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

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

According to Lippincott's Pharmacology, doxorubicin (often referred to by its trade name; Adriamycin) and daunorubicin are classified as anthracycline antibiotics. Anthracyclines have three major activities that may vary with the type of cell; all are maximal in the S and G_2 phases. The drugs insert nonspecifically between adjacent base pairs and bind to the sugar-phosphate backbone of DNA causing a local uncoiling, thus blocking DNA and RNA synthesis. Intercalation can interfere with the topoisomerage II-catalyzed breakage-reunion reaction of DNA strands to cause unreparable breaks. The binding of the cell membrane alters the function of transport processes coupled to phosphatidylinositol activation. The generation of oxygen radicals through lipid peroxidation include Cytochrome P-450 reductase, which is present in the cell nuclear membranes, catalyzes reduction of the anthracyclines to semiquinone free radicals. These in turn reduce molecular O_2, producing superoxide ions and hydrogen peroxide that mediate single strand scission of DNA. Tissues with ample superoxide dismutase (SOD) or

glutathione peroxidase activity are protected. Tumors and the heart are generally low in SOD. In addition, cardiac tissue lacks catalase and thus cannot dispose of hydrogen peroxide. This may explain the cardiotoxicity of anthracyclines. Doxorubicin is one of the most important and widely used anticancer drugs. It is used for treatment of sarcomas and a variety of carcinomas, including breast and lung. The pharmacokinetics; both drugs must be administered intravenously since they are inactivated in the gastrointestinal tract. Extravasation is a serious problem that can lead to tissue necrosis. These drugs bind to plasma proteins as well as to tissues where they are widely distributed. They do not penetrate into the CNS. Both drugs undergo extensive metabolism. The bile is the major route of excretion, and the drug dose must be modified in patients with impaired hepatic function. Some renal excretion also occurs, but the does generally need not be adjusted in patients with renal failure. The drugs impart a red color in the urine. Adverse effects are irreversible, dose-dependent cardiotoxicity, apparently a result of the generation of free radicals, is the most serious adverse reaction. Irradiation of the throax increases the risk of cardiotoxicity. There has been some success with the iron chelator, dexrazone, in protecting against the cardiotoxicity of doxorubicin. Doxorubicin can also cause a transient bone marrow suppression, stomatitis, and GI tract disturbances. Alopecia is usually severe. Harvey, Champe, Mycek. Lippincott's Illustrated Reviews "Pharmacology" 2nd edition. In the review article "Treatment of intermediate stage hepatocellular carcinoma: a review of intrahepatic doxorubicin drug-delivery systems", Doxorubicin (DOX) is the only cytostatic agent that is used in both Lipiodol (LIP) emulsion and DC bead drug delivery system and is the dominating cytotoxic agent for intermediate-stage hepatocellular carcinoma. DOX is an amphiphilic active pharmaceutical ingredient often used as its HCl salt in pharmaceutical formulations. DOX is an anthracycline, antibiotic, antineoplastic drug, which is indicated for multiple forms of cancer. The pharmacological effects of DOX appear to be mediated by at least three antitumor mechanisms: reversible binding to topoisomerare I and II, intercalation to DNA base pairs, and free-radical generation, which causes DNA damage. When DOX is administered its active metabolite DOXol increases the risk of severe side effects such as cardiomyopathy. Dubbelboer IR[1], Lilienberg E, Ahnfelt E, Sjögren E, Axén N, Lennernäs H. "Treatment of intermediate stage hepatocellular carcinoma: a review of intrahepatic doxorubicin drug-delivery systems." Ther Deliv. 2014 Apr;5(4):447-66. doi: 10.4155/tde.14.11.

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) CYP3A4

(b) CYP2C9
(c) CYP1A1
(d) CYP2D6

According to "UNDERSTANDING THE MECHANISM OF CYTOCHROME P450 3A4": by Irina F. Sevrioukova and Thomas L. Poulos; Cytochrome P450 enzymes perform an assortment of modifications on a variety of ligands, utilizing its large active site and its ability to bind more than one substrate at a time to perform complicated chemical alterations in the metabolism of endogenous and exogenous compounds. These include hydroxylation, epoxidation of olefins, aromatic oxidation, heteroatom oxidations, N- and O- dealkylation reactions, aldehyde oxidations, dehydrogenation reactions, and aromatase activity.

