of 0.5 - 1.5 mg/L) was compared to fixed 200 mg/d (Fig) using R software.
Results: A 2-compartment model gave superior prediction to 1-compartment. The ideal result (i.e. rule-determined-[steady-state] = [target]) was contained within dosing-rule 95% confidence interval. Pop-PK showed dominance of the peripheral compartment and relatively little variation in its typical half-life. The effect of personal-PK on absorption and clearance can be estimated proportionally from their influence on time-to-clinical end point. By knowing the drug's dosing rate and the duration it took to produce a desired response, the maintenance dose required to maintain that exposure can be estimated.
Conclusions: A decision rule using input of Time-to-Effect during standard loading dose can predict individualized maintenance dose without requiring precise knowledge of their PK profile. This is a more rigorous framework for dose estimation than experience-based guessing, and is intrinsically better than any "one-dose-fits all" strategy. Replacing arbitrary dosing regimens with an individually calculated maintenance dose moves closer to Personalized Medicine.



Keywords: Drug Dose Prediction; Personalized Medicine, Cardiac Arrhythmias

#31

Ionization status of drugs has poor association with their transmembrane diffusion

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Background: Ionization of drugs in solution can be calculated from Henderson-Hasselbalch Equation (HHEq), knowing their pKa and pH of medium. HHEq reveals that drugs should have similar transmembrane diffusion when pKa and pH of medium are same. But drugs with similar pKa/ionization had poor correlation with their pH dependent buccal absorption (BA) and urine excretion (UE). Similarly, quaternary amines (QA), ionized, had similar effects as tertiary amines (TA), not ionized, on pupil size.
Objectives: To demonstrate that ionization of drugs does not affect their transmembrane diffusion.
Methods: (a) Ratio unionized/ionized (U/I) at pH4 calculated from HHEq was correlated with %BA at pH4 (Beckett & Triggs, 1967) for 7 acidic drugs with similar pKa (3.5-5.6). (b) Ratio U/I at pH9 was correlated with %BA at pH9 for 10 basic drugs with similar pKa (9-9.6). (c) Ratio acid/alkaline (Ac/Al) for 24-hour urine excretion (UE), (Randhawa & Turner, 1988), of 10 basic drugs was correlated with their pKa. (d) Onset and duration of action of 10 QA and TA was determined on pupil size of rabbit eye.
Results: Correlation (R^2) between parameters mentioned above in methods (a-c) was 0.0527, 0.0583 and 0.0192, respectively. QA and TA had similar onset and duration of action on pupil size.
Conclusions: Poor correlation between BA and UE of drugs with their pKa values, ratio U/I, or Ac/Al; and similar effects of QA and TA on pupil size indicate that ionization of drugs in not associated with their transmembrane diffusion.
Keywords: Ionization, acidic/basic drugs, poor correlation, transmembrane diffusion.

#32

Long term benefits of a new life-style change for the management of GERD – A case report

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Background: Gastro-Esophageal Reflux Disease (GERD) is a challenge for medical profession. Proton pump inhibitors (PPIs) are prescribed but have many risks, especially with prolonged use. In earlier pilot study, benefits of short-term practice of new life-style change, two meals a day with only soft-drinks in between, were reported for management of GERD. Present case-report demonstrates benefits of its long-term practice.
Objectives: To report long-term benefits of new life-style change in a patient with severe GERD.
Methods: A 61 year old patient complaining of night refluxes was endoscopically diagnosed to suffer from severe GERD, with ulcerations at gastroesophageal junction, Barrett's esophagus and hiatus hernia. Besides, he had mild erosions in stomach. He practiced suggested dietary regimen, two meals a day with only soft drinks in between (Water, fruit juice, tea and milk), whenever he felt hungry or thirsty. His reflux symptoms improved within fortnight. During this period he took only antacid mixture for 4-5 days and continued suggested dietary regimen.
Results: For 7 years he had no complaint, except that whenever he took solid food during day suffered from mild reflux symptoms, which were relieved by antacid mixture for 1-2 days. Recently, his endoscopy was done to see prognosis. His gastroesophageal junction was clear without any ulcers or inflammation. However, he had mild sliding hiatus hernia and erosions in stomach.
Conclusions: Two meals a day with only soft drinks in between is very useful dietary regimen for management of GERD.
Keywords: Case report, severe GERD, new life-style modification, long-term benefits.

