

Pathology 438
Spring 2015
NAME _____

Midterm Examination

due: by 1:00 PM, 6 May 2015

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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

SCORING

PK: A/D=4 M=6 E=4

PD: 10

Ref: 5 T=29

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
 - 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

SCORING

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 4 REF=5 T=27

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 that 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?

SCORING

Content = 20 REF= 5 T=25

GRAND TOTAL = 29 + 27 + 25 = 81

1. Select one of the substances below: 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.

VALPORIC ACID (VPA): Valporic acid is a short chain fatty acid that is derived from the natural occurring substance valeric acid and is mostly used for the treatment of epilepsy and seizures. 87-95% of VPA is bound to protein which gives it a low clearance rate according to Leppik and Birnbaum (1).

There are three pathways of VPA metabolism that have been identified in humans which include glucuronidation, beta oxidation, and cytochrome P450 mediated oxidation. As previously stated, VPA is a fatty acid so it can be metabolized in the mitochondria which is illustrated wonderfully in figure 1 in the article by Ghodke-Puranik, Thorn, Lamda, Leeder, Song, Birnbaum, Altman and Klein (2).

VPA can act on the gamma amino butyric acid aka GABA levels in the brain, it can block voltage-gated ion channels, and can also act as an HDAC inhibitor which is a new exciting revelation according to Ghodke-Puranik and associates in there article on the pharmodynamics and pharmokinetics of VPA (2).

If the GABA inhibitory mechanism is impaired or malfunctioning this can lead to convulsions and seizures. This of course, is exactly why efforts to control this pathway through anti epileptic drugs has been such a priority. VPA may also have anti epileptic capabilities by blocking voltage-gated sodium, potassium, and calcium channels which would reduce the high frequency firing of neurons and thus decrease seizures which was highlighted in the review by Johannessen in 2003 (3). VPA is currently being looked into for its anti tumor properties as well with its ability to inhibitor of HDAC1.

References

1. Review: Epilepsy in the elderly.
Leppik IE, Birnbaum AK
Ann N Y Acad Sci. 2010 Jan; 1184(1):208-24. PK: AD=3 M=5 E=2
PD: 10
Ref: 5 T=25
2. Valproic acid pathway: pharmacokinetics and pharmacodynamics
Yogita Ghodke-Puranik, Caroline F. Thorn, Jatinder K. Lamba, J. Steven Leeder, Wen Song, Angela K. Birnbaum, Russ B. Altman and Teri E. Klein
3. Review: Valproate: past, present, and future.
Johannessen CU, Johannessen SI
CNS Drug Rev. 2003 Summer; 9(2):199-216.

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CYP1A1 (i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 2
REF=4 T=24

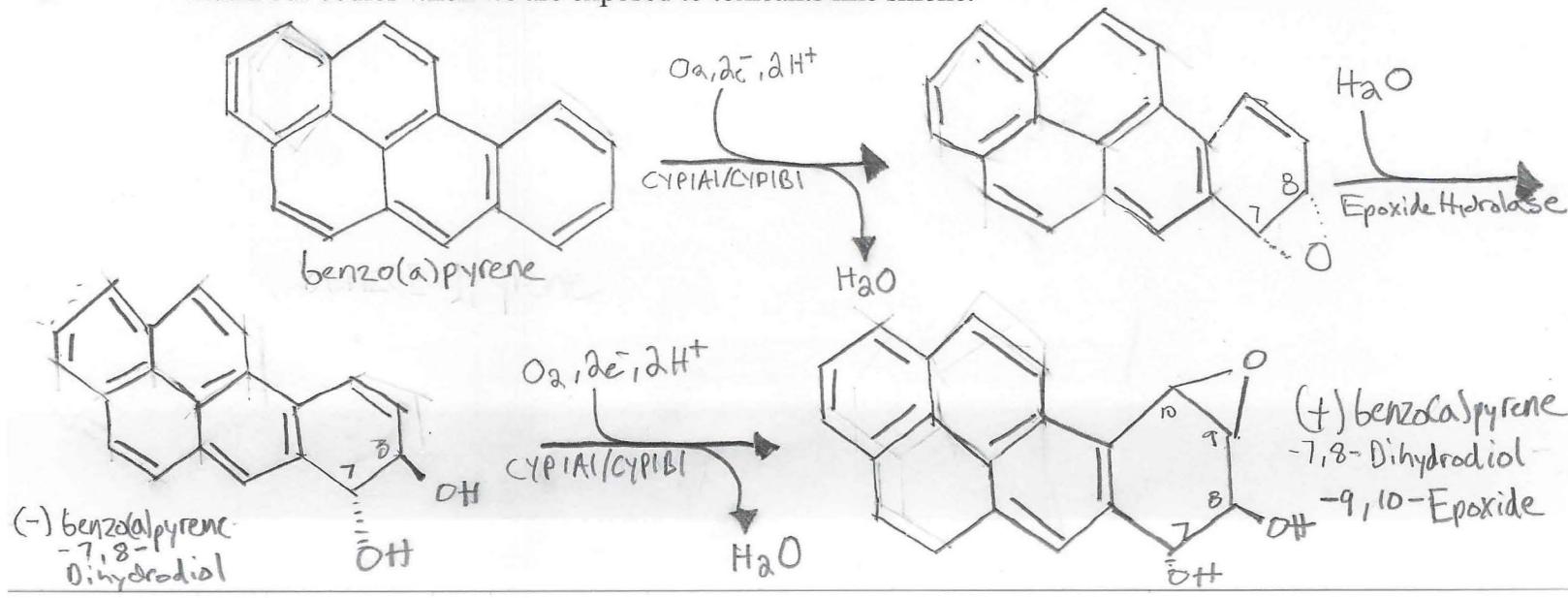
- i. Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize.

According to Walsh, Scklarz, and Scott CYP1A1 is an extrahepatic monooxygenase that is involved in the metabolism of drugs and endogenous substrates but it also is responsible for the activation of certain toxins and environmental pollutants. Some of these substrates include arachidonic acid, eicosapentoic acid, estradiol, and melatonin. CYP1A1 detoxifies polycyclic aromatic compounds. While CYP1A1 is responsible for the metabolism of these substrates it is more well known for being one of the most important enzymes in bioactivation of procarcinogens to generate reactive metabolites. CYP1A1 is also the primary cytochrome P450 enzyme that activates benzopyrene which is highlighted in my drawing below under section 2.

I found it very interesting that CYP1A1 is the main microsomal enzyme responsible for activation of polycyclic aromatic hydrocarbons which is what happens when you smoke, eat charbroiled meat, or are exposed to smog or exhaust. CYP1A1 has been discovered to play a significant role in this carcinogenic process.

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

According to the text, Cytochromes P450 Metabolic and Toxicological Aspects edited by Ioannides, CYP1A1 is an inducible enzyme which is upregulated by the aryl hydrocarbon receptor. In humans it can be induced by consuming charbroiled meat or vegetables or by smoking cigarettes or being exposed to too much car exhaust. Below is a drawing of how CYP1A1 is involved in the metabolism of benzoapyrene which then yields the carcinogenic benzoapyrene-7,9-dihydrodiol-9,10-epoxide. This is the same carcinogenic that is taking place within our bodies when we are exposed to toxicants like smoke.