Hydroxylation of an sp^3 C-H bond is one of the ways in which CYP3A4 (and cytochrome P450 oxygenases) affects its ligand. In fact, hydroxylation is sometimes followed by dehydrogenation, leading to more complex metabolites. An example of a molecule that undergoes more than one reaction due to CYP3A4 includes tamoxifen, which is hydroxylated to 4-hydroxy-tamoxifen and then dehydrated to 4-hydroxy-tamoxifen quinone methide. Two mechanisms have been proposed as the primary pathway of hydroxylation in P450 enzymes.

CYP3A4 is induced by a wide variety of ligands. These ligands bind to the pregnane X receptor (PXR). The activated PXR complex forms a heterodimer with the retinoid X receptor (RXR), which binds to the XREM region of the *CYP3A4* gene. XREM is a regulatory region of the *CYP3A4* gene, and binding causes a cooperative interaction with proximal promoter regions of the gene, resulting in increased transcription and expression of CYP3A4. Activation of the PXR/RXR heterodimer initiates transcription of the CYP3A4 promoter region and gene. Ligand binding increases when in the presence of CYP3A4 ligands, such as in the presence of aflatoxin B1, M1, and G1. Indeed, due to the enzyme's large and malleable active site, it is possible for the enzyme to bind multiple ligands at once, leading to potentially detrimental side effects.

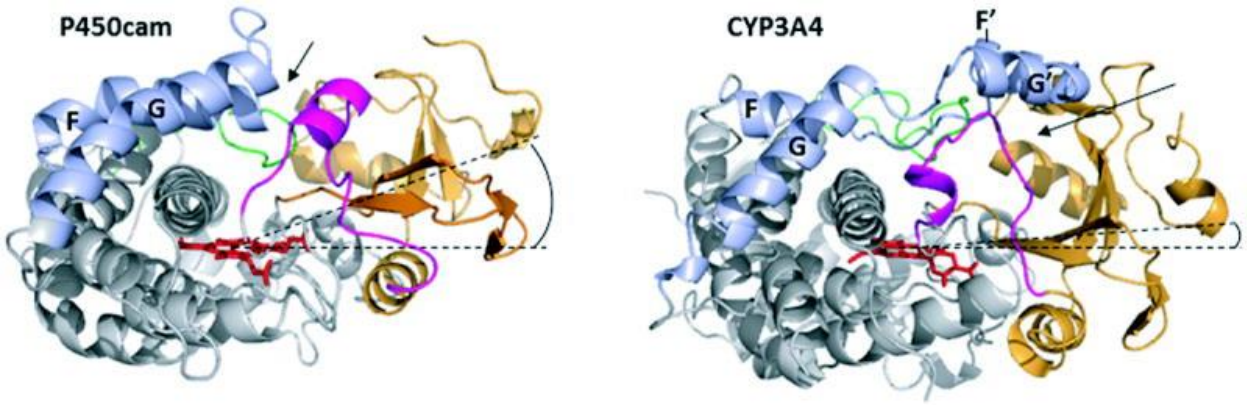
Induction of CYP3A4 has been shown to vary in humans depending on gender. Evidence shows an increased drug clearance by CYP3A4 in women, even when accounting for differences in body weight. A study by Wobold et al. (2003) found that the median CYP3A4 levels measured from surgically removed liver samples of a random sample of women exceeded CYP3A4 levels in the livers of men by 129%. CYP3A4 mRNA transcripts were found in similar proportions, suggesting a pre-translational mechanism for the up-regulation of CYP3A4 in women. The exact cause of this elevated level of enzyme in women is still under speculation, however studies have elucidated other mechanisms (such as CYP3A5 or CYP3A7 compensation for lowered levels of CYP3A4) that affect drug clearance in both men and women (2).

