Shania Henry

Senior

Forensic Science & Chemistry

Evaluation of Ethanol Inhibition of Hepatic Carboxylesterase

Mentor: Robert H. Powers, Ph.D., F-ABFT

A number of drugs are metabolized in the body by the action of "de-esterifying" enzymes, primary "carboxylesterases." Examples of the substrates for these enzymes vary from drugs of abuse, such as cocaine, through sympathomimetic amines such as methylphenidate ("Ritalin") local anesthetics (e.g. bupivicaine) and even over-the-counter analgesics, such as aspirin. Because of the structural similarity between ethanol and the hydrolyzed reaction products (e.g. ethanol, methanol or other alkyl-substituents), we have hypothesized that co-ingestion of ethanol with substrates for the carboxylexterases will competitively inhibit the reaction of the drug, resulting in an longer half-life in the body, with a corresponding increase in the pharmacologic parameter "area under the curve" or AUC.

The question of ethanol interference was addressed via an enzyme assay using porcine hepatic carboxylesterase, with various substrates in "substrate-velocity" experiments . Substrate, (e.g. acetylsalicylic acid, methylphenidate, cocaine) was incubated at concentrations of 0mM, 25mM, 50mM, 100mM, and 200mM with 2 units of carboxylesterse/mL, in a final volume of 120 uL in a micro centrifuge vial. Each concentration incubation was run in triplicate and repeated entirely in the presence of ethanol (0.2 g/dL) to evaluate the effect of the presence of ethanol on the reaction velocity. The assay was incubated at 25^{o}C for 45 minutes, and the reaction was terminated by the addition of with 100uL of acetonitrile. An aliquot of the mixture was transferred into an GCMS vial and analyzed via DAD-HPLC (210 & 270nM) to determine amount of hydrolysis product present, as a measure of enzyme reaction velocity. The mobile phase consisted of acetonitrile and 0.1M sodium acetate (pH 4.0), in a gradient elution profile of 15:85, increased to 40:60 over 14 minutes, it held for 2 minutes and then decreased to 15:85 over 2 minutes, with a 5 minute equilibrium period. Reaction product peak heights were plotted against the substrate concentration Michaelis-Menten plot. The reciprocal of the substrate concentration and velocity were plotted, generating a "Lineweaver-Burk" plot, from which enzymatic kinetic constants could be graphically determined.

Evaluation of the Lineweaver Burk data showed similar y-intercepts with or without ethanol which suggests that the ethanol did not interfere with the functionality of the enzyme. The Km however (Michaelis-Menten Constant), increased with the addition of ethanol, which indicates that ethanol affected the ability of the enzyme to bind the substrate. This indicates that the presence of ethanol affected the ability of the carboxylesterase to bind and metabolize the various substrates tested.

Continued efforts on this project will further characterize the nature of the effect of ethanol on the Carboxylesterase-mediated metabolism of various drugs, with the goal of elucidating the extent to which co-ingestion of ethanol with specific compounds may have significant impacts on resulting drug levels, half-life and AUC in the body.