- iii. Provide the names of any substances known to inhibit the cytochrome, if any
Dietary Flavanoids, fluoroquinolones, Ellipticine and macrolides are all inhibitory
and oxidative stress can also down regulate CYP1A1.
- 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

Walsh, Scklarz, and Scott described the structure of CYP1A1 as a 2.6 degree alpha structure with the inhibitor alpha naphthoflavone (ANF). They go on to describe the binding of ANF at an enclosed active site, and these active sites have distinct features that may underlie the functional variability of these enzymes. They also note a 5 residue disruption of the F helix but I was not able to really understand what they were talking about here but it was deemed significant by Walsh and colleagues.

- v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc

I imagine if you continue to be exposed to toxic substances like cigarette smoke, smog, exhaust and charbroiled foods then the turnover rate would continually increase. On the other hand if you reduce your exposure to these toxins then you can greatly reduce your risk of developing cancer in the future. I do not believe I am fully grasping the enzymes effects or kinetic parameters but I did take away a good bit of information from this midterm that I can research further and apply in my practice.

References

1. Human Cytochrome P450 1A1 Structure and Utility in Understanding Drug and Xenobiotic Metabolism. Agnes A. Walsh, Grazyna D. Szklarz, and Emily E. Scott
First Published on March 18, 2013, doi: 10.1074/jbc.M113.452953
May 3, 2013 The Journal of Biological Chemistry, 288, 12932-12943.
2. Cytochromes P450 Metabolic and Toxicological Aspects, Edited by Costas Ioannides
- 3.

- 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.

Sex, Drugs, and Cognition: Effects of Marijuana
Beth M. Anderson, Ph.D., Matthew Rizzo, M.D., Robert I. Block, Ph.D., Godfrey D. Pearlson, M.D., and Daniel S. O'Leary, Ph.D.
Journal Psychoactive Drugs. 2010 dec;42(4):413-24

The aim of this study was to compare the effects of marijuana on cognition, specifically the differences in cognition between men and women after smoking marijuana. The authors talk

about the fact that there is knowledge that many drugs affect men and women differently, however there are few studies that look at effect of marijuana use on cognition in women. This is one of the reasons the authors chose this study, they wanted to examine the sex differences in the acute effects of marijuana on cognition in 35 males and 35 females who were occasional marijuana users.

Participants for this study were recruited by fliers and by word of mouth in the Iowa City area. I thought it was interesting that they excluded non marijuana users from the study for fear that it may be “the gateway drug,” even though I have seen no research substantiating this claim. The participants were asked to smoke marijuana cigarettes where they were instructed to inhale for 3 seconds, hold their breath for 5 seconds, and give 27 seconds between inhalations. The participants were told to continue this procedure until they finished the marijuana cigarette or until they reached an “uncomfortable” level of highness. This brings up one problem with this study. Not everyone consumed the same amount of marijuana due to the choice to stop the smoking session early if they felt too high. This is an interesting fact to keep in mind because one thing they did find linked to females over males, was the urge to stop the smoking session earlier which could have tainted there results. The study shows that 0% of the men requested to discontinue the active cigarette while 44.4% of the women chose to discontinue the cigarette. This is a big difference and I think the study needs to be conducted again with no option to discontinue use.

The marijuana cigarettes used in this study were provided by the national institute of drug abuse and were given in between subjects, with sex, randomized, double blinded, design. There were two types of cigarettes, one with no THC and one with 2.9% THC. I do like how they used a control group with no THC but I think 2.9% THC is too low and they should have used a THC content closer to 10%. The study had the patients smoke, wait 30 minutes and then perform a variety of tests which included visuospatial processing, trail making, time estimation, and cognitive flexibility which involved task switching. One important and interesting bit of information I found in this article was that there were no differences in heart rate, or cognitive test performance found in those that completed the marijuana cigarette versus those that did not complete it and there were also no sex differences in heart rate found at any time. One interesting fact reported in the article was the fact that women reported more sleepiness than men before cognitive testing but no differences were found at any other point. The final interesting piece I took away was on completion time of tasks. When you asked the control group how long it took them to complete the tasks they underestimated the time compared to the group that smoke the marijuana cigarette who overestimated the time it took for completion.

In conclusion the study found that marijuana did have an impact on selective attention, divided attention, cognitive flexibility, and time estimation. The study found no sex by drug interactions for cognitive testing, but, psychological response to the smoking session was remarkable in that the women wanted to discontinue use at such a greater rate than men. One opportunity for future testing on marijuanas effect on different sexes could be on cognitive flexibility since this study found women that smoked the marijuana cigarette had a slower reaction time to task switching.

Report=20 Ref=5
T=25

Shepherd TOTAL
=25+24+25 = 74

Pathology 438

Spring 2015

NAME Christina Lowenthal

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 - 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 G₂ 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 topoisomerase 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 O₂, producing superoxide ions and hydrogen peroxide that mediate single strand scission of DNA. Tissues with ample superoxide dismutase (SOD) or

catalase probably
in peroxisomes
of human
cardiomyocytes

PK: AD=3 M=4 E=4

PD: 9

Ref: 4

T=24

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, dextrazone, 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 topoisomerase 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 IR¹, 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 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
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(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.

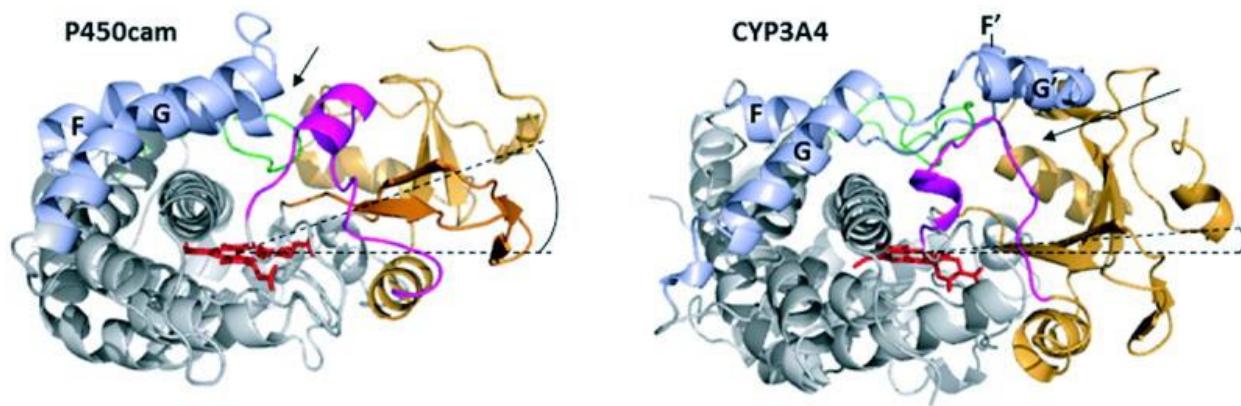
Induction of CYP3A4 has been shown to vary in humans depending on gender. Evidence shows an increased drug clearance by CYP3A4 in women, even when accounting for differences in body weight. A study by Wobold et al. (2003) found that the median CYP3A4 levels measured from surgically removed liver samples 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 16 α -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