#34

The effect of chronic kidney disease on CYP2B expression and activity in male Wistar rats

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Background: Chronic Kidney Disease (CKD) is characterized by a progressive reduction in kidney function over time. CKD affects greater than 10% of the population and its incidence is on the rise due to the growing prevalence of its risk factors. Previous studies demonstrated CKD alters non-renal clearance of drugs in addition to reducing renal clearance. While CKD has been shown to decrease hepatic CYP3A and CYP2C mediated metabolism, it is unknown whether it alters CYP2B mediated metabolism.
Objective: To assess the function and expression of hepatic CYP2B enzymes using a rat model of CKD.
Methods: CKD was induced in Wistar rats by supplementing their chow with adenine and confirmed by measuring creatinine, urea and other uremic toxins in plasma. Bupropion was used as a probe substrate for hepatic CYP2B function using rat liver microsomes. The resulting metabolite, hydroxy-bupropion, and bupropion were quantified by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Levels of CYP2B mRNA and protein were determined by RT-PCR and Western Blot, respectively.
Results: CYP2B1 mRNA level was significantly decreased (88%, $p<0.001$) in CKD relative to control. Similarly, maximal enzymatic velocity (V_{max}) for CYP2B was decreased by 46% in CKD relative to control ($p<0.0001$).
Conclusions: Previous studies involving patients with CKD demonstrated altered bupropion pharmacokinetics compared to control. Hence, our results suggest that these alterations may be mediated by attenuated CYP2B hepatic metabolism. This finding may partially explain the alterations in pharmacokinetics and non-renal drug clearance frequently observed in patients with CKD.
Keywords: chronic kidney disease, uremic toxins, non-renal clearance, hepatic CYP metabolism

#35

Human pluripotent stem cell-derived liver organoid model to assess metabolism and cardiac function in mental health disease

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Background: Current treatments for mental health disease often show adverse drug reactions (ADRs), most commonly within the context of heart inflammation. Organoids derived from human pluripotent stem cells (hPSCs) are quickly becoming powerful *in vitro* models for pharmacological testing and may prove effective at predicting ADRs.
Objectives: Here we present a scalable system for the production of liver organoids and the utility of this system to elucidate the effects of liver-mediated metabolism of psychotropics on hPSC-derived cardiomyocytes.
Methods: Liver organoids were generated from aggregated hPSCs through a step-wise differentiation procedure. Expression of drug-metabolizing CYP enzymes were assessed at both a protein and transcriptional level. After three weeks, liver organoids were treated with diazepam for one day. Cardiomyocytes were then incubated with liver-conditioned media containing metabolites for an additional day. Organoids, cardiomyocytes and media were analyzed for pro-inflammatory markers.
Results: We were successful in generating tens of thousands of liver organoids in parallel using our novel differentiation protocol. By the end of differentiation, we detected robust expression of CYP3A4, a key metabolizing enzyme of diazepam. Conditioned media from liver organoids treated with diazepam prevented upregulation of the pro-inflammatory marker CCL2 in cardiomyocytes, compared to treating cardiomyocytes with diazepam directly. This data suggests that our liver organoid system is able to convert diazepam to the heart-safe metabolites temazepam and nordazepam.
Conclusions: As we continue to characterize our hPSC-derived liver organoids, this platform provides an attractive starting point for precision screening of ADRs.
Keywords: Human pluripotent stem cell, liver organoid, cardiac organoid, adverse drug reaction

#36

Equilibrative nucleobase transporter 1 mediates the cellular efflux of 6-mercaptopurine

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Background: 6-mercaptopurine (6-MP) is a nucleobase analog drug used in inflammatory bowel diseases and acute lymphoblastic leukemia. We have established that transfection of cells with SLC43A3, which encodes for equilibrative nucleobase transporter 1 (ENBT1), increases both 6-MP influx and its cytotoxicity. This system may also contribute to 6-MP resistance if it is capable of mediating the transport of 6-MP out of cells.
Objectives: To determine if ENBT1 is capable of mediating the efflux of 6-MP, and how this compares with established 6-MP efflux by multidrug resistance proteins MRP4 and MRP5, using a recombinant expression model.
Methods: HEK293 cells stably transfected with SLC43A3 and un-transfected HEK293 cells were loaded with 100 µM [^{14}C]6-MP. Cells were then pelleted and the supernatant removed, and efflux measurements initiated upon suspension of the cells in buffer containing different concentrations of adenine with or without the MRP inhibitors, ceefourin-1 (MRP4) and zaprinast (MRP5). Aliquots of cells were taken at times from 10 – 180 sec and assessed for intracellular [^{14}C]6-MP.
Results: Un-transfected cells displayed minimal efflux with ~80% of initial 6-MP remaining after 3 min. Transfected cells displayed rapid efflux of 6-MP (rate constant >0.35 s^{-1}) with maximum release achieved within 10 sec. Adenine (100 µM or 1 mM) decreased the rate of efflux significantly (rate constants: 100 µM; 0.15±0.02s^{-1}, 1 mM; 0.02±0.002s^{-1}). Ceefourin-1 also decreased the efflux of 6-MP from these cells, while zaprinast did not.
Conclusions: ENBT1 mediates the efflux of 6-MP. MRP4 also contributes to 6-MP efflux in this model but is less efficient in that regard than ENBT1.
Keywords: nucleobase, 6-mercaptopurine, efflux, MRP4, MRP5