CYP3A4 substrate activation varies amongst different animal species. Certain ligands activate human PXR, which promotes CYP3A4 transcription, while showing no activation in other species. For instance, mouse PXR is not activated by rifampicin and human PXR is not activated by pregnenalone 16α-carbonitrile. In order to facilitate study of CYP3A4 functional pathways *in vivo,* mouse strains have been developed using transgenes in order to produce null/human CYP3A4 and PXR crosses. Although humanized hCYP3A4 mice successfully expressed the

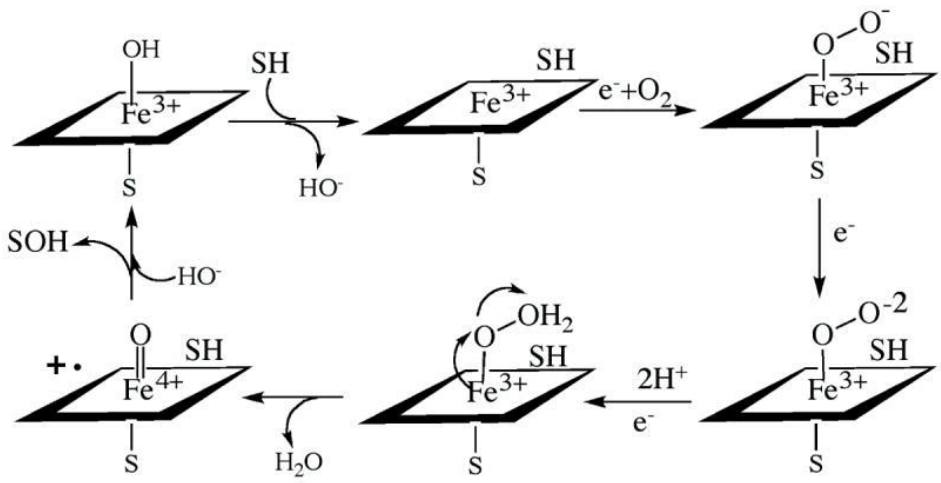
enzyme in their intestinal tract, low levels of hCYP3A4 were found in the liver. This effect has been attributed to CYP3A4 regulation by the growth hormone signal transduction pathway. In addition to providing an *in vivo* model, humanized CYP3A4 mice (hCYP3A4) have been used to further emphasize gender differences in CYP3A4 activity (3).

CYP3A4 activity levels have also been linked to diet and environmental factors, such as duration of exposure to xenobiotic substances. Due to the enzyme's extensive presence in the intestinal mucosa, the enzyme has shown sensitivity to starvation symptoms and is upregulated in defense of adverse effects. Indeed, in fatheaded minnows, unfed female fish were shown to have increased PXR and CYP3A4 expression, and displayed a more pronounced response to xenobiotic factors after exposure after several days of starvation. By studying animal models and keeping in mind the innate differences in CYP3A4 activation, investigators can better predict drug metabolism and side effects in human CYP3A4 pathways (4).