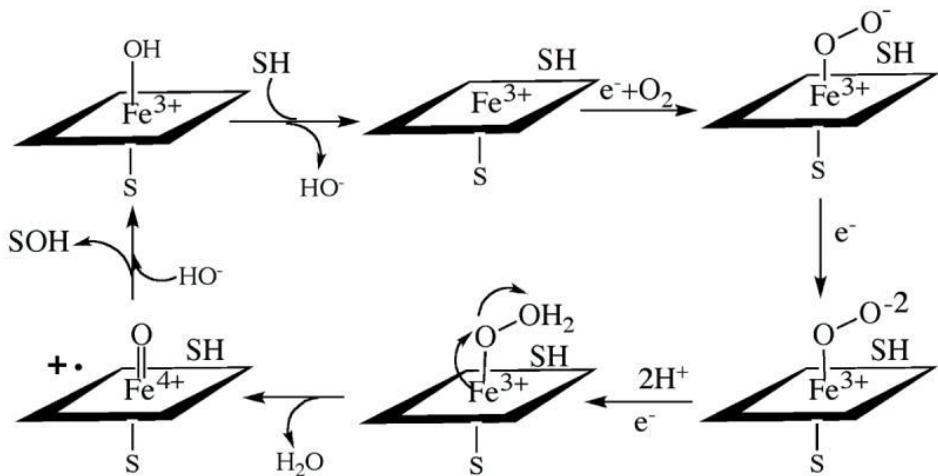
enzyme in their intestinal tract, low levels of hCYP3A4 were found in the liver. This effect has been attributed to CYP3A4 regulation by the growth hormone signal transduction pathway. In addition to providing an *in vivo* model, humanized CYP3A4 mice (hCYP3A4) have been used to further emphasize gender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

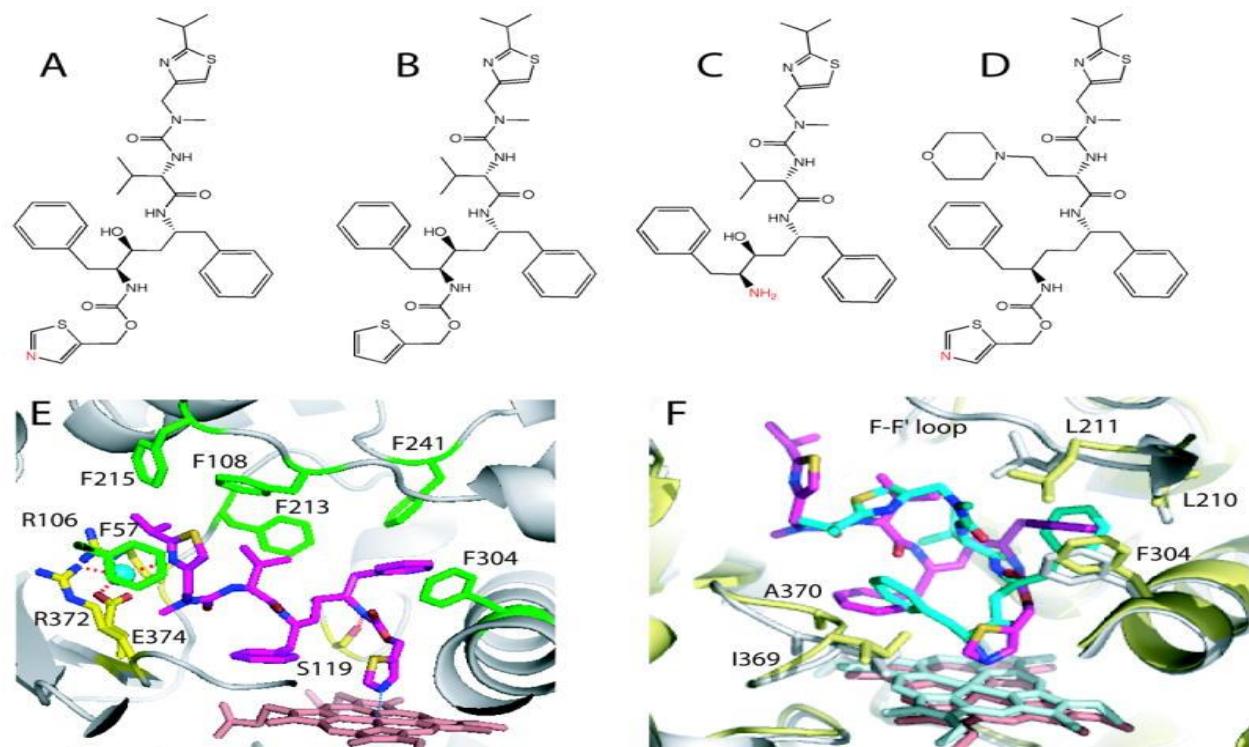
In 1998, various researchers showed that grapefruit juice, and grapefruit in general, is a potent inhibitor of CYP3A4, which can affect the metabolism of a variety of drugs, increasing their bioavailability. In some cases, this can lead to a fatal interaction with drugs like astemizole or terfenadine. 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.

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 R¹, 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 J¹, 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 DG¹, Dresser GK. "Interactions between grapefruit juice and cardiovascular drugs". Am J Cardiovasc Drugs. 2004;4(5):281-97.

(i) 2 (ii) 2 (iii) 2 (iv) 4 (v) 2
REF = 5 T = 17

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 that 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. Koepp, 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

Report=13 Ref=5
T=18

Pathology 438

Midterm Examination

due: by 1:00 PM, 6 May 2015

Spring 2015

NAME: Erick Dobrzynski

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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: Is thought to inhibit DNA synthesis causing cell death.

Distribution:

Doxorubicin is measured to be 90% liposomally encapsulated, after being steadily administered over an extended period of time. The small steady state volume of distribution suggests that Doxorubicin is confined to the vascular fluid. Doxorubicin gets into the vascular system after it becomes activated, this happens when the liposomes become extravasated.

Metabolism:

It is estimated that 50% of the Doxorubin exits the body unchanged. There are 3 ways Doxorubicin is metabolized; one-electron reduction, two-electron reduction and deglycosidation. Doxorubicin enters the cell with a carnitine transporter. It then is metabolized further through dehydrogenase enzymes and reduction enzymes. After these enzymes react with Doxorubicin, it is broken down into Doxorubicin semiquinone, Doxorubicin Deoxyaglycone, Doxorubicin hydroxyaglycone, Doxorubicinol. The remaining Doxorubicin molecule is than bound to an

active transporter protein molecule and proceeds to inhibit the DNA synthesis in the nucleus of the cell.

Elimination

40% of the Doxorubicin appears in bile after 5 days, and only 5-12% of Doxorubicin appears in urine. Elimination of Doxorubicin is decreased in obese patients and decreased to a greater degree in obese women. This decreased elimination in obese patients suggests that some Doxorubicin stays in the liposomes and is not extravasated as well in obese patients.

Pharmacodynamics:

Doxorubicin is known to have a cardiotoxic effect after administration. Studies have been done on rats, Ca²⁺-ATPase was used as a biological marker to measure cardiotoxicity. By using the drug with multiple doses studies have found that plasma concentration of Doxorubicin is higher and will hopefully help eliminate cancer.

PK: AD=3 M=6 E=4

<https://www.doxil.com/shared/product/doxil/prescribing-information.pdf>

PD: 9

<https://www.pharmgkb.org/pathway/PA165292177#>

Ref: 5

T = 27

<http://www.drugs.com/pro/doxorubicin.html>

Cheng, Rong C. "Total Flavonoids from Clinopodium Chinense (Benth.) O. Ktze Protect against Doxorubicin-Induced Cardiotoxicity In Vitro and In Vivo." Evidence-Based Complementary and Alternative Medicine (2015): n. pag. PubMed. Web. 3 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

CYP3A4

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

The purpose of Cytochrome P450 3A4 is to oxidize small foreign organic molecules such as xenobiotics to be removed from the body. CYP 3A4 belongs to a class of heme containing mono-oxygenase enzymes. Cytochrome P450 3A4 is responsible for metabolizing nearly 50% of clinical drugs. CYP3A4 is significantly involved in metabolizing cyclic peptide cyclosporine A and macrolide antibiotics. CYP3A4 does not fit the Machelis-Menton type kinetics and has been shown to bind more than one substrate during in-vivo and in-vitro studies. CYP3A4 modifies the substrate through hydroxylation, aromatic oxidation, heteroatom oxidation, and dealkylation reaction processes.

