Online Proceedings

POSTER SESSION 3

MGH 258, Easel 189

2:30 PM to 4:00 PM

Modeling Nephrotoxicity of Aristolochic Acid Using Microphysiological Systems

Phoenix Chen, Senior, Biochemistry
Jacelyn Danielle Bain, Junior, Engineering Undeclared
Mentor: Kendan Jones-Isaac, Pharmaceutics
Mentor: Edward Kelly, Pharmaceutics

Microphysiological systems (MPS), also known as "organs-on-chips," provide a novel, *ex vivo* approach for evaluating the risk of drug induced toxicity and the impact of environmental toxins. Evaluating the chronic impact of exposure to environmental toxins in humans is difficult, due to ethical concerns, but the MPS provide an approach to examining the toxicological effects on human cells, without the need for human subjects. Aristolochic acid I (AA-I) is a potent, plant-derived nephrotoxin and carcinogen that has been implicated as the causative agent in both Balkan Endemic Nephropathy and Chinese Herb Nephropathy. The contribution of hepatic metabolism to the bioactivation of AA-I was previously demonstrated by microfluidically linking a hepatocyte containing MPS and a human renal proximal tubule eptileial cell (PTEC) containing MPS. PTECs are kidney cells from the nephron that lie between the glomerulus and the Loop of Henle. We studied the cytotoxic effects of AA-I on PTECs and human hepatocytes in MPS by exposing the MPS to concentrations of AA-I ranging from 0 μM to 10 μM for longer than seven days. AA-I was perfused into the MPS into either a linked hepatocyte-PTEC MPS or an unlinked PTEC only MPS. The chronic, low-dose effect of AA-I resembles Balkan Endemic Nephropathy, where individuals were exposed to low concentrations of Aristolochic acid from tainted wheat. Both PTEC only and linked hepatocyte-PTEC MPS provide experimental methods for further investigating how organ-organ interactions affect drug metabolism and xenobiotic toxicity.

POSTER SESSION 4

MGH 258, Easel 185

4:00 PM to 6:00 PM

Optimization of an FABP5 Ligand Binding Assay and Determination of Xenobiotic Affinities

Wendy Ni, Senior, Biochemistry
UW Honors Program
Mentor: Nina Isoherranen, Pharmaceutics
Mentor: King Yabut

Fatty Acid Binding Proteins (FABPs) are intracellular proteins that facilitate the transport of fatty acids and other lipophilic substances into the cell for various biological functions. For example, FABP5 is important for binding endocannabinoid anandamide (AEA) which helps regulate cognition, learning, and memory. Compounds such as tetrahydrocannabinol (THC), the psychoactive component of cannabis, competitively inhibit FABP5 and prevent catabolism of AEA in the cytosol by fatty acid amide hydrolase (FAAH). Because many drugs (like ibuprofen and progesterone) are also lipophilic and poorly water-soluble, we predict that FABP5 has a role in their metabolism. The goal of this investigation is to better understand how FABP binding may affect drug metabolism by determining the binding affinities of different drugs to FABP5 using a fluorescence displacement assay. We first validated an assay for screening drugs and determining drug-FABP5 binding. Both ANS and NBD-Stearate, molecular probes that generate a fluorescence signal upon binding, were evaluated. FABP5 was titrated with increasing concentrations of each probe and the resulting changes in fluorescence plotted a hyperbolic curve used to calculate Kd (dissociation constant). In these trials, NBD-Stearate was found to have high background fluorescence and low sensitivity to changes in fluorescence upon alternative ligand binding, so ANS was selected for further method development. We found that factors such as mixing and time to equilibrate affected the Kd, possibly from non-specific binding. The binding affinities for ANS were 1.177 $\pm$ 1.005 μM (Morrison equation fit average), and 4.744 $\pm$ 1.387 μM (One Site Specific average). The preliminary data showed that fluorescence decreased by 48.28% with the addition of arachidonic acid (positive control) which confirmed ANS as a suitable assay. By adding increasing concentrations of various drugs to competitively inhibit the binding of ANS, a decrease in fluorescence will be measured and used to calculate Ki (inhibitor binding affinity).

POSTER SESSION 4

Commons West, Easel 35

4:00 PM to 6:00 PM

Aldehyde Oxidase Contributes to All-*trans* Retinoic Acid Biosynthesis in Human Liver

Christopher James (Chris) Seaman, Senior, Chemistry
Mentor: Guo Zhong, Pharmaceutics
Mentor: Nina Isoherranen, Pharmaceutics

All-trans-retinoic acid (RA) serves an important role in maintaining tissue health, either deficient or excessive levels can lead to health issues. Aldehyde dehydrogenase 1A1 (ALDH1A1), is generally believed to be the main enzyme responsible for the conversion of retinaldehyde (RAL) to RA in the liver, requiring the cofactor nicotinamide adenine dinucleotide (NAD+). However, previous studies indicated that after WIN18,446, a potent inhibitor of ALDH1A enzymes, was administered to mice; liver RA concentrations were not significantly altered, and in vitro the RA formation in mouse liver was only inhibited by about 50% suggesting other enzymes except ALDH1A1 synthesis RA in mouse liver. Mouse aldehyde oxidase has previously been proposed to synthesize RA. Hence, in the current study we tested the hypothesis that aldehyde oxidase (AOX) also contributes to the formation of RA in human liver. Our data shows that purified human recombinant AOX catalyzes the oxidation of RAL to RA. The K_m (indicating substrate binding affinity to the enzyme) and k_cat(indicating maximum enzyme velocity) values were determined by enzyme kinetic assays as 1.4 μM and 3.5 min-1. In the absence of NAD+(AOX mediated activity), RA formation was observed in human liver S9 fractions (HLS9) and the RA formation rate was, on average, 60% lower than that measured in the presence of NAD+(n=4). In addition, hydralazine, a selective AOX inhibitor, inhibited about 55% of RA formation in HLS9 in the presence of NAD+ while combining hydralazine and WIN18,446, more than 85% of RA formation in HLS9 was inhibited when compared to the control. In conclusion, this data shows that AOX and ALDH1A1 each contribute about 50% of RA biosynthesis in human liver. This research helps us better understand the regulation of retinoid homeostasis in humans.