Pathology 438 Midterm Examination due: by 1:00 PM, 6 May 2015
Spring 2015
NAME _____**JOE BOTKIN**_____

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.*

Valproic Acid (VPA)

Absorption: Valproic acid is rapidly absorbed in the gastrointestinal tract. It may be administered orally, rectally or injected. When administered as an enteric-coated tablet the peak concentrations of the drug at 1 to 2 hours after administration.

Distribution: Due to high plasma protein binding, the apparent volume of distribution is relatively small at 0.1 to 0.4 L/kg. The total plasma clearance of valproic acid is in the range of 5-10 ml/min and the plasma elimination half-life is between 10-16 hours. Renal excretion of valproic acid accounts for only 1-3% of the total dose administered. It is present in the cerebrospinal fluid. The drug is excreted into breast milk and there is evidence suggesting that it also crosses the placenta.

Metabolism: Beta-Oxidation is the most significant pathway of oxidative metabolism of therapeutic VPA levels, accounting for nearly 70% of phase I reactions. In the mitochondrial matrix the first oxidative step in the sequence is the conversion of valproyl-CoA to D2(E)-

valproyl-CoA, a reaction that has been shown to be mediated by 2-methyl-branched-chain acyl-CoA dehydrogenase. The second step of the b-oxidation involves hydration of D2(E)-valproyl-CoA to 3-hydroxyvalproyl-CoA. The final reaction of the b-oxidation cycle of straight-chain fatty acids consists of the thiolytic cleavage of 3-ketoacyl-CoA derivatives, a reaction catalyzed by the 3-ketoacyl-CoA thiolases producing a chain-shortened acyl-CoA and acetyl-CoA. Through another cycle of b-oxidation, pentanoyl-CoA will be further cleaved into acetyl-CoA and propionyl-CoA as shown in Fig.2. Both metabolites will ultimately enter the tricarboxylic acid cycle to complete oxidation to CO2 and H2O.

Elimination: Valproic acid is eliminated by first order kinetics. Plasma clearance after a therapeutic dose is 5 to 10 mL/min and is independent of liver blood flow. The free drug is cleared much more rapidly about 77 mL/min. Excretion occurs partially in the form of ketone bodies.

Pharmacodynamics: The mechanism of action of valproic acid is unknown. Effects of the drug may be related, at least in part, to increased brain concentrations of the inhibitory neurotransmitter GABA. Animal studies have shown that valproic acid inhibits GABA transferase and succinic aldehyde dehydrogenase, enzymes which are important for GABA catabolism. Results of one study indicate the drug inhibits neuronal activity by increasing potassium conductance. In animals, valproic acid protects against seizure induced by electrical stimulation, as well as those induced by pentylenetetrazol.

- Ghodke-Puranik Yogita, Thorn Caroline F, Lamba Jatinder K, Leeder J Steven, Song Wen, Birnbaum Angela K, Altman Russ B, Klein Teri E. "Valproic acid pathway: pharmacokinetics and pharmacodynamics" *Pharmacogenetics and genomics* (2013).
- Mercedes, Maat M. "Valproic Acid." Cardiff, 1 Dec. 1991. Web. 05 May 2015. http://www.inchem.org/documents/pims/pharm/pim551.htm
- Silva, M. F. "Valproic Acid Metabolism and Its Effects on Mitochondrial Fatty Acid Oxidation: A Review." *Research Gate*. Journal of Inherited Metabolic Disease, 11 Dec. 2007. Web. 5 May 2015.

2. *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*

CYP2C9

   i. *Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize.*

CYP2C9 is the primary enzyme responsible for metabolizing nonsteroidal anti-inflammatory drugs (NSAIDs), oral antidiabetic agents, and angiotensin II receptor blockers. CYP2C9 also is the major enzyme involved in the disposition of warfarin.

ii. Explain the mechanism of catalysis (you can even draw the steps).

Some drugs induce CYP2C9, and they may reduce the efficacy of CYP2C9 substrates. Such interactions tend to be insidious, because they result in lack of efficacy, rather than more apparent adverse effects. One of the dangers is that, not knowing that an interaction is occurring, the dose of the CYP2C9 substrate is increased to compensate for the CYP2C9 induction, and then the CYP2C9 inducer is discontinued. This sequence of events can result in a substantial increase in the plasma concentrations of the CYP2C9 substrate, leading to toxicity.

iii. Provide the names of any substances known to inhibit the cytochrome, if any.

CYP2C9 is inhibited by amiodarone, fluconazole, and sulphaphenazole.

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.

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. CYP2C9 has a crystal structure that shows unanticipated interactions between CYP2C9 and Warfarink, revealing a new binding pocket, suggesting that CYP2C9 may simultaneously accommodate multiple ligands during its biological function. Structural analysis suggests that it may undergo an allosteric change when binding to Warfarin.

- Van Booven, Derek et al. "Cytochrome P450 2C9-CYP2C9." *Pharmacogenetics and genomics* 20.4 (2010): 277–281. *PMC*. Web. 6 May 2015.

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

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?

Lead

Lead has been a very common cause of acute or chronic renal failure in the past. The renal effects of lead are primarily tubular or tubulo-interstitial and they may be both acute and chronic. However, the acute effects of lead differ from those of most of the other metals in that cell injury is for the most part reversible and necrosis is uncommon. Cells of the proximal tubule are most severely affected, and this effect is characterized by a reduction in resorptive function leading to a generalized amino-aciduria, glycosuria, and hyperphosphaturia. It isn't quite clear the effect of lead on renal tubular cells and sodium reabsorption. The renal effects of lead may

also be influenced by interactions with calcium. Decreasing dietary calcium increases lead retention, possibly due to a decrease in lead excretion.

The major fraction of lead in the kidney during the acute phase of lead toxicity is bound in the inclusion bodies. For this reason, the inclusion bodies have been interpreted as serving as an intracellular depot for lead. Nevertheless, proximal renal tubular cells during the acute phase of lead toxicity are usually swollen, and the mitochondria show a decrease in matrical granules and altered cristae.

The rate of excretion of lead is low. Renal clearance of unchanged lead occurs essentially by glomerular filtration but at high levels some active tubular transport occurs. Urinary excretion accounts for 76% of daily losses, while gastrointestinal secretions for 16% and hair, nails, sweat and other routes for 8%. Chelation therapy following lead toxicity produces a marked increase in lead excretion. This is accompanied by reversal of the acute morphological effects of lead on proximal renal tubular cells, loss of inclusion bodies from nuclei, and restoration of normal renal cell morphology and function.

The precise toxic level in man isn't really known, however it is reported somewhere between 100-350 μg/dl in blood and urine. The lowest reported lethal dose in man of tetraethyl lead is 1470μg/kg. The lowest toxic dose orally in a mouse of tetraethyl lead is 11 mg/kg compared to tetramethyl lead is 112 mg/kg.

- Rahde, Alberto F. "Lead, Organic." International Programme on Chemical Safety, 1 Dec. 1991. Web. 05 May 2015. http://www.inchem.org/documents/pims/chemical/organlea.htm