Fa, Batao. "Pi-pi Stacking Mediated Cooperative Mechanism for Human Cytochrome P450 3A4." Molecules (2015): 7558-573. Web. 4 May 2015.

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

First CYP3A4 forms a strong hydrogen bond with an amino acid in its structure. The hydrogen bond along with hydrophobic interactions of the molecules allows the CYP3A4 enzyme to position itself in an optimal position for binding the substrate molecule and facilitating the reaction.

Fa, Batao. "Pi-pi Stacking Mediated Cooperative Mechanism for Human Cytochrome P450 3A4." *Molecules* (2015): 7558-573. Web. 4 May 2015.

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

Studies have shown that grapefruit juice is an inhibitor of the CYP3A4 enzyme. Grapefruit juice is known to inhibit first pass enzymes, CYP3A4 is an enzyme that breaks down most substrates on the first pass.

Bressler R (November 2006). "Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs". *Geriatrics* **61** (11): 12–8. PMID 17112309.

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

The CYP3A4 molecule is divided up into 13 exons and 12 introns. GT and AG were found at the boundaries of all introns. Arg372 formed a strong hydrogen bond and hydrophobic forces were present in order to put the substrate in an optimal binding site for further metabolism.

Fa, Batao. "Pi-pi Stacking Mediated Cooperative Mechanism for Human Cytochrome P450 3A4." *Molecules* (2015): 7558-573. Web. 4 May 2015.

Hashimoto, Hasashi. "Gene Structure of CYP3A4, an Adult-specific Form of Cytochrome P450 in Human Livers, and Its Transcriptional Control." *European Journal of Biochemistry* 218.2 (1993): 585-95. Web.

v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc

CYP3A4 has the highest rate of catalysis among all cytochrome p450 enzymes. CYP3A4 has shown in studies that it requires a phenolic group for ortho hydroxylation of estradiol and mono-O-demethylated methoxychlor. I don't quite understand this chemistry but it was the best that I could find.

Stresser, David M., and David Kupfer. "Catalytic Characteristics of CYP3A4: Requirement for a Phenolic Function in Ortho Hydroxylation of Estradiol and Mono-O-demethylated Methoxychlor." *Biochemistry* (1997): 2203-210. ACS Publications. Web.

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 4

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

REF = 5 T = 27

- 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.

This was a study done on men and women and compared brain activity after using alcohol, cocaine, and both alcohol and cocaine. PET scans of the brain were done on patients that participated in the study.

The results showed that men and women responded similarly to alcohol consumption. The participants scored similarly. It was noted that women had a greater increase in heart rate than men did.

The second part of the study recorded how women responded to both alcohol and cocaine at the same time. The doses were delivered at 30 minute intervals for an 8 hour period. Both men and women responded similarly to the combination of alcohol and cocaine.

When testing Cocaine consumption alone, the study showed that women had a greater response to the cocaine by itself than the men did. They were observed to have a greater feel good rating 36-54, and the men in the study had a feel good rating of 20-34. This shows that women metabolize cocaine different than men do. It also provides evidence to the high amount of female deaths that reveal traces of cocaine in the autopsy report.

This study suggests that drugs should be studied on both men and women and data collected cannot be generalized for both men and women. Discovering different metabolic pathways for men and women will improve effectiveness of treatments for pathologies and will benefit human kind as a whole.

Report=20 Ref=5 T=25

http://archives.drugabuse.gov/NIDA_Notes/NNVol20N6/Drugs.html

Question 1) a:doxorubicin

Doxorubicin is an anthracycline and normally this drug used for treatment of different cancers including breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma. One very important restriction for this one is for cardiotoxicology. Unfortunately this drug is anti cancer and also has cardiotoxicity as well, if we know better information about the pharmacogenomics of usage of this drug we can use it in lower dose for cardiotoxicology and better result for anticancer.

"doxorubicin the cancer cell do intercalation into DNA and interference of topoisomerase-II-mediated DNA repair and creation of free radicals and their loss to cellular membranes, DNA and proteins . doxorubicin is oxidized to semi Quinone, an uneven metabolite, which is converted back to doxorubicin in a process that releases reactive oxygen species. Reactive oxygen species can lead to lipid peroxidation and membrane damage, DNA damage, oxidative stress, and triggers apoptotic pathways of cell death."

Doxrubicinol is made when doxorubicin is decreased.Metabolism of doxorubicin inside the mitochondria can interrupt and block any respiration that can finally leads to the release of cytochrome-C initiating apoptosis.

impounding of iron blocking free radical formation can affects mechanism of the action of dextrazoxane protection against cardiotoxicity .

the most noticeable result of drug–drug interactions causing to in the cardiotoxicity from cotreatment and coeffecton with doxorubicin and trastuzumab or taxanes such as paclitaxel and docetaxel. No using and Without this endogenous cardioprotection, doxorubicin treatment can be more distructed and bad and damaging .The interaction with taxanes is through a different mechanism.the way using and helpful for the taxane-is try to increase in cardiotoxicity is by increased making of doxorubicin, by the changing and making of the catalytic activity of aldehyde reductase.

"doxorubicin mechanism many of the genes that modulate the doxorubicin response. However, PGx studies that implicate variants in these genes are still in their infancy. As with many antineoplastic drugs, the PGx can be complicated by combined treatments. However, there are clear benefits for identifying individuals at risk for toxicity and response".

this drug has a very wide antitumor spectrum, compared with other anticancer drugs; however, just not for Hodgkin's disease, it is not associated and related with therapeutic chemotherapy. Doxorbucin used several years but now and only recently, recognized that the cytotoxic effect and result is produced at the cellular level by multiple mechanisms which have not yet been finally well-known.

Important thing over here are a combination of doxorubicin-induced free radical formation and this is because of metabolic activation, toxic actions at the level of the membrane, and drug-intercalation into DNA.

The problem is clinician found a lot of concentration of doxorubicin in hematopoietic cells and other tissues.

PK: AD=2 M=6 E=1

PD: 10

Ref: 5

T=24

Question 2

Cyp2d6

1) When we want to talk specifically about this one, this is shown in researchers that display genetic polymorphism. Metabolization of the substrate in low limit can result from mutation in Cyp2d6. You can find this gene in liver or CNS in substantia nigra.

"some individual for example obtain no benefit from the opioid analgesic codeine, because they lack cyp2d6 enzyme that o-demethylates and activates the drug"

2) in first step tamoxifen change to cyp3a4 AND cyp3a5 and in other hand tamoxifen change to cyp2d6 to 4-OH-TAMOXIFEN and next step the third step is goes from here and cyp3a4 and cyp3a5 goes to 4-OH-DES METHYL-TAMOXIFEN and in other hand from step a from cypa4 and cyp3a5 goea to N-desmethyl-tamoxifen and then by step D cyp2d6 goes to 4-ch-n-desmethyl-tamoxifen , we have this one from step c and d together and its both from the affect of cyp2d6 from step b and step d

3) omeperazol, erythromycin, ketoconazole, ritonavir.

