Pathology 438 Midterm Examination due: by 1:00 PM, 6 May 2015
Spring 2015
NAME ____Mary Whalen_____

The electronic responses to this examination are due at 1:00 PM on Wednesday, 6 May 2015. Submit them to shalloran@lifewest.edu.

You are not allowed to consult with classmates or any individuals *other than* the instructor as you research, prepare and compose your responses to the questions posed in this examination. You may use the information available from lecture content (slides) in MOODLE, the LCCW library, reference books and course text books, and on-line resources. Please proofread and organize your work and assemble the exam before submitting it.

Some answers require you to include a citation of the sources you consult to formulate your response. Format your citation according to MLA or APA standards. (If you wish, you can use the built-in Word feature that formats your references: under the References tab, use Insert Citation and fill in the fields as much as possible. Later you will use Bibliography->Insert Bibliography at the point of the cursor. You might learn how to use Section Break too in order to insert bibliographies under separate answers. I have put in section breaks in this document between questions.)

By working the examination and submitting it for grading you are agreeing to work independently of all other individuals and you are certifying that all the responses and answers to the examination questions are your own work.

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1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxico/pharmacokinetics and toxico/pharmacodynamics as possible. Support your description with at least two references, one of which must be from a published book or a journal article.

Clonidine is an antagonist at alpha 2 adrenergic receptors, which are presynaptic, P1 purinergic receptors and H2 histamine receptors. Antagonists block the binging of an agonist at a receptor thus inhibiting the signal that would be produced by the receptor-agonist binging. So Clonidine decreases the firing rate of the sympathetic nerves and the amount of epinephrine release. It is primarily used as a central antihypertensive drug. It also abolishes most symptoms of opiate withdrawal.[1,2]

The pharmacokinetics of clonidine is dose-proportional. It is usually orally administered and the absolute bioavailability on oral administration is 70% to 80%. Peak plasma clonidine levels are attained in approximately 1 to 3 hours after administration. Following oral administration about 40% to 60% of the absorbed dose is recovered in the urine as unchanged drug in 24 hours. About 50% of the absorbed dose is metabolized in the liver. Neither food nor the race of the patient influences the pharmacokinetics of clonidine. The antihypertensive effect is reached at plasma concentrations between about 0.2 and 2.0 ng/mL in patients with normal excretory function. A further rise in the plasma levels will not enhance the antihypertensive effect.[3]

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[1] Smith, A. (2003). Oxford dictionary of biochemistry and molecular biology (Rev. ed.). Oxford [England: Oxford University Press.

[2] Clark, M. (2012). Lippincott's illustrated reviews: Pharmacology (5th international ed.). Baltimore: Wolters Kluwer/Lippincott William & Wilkins.

[3] Clonidine Tablets - FDA prescribing information, side effects and uses. (n.d.). Retrieved May 5, 2015, from http://www.drugs.com/pro/clonidine-tablets.html

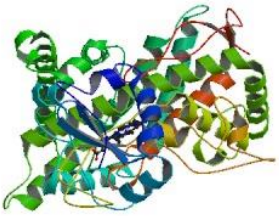
2. Select <u>one</u> of the cytochrome P450 enzymes below in the (a) through (d) list, and describe it as thoroughly as possible in points (i) through (v) below. You should cite at least one reference to a peer-reviewed publication or to a monograph for the course. For any information you put in your response, ensure that it is sourced/referenced. Your response will be compared to the information in the reference

i. Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize.
ii. Explain the mechanism of catalysis (you can even draw the steps)
iii. Provide the names of any substances known to inhibit the cytochrome, if any
iv. If its gene and/or protein structure is known, describe the domains (functional parts or features) of the enzyme, and any molecular detail/features that are interesting or significant to the enzyme's function
v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc

A) CYP2C9 is a phase I drug-metabolizing cytochrome P450 (CYP450) enzyme isoform that plays a major role in the oxidation of both xenobiotic and endogenous compounds. CYP enzymes bind the substrate and O_2 molecule, receives electrons from another enzyme handling NADPH reductant and result in a product, H_2O and $NADP^+$.

