Pathology 438 Midterm Examination due: by 1:00 PM, 6 May 2015
Spring 2015
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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.**

a) doxorubicin
b) clonidine
c) valproic acid

- Valproic acid is an antiepileptic medication used to combat petit mal and complex absence seizures, sometimes in combination with other anticonvulsant drugs.
- Pharmacokinetics
  - Absorption Oral dose: Valproic acid is rapidly absorbed fully from the GI tract and has immediate release with a peak serum concentration occurring just 6 hours after immediate release. The tmax for valproic acid in this form is 4 hours.
  - Absorption Extended release: Valproic acid with extended release has a peak serum concentration of up to 24 hours with a half-life between 9-16 hours.
  - Absorption Intravenous: Valproic acid in this form has a tmax after 1 hour of infusion.

    - Distribution: Valproic acid binds roughly 90% to protein, with a volume distribution of 0.1-0.2 /kg.
    - Metabolism: Valproic acid is metabolized in the liver.
    - Excretion: The half-life of valproic acid is between 6-18 hours.
    - The mechanism of valproic acid is not fully known. It has been shown that it inhibits both voltage-gated sodium channels and T-type calcium channels, and is a competitive antagonist of NMDA. Is has also been shown that it increases the brain concentration of GABA, an inhibitory NT, by inhibiting GABA transaminase.
- Pharmacodynamics: what a drug does to the body
    - Valproic acid depresses the CNS, also depletes hepatic carnitine stores and coenzyme A. Depletion of carnitine stores leads to chronic fatty liver due to the lack of metabolism of fatty acids. This also leads to an association with fatal hepatic failure in chronic use. Depletion of CoA affects the body's ability to incorporate ammonia into the urea cycle, leading to hyperammonemia. With a normal therapeutic dosage, pancreatitis is common. Also associated with valproic acid use is thrombocytopenia, abnormal bleeding time and decreased fibrinogen levels which leads to bruising, petechiae, hematoma, and epistaxis. Valproic acid can cross the placental barrier and has been found in breast milk, and has not been proven safe during pregnancy or nursing due to a possible association to neural tube defects.
    - Common side effects include: drowsiness, apathy, withdrawal, confusion, restlessness, hyperactivity, rashes, alopecia, anorexia, nausea, weight gain, and altered thyroid function. Coma and seizures may occur but are not common. Sedative effects are more prominent when other anti-epileptic drugs are used in conjunction with Valproic acid.
    - Death is rare, but is usually due to cardiopulmonary arrest secondary to hepatic failure.

Ghodke-Puranik, Yogita. "Valproic Acid Pathway: Pharmacokinetics and Pharmacodynamics." Pharmacogenet Genomics April 23.4 (2013): 236-41. National Institute of Health. National Library of Medicine, 1 Apr. 2014. Web. 1 May 2015. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696515/pdf/nihms465332.pdf>.

"VALPROIC ACID." TOXNET. US National Library of Medicine, 18 Feb. 2015. Web. 01 May 2015. <http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs%2Bhsdb%3A%40term%2B%40DOCNO%2B3582>.

2. <u>Select one 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</u>

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) CYP3A4

i. CYP3A4 oxidizes small foreign organic molecules, such as xenobiotics, toxins or drugs. Some examples of substrates it is known to metabolize are: tamoxifen, acetaminophen, codeine, cyclosporine, diazepam, erythromycin, some steroids & carcinogens.
ii. CYP3A4 catalysis reactions are involved in drug metabolism as well as synthesis of cholesterol, steroids, and some lipid components. It is used in roughly half of all commonly used drugs today. Expression of CYP3A4 is induced by the presence of glucocorticoids and catalysis is localized to the endoplasmic reticulum. Catalysis happens with hydroxylation of an sp^3 C-H bond that affects a ligand. This can be followed by the dehydrogenation of the substrate, leading to a more complex metabolite.
iii. Some substances known to inhibit CYP3A4 are: protease inhibitors (ritonavir, indinavir, nelfinavir, saquinavir), macrolide antibiotics (clarithromycin, telithromycin), chloramphenicol (antibiotic), azole antifungals (ketoconazole, itraconazole), nefazodone (antidepressant), and cobicistat. The most widely known inhibitor is grapefruit juice, whose effects can last 3-7 days and has its greatest effect when taken in conjunction with the drug CYP3A4 is trying to metabolize. Pomegranate and other citrus juices can have the same effect, however grapefruit juice is the most widely known inhibitor of the cytochrome P450 family.
iv. CYP3A4 is the most common and most versatile cytochrome of the P450 family. It is a hemoprotein and monooxygenase, containing over 28 single nucleotide polymorphisms. In addition, the alleles have minimal function as compared to other P450 cytochrome family members. CYP3A4 also has been observed to have decreased catalytic activity with ligands such as nifedipine and testosterone.
v. The turnover rate of CYP3A4 varies widely, the half-life ranging from 70-140 hours in vivo, and between 36-79 hours in vitro. The turnover rate is a function of the rate of enterocyte renewal, and can be seen between 12-33 hours when grapefruit juice has been ingested in conjunction with a substrate.