4) This quality encodes an individual from the cytochrome P450 big groups of chemicals enzyme. This one can catalyze a lot of responses and they are monooxygenases and have include in for drug for digestive system and making cholesterol and steroids and some lipids .This protein limits to the endoplasmic reticulum and is referred to metabolize to approximatly of 20% of regularly recommended medications. This gene is really has high number in polymorphic in the world. Some special alleles has bad metabolize phenotype and this is their characteristic to reduce the quality of metabolism of the enzyme substrate.

(i)4 (ii)2 (iii)4 (iv)3 (v)1
REF=4 T=17

Question 3 part c)

Aminoglycosides used for along period of time one of the most reason for drug induced nephrotoxicity. Aminoglycosides increased nephrotoxicity but this effects is as nonoliguric renal failure. This is also has an small and slow increase in the serum creatinine and hypoosmolar urinary output developing even after a period of time from treatment.

Aminoglycosides is highly nephrotoxic because a alittle dose of this drug is hold inside of the epithelial cells lining of the S1 and S2 segments of the proximal tubules and then after glomerular filtration. Aminoglycosides gatherd inside of epithelial cells are really in one special places with endosomal and lysosomal vacuoles but they are also located with the Golgi complex .

aminoglycosides has a noticeable changes in lysosomes of proximal tubular cells steady with the buildup of polar lipids .These changes has a result of tubular dysfunction or changes which can decreased reabsorption of the protein,k and mg and ca and glucose when these happens in the human body it will result as renal failure mostly because of nonoliguric or deacrese of polyuric hypoosmotic in creatinine clearance.

When we use very high dose in animal very fast affected to increased cortical necrosis and overt renal dysfunction. one of the most important affect is for the primary steps for uptaking in proximal tubular cells. Although several effects like decreasing of protein synthesis and modulation of gene expression and changing

in mitochondrial .

Reseaches proved that the first and most important cause of functional toxicity is tubular necrosis. aminglycosides toxicity is mostly because of local concentration. This is because of lysosomal change as a most imporant reason for toxicity. Actually all these are still hypothesis for the cell damage and just some has laboratory result finding but it still strongly mentioned for renal impairment.

While the determinants of cell damage still remain undefined, more knowledge concerning the mechanisms causing the impairment of the renal function is available. It causes the renin angiotensin system to work again and then vasoconstriction comes up for deacreasing in glomerular filtration part.we can understand explanation of when this drug reduced production of vasodilatory prostaglandis pge2 , how does it works for aggravating effect of nonsteroidal anti inflammatory drugs on aminoglycoside nephrotoxicity. An increase in proximal intratubular and some pressure of single nephrons, can be related to necrotic obstruction, reducing of glomerular filtration has a multifactorial origin and involves a mixture of tubular and nontubular mechanisms. The hypo osmotic polyuria, typical of the aminoglycoside toxicity has some result from the reducing fluid reabsorption by proximal tubules, secondary to an impaired solute reabsorption.

Human body kidney has a large capacity to compensate for tubular insults so that an ongoing cell death process may long remain undetected by functional explorations.

“Decreasing or preventing aminoglycoside accumulation by the kidneys would represent one of the most simple and radical approaches to reduce aminoglycoside nephrotoxicity, since it should goes to success whatever the targets of aminoglycosides are in the kidney. Aminoglycoside build up has to decreased either by impairing their uptake or by increasing their release.

Reducing and decreasing of uptake has been obtained by two strategies. The first one is goes at complexing the aminoglycosides extracellularly, and the second one is at competing with or decreasing drug binding to the brush-border membrane. An explanation for this unexpected behavior came from the finding that aminoglycoside uptake by kidney tubular cells is saturable ,so that much of the drug that passes in the lumen will not be reabsorbed if the drug is too concentrated. Because saturation was shown to occur at a clinically meaningful range of concentrations, this observation triggered a large number of studies comparing the toxicities of various drug administration schedules.”

between the various approaches applicable to the presently available aminoglycosides, onlyjust one dose a day is good and successfully to the clinic. Other protective approaches such as the coadministration of polyaspartic acid or deferoxamine deserve preclinical and clinical development, and many more could certainly be explored. Recently discover and improvement in molecular modeling and an improved knowledge of the meaningful differences in structure-activity and structure-toxicity relationships for aminoglycosides could also bring us,intrinsically less toxic aminoglycosides.

1. Lal S, Mahajan A, Chen WN, Chowbay B. Pharmacogenetics of target genes across doxorubicin disposition pathway: a review. *Curr Drug Metab.* 2010;11:115–128. [\[PubMed\]](#)
 2. Simunek T, Sterba M, Popelova O, Adamcova M, Hrdina R, Gersl V. Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacol Rep.* 2009;61:154–171. [\[PubMed\]](#)
 3. Mordente A, Meucci E, Silvestrini A, Martorana GE, Giardina B. New developments in anthracycline-induced cardiotoxicity. *Curr Med Chem.* 2009;16:1656–1672. [\[PubMed\]](#)
 4. Ademuyiwa O., Ngaha E. O., Ubah F. O. (1990) Vitamin E and selenium in gentamicin nephrotoxicity. *Hum. Exp. Toxicol.* 9:281–288. [Medline](#)[Google Scholar](#)
 5. Ali M. Z., Goetz M. B. (1997) A meta-analysis of the relative efficacy and toxicity of single daily dosing versus multiple daily dosing of aminoglycosides. *Clin. Infect. Dis.* 24:796–809.
 6. Appelkvist E. L., Soderstrom M., Nassberger L., Damberg C., Dallner G., DePierre J. W. (1991) Characterization of the lipid and protein contents of myelin bodies isolated from the renal cortex of gentamicin-treated rats. *Biochem. Biophys. Res. Commun.* 181:894–901. [CrossRef](#)[Medline](#)[Google Scholar](#)
 7. Assael B. M., Chiabrandi C., Gagliardi L., Noseda A., Bamonte F., Salmona M. (1985) Prostaglandins and aminoglycoside nephrotoxicity. *Toxicol. Appl. Pharmacol.* 78:386–394. [CrossRef](#)[Medline](#)[Google Scholar](#)
 8. lippincott's pharmacology ,5th edition
- .

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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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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 t_{max} 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 t_{max} 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. It 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>](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. 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**

- 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>>. (i)6 (ii)4 (iii)4 (iv)4 (v)4
REF=5 T=27

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 that 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.

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Pathology 438
Spring 2015

Midterm Examination

due: by 1:00 PM, 6 May 2015

NAME _Jereme Sommers_____

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

Valproic Acid

Valproic acid is used as an anticonvulsant for epilepsy patients primarily. It has a very narrow therapeutic index (TI) of 50-125 µg/mL. Toxicity > 150 µg/mL is due to metabolites mostly from beta oxidation. These metabolites cause death of hepatocytes which can be detected by increased GSH.

Absorption: Valproic acid is administered orally and is rapidly absorbed in the GI tract. It is absorbed at an estimated rate of 100% and fully absorbed within 4 to 8 hours depending on what form it is taken in.

Distribution: It is highly protein bound (87-95%) and can cross the mitochondrial membrane via carnitine. It also appears in the breast milk and can cross the placenta which poses a concern for pregnant and breastfeeding mothers as it would be dangerous for the child.

Metabolism: There are at least 3 routes of absorption in humans: Glucuronidation (50%), beta oxidation in the mitochondria (40%) and cytochrome P450 mediated oxidation (10%)

Elimination: Plasma elimination half-life is 10-16 hours but can be as short as 6-8 hours when used with other antiepileptic drugs. This necessitates careful monitoring especially when pairing valproic acid with other medications.

