UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

1D

MEDICAL THERAPEUTICS AND ENDOCRINOLOGY

Session Moderator: Ian Sweet, Medicine

231 MGH

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Single Nucleotide Polymorphisms and Subcellular Localization of Glucocorticoid Receptor-Associated Signaling Proteins Associated with Cortisol-Mediated Stress Response

Olivia Joy (Olivia) Fox, Sophomore, Computer Science
Mentor: Patrick Murphy, Interdisciplinary Health Sciences, Seattle University

Pharmacogenetics is the study of how variations in a person's DNA affect their response to drugs. It is the foundation of 'personalized medicine' and could one day enable drug therapies for individual patients based on genetic composition. Glucocorticoids are a group of cholesterol-based signaling molecules including endogenous hormones such as cortisol, which the body produces naturally in response to various forms of stress, and commonly prescribed anti-inflammatory drugs, which are used to treat diseases ranging from asthma and cancer to autoimmune disorders and psoriasis. For a glucocorticoid to produce its physiologic effects in any cell of the body, it must first bind to the glucocorticoid receptor (GR) and translocate with the GR to the cell nucleus. Both of these biologic processes are dependent on the molecular chaperone protein hsp90. A necessary first step in glucocorticoid-based pharmacogenetics research is better understanding the genetic variation associated with alterations in endogenous cortisol signaling. Our goal was to identify correlations between individuals' variations in endogenous cortisol signaling and underlying genetic variability. Salivary cortisol (CORT) concentrations were measured in healthy volunteers (n=66) before and after CORT stimulation to determine variability in circulating CORT levels and CORT response. Statistical analysis revealed that inter-participant CORT variability in concentration or percent change was not exclusively correlated to participant demographics, health history, or GR single nucleotide polymorphisms (SNPs). Interestingly, nuclear localization of hsp90 correlated with an elevated pre-stimulus CORT concentration and a decreased post-activity CORT concentration. To investigate the observed CORT response and hsp90 localization association further, coding regions of the hsp90 gene were sequenced to identify possible genetic linkages. Eight hsp90 SNPs were detected, four previously unreported. All participants expressed between two and five of these SNPs. These data demonstrate inter-participant differences in CORT response, nuclear hsp90 concentrations, and GR/hsp90-associated SNPs are readily detected and potentially interrelated.

Maternal Low Protein Diet Alters Clearance of Uric Acid and Creatinine in Rat Offspring

Tahir Mahmood, Senior, Biochemistry
Mentor: Ganesh Cherala, Pharmacy Practice, Oregon State University
Mentor: Barent DuBois, College of Pharmacy, Pharmacy Practice, Oregon State University
Mentor: Jacob Pearson

Abnormal perinatal environment, commonly manifested as low birth weight, has been associated with birth defects as well as chronic disease. In particular, studies have shown low birth weight to have a deleterious association with type II diabetes, hyperlipidemia, hypertension, cardiovascular disease, renal disease, and renal failure. Of specific interest are the mechanistic details concerning low birth weight subjects' altered excretion. Maternal low protein diet (LPD) during gestation and lactation results in offspring with significantly lower birth weight. Using the maternal LPD rat model, we determined renal excretion of select endogenous compounds by collecting blood and urine at various times of age. Clearance of creatinine and uric acid are indicators of glomerular filtration rate and reabsorption, respectively. High Performance Liquid Chromatography assays were used to simultaneously quantify creatinine and uric acid in the urine and in the blood of the rat. It was found that creatinine clearance and uric acid clearance decreased in LPD rats in an age-dependent and sex-specific fashion. Specifically, there were observed significant decreases in both creatinine clearance and uric acid clearance in LPD male offspring at both age's day 90 and day 120 old. No differences were observed in both creatinine clearance

and uric acid clearance in LPD female offspring until age day 120 old indicating an age-dependent programming of females as opposed to males. The results strongly correlate with decreased and altered excretion observed in low birth weight subjects and suggest that perinatal LPD has a long-term programming of renal excretion. These findings could partially explicate the underlying mechanisms that lead to higher incidence of renal disease/dysfunction in perinatally growth restricted subjects. Furthermore, altered renal function could affect the renal handling of therapeutic agents and thus affecting the overall treatment outcomes in low birth weight subjects.