#37

Transcriptome-wide association study of anthracycline-induced cardiotoxicity in pediatric cancer patients

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Background: Anthracyclines are highly effective chemotherapeutic agents; however, their clinical utility is limited by severe anthracycline-induced cardiotoxicity (ACT). Genome-wide association studies (GWAS) have uncovered several genetic variants associated with ACT, but these findings explain only a portion of its heritability.
Objectives: To provide an in-depth examination of the genetics underlying ACT.
Methods: We conducted a transcriptome-wide association study (TWAS) using our previous GWAS summary statistics (n=280 patients) to identify gene expression-related associations with ACT. Next, we conducted gene set enrichment and pathway analyses to investigate biological mechanisms underlying ACT.
Results: Three genes expressed in heart/arterial tissues were significantly associated with ACT in the TWAS: *GDF5* (*Z*-score = -4.30, $P = 1.70 \times 10^{-5}$), *FRS2* (*Z*-score = 4.07, $P = 4.67 \times 10^{-5}$), and *HDDC2* (*Z*-score = 4.01, $P = 6.08 \times 10^{-5}$). Significantly enriched gene sets consisted of genes essential in: mice ($P = 2.0 \times 10^{-7}$), culture ($P = 0.036$), and humans ($P = 1.36 \times 10^{-5}$), as well as genes differentially expressed upon treatment with the cardioprotectant all-trans retinoic acid (ATRA; $P = 1.53 \times 10^{-4}$) and genes up-regulated at lower doses of anthracyclines ($P = 3.60 \times 10^{-4}$). Pathway analyses revealed an overrepresentation of genes involved in ribosomal, spliceosomal, and cardiomyopathy pathways.
Conclusions: In summary, we have identified three novel genetic associations with ACT; determined that genes essential for survival, cardioprotection, and response to anthracyclines are important for development of ACT; and uncovered pathways dysregulated in patients with ACT, providing further insight into the mechanism of ACT.
Keywords: Anthracycline-induced cardiotoxicity; Pharmacogenomics; Bioinformatics; Gene expression/regulation; Transcriptome-wide association study

#38

Development and assessment of novel HEK293 cell lines for the study of equilibrative nucleoside transporter regulation

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Background: Equilibrative nucleoside transporters (ENT1, ENT2) mediate the transmembrane flux of endogenous nucleosides along with nucleoside-analogue drugs used in anti-cancer and anti-viral therapies. An understanding of how ENT function is regulated is critical to controlling/exploiting its impact on therapies using nucleoside analogues.
Objectives: Previous work indicates that ENT1 is regulated by PKC and may involve interactions between ENT1 and ENT2. To assess underlying mechanisms, we have developed novel HEK293 mutants (using CRISPR/cas9) lacking both ENT1 and ENT2 (HEK293-NTD), or just lacking ENT2 (HEK293-E2KO). We now report on the characteristics of these cell lines, and the effects of PKC activator-PMA thereon.
Methods: ENT1 function was assessed by measuring the initial rates of [^{3}H]2-chloroadenosine uptake (1 – 150 µM) ± PMA (100nM, 15 min, 37°C). Protein expression was assessed by immunoblotting using ENT-specific antibodies.
Results: HEK293-E2KO had a similar level of ENT1 as wild-type HEK293 cells (Km-38.7±6.9 µM, Vmax-5.4±0.6 pmol/µl/s). Treatment of HEK293 cells with PMA resulted in a significant decrease in ENT1 (Vmax 4.7±1.0 to 1.9±0.7 pmol/µl/s). ENT2 activity (Vmax) also decreased from 0.7±0.2 to 0.2±0.3 pmol/µl/s. PMA had no effect on the Km of 2-chloroadenoisne in HEK293 cells. In contrast, treatment of the HEK293-E2KO cells with PMA had no significant effect on the Vmax of ENT1-mediated 2-chloroadenosine transport (Vmax, 2.06±0.15 and 2.164±0.13 pmol/µl/s, ± PMA), but did cause a significant 2-fold increase in the Km.
Conclusions: These data suggest that ENT2 contributes to the effect of PKC on ENT1. Further exploitation of these new cell models may reveal further mechanisms of ENT regulation.
Keywords: nucleoside transporter, PKC, adenosine, CRISPR/Cas9