PK: AD=4 M=4 E=4

PD: 4

Ref: 5

T=21

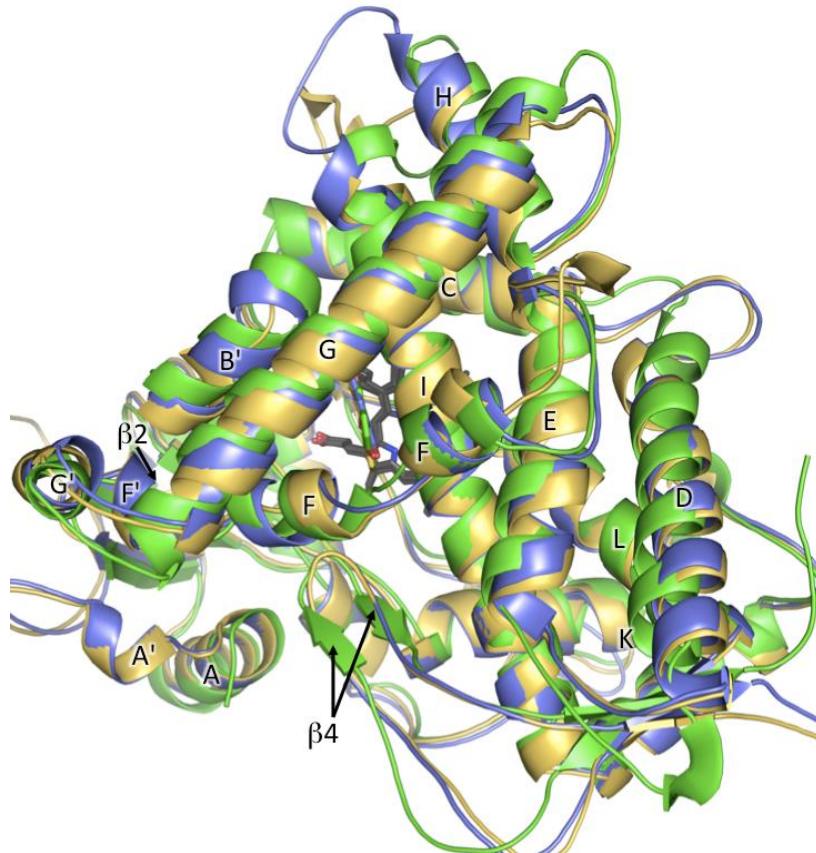
2.CYP1A1

CYP1A1 is a cytochrome P450 that is especially important for the metabolism of xenobiotics in the fetal placenta. It oxygenates polycyclic aromatic hydrocarbons such as benzo(a)pyrene so that they can be made water soluble for elimination. It is activated by AhR and Amt proteins which induce CYP1A1 transcription. It can be inhibited by the flavonoid galangin.

It seems that the unique functions of CYP1A1 are not well known. The only information I could find specific to this Cytochrome P450 is in its induction by AhR.

Below is a proposed drawing of the CYP1A1.

(i)5 (ii)2 (iii)4 (iv)2 (v)0
REF=5 T=18



Report=20 Ref=5

Acetaminophen is a nephrotoxic substance that is classified as a non-steroidal anti-inflammatory. It affects the proximal renal tubules and causes a decrease in glomerular filtration rate. Metabolites of Acetaminophen are produced in the form of quinones which are highly reactive. These quinones react with glutathione and sulphydryl groups on proteins and cause cellular dysfunction. This cellular dysfunction leads to renal failure.

Normally Acetaminophen is not toxic and is metabolized by cytochrome P450 and excreted. It is toxic when given in doses >2,000mg/kg.

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Pathology 438

Spring 2015

NAME _____

Midterm Examination

due: by 1:00 PM, 6 May 2015

Jessica Cheung

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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**

Pharmacokinetics –

- Time course of drug absorption – In clinical studies, patients begin responding after 5 – 10 days of initial treatment of this drug. (Narayanaswamy)
- Distribution – Volume distribution is 0.2L/kg with a half-life of 10-14 hours in adults. The drug, which is a fatty acid, is also 90% protein bound which results in low clearance (6-20ml/hour/kg).
- Metabolism – There are 3 major ways the body metabolizes valproic acid. The major routes are via glucuronidation which accounts for 50% of dose and beta oxidation which accounts for 40% of dose. Cytochrome P450 – mediated oxidation is also used by only accounts for about 10% of dose.
 - Some of the mitochondrial metabolites generated are hepatotoxic
 - This cytotoxic metabolic eventually becomes conjugated with glutathione to form thiol conjugates
 - This will deplete glutathione and form conjugates with CoA which is when the beta-oxidation pathway is used
- Excretion - 30-50% of the drug appears in urine as glucuronide conjugate.

Pharmacodynamics - What a drug does to the body

- Valporic acid is used for simple and complex absence seizures
- Starting dose is 15mg/kg/day and can be increased up to a maximum of 60mg/kg/day.
- Hepatic failure and Reyes-like symptoms have been reported which is why it is recommended that careful observation of liver function is required. This is due to the cytotoxic metabolites that are created during its metabolism.
- The drug acts on GABA (gamma amino butyric acid) levels in the brain, blocks voltage-gated ion channels and acts as an HDAC (Histone deacetylase) inhibitor
 - GABA levels in brain: Valproic acid inhibits GABA transaminase and succinate semialdehyde dehydrogenase which are involved in the GABA degradation pathway. This increases overall GABA levels which results in antiepileptic activity.
 - Blocking voltage gated ion channels: This will reduce the high frequency of the neuron firing, also resulting in antiepileptic activity.
 - HDAC (Histone deacetylase) inhibitor: This may potentially increase gene expression for apoptosis and antitumor functions. By increasing this gene expression, it can help promote killing tumor cells.

PK: AD=4 M=6 E=4

PD: 10

Ref: 5

T=29

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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

(a) **CYP3A4**

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

They are monooxygenases. It helps catalyze reactions involving drug metabolism, steroid hormone termination, elimination of phytochemicals in food and bile acid detoxification. Example of a drug it metabolizes includes Sildenafil (Viagra).

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

CYP3A4 activity is initiated by various receptors including the pregnane X receptor, constitutive androstane receptor and peroxisome proliferator-activated receptor. CYP3A4 will cause hydroxylation of the sp³ C-H, which is sometimes followed by dehydrogenation to create the metabolites which is then excreted. (Shahrokh K)
It will perform oxidation reactions and will hydroxylate etoposide.

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

Substances that inhibit CYP3A4 will increase the plasma concentration of it.