Substrate (RH) + O_2 + NADPH + H^+ → Product (ROH) + H_2O +$NADP^+$

CYP2C9's common substrates include Angiotensin II blockers (Irbesartan and Losartan), Nonsteroidal Anti-Inflammatory drugs, or NSAIDs (eg Ibuprofen), Sulfonylurea's (eg Tolbutamide, which is also known as an oral hypoglycemic), the antiocoagulant Warfarin, and the antiepileptic Phenytonin.[4,5] CYP2C9 is the enzyme responsible for the metabolism of the S-isomer of warfarin that is principally responsible for the anticoagulant effect of the drug.[5]



The structure of CYP2C9 can be found in the protein databank online and I've included a picture of it to the right.[6] I was unable to find information detailing the specifics of the domains of the enzymes and/or was unable to fully understand the articles that talked about the structure.

CYP2C9's inducers are phenobarbital and rifampin.[7] Treatment with rifampicin has been shown consistently to increase the clearance of drugs eliminated by CYP2C9.[5]

CYP2C9 is inhibited by amiodarone, bishydroxycocoumarin, chloramphenicol, cimetidine, fluconazole, fluvastatin, miconazole, phenylbutazone, sulphinpyrazone, sulphadiazine, sulphamethizole, sulphamethoxazole, sulphaphenazole, trimethoprim, and zafirlukast. [8]

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[4] Booven, D., Marsh, S., McLeod, H., Carrillo, M., Sangkuhl, K., Klein, T., & Altman, R. (n.d.). Cytochrome P450 2C9-CYP2C9. Retrieved May 5, 2015, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3201766/table/T1/

[5] Booven, D., Marsh, S., McLeod, H., Carrillo, M., Sangkuhl, K., Klein, T., & Altman, R. (n.d.). Cytochrome P450 2C9-CYP2C9. Retrieved May 5, 2015, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3201766/

[6] RCSB Protein Data Bank - RCSB PDB - 1OG2 Structure Summary. Retrieved May 5, 2015, from http://www.rcsb.org/pdb/explore/explore.do?structureId=1OG2

[7] Clark, M. (2012). Lippincott's illustrated reviews: Pharmacology (5th international ed.). Baltimore: Wolters Kluwer/Lippincott William & Wilkins.

[8] Miners, J., & Birkett, D. (n.d.). Cytochrome P4502C9: An enzyme of major importance in human drug metabolism. Retrieved May 5, 2015, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1873650/

2. Select (a) or (b) or (c) to answer:

a) Find at least one report/article that discusses the differences in how men and women respond to toxicants or drugs. Your search for an article may focus on one particular toxicant/drug or you may summarize an article that treats these differences in a broad survey. In any article you obtain, be sure to indicate at least three significant points, but list all of them if there are more.

The article that I found was titled: *Sex Differences in Liver Toxicity—Do Female and Male Human Primary Hepatocytes React Differently to Toxicants* In Vitro*?*

The objective of this study was to investigate whether sex-specific differences in acute hepatotoxicity can be observed at a cellular level by comparing the effects of well-known hepatotoxic drugs on male and female primary human hepatocytes pooled from different donor groups.

In this article they mentioned that there are marked sex-based differences in the epidemiology, clinical manifestations, progression and treatment of disease, as well as pharmacodynamics, -kinetics, and adverse drug effects. They state that these differences are derived from the fundamental biological differences between sexes and are only partially understood in molecular and cellular terms. Females are under-represented in basic research as well as in animal tests, and more importantly, in human clinical trials. Males are preferred because they are thought to be less variable due to their constant hormone levels. Also, they state that currently, the sex of experimental animals or cells is not regularly reported in scientific publications.