#39

Vancomycin Pharmacokinetics in Pediatric Patients with Febrile Neutropenia

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Background: Vancomycin is recommended in guidelines for febrile neutropenia following Hematopoietic Stem Cell Transplantation (HSCT). Recent published studies suggest that empirical vancomycin dosing 60 mg/kg/day does not reach serum trough concentration targets of 15-20 mg/L for serious or complicated infection and higher dosing regimen is required in pediatric patients. However, vancomycin pharmacokinetics in pediatric patients with febrile neutropenia has not been fully characterized.

Objectives: The purpose of this study was to investigate the pharmacokinetics of vancomycin in pediatric patients with febrile neutropenia following HSCT.

Methods: The inclusion criteria were pediatric patients (age 0-17) and treatment with vancomycin for febrile neutropenia following HSCT from 2009 to 2014. Population pharmacokinetics analysis was performed with NONMEM 7.4 to evaluate potential covariates including demographic information and laboratory parameters.

Results: A total of 122 patients had 255 vancomycin concentrations were used in the population pharmacokinetics analysis. A one compartment structure model best described data and objective function value (OFV) decreased in the model includes body weight using allometry and maturation factor. Regarding maturation factor, TM_{50} which describes the maturation half-time was 47.4 weeks and the value approximates TM_{50} of GFR; 47.7 weeks. Estimated vancomycin clearance was 0.18 (0.07-0.28) L/h/kg that demonstrated enhanced clearance compared with previous reports.

Conclusions: Vancomycin clearance in pediatric patients with febrile neutropenia was enhanced probably due to augmented renal clearance. In addition, age-appropriated vancomycin dosage based on develop and maturation is required for neonates and infants

Keywords: vancomycin, population pharmacokinetics, children, febrile neutropenia

#40

Investigation of TMAP as a novel biomarker of kidney function

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Background: Although kidney function is critical for determining drug dosing and diagnosing chronic kidney disease (CKD), there is a lack of sensitive biomarkers for detecting early changes in kidney function. While the current gold standard is creatinine, serum levels of creatinine do not change until approximately 50% of kidney function has been lost. We previously used metabolomics to discover new biomarkers and identified *N,N,N*-trimethyl-L-alanyl-L-proline betaine (TMAP) as a novel biomarker of kidney function.
Objectives: Develop a liquid chromatography coupled to mass spectrometry (LCMS) method for the absolute quantitation of TMAP in CKD patient and control plasma samples, and evaluate changes in plasma concentration as CKD progresses.
Methods: Plasma samples were spiked with varying concentrations of TMAP to generate a standard curve. Liquid chromatography coupled to quadrupole – time of flight (QToF) mass spectrometry was used to determine the concentration of TMAP in plasma of healthy controls and patients with CKD.
Results: The standard curve of TMAP was linear ($R^2 = 0.99971$) within the range of 1.9 and 500 ng/mL. Preliminary data indicate plasma TMAP is 4.9- fold higher in CKD patient plasma when compared to controls with normal kidney function.
Conclusions: TMAP has previously been shown to be a more sensitive indicator of decreased renal function than serum creatinine, the current standard for renal function evaluation. Quantitation of absolute levels of TMAP in plasma shows that as CKD progresses, TMAP plasma concentration increases.
Keywords: chronic kidney disease, metabolomics, biomarker

#41

Equilibrative nucleoside transporter-4 (ENT4) mediated efflux of adenine nucleosides: a potential contributor to ischaemia-reperfusion injury

