Online Proceedings

Poster Session 2

MGH 241, Easel 142

1:00 PM to 2:30 PM

Further Investigation into the Influence of UGT1A4*3 Genotype on The Glucuronidation of 25-Hydroxyvitamin D_3

Huanbin Huang, Senior, Physics: Biophysics
Mentor: Kenneth Thummel, Pharmaceutics
Mentor: Tim Wong, Pharmaceutics

Uridine diphosphate glucuronosyltransferase 1A4 (UGT1A4) is a phase II drug metabolizing enzyme that plays an important role in the conjugation of various xenobiotics and endogenous compounds. In the context of vitamin D metabolism, conjugation of 25-hydroxyvitamin D3 (25(OH)D3), the main circulating form of vitamin D, may function as a pathway to help facilitate the enterohepatic circulation of vitamin D. Allele variants of the UGT1A4 gene are reported to contribute to inter-individual differences in enzyme activity, with the UGT1A4*3 variant gene product exhibiting increased glucuronidation activity. Previous work in our lab evaluated the polymorphic glucuronidation of vitamin D using human liver microsomes isolated from liver samples that were procured by St. Jude Children's Research Hospital and University of Washington. The study found that carriers of the UGT1A4*3 allele showed increased 25(OH)D3 glucuronidation activity. While the samples used in this previous work was limited to a smaller subset of livers with the UGT1A4*3 genotype, we sought to further our investigation to include gene expression data obtained through RNA-Seq analysis and UGT1A4 protein quantification in LC-MS data. In addition, the current analysis was expanded to include a significantly greater portion of both the St. Jude and UW liver banks. From this study, we hope to gain greater insight into the impact that the *3 allele may have on the RNA and protein expression of UGT1A4. In addition, by assessing the relationship between UGT1A4*3 genotype and 25(OH)D3 glucuronidation, we may develop a better understanding of factors that may contribute to the variability in the biliary excretion and/or plasma levels of vitamin D glucuronides.

Poster Session 2

MGH 241, Easel 151

1:00 PM to 2:30 PM

Comparing Immune Cell Phenotype and Function Between HIV Positive, Cannabis Users and Non-users

Toni Gott, Recent Graduate, Integrated Sciences
Mentor: Nichole Klatt, Pharmaceutics

Cannabis is a widely used drug, however the impact of cannabis use in HIV-infected individuals is unknown. While current antiretroviral treatments (ART) can successfully suppress viral burden in HIV-infected individuals, treatment alone does not always completely restore health. We aimed to assess how cannabis may affect HIV pathogenesis to better inform how to advise HIV-infected patients. Previous studies in animal models have demonstrated anti-inflammatory effects of cannabis, and thus we aim to assess the immunological implications associated with cannabis use in HIV-infected individuals. This retrospective study assessed peripheral immune cell frequency and phenotype in HIV-infected individuals (n=185) that either frequently use cannabis (n=57) or do not use any drugs of abuse (n=128). Expression of protein markers associated with cell activation (HLA-DR+ CD38+), proliferation (Ki-67+), and exhaustion (PD-1+) was assessed via flow cytometry and differences between groups were analyzed using Mann-Whitney T-tests. No statistical differences in CD4+ and CD8+ T-cell population frequencies were found between cannabis users and non-users, nor did we find differences in plasma biomarkers of inflammation or microbial translocation. Cannabis users were stratified into lower, middle, and upper quartiles of cannabis metabolite concentration (11-nor-carboxy-THC), as measured by mass spectrometry. Comparing high-use cannabis users, defined as above the 75th percentile (>251.8nM), to non-users indicated statistically significantly lower frequencies of activated CD4+ and CD8+ T-cell (HLA-DR+ CD38+ expression; p=0.0307 and p=0.0454, respectively) and CD4+ and CD8+ T-cell exhaustion (PD-1+ expression; p=0.0184 and p=0.0254, respectively.) These data suggest that cannabis use in HIV-infected individuals does not increase markers of HIV pathogenesis, but may actually reduce activation and exhaustion of T cells. Thus, further studies to determine how cannabis impacts systemic and tissue inflammation and disease progression in HIV-infected individuals are warranted.