The In Vivo Effects of BDE-47 in Hepatic Metabolism

Rebecca Lynn (Rebecca) Mc Intyre, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Raymond Yeung, surgery
Mentor: Heidi Kenerson, Surgery

Obesity is a major cause of health-related problems in the developed world and recently studies have demonstrated a link between environmental factors and obesity. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) is one toxic chemical used as a flame-retardant in consumer products. BDE-47 contaminants are commonly found in dust, air, and soil and their accumulation in the food chain contributes to significant levels found in humans, particularly children. Recent studies have linked BDE-47 to a range of toxic impacts, including disruption of insulin signaling. The objective of this study is to investigate the in vivo effects of BDE-47 in hepatic metabolism. We hypothesize that the drug will cause increased cell injury, inflammation and accumulation of fat within the liver (hepatic steatosis) and will have significant effects on individuals predisposed to disruption in insulin signaling. In order to disrupt insulin signaling in vivo, we have generated mouse models with liver-specific deletion of Tsc1 and Pten, negative regulators of mTORC1 and Akt respectively. Both of these complexes play critical roles in the cell cycle and insulin metabolism within the liver. Cohorts of Tsc1-/-, Pten-/-, and control mice were exposed to BDE-47 or placebo for six weeks within their first 10-12 weeks of life. During exposure, the mice were tested for insulin sensitivity and glucose tolerance. Liver tissue was also tested for several markers of hepatic insulin signaling post-mortem. Differences in impacts on these genetic mutants could indicate if the BDE-47 targets the Akt or mTOR cascades differently and if those predisposed to aberrations in insulin signaling may be more sensitive to the toxic effects of BDE-47. This study will determine if a significant interaction between the environmental influence of BDE-47 and the genetic deregulation of insulin signaling occurs, which could have deleterious implications for the use of these compounds in consumer products in the future.

Pathologic Onset of Megaesophagus in the Aging Mouse Model of Duchenne Muscular Dystrophy

Ladan Laurel (Ladan) Mukherjee, Senior, Biochemistry
Mentor: Jeffrey S Chamberlain, Neurology
Mentor: John Hall, Neurology

Pathologic enlargement of the esophagus, termed megaesophagus, results in a failure to complete peristalsis leading to vomiting, severe weight loss and potential death. Megaesophagus is characterized in a number of disease states including parasitic (Chaga's disease), autoimmune (myasthenia gravis), and neuromuscular (muscular dystrophies), however, a detailed cellular and mechanistic understanding is lacking. To address this deficit, we performed a comprehensive examination of the onset, pathology and cellular composition of megaesophagus in the mouse model of Duchenne muscular dystrophy (DMD). DMD affects ˜1/3500 male births and is a catastrophic and ultimately fatal muscle wasting disease resulting from a mutation in the gene encoding the integral skeletal muscle protein dystrophin. Although poorly defined in human DMD patients, megaesophagus is documented in canine and mouse models of DMD. Advancements in DMD therapies has increased patient lifespan and underscored a need to assess the impact of the disease in a range of organ systems, including the esophagus. We find that older dystrophic mice can develop severe megaesophagus, but that the timing of onset depends on the nature of dystrophin expression in muscle and non-muscle tissues and the genetic background of the mice. Further studies are in progress to delineate the precise dystrophin expression pattern in different cell types of the esophagus that contribute to pathology. Work presented here will be essential for future work aimed at identifying new therapeutic targets for DMD.

Nalfurafine as a Novel Treatment for Hot Flashes

Lee Wohlen (Lee) Organick, Junior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Robert Steiner, Obstetrics And Gynecology
Mentor: Amy Oakley, Physiology and Biophysics

Hot flashes affect 75% of menopausal women, leaving them with recurring flushing, sweating, skin blotching, and occasional anxiety, palpitations, and sleep disturbances. 20% of those affected describe the symptoms as "intolerable," and current treatment methods carry too many side effects or are insufficiently effective. We propose the drug nalfurafine to prevent these symptoms. Nalfurafine has the distinct clinical advantage of being taken orally and targeting κ-opiod receptors outside of the blood-brain barrier, leading to minimal side effects. κ-opiod receptors decrease the activity of Kiss1 neurons, and we believe kappa agonists such as nalfurafine inhibit the Kiss1 neuron activity we hold responsible for hot flashes. To test this hypothesis, we injected nalfurafine in mice and collected blood and brain samples, expect-

ing reduced levels of specific hormones and proteins associated with Kiss1 neuronal activity. Analysis of data collected in this study is ongoing. If our hypothesis is incorrect, we will have a new understanding of how κ-opiod receptors regulate Kiss1 neuronal activity. However, if we find evidence that nalfurafine inhibits Kiss1 neuronal activity, it may alleviate hot flashes in women, thereby providing rationale for clinical studies and potentially leading to a therapy that would improve quality of life for millions of women.