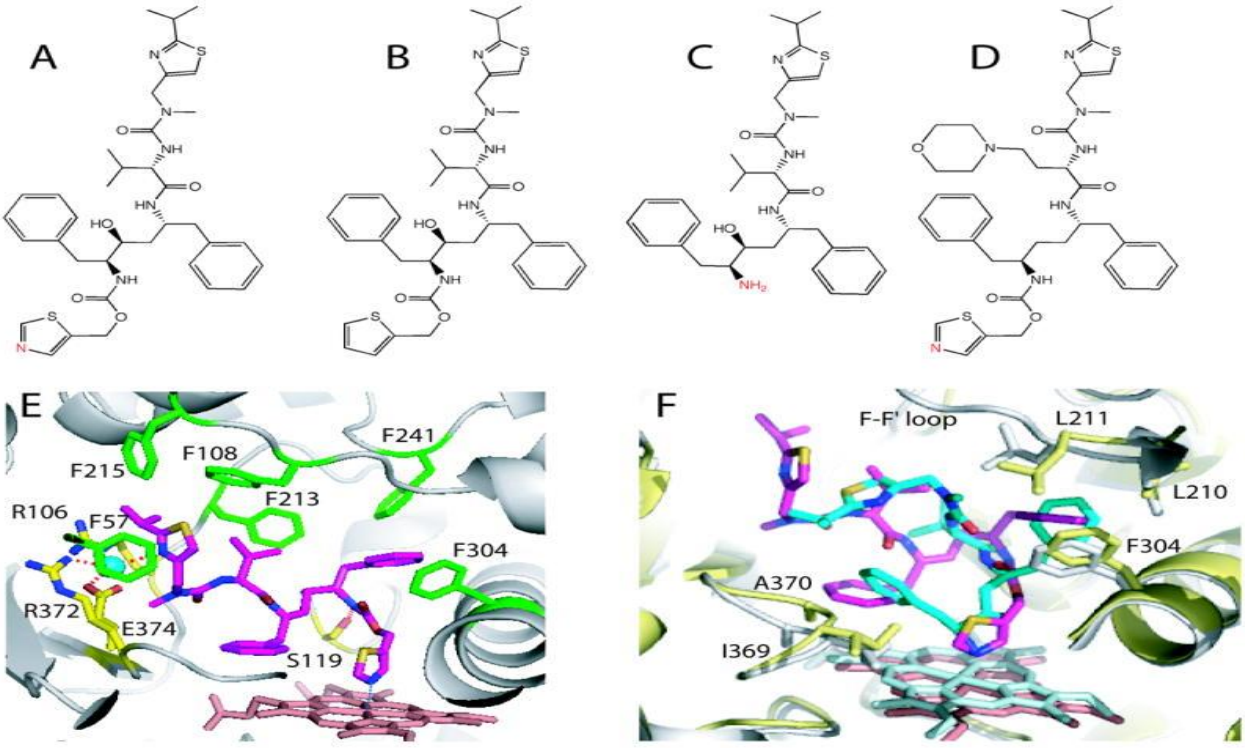
In 1998, various researchers showed that grapefruit juice, and grapefruit in general, is a potent inhibitor of CYP3A4, which can affect the metabolism of a variety of drugs, increasing their bioavailability. In some cases, this can lead to a fatal interaction with drugs like astemizole or terfenadine. The effect of grapefruit juice with regard to drug absorption was originally discovered in 1989. The first published report on grapefruit drug interactions was in 1991 in the Lancet entitled "Interactions of Citrus Juices with Felodipine and Nifedipine" and was the first reported food-drug interaction clinically. The effects of grapefruit last from 3–7 days, with the greatest effects when taken simultaneously with the drug. In addition to grapefruit, other fruits have similar effects. Noni (*M. citrifolia*), for example, is a dietary supplement typically consumed as a juice and also inhibits CYP3A4; pomegranate juice has this effect as well (5),(6).



Comparison of the x-ray structures of soluble bacterial CYP101 (P450cam, PDB ID 1DZ4) and human CYP3A4 (1TQN). The beta-domain is depicted in orange, the B-B' loop and B' helix in magenta, the F, F', G' and G helices and connecting loops in light blue, the C-terminal loop in green, and the heme cofactor in red sticks. Dashed lines pass through the heme plane and the center of the beta-domain. The F'-G' helix/loop insertion in membrane-bound mammalian CYPs shifts the beta-domain toward the heme plane (compare angles between the dashed lines), which opens a channel located between the B-B' loop and the β_1 and β_3 sheets of the beta-domain (shown by an arrow). In contrast, in CYP101 and other soluble P450s substrates access the active site primarily through a channel formed by the F-G loop and B' helix (indicated by an arrow) (1).



Cytochrome P450 catalytic cycle (1).



A-D, Chemical structures of ritonavir, deaza-ritonavir, DTMCR, and cobicistat, respectively. The heme-ligating primary amino group and thiazole nitrogens are shown in red. *E*, Ritonavir (magenta) bound to the active site of CYP3A4 (3NXU structure).Phenylalanine residues surrounding ritonavir are in green, whereas residues comprising the polar 'umbrella', a cluster of charged residues connected to the isopropyl moiety *via* a water molecule (cyan sphere), and the H-bond forming Ser119 are in yellow. *F*, Superposition of the ritonavir-(magenta, pink and gray) and DTMCR-bound (cyan, light cyan and yellow; 3TJS) structures of CYP3A4. To optimize hydrophobic interactions *via* phenyl side groups, DTMCR rotates by 180° relative to ritonavir. Since DTMCR is shorter than ritonavir, it cannot interact with the polar 'umbrella' and F-F'

loop. As a result, the F-F' loop becomes disordered and the active site is solvent accessible in the DTMCR-bound structure. Also, DTMCR has differently oriented phenyl groups and does not clash with the 369-370 peptide. This is in contrast to the CYP3A4-ritonavir complex, where the heme shifts downwards and the Fe-N bond is slightly elongated because of steric hindrance with the 369-370 peptide (1).