Poster Session 4

MGH 241, Easel 161

4:00 PM to 6:00 PM

Single Nucleotide Polymorphisms in SULT1A1 and Their Impact on SULT1A1 RNA and Protein Expression in Humans

Gregory Tong, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Tim Wong, Pharmaceutics
Mentor: Kenneth Thummel

Sulfate conjugation is an important metabolic pathway which has been shown to play a vital role in modulating the biological activity and disposition of drugs and endogenous compounds. Studies have shown that sulfotransferases (SULTs) have a broad substrate selectivity that includes hormones, neurotransmitters and xenobiotics. SULT proteins are expressed ubiquitously in human tissue, but is largely expressed in the gastrointestinal tract, liver and bone marrow. Numerous SULT isoforms exist in humans and the most predominate of these isoforms in the liver is SULT1A1. In this study, we evaluate the impact of various single nucleotide polymorphisms (SNPs) present in the SULT1A1 gene using samples obtained by St Jude Children's Research Hospital and the University of Washington. Of particular interest is the effect these SNPs have on the RNA and protein expression of SULT1A1. In total, 17 SNPs within the SULT1A1 gene was identified. A subsequent analysis using RNA-seq data found that 7 out of the 17 SNPs were common variants with an allele frequency greater than 5%. To assess the effects of these SNPs on the protein expression of SULT1A1, samples of human liver cytosol was analyzed using a mass spectrometry based protein quantification method. While this protein analysis continues to be an ongoing effort, we expect results from this analysis to be consistent with genotypic trends observed in our RNA-seq dataset. By evaluating the relationship between various SNPs in the SULT1A1 gene and their effect on SULT1A1 RNA and protein levels, we hope to expand on our knowledge of the impact SNPs have on the disposition of substrates that undergo sulfonation.

POSTER SESSION 4

MGH 241, Easel 160

4:00 PM to 6:00 PM

Influence of Single Nucleotide Polymorphisms in SULT2A1-mediated Sulfonation of 25-Hydroxyvitamin D_3

Sara Soofian, Senior, Public Health-Global Health
Mary Gates Scholar, UW Honors Program
Mentor: Tim Wong, Pharmaceutics
Mentor: Kenneth Thummel, Pharmaceutics

Vitamin $D_3$is an essential hormone involved in the regulation of calcium and phosphate homeostasis. The major circulating form of vitamin D_3 is 25-hydroxyvitamin D_3 ($25(OH)D_3$) which is the biomarker often used as a measure of vitamin D status. In addition, the sulfonated form ($25(OH)D_3$-sulfate) has also been found to be a major metabolite, often circulating in plasma at concentrations comparable to, and at times exceeding, levels of the unconjugated form. Sulfate conjugation occurs primarily in the liver and is catalyzed by the enzyme sulfotransferase 2A1 (SULT2A1). In this study, we evaluated allele variants of the SULT2A1 gene in an effort to better understand factors that may contribute to the inter-individual variation of plasma vitamin D sulfate concentrations. Using liver samples obtained by St. Jude Children's Research Hospital and the University of Washington, our analysis revealed the presence of three common single nucleotide polymorphisms (SNPs). Of the three SNPs, only one SNP (rs296361) was found to be significantly associated with SULT2A1 mRNA and protein expression as well as vitamin D sulfonation activity. Specifically, we found that homozygous carriers of the mutant allele had significantly reduced expression of SULT2A1 both at the RNA and protein level as well as significantly reduced sulfonation activity. The results of this study provide valuable insight into the role that genetics plays in the disposition of vitamin D, and allows us to better understand factors that may impact inter-individual differences in plasma vitamin D levels.