"CYP3A4 Cytochrome P450, Family 3, Subfamily A, Polypeptide 4 [Homo Sapiens (human)]." National Center for Biotechnology Information. U.S. National Library of Medicine, 3 May 2015. Web. 3 May 2015. <http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=1576>.

Shahrokh, K., T. E. Cheatham, 3rd, and G. S. Yost. "Conformational Dynamics of CYP3A4 Demonstrate the Important Role of ARG212 Coupled with the Opening of Ingress, Egress and Solvent Channels to Dehydrogenation of 4-hydroxy-tamoxifen." Biochimica Et Biophysica Acta. 1820.10 (2012): 1605-617. National Center for Biotechnology Information. US National Library of Medicine, 4 June 2012. Web. 3 May 2015. <http://www.ncbi.nlm.nih.gov/pubmed/22677141>.

Schmiedlin-Ren, P. "Mechanisms of Enhanced Oral Availability of CYP3A$ Substrates by Grapefruit Constituents. Decreased Enterocyte CYP3A4 Concentration and Mechanism-based Inactivation by Furanocoumarins." Drug Metabolism and Disposition: The Biological Fate of Chemicals 25.11 (1997): 1228-233. National Center for Biotechnology Information. U.S. National Library of Medicine, Nov. 1997. Web. 03 May 2015. <http://www.ncbi.nlm.nih.gov/pubmed/9351897>.

Yang, J., M. Liao, M. Shou, M. Jamei, K. R. Yeo, G. T. Tucker, and A. Rostami-Hodjegan. "Cytochrome P450 Turnover: Regulation of Synthesis and Degradation, Methods for Determining Rates, and Implications for the Prediction of Drug Interactions." Current Drug Metabolism 9.5 (2008): 384-94. National Center for Biotechnology Information. U.S. National Library of Medicine, June 2008. Web. 01 May 2015. <http://www.ncbi.nlm.nih.gov/pubmed/18537575>.

3. 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.

b) Hepatocytes have several different efflux transporters in the plasma membrane that forms the canalicular wall. In the literature there are many original articles and reviews of these canalicular efflux transporters. Pick two of the transmembrane proteins, give their names, describe what substances are known to be transported by them (or class of substances). Explain what is known about their function and include any details of known mechanisms (the "molecular machinery and gears"), such as cellular substrates required to make them work. Summarize what is known about how they are regulated: what turns them on or off, or what increases or decreases their activity, including regulation of gene expression, or signaling pathways the modify protein activity and/or de novo synthesis.

c) Search for a nephrotoxic substance (toxicant, poison or drug). Explain what part(s) of the nephron it disrupts (describe the mechanism of toxicity). Describe how normal kidney physiology would be disrupted for the parts of the nephron affected. Describe how the nephrotoxic substance is detoxified (metabolism? elimination? both?) What doses or concentration levels are required to obtain the toxic effect?

- Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, and naproxen are common over-the-counter drugs used for pain mediation.
- NSAIDS change renal hemodynamics by affecting prostaglandins via COX-1 and COX-2. Whereas normally prostaglandins help to vasodilate the afferent arterioles of the glomerulus, NSAIDs block prostaglandins and in turn constrict the afferent arteriole and decrease renal perfusion pressure. This decrease in renal perfusion pressure decreases glomerular filtration rate, decreasing overall kidney function.
- NSAIDs are metabolized in the liver and turned into inactive metabolites, which are then excreted either in urine or bile. Accumulation can occur even in normal doses, and metabolism can be abnormal depending on other immune system compromises.

- A dose of 100 mg/kg or less should not produce symptoms. Symptoms are usually non-life-threatening unless there is a dose of 400 mg/kg or more ingested.

Botting, R. M. "Inhibitors of Cyclooxygenases: Mechanisms, Selectivity and Uses." Journal of Physiology and Pharmacology 57.5 (2006): 113-24. Print.

Knights, Kathleen M., Arduino A. Mangoni, and John O. Miners. "Defining the COX Inhibitor Selectivity of NSAIDs: Implications for Understanding Toxicity." Expert Rev Clin Pharmacol. 3.6 (2010): 769-76. Medscape. Web. 1 May 2015. <http://www.medscape.com/viewarticle/733075_5>.

Rossi, Simone. Australian Medicines Handbook 2006. N.p.: Australian Medicines Handbook Pty, 2006. Web. 01 May 2015.

Smolinske, S. C., A. H. Hall, S. A. Vandenberg, D. G. Spoerke, and P. V. McBride. "Toxic Effects of Nonsteroidal Anti-inflammatory Drugs in Overdose. An Overview of Recent Evidence on Clinical Effects and Dose-response Relationships." Drug Safety 5.4 (1990): 252-74. National Center for Biotechnology Information. U.S. National Library of Medicine. Web. 1 May 2015. <http://www.ncbi.nlm.nih.gov/pubmed/2198051>.