1. Irina F. Sevrioukova and Thomas L. Poulos. "UNDERSTANDING THE MECHANISM OF CYTOCHROME P450 3A4: RECENT ADVANCES AND REMAINING PROBLEMS."Dalton Trans. 2013 March 7; 42(9): 3116–3126. doi:10.1039/c2dt31833d

2. Wolbold R[1], Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, Schwab M, Zanger UM. Sex is a major determinant of CYP3A4 expression in human liver. Hepatology. 2003 Oct;38(4):978-88.

3. Gonzalez FJ. "CYP3A4 and pregnane X receptor humanized mice". J Biochem Mol Toxicol. 2007;21(4):158-62.

4. Crago J[1], Klaper RD. "Influence of gender, feeding regimen, and exposure duration on gene expression associated with xenobiotic metabolism in fathead minnows (Pimephales promelas)". Comp Biochem Physiol C Toxicol Pharmacol. 2011 Sep;154(3):208-12. doi: 10.1016/j.cbpc.2011.05.016. Epub 2011 Jun 2.

5. Muneaki Hidaka, Manabu Okumura, Ken-ichi Fujita, Tetsuya Ogikubo, Keishi Yamasaki, Tomomi Iwakiri, Nao Setoguchi and Kazuhiko Arimori. "EFFECTS OF POMEGRANATE JUICE ON HUMAN CYTOCHROME P450 3A (CYP3A) AND CARBAMAZEPINE PHARMACOKINETICS IN RATS". January 26, 2005, doi: 10.1124/dmd.104.002824 *DMD May 2005 vol. 33 no. 5 644-648.*
6. Bailey DG[1], Dresser GK. "Interactions between grapefruit juice and cardiovascular drugs". Am J Cardiovasc Drugs. 2004;4(5):281-97.

3. Select (a) <u>or</u> (b) <u>or</u> (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?

In the article μ-Opioid Receptor-Mediated Antinociceptive Responses Differ in Men and Women, they performed a study to test the pain threshold between men and women. They had a total of 28 volunteers; 14 men and 14 women between the age 20 and 30. They were all right handed, nonsmokers who had no history of medical illness, psychiatric illness or substance abuse. The women were not on any form of birth control for the past 6 months and have a normal/regular menstrual cycle. They screened for follicular phase in their menstrual cycle to assert the levels of estradiol and progesterone where low. There were two groups in the study; one half received pain first and one half received a saline solution. A steady muscle pain was applied to the masseter muscle. This was a model of sustained deep somatic pain, the intensity of the painful stimulus was standardized across the subjects. After a brief standard 15 second bolus was administered for an electronic version of a visual analog scale to rate the pain intensity every 15 secs, then sends a signal to the computer to record information. A PET scan and MRI were also performed on each volunteer to visualize the effects of pain on the brain between each sex. The areas of the brain that perceived pain in both sexes include; anterior thalamus, contralateral amygdala, ipsilateral ventral pallidum/substantia innominate, and contralateral anterior insular cortex. It showed that the pain tolerance in women was higher than in males. All areas of the brain listed above were triggered in both sexes, however, there were increased pain tolerance in these areas for women than in the men. The article reference a study done on male and female rodents also correlated with the research done on humans.

Jon-Kar Zubieta, Yolanda R. Smith, Joshua A. Bueller, Yanjun Xu, Michael R. Kilbourn, Douglas M. Jewett, Charles R. Meyer, Robert A. Koeppe, and Christian S. Stohler. "μ-Opioid Receptor-Mediated Antinociceptive Responses Differ in Men and Women." The Journal of Neuroscience, 15 June 2002, 22(12): 5100-5107