This article states that in 2012, the American Physiological Society (APS) was one of the first bodies within the scientific publication community to announce that sex indication of the experimental material, derived from animals or humans, is required for publication in their journals. The NIH also plans to address the issue of sex and gender inclusion across biomedical research multi-dimensionally, pointing out the need to indicate the sex of cell lines studied *in vitro* and has launched a formal Request for Information (RFI) from the research community.

Clinically, women have been reported to have a 1.5–1.7 fold greater risk than men of experiencing an adverse drug reaction (ADR). Despite these reports on sex-based differences in ADR for marketed substances, the evaluation of sex differences in efficacy and toxicity has not been fully instituted for new drugs in development. The article states that acute liver failure is a rare but very serious ADR that occurs more frequently in women. Adverse liver effects typically show acute centrilobular necrosis and the mechanism of hepatic injury is still unknown. Statistics show that females were adversely affected in 52% and males in 45% of cases (3% unknown sex) with an age maximum for all groups of 59 years. And at the molecular level, many studies have reported sex differences in gene expression, protein product, or enzyme activity for cytochrome P450 and transferases without showing a clear distinct pattern.

To the author's knowledge, the effect of known hepatotoxic drugs on primary human hepatocytes of both sexes has not been compared yet in a systematic manner. So, as stated before they set out to investigate whether sex-specific differences in acute hepatotoxicity can be observed at a cellular level. To do this they selected five drugs with varying mechanisms of toxicity and documented sex-related differences in their adverse effects: Diclofenac, Chlorpromazine, Acetaminophen, Verapamil, and Omeprazole. Also, caffeine was selected as a

negative control for the study because it is a “non-hepatotoxic” compound. Caffeine is a xanthine alkaloid that is metabolized in the liver by cytochrome P450 (1A2 isozyme) into three dimethylxanthines.

In this article they looked at ATP levels, changes in nuclear intensity ROS accumulation, mitochondrial damage, plasma membrane permeability modification, intracellular calcium accumulation, and endoplasmic reticulum status in pooled primary hepatocytes.

The ATP measurement showed statistically significant differences between male and females only when hepatocytes were treated for 30 min with Chlorpromazine, or for 5h with Acetaminophen, or for 30 min with Verapamil. For the Mitochondrial damage the data showed that post-menopausal female hepatocytes exposed to either Diclofenac, or Acetaminophen, or Chlorpromazine, or Verapamil are more sensitive to mitochondrial damage than pre-menopausal female and male cells. For the ER modifications they showed that male hepatocytes exposed to either Diclofenac, or Acetaminophen, or Chlorpromazine are more sensitive to ER modifications compared to female hepatocytes.

In correlation with the hypothesis that female hepatocytes are more sensitive to hepatotoxicant damage, they observed that in terms of nuclear condensation, Verapamil and Diclofenac are more toxic in the post-menopausal female group. Also, for substances such as Verapamil and Chlorpromazine they showed that the plasma membrane permeability, which is an indicator for cell death, is more compromised in female hepatocytes than male cells. And finally, they showed that with Acetaminophen treatment reactive oxygen species accumulation is occurring in female hepatocytes at lower concentration than in male cells. Overall this study showed significant differences in mitochondrial injury, nuclear condensation, ER status, and plasma membrane permeability between sexes presenting female cells as being more sensitive, at certain exposure times, for some of the tested drugs.

The article states that this study is the first step to elucidate cell-based sex differences in response to toxicants and the molecular pathways affected, but further experiments are needed to confirm the results and extend evidence for the observations. Also, this article demonstrated that this type of research might not only yield deeper insight into the effects of the karyotype of our basic structural and functional unit of life, but could also contribute to more accurate screening methods for risk assessment that consider the varying susceptibility of male and female populations.

Mennecozzi, M., Landesmann, B., Palosaari, T., Harris, G., & Whelan, M. Sex Differences in Liver Toxicity—Do Female and Male Human Primary Hepatocytes React Differently to Toxicants In Vitro? Retrieved May 5, 2015, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4388670/