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Background: Cardiac ischaemia-reperfusion injury remains a life-threatening condition with no effective treatments. Though the mechanisms remain unknown, we know that ischaemic tissues are acidic and that adenosine is protective. Equilibrative nucleoside transporter 4 (ENT4), expressed in cardiomyocytes and the vasculature, transports adenosine specifically under acidic conditions. Therefore, we hypothesise that ENT4 activity in ischaemic tissues regulates the protective actions of adenosine and may mediate the loss of intracellular adenosine during reperfusion.
Objectives: To investigate whether ENT4 is capable of mediating nucleoside efflux, and whether ENT4 activity/expression changes under ischaemic conditions.
Methods: hENT4-transfected pig kidney epithelial nucleoside transporter deficient (PK15-ENT4) cells were loaded with [^{3}H]2-chloroadenosine for 12 min at pH 6.0, and efflux was initiated by exposing the cells to a substrate-free buffer. HL-1 cardiomyocytes were exposed to ischaemia-mimetic conditions for 4 hours and reperfused with culture media for 2 hours before assessing [^{3}H]nucleoside uptake. mRNA levels were analysed using real-time quantitative PCR.
Results: ENT4 mediated 2-chloroadenosine efflux under acidic conditions in PK15-ENT4 cells ($k = 0.050\pm0.009$ min^{-1}). HL-1 cardiomyocytes exhibited pH-dependent uptake of 10 μM 2-chloroadenosine ($k = 0.218\pm0.185$ min^{-1}) and 100 μM adenosine ($k = 0.280\pm0.042$ min^{-1}), characteristic of ENT4. Following 4 hr simulated ischaemia, this pH-dependent uptake was reduced, but recovered with 2 hr reperfusion. ENT4 mRNA levels were also reduced with ischemia, and remained suppressed even after reperfusion.
Conclusions: While ENT4 appears to be down-regulated under ischaemic conditions, our efflux data suggests that ENT4 might contribute to the loss of intracellular adenosine upon reperfusion.
Keywords: Nucleoside transporters, ischaemia-reperfusion

#42

Thresholds of evidence required for the prioritization of robust and actionable pharmacogenomic biomarkers.

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Background: Pharmacogenomic studies have identified genetic variants related to adverse drug reactions (ADRs). However, many pharmacogenomic biomarkers have weak associations or conflicting results between the drug outcome and genetic variant, or are at the margin of clinical relevance. The identification of actionable biomarkers in the prediction of ADRs is therefore important. Some investigators have suggested that randomized controlled trials are necessary to determine clinical relevance. However, randomized controlled trials may not be feasible due to the low incidence of the ADRs or ethical concerns with placing patients at unnecessary risk of ADRs.
Objectives: The objective of this presentation is to propose thresholds of evidence for the prioritization of clinically actionable pharmacogenomic biomarkers for the prediction of serious ADRs.
Methods: A group of experts in medical genetics, regulatory science, clinical pharmacology and clinical medicine was formed. The relevant literature, regulatory actions, and clinical practice guidelines were assessed to determine the most relevant thresholds for the identification of clinically actionable variants.
Results: Levels of evidence were selected according to published evidence, and we concluded a pharmacogenomic biomarker should meet one of the three following criteria to be considered clinically relevant:
1. Drug label recommendations by regulatory agencies.
2. Clinical practice guidelines by expert consortia published in peer-reviewed journals.
3. Evidence of a drug outcome-gene association that is replicated in at least three independent patient populations with an odds ratio of at least three.
Conclusions: Establishing a high threshold for clinical action is essential when bringing a new technology into clinical care.
Keywords: pharmacogenomics, clinical implementation, biomarker, drug outcome

#43

Proteinase Activated Receptor 4 (PAR4) agonist peptide structure and conformational dynamics elucidated by 2D NMR and molecular dynamics simulations

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Background: Proteinase-activated receptor 4 (PAR4) is a member of the proteolytically-activated PAR family of GPCRs and is activated through limited proteolysis by enzymes such as thrombin to unmask a receptor activating tethered-ligand sequence. PAR4 can also be activated by tethered-ligand mimicking synthetic peptides such as AYPGKF-NH_2. Since PAR4 is a key regulator of platelet activation, there is much interest in discovering novel compounds targeting PAR4 for development as anti-thrombotic agents. In a previous study we determined structure activity relationships (SAR) of PAR4 with a library of novel peptide agonists to determine peptide properties that enable PAR4 activation.
Objectives: To understand peptide properties enabling PAR4 activation, we investigate structural and conformational dynamics of the PAR4 agonist peptide AYPGKF-NH_2.
Methods: 2D nuclear magnetic resonance spectroscopy was utilized to determine the structure of AYPGKF-NH_2. The structures were then used as input for molecular dynamics simulations (MD) using gromacs with AMBER99sb-ILDN forcefield for simulation and internal routines for analysis.
Results: 2D NMR reveals two distinct conformations that we have termed major and minor. Both conformations were used as inputs for 100ns MD simulation. Data analyses comparing major and minor conformations reveal significant differences in hydrogen bonding, conformational dynamics, and diffusion coefficients.
Conclusions: These data represent what we believe are two distinct conformations of AYPGKF-NH_2 in solution that may possess varying degrees of efficacy. Current studies are aimed at characterizing peptides from our SAR study that may adopt similar conformations to major or minor AYPGKF-NH_2 to elucidate how the peptide structure contributes to PAR4 activation.
Keywords: Proteinase Activated Receptor 4 (PAR4), 2D NMR, MD Simulation

#44

Efficient, multi-dimensional optimization of organoid production from pluripotent stem cells enables combinatorial drug screening

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5. Alberta Diabetes Institute, University of Alberta

Background: Effective and economically viable organoid-based drug screening will depend on the establishment of optimized protocols for the production of the necessary cell types. Our ability to do this will depend in turn on the capacity to efficiently screen multiple variables in a timely and cost-effective manner.