Divergent Effects of High- and Low-Molecular Weight Hyaluronan on Glucose-Stimulated Insulin Release from Rat Islets

Len Tran, Senior, Biology (Physiology), Neurobiology
Mary Gates Scholar
Mentor: Rebecca Hull, Medicine
Mentor: Michael Peters, Metabolism, Seattle Institute for Biomedical and Clinical Research

The pancreatic islet β-cell maintains blood glucose homeostasis by releasing insulin, a process that becomes dysfunctional in type 2 diabetes mellitus. Thus, maintaining normal β-cell function is critically important and occurs through many mechanisms including interactions with the surrounding extracellular matrix (ECM). We have recently shown that the polysaccharide hyaluronan is a normal component of the islet ECM. Under normal conditions, hyaluronan exists in a high molecular weight (HMW) form ($>$500 kDa) which exerts anti-inflammatory effects on surrounding cells. One study has shown that HMW hyaluronan can potentiate β-cell glucose-stimulated insulin release (GSIS). Our preliminary data suggest, in diabetic islets, hyaluronan can become fragmented. Low molecular weight (LMW) hyaluronan ($<$10 kDa) is pro-inflammatory. Furthermore, agents that can activate the same intracellular signaling pathways as LMW hyaluronan (e.g. free fatty acids and lipopolysaccharide) have been shown to impair GSIS, suggesting LMW hyaluronan may have a similar effect. I hypothesize that HMW hyaluronan potentiates GSIS, whereas LMW hyaluronan inhibits GSIS in vitro. Rat pancreatic islet cells were cultured on plates coated with HMW hyaluronan, LMW hyaluronan, or L-ornithine (control). After 48 hours GSIS was determined by comparing insulin release under basal (low glucose) and stimulated (high glucose) conditions. Our preliminary data show that as expected HMW hyaluronan potentiates GSIS 2 fold while LMW hyaluronan decreases GSIS by 30% compared to control. Thus it appears HMW hyaluronan is beneficial for β-cell function whereas LMW hyaluronan lacks this effect. Hence, hyaluronan fragmentation in the islet under diabetic conditions may contribute to impaired β-cell function.

Expression of Costimulatory Molecules in Juvenile Idiopathic Arthritis versus Severe Gingivitis

Megan Christine Yuasa, Senior, Biology (Physiology)
Mentor: Anne Stevens, Pediatrics

The most common rheumatological disorder found in children is juvenile idiopathic arthritis (JIA), a disabling disease of unknown etiology and with no cure to date. Previous studies have suggested autoantibodies of adult rheumatoid arthritis (RA) cross-react with oral pathogens, suggesting that oral infection could trigger arthritis. Severe gingivitis, an inflammatory disease of the gums, is present in 50-100% of adolescents, and may exhibit this same interaction with JIA. Costimulatory molecules on antigen presenting cells are induced during an inflammatory response to regulate T lymphocytes. Specifically, programmed death ligand-1 (PD-L1) known to be expressed during infection, is also highly upregulated in JIA. To test the hypothesis that PD-L1 expression is induced by oral pathogens associated with gingivitis, peripheral blood cells were isolated from JIA patients and healthy children. Gingivitis was scored by oral examination. PD-L1 expression was assayed on myeloid DCs and monocytes by flow cytometry. Preliminary data on a subset of subjects (n=7) showed a higher percentage of monocytes with PD-L1 in JIA patients compared to controls; however JIA patients had a lower density of PD-L1 per cell. There was no association between extent of gingivitis and PD-L1 expression. The results of this study could contribute to a new field of JIA therapy targeted at costimulatory molecules and oral hygiene.