The following table is from Pharmacy Times (John R. Horn and Philip D. Hansten):

Amiodarone	Imatinib
Amprenavir	Indinavir
Aprepitant	Isoniazid
Atazanavir	Itraconazole
Chloramphenicol	Ketoconazole
Clarithromycin	Lapatinib
Conivaptan	Miconazole
Cyclosporine	Nefazodone
Darunavir	Nelfinavir
Dasatinib	Posaconazole
Delavirdine	Ritonavir
Diltiazem	Quinupristin
Erythromycin	Saquinavir
Fluconazole	Tamoxifen
Fluoxetine	Telithromycin
Fluvoxamine	Troleandomycin
Fosamprenavir	Verapamil
Grapefruit juice	Voriconazole

- 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**

Protein structure: It is part of a group of heme-thiolate monooxygenases. It is a monomer, and a hemoprotein (a protein that contains a cofactor, heme, and an iron group). It has one metal binding group for the iron. It is an enzyme that is NADH/NADPH dependent. It is a helical, transmembranous enzyme. (UniProt Consortium)

Genes: The full name is cytochrome P450, family 3, subfamily A, polypeptide 4. This is in homosapiens, gene family is cytochrome P450. (National Center for Biotechnology Information). There are about 28 SNPs that code for this enzyme that we know of at this time. The gene has 13 exons and 12 introns and has a length of 27 kb. (M. Whirl-Carrillo)

- v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc**
For hepatic turnover it varies between humans. In vivo studies showed that half-life was between 70 – 140 hours. In vitro studies estimated half-life to be 26 – 79 hours. (Med Safe)

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(i)6 (ii)4 (iii)4 (iv)4 (v)4
REF=5 T=27

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 that modify protein activity and/or de novo synthesis.

c) Search for a nephrotoxic substance (toxicant, poison or drug).

Vancomycin

Explain what part(s) of the nephron it disrupts (describe the mechanism of toxicity).

Tubular cell toxicity is the result of mechanism of injury. The tubule cells are effected by the toxic because they concentrate and reabsorb the glomerular filtrate. Since these cells are consistently exposed to the drug, it interferes with mitochondrial function, tubular transport, and increasing oxidative stress. (Zager) (Markowitz and Perazella)

Describe how normal kidney physiology would be disrupted for the parts of the nephron affected.

When the tubular cells are effected, it will disrupt the kidneys ability to concentrate and reabsorb the glomerular filtrate. Since it interferes with mitochondria function, the ability for the active transport of solutes will also be disrupted. This will result in ion and water imbalances in the body.

Describe how the nephrotoxic substance is detoxified (metabolism? elimination? both?)

- Half life of initial phase – 7 minutes, second phase is 0.5 – 1 hour and the elimination ranges between 3 – 9 hours in people with normal renal function
- Renal clearance of 0.5 – 0.8 determined by creatinine which show that glomerular filtration is the main way to excreting this drug (Matzke, Zhanel and Guay)

What doses or concentration levels are required to obtain the toxic effect?

- General dosing recommendation: 2 g/day via IV over 6 to 12 hours, may change depending on body weight
- Toxicity increases at greater than 4 grams per day (MedScape)

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NAME _____ **JOE BOTKIN**_____

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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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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)-

PK: AD=4 M=6 E=4

PD: 10

Ref: 5

T=29

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).
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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 Warfarin, 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:

(i)6 (ii)2 (iii)4 (iv)4 (v)4
REF=5 T=25

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.

Report=20 Ref=5

T=25

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Pathology 438

Midterm Examination

due: by 1:00 PM, 6 May 2015

Spring 2015

NAME Justine Bellefeuille

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

PK: AD=3 M=5 E=3

PD: 9

Ref: 5 T=25

1.

b) clonidine: Is usually administered orally as treatment for patients with high blood pressure medication to decrease the risk of stroke and heart attack. It is also an anti-hypersensitive drug class used for ADHD (attention deficit hyperactive disorder). Also categorized in the class of Central Alpha-2 Adrenergic Agonist drugs. Can be used to help with withdrawal symptoms from tobacco, opiates and benzodiazepines.

-An article from the European Journal of Clinical Pharmacology reported the action of this drug is mediated by alpha adrenoceptor agonist for both the central and peripheral nervous system. It has a high lipid solubility that makes the side effects correlated directly with how much serum clonidine is in the plasma. The absorption half life of a dose of 300 µg was about .6 hours and only able to be studied now because of specific and sensitive mass-fragmentographic studies. The two most commonly experienced side-effects are severe sedation and dry mouth. [Pharmacokinetics and side-effects of clonidine]

-Another study done showed that most studies stated that the elimination half-life was 20 hours but that this was actually not long enough, it is closer to over 25 hours. When it is prescribed for antihypertensive therapy it is given in very small doses that are difficult to study effects in the plasma. They used a radioimmunoassay analysis with normotensive subjects. They found that 62% of a single doses is excreted in the urine, independently of quantity of dose given.

[New Aspects of Pharmacokinetics and Pharmacodynamics of Clonidine in Man]

-Clonidine changes the amount of noradrenaline that is released by stimulating presynaptic receptors in the medulla that decrease the sympathetic output to the peripheral blood vessels by means of vasoconstriction. This leads to a decrease in blood pressure. Should not be used with BPH or Urinary Incontinence and adverse side-effects include drowsiness, depression and postural hypertension.

Thorp, Christine M., Pharmacology for the Health Care Professions. Wiley-Blackwell, Oxford, UK; 2008.

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

- 1.i. Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize.
- 1.ii. Explain the mechanism of catalysis (you can even draw the steps)
- 1.iii. Provide the names of any substances known to inhibit the cytochrome, if any
- 1.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
- 1.v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc

CYP2C9

1.
 1. There are several substrates metabolized by this enzyme including NSAIDs (diclofenac, ibuprofen, naproxen and piroxicam), Oral Hypoglycemic (tolbutamide, glipizide and glyburide), Angiotensin II Blocker (Iosartan, irbesartan) and other drugs for essential hypertension (celecoxib, fluvastatin, phenytoin, rosiglitazone, torsemide, valproic acid, warfarin, zafirlukast). [P450 Drug Interactions]
 2. In the Journal of Biological Chemistry, they studied the structure of this enzyme with Fluriprofen. This study showed the catalytic efficiency of this enzyme when it regioselectively oxidizes NSAIDs. This enzyme aids in hepatic metabolism and can decrease the metabolic capacity of other substrates with low therapeutic margins and lead to toxicity at normal therapeutic doses. Other structural bindings are shown on this article but too confusing to try to draw. [CYP2C9]

“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. The crystal structure of human CYP2C9 was described by Williams et al. [46], for both CYP2C9 in complex with warfarin and unliganded CYP2C9 (Protein Data Bank ID: 1OG2 and 1OG5, respectively). The structure showed unanticipated interactions between CYP2C9 and warfarin, revealing a new binding pocket, suggesting that CYP2C9 may simultaneously accommodate multiple ligands during its biological function [46]. Structural analysis suggested that CYP2C9 may undergo an allosteric change when binding warfarin [46].” [CYP2C9]

3. Moderate inhibitors include amiodarone and efavirenz. Strong inhibitors include: fluconazole, isoniazid, metronidazole, paroxetine, sulfamethoxazole and voriconazole. This means that you would need to increase the dosage if you are taking one of these at the same time. [P450 Drug Interaction]
4. Inducers associated with this include: carbamazepine, nevirapine, phenobarbital, rifampin and St. John's Wort. [P450 Drug Interaction]

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 0

REF=4 T=22

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

- 1.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 I found studied the differences between men and women hepatocyte function with 5 drugs: Diclofenac, Chlorpromazine, Acetaminophen, Verapamil and omeprazole. Caffeine was used as a negative control. Women were found to be more sensitive to drugs invitro. Findings showed differences in plasma permeability, nuclear condensation, endoplasmic reticulum function and mitochondria. There was also evidence that there might be a difference with post-menopausal women's cells and women who have menses. There is little understood on this topic still because the studies are primarily on animals and not also directly correlated to human genome. Also the number of women that are participating in the study was smaller sample size and takes away from the findings.[men vs women]

Report=20 Ref=2

1) Valproic Acid (VPA) - Used to treat seizures and epilepsy.Pharmacokinetics:

Absorption:

Valproic acid is absorbed in the GI tract quick, with time depending on the type of tablet consumed. In the GI tract, valproic acid breaks into valproate ion. The time after consumption when the absorption rate is equivalent to the elimination rate, also known as the time when maximum concentration in the blood is reached (Tmax), is 4-17 hours depending on the type and dose of the tablet (Wolters Kluwer Health, 2009).