Objectives: Here, we present a generalizable, integrated strategy employing statistical design of experiments to efficiently produce optimized hepatic organoids from pluripotent stem cells and in turn use them to screen for drug metabolic activity.

Methods: We have developed a liquid-handing and mathematical modeling approach to enable us to screen an arbitrary number of soluble factors using liquid-handling robotics to permit complex experimental designs and minimize errors. Importantly, we are able to identify factor-factor interactions that can be missed in typical single-factor high-throughput screening approach. We used this pipeline to optimize the differentiation of hepatic organoids for drug metabolizing activity.

Results: Initial validation of this platform resulted in the establishment of a novel 3D hepatocyte differentiation protocol. We further demonstrated that organoids differentiated in this manner express the drug-metabolizing cytochrome P450 enzymes CYP3A4 and CYP2D6 and are able to metabolize the anti-anxiety medication diazepam. Further rounds of optimization identified factor combinations that were able to increase expression of key hepatic functional markers. With these optimized organoids we plan to perform a combinatorial screen to identify significant drug-drug interactions.

Conclusions: This broadly applicable approach to multifactorial, high-throughput optimization and screening provides a foundation to develop functional organoid models and improve precision pharmacology.

Keywords: Organoids; High-throughput screening; Drug metabolism

#45

Effect of chronic kidney disease on drug disposition in rodent models

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Background: Over 40% of chronic kidney disease (CKD) patients experience adverse drug reactions. Cytochrome P450 (CYP) metabolism and elimination by transporters are major components influencing drug disposition. Although rodent models of CKD exhibit reduced CYP activity, the impact of CKD on transporters, namely organic anion transporting polypeptides (OATPs), is not well elucidated. In addition, cannabinoid metabolism by CYP enzymes has not been investigated in the setting of CKD.
Objectives: 1) To study the effects of CKD on drug transporter activity, in a humanized OATP1B1/1B3 mouse model. 2) To investigate cannabinoid metabolism in a rat model of CKD. It was hypothesized that CKD would decrease the activity of OATP transporters and reduce cannabinoid metabolism.
Methods: 1) FVB/N mice humanized for OATP1B1/1B3 were fed chow supplemented with 0.2% adenine (n=6) to induce CKD. Plasma creatinine levels and the liver to plasma ratio of the OATP probe drug, fexofenadine ($L:P_{FEX}$), were measured using ultra performance liquid chromatography coupled to mass spectrometry (UPLC-MS). 2) Hepatic microsomal fractions, isolated from CKD-induced rats, will be incubated with cannabinoids and metabolite formation will be measured by UPLC-MS.
Results: Plasma creatinine was doubled in adenine-fed mice compared to controls ($p=0.0011$), and there was a modest 15% decrease in $L:P_{FEX}$ ($p=0.1870$).
Conclusions: Induction of CKD did not significantly alter the liver distribution of fexofenadine. Further analysis of OATP expression levels, as well as cannabinoid metabolism, in rodent models will help to elucidate the impact of CKD on drug disposition.
Keywords: Chronic Kidney Disease, Drug Disposition, Drug Transporters, Cytochrome P450s

#46

Urinary diacetylspermine as a metabolic biomarker of doxorubicin effectiveness in triple negative breast cancer patient-derived xenografts

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Background: Triple negative breast cancer (TNBC) patients have the worst clinical outcomes and highest rate of recurrence compared to other breast cancer molecular subtypes. Treatment of TNBC requires non-specific chemotherapy and therefore, early detection of drug effectiveness would be highly beneficial to improve outcomes.
Objectives: The objective of this study was to identify metabolic biomarkers of doxorubicin effectiveness using TNBC patient-derived xenografts (TNBC-PDXs).
Methods: NSG mice were engrafted with a doxorubicin sensitive or a doxorubicin resistant TNBC-PDX and administered intravenous doxorubicin (2 mg/kg) or vehicle control once weekly for three weeks. Longitudinal urine samples were collected after each dose followed by a final 24-hour urine collection in the absence of drug (n=8-9).
Results: Untargeted metabolomics revealed diacetylspermine as the most significantly altered metabolite in urine samples by two-way repeated measures ANOVA ($p=1.26x10^{-11}$, $q=4.82 \times 10^{-8}$). Diacetylspermine correlated with tumor growth in the doxorubicin sensitive model. Interestingly, when these mice were treated with doxorubicin, there was a further increase in diacetylspermine despite the decrease in tumor size, which persisted for one week following the final dose. Spermine/spermidine acetyl-transferase 1 (SAT1) expression, which is responsible for spermidine and spermine acetylation, was significantly elevated in the doxorubicin sensitive tumor and further increased with doxorubicin treatment. Diacetylspermine, N-acetylspermine, and N1-acetylspermidine were also increased in tumor samples when doxorubicin sensitive mice were treated with doxorubicin compared to vehicle.
Conclusions: These data suggest that doxorubicin increases diacetylspermine in doxorubicin sensitive tumors and may be a useful non-invasive urinary biomarker for evaluating doxorubicin effectiveness during treatment.
Keywords: metabolomics, polyamines, triple negative breast cancer, drug resistance

#47

A pharmacogenomic investigation of the cardiac safety profiles of 5-HT$_3$ antagonists in children and pregnant women

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Background: 5-HT$_3$ receptor antagonists such as ondansetron are highly effective medications for the treatment of nausea and vomiting. However, these medications are also associated with severe cardiac adverse drug reactions. Pharmacogenomic information for response to ondansetron exists, particularly pertaining to *CYP2D6*, but no study has been performed on genetic factors that influence the cardiac safety of this medication.
Objectives: Determine ondansetron-related cardiac safety profiles in three diverse cohorts and assess whether pharmacogenomic information can be used to predict at risk patients.
Methods: Three patient groups who received ondansetron for the prevention of nausea and vomiting were recruited via active surveillance (pediatric surgery *n*=100; pediatric oncology *n*=96; pregnant women *n*=61). Electrocardiograms were conducted at baseline and post-ondansetron administration. Pharmacogenomic associations were then assessed via analyses of comprehensive *CYP2D6* genotyping data and imputed genome wide information.
Results: In the entire cohort, 62 patients (24.1%) were defined as cases based on Bazett-corrected QTc values. The most significant shift from baseline occurred at five minutes post-administration (P=9.8 x10^{-4}). Although CYP2D6 activity score was not associated with case-control status, genome-wide analyses identified novel candidate genes for this drug-induced phenotype. The candidate genes that were identified through these analyses (rs5896569 *MPDZ* and rs73111553 *PTPRG*) have been previously implicated in serotonin-related traits and therefore represent biologically relevant findings.
Conclusions: The results of this study provide the first step towards pharmacogenomically-informed therapeutic decision-making regarding the use of 5-HT$_3$ antagonists in children and obstetric populations, with the overall goal to improve the safety of these commonly used antiemetic medications.
Keywords: CYP2D6; cardiac safety; GWAS; ondansetron; pharmacogenomics

#48

Modulation of SSAT1 Expression for Cancer Cell Sensitization and Therapy

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Background: The regulation of polyamine levels in cells is highly regulated and dependent upon both synthesis and catabolism processes within the cell. The enzyme Spermine/spermidine acetyl transferase 1 (SSAT1) is critical for the metabolism of polyamines and is overexpressed in many cancers including glioblastoma. Preliminary studies suggest SSAT1 overexpression in glioblastoma (GB) may contribute to radiation resistance. Whether similar advantages are present for chemotherapeutic responses is unclear. The present study examined the effects of, lipid nanoparticles (LNPs) to selectively knockdown (KD) SSAT1 expression in GB cells.
Objectives: To identify the survival advantages of SSAT1 in GB cells and develop a cellular "tuned" nanoparticle-based delivery system tailored to modulate SSAT1 expression.
Methods: SSAT1 specific siRNA was loaded in 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) LNPs prepared using the microfluidics method. The SSAT1 knockdown efficiency of LNPs was quantified in the U251 GB cell line using qPCR and WB. The effects of SSAT1 knockdown on both DNA double strand breaks and cytotoxic response to doxorubicin (DOX) and carmustine (BCNU) was evaluated using MTT assay.
Results: A SSAT1 knockdown in U251 cells of approximately 70% was achieved at the messenger level and 50% at the protein level using LNPs. An enhancement of toxicity of around 15% towards Dox and BCNU was observed in U251 cells following SSAT1 knockdown.
Conclusions: Using LNPs to deliver siRNA is a promising strategy for selectively knocking down SSAT1 and sensitize GB cells towards chemotherapy.
Keywords: (SSAT1, Lipid nanoparticles, glioblastoma)

#49

Crataegus songarica Causes Endothelium-dependent Relaxation via a Redox-sensitive Src- and Akt-dependent Activation of Endothelial NO Synthase mediated by Estrogen Receptors

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Background: Fruits of *Crataegus songarica* are commonly used in folk medicine for the treatment of hypertension, vascular insufficiency and heart problems.
Objectives: The aim of current study was to determine the mechanisms underlying the vasorelaxant properties of *C. songarica.*
Methods: Segments of porcine distal coronary artery were set up in a wire myograph for isometric force measurements. Extract/fractions of *C. songarica* were tested for vasodilator activity by measurement of changes in tone after pre-contraction with the thromboxane mimetic U46619 in the presence or absence of inhibitors of intracellular signaling cascades. The formation of reactive oxygen species was accessed by dihydroethidine staining and the level of eNOS and AKT phosphorylation was measured by immunohistochemical staining in sections of coronary arteries.
Results: Vasorelaxant effect of *C. songarica* was reduced by pretreatment with L-NAME (a competitive inhibitor of NO synthase), ODQ (soluble guanylyl cyclase inhibitor), KCl And tetraethylammonium (non-selective K+ channel inhibitor). Relaxations were also attenuated by LY249002 (PI3-kinase inhibitor), PP2 (Src tyrosine kinase inhibitor) and intracellular ROS scavengers, PEG-catalase (hydrogen peroxide inhibitor), diethyldithiocarbamate (superoxide dismutase inhibitor) and MnTMPyP. Inhibitors of muscarinic, bradykinin B2 and tachykinin NK1 receptors had no effect on the relaxation response but both estrogen receptor antagonists (tamoxifen and ICI 182,782) reduced the AS-CS mediated vasorelaxation. *C. songarica* stimulated the endothelial formation of ROS and phosphorylation of Akt and eNOS in coronary artery sections.
Conclusions: Current data validated that *C. songarica* produces a noteworthy, endothelium-dependent vasorelaxation of coronary artery rings owing to activation of estrogen receptors leading to initiation of ROS/Src/PI3K/NO/cGMP pathway. Stimulation of this pathway possibly lead to inhibition of calcium influx, either directly or through activation of K^+ channels.
Keywords: Crataegus, endothelium-dependent relaxation, PI3-kinase/Akt, coronary artery

#50

Establishing the HepaRG cell line as a model for studying the influence of selenium on arsenic hepatobiliary transport

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Background: Worldwide, >200 million people are exposed to the proven human carcinogen arsenic at levels exceeding the World Health Organization guideline (10 µg/L). In animal models selenium and arsenic are mutually protective via the formation and biliary excretion of the seleno-bis (S-glutathionyl) arsinium ion $[SeAs(GS)_2]^-$. Despite ongoing human selenium-supplementation trials in arsenic-endemic regions, the influence of selenium on human hepatic handling of arsenic is not adequately understood. We hypothesized that selenium would increase the biliary excretion of arsenite (As^{III}) from human HepaRG cells, an immortalized cell line used as a surrogate for primary human hepatocytes (PHH).
Objectives: To study the influence of selenite (Se^{IV}) and selenide (Se^{II}) on arsenic efflux from HepaRG cells.
Methods: The formation of canalicular networks and function of multidrug resistance protein 2 (MRP2) (the canalicular transporter of $[SeAs(GS)_2]^-$) were evaluated using fluorescence imaging of cells after treatment with 5(6)-carboxy-2',7'-dichlorofluorescein (CDF) diacetate. $^{73}As^{III}$ (1 µM) efflux was measured across sinusoidal and canalicular membranes using B-CLEAR® technology in the presence or absence of Se^{IV} or Se^{II-} (1 µM). Biliary excretion indices (BEIs) were calculated to quantify biliary excretion.
Results: CDF accumulated in canalicular networks, suggesting the presence of functional MRP2. Biliary excretion of $^{73}As^{III}$ at 10 min was 4%. In the presence of Se^{IV} and Se^{II-}, BEIs were increased to 22% and 26%, respectively. Therefore, selenium increased arsenic biliary excretion.
Conclusions: This work will lead to a better understanding of the influence of selenium on arsenic handling by human liver and provide valuable information for ongoing selenium-supplementation trials.
Keywords: Arsenic, selenium, hepatobiliary transport, HepaRG, multidrug resistance protein 2