Distribution:

The volume of distribution (Vd) is known as the volume of fluid that is needed to keep a drug in the body with an equal concentration to the plasma. The lower the Vd means that the substance can be found mainly in the vascular system, whereas a higher Vd means it is absorbed more by the tissues. Vd of valproic acid is 11L/1.73m² for total amount, and 92L/1.73m² for the free amount. These values match Leppik & Birnbaum's (2010) statement that VPA is "highly protein bound (87-95%)", as we see the low total VPA Vd showing primary isolation in the vascular system as bound to plasma proteins, and the total free amount as moving to the tissues because it is not bound to proteins.

Metabolism:

Valproic acid (VPA) is branched short-chain fatty acid that is metabolized in the mitochondria of the liver. The 3 well-known pathways of VPA metabolism are glucuronidation (50%), beta oxidation in the mitochondria (40%) and oxidation by cytochrome P450 (CYP) (10%). Prior to entrance into the mitochondria, VPA is activated into several compounds, including 4-ene-VPA. This 4-ene-VPA enters the mitochondria and is converted to 2,4-diene-VPA-CoA via B-oxidation. 2,4-diene-VPA-CoA is hepatotoxic and is therefore conjugated with glutathione to form thiol conjugates, which are excreted from the liver mitochondria (Ghodke-Puranik et al., 2013).

Elimination:

The excretion of thiol conjugates serves as detoxification for the liver. Since VPA is protein bound, the clearance rate is slow, approximately 6-20 mL/hr/kg. Valproate-glucuronide is approximately 30-50% of VPA urinary metabolites (Argikar et al., 2008).

Pharmacodynamics:

According to Ghodke-Puranik et al. (2013), VPA acts on gamma amino butyric acid (GABA) in the brain, it blocks voltage-gated ion channels and also inhibits histone deacetylase. With epilepsy, GABA receptors are impaired and can not be inhibited, which leads to the convulsive symptoms. VPA has been found to inhibit key enzymes that function in the pathway of GABA degradation. By having more circulating GABA, and by decreasing firing of neurons through the blocking calcium, potassium and sodium voltage-gated channels, this decreases epileptic instances (Johannessen et al., 2003).

PK: AD=4 M=6 E=4

PD: 10

Ref: 5

T=29

2. CYP3A4

i) CYP3A4 is a cytochrome P450 enzyme that metabolizes xenobiotics, seen as clinical drugs such as antibiotics and endogenous compound bile acids. CYP3A4 is the most abundant hepatic and phase I intestinal enzyme, known to metabolize approximately 50% marketed drugs and detoxify bile acids, as high levels of bile acids can injure tissues, especially the liver (Zhou, 2008). Erythromycin is an example of an antibiotic that is metabolized by CYP3A4.

ii) CYP3A4 catalysis reactions are an integral part in the metabolism of drugs, and the synthesis of cholesterol, steroids, and various lipids. CYP3A4 is induced by glucocorticoid presence and catalysis of the substrates is localized at the endoplasmic reticulum. Catalysis begins with sp^3 C-H bond hydroxylation, which in turn affects the ligand. Further mechanism includes substrate dehydrogenation, which creates further complex metabolites.

iii) Some examples of strong CYP3A4 inhibitors, meaning they decrease clearance of substrates by at least 80%, are: protease inhibitors, and some macrolide antibiotics and azole antifungals (Flockhart, 2007). (i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 4

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 4
REF=5 T=27

iv) CYP3A4 is a cytochrome P450 oxidizing enzyme, which contains a hemeoprotein, meaning that it is a protein with a heme group containing an iron atom. This CYP3A4 protein is encoded by the CYP3A4 gene (Hashimoto et al., 1993), has a large active site, and has the ability to bind more than one substrate at a time. The ability to bind multiple substrates allows CYP3A4 to work on several endogenous and exogenous substrates through various reactions including hydroxylation, aromatic oxidation, and dehydrogenation to name a few (Shahrokh et al., 2012).

v) Turnover rate estimates of the CYP3A4 enzyme have extreme varying values. With hepatic in vivo methods, the half-life has been estimated as 70-140 hours. Hepatic in vitro methods have estimated 26-79 hours. CYP3A4 in the GI tract also has its own estimates, however likely has additional role-playing factors to consider, such as the enterocyte renewal. One study did get results of the GI tract half-life estimates, seen as 12-33 hours (Yang et al., 2008).

3. Nephrotoxic substances I am interested in are non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen. NSAIDs can cause inflammatory changes in the glomerulus, renal tubules, and interstitium, leading to further problems including fibrosis and scarring of the kidney. In healthy young adults, the glomerular filtration rate (GFR) is usually close to 120 mL/min. The kidney regulates intraglomerular pressure by constriction and dilation of the afferent and efferent arterials, to optimize urine output and GFR, through the action of angiotensin-II. There are two known effects that NSAIDs have on the kidney; acute kidney injury and acute interstitial nephritis. Acute kidney injury (AKI) from NSAIDs is seen as decreased prostaglandin production, which resultantly reduces renal plasma flow because these prostaglandins regulate glomerular vasodilation. By decreasing the substance doing vasodilation, vasoconstrictor hormones have a greater effect on the vessels (Whelton, 2009). Inhibition of prostaglandins begins acute renal function deterioration. AKI is also acute interstitial nephritis (AIN), but it is characterized by inflammatory cells in the kidney interstitium. AIN is caused by an immunological reaction after approximately a week of NSAID usage, and it causes 15% of ANI (Dixit et al., 2007).

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Rates, and Implications for the Prediction of Drug Interactions. *Current Drug Metabolism*, 9(5) 384-393. doi:10.2174/138920008784746382

Zhou, S. (2008). Drugs Behave as Substrates, Inhibitors and Inducers of Human Cytochrome P450 3A4. *Current Drug Metabolism*, 9(4), 310-322.

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

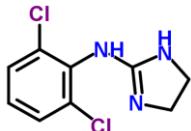
You are not allowed to consult with classmates or any individuals *other than* the instructor as you research, prepare and compose your responses to the questions posed in this examination. You may use the information available from lecture content (slides) in MOODLE, the LCCW library, reference books and course textbooks, and online 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.

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



PK: AD=4 M=3 E = 4

PD: 6

Ref: 5

T=22

Toxicokinetics:

Absorption: offered orally, transdermally, and intravenously; absorption unaffected by food or race of patient

Distribution: antihypertensive effects noted between 0.2 – 2.0 ng/mL, excreted in human milk

Metabolism: 50% of absorbed dose metabolized in the liver

Elimination: 40-60% of absorbed dose eliminated in urine unchanged

Toxicodynamics:

Protonated clonidine is the active form.

Effects of Clonidine are reduced when taken with tricyclic antidepressants. Corneal lesions developed in rats after five days with Clonidine was taken in combination with amitriptyline.

Qin J, Wang L, Wu L, Chen J, Shen T, Li Y, Han L, Wang J. "Development of an LC-MS/MS method for determining the pharmacokinetics of clonidine following oral administration of Zhenju antihypertensive compound." *National Center for Biotechnology Information*. U.S. National Library of Medicine, n.d. Web. 03 May 2015.

<http://www.ncbi.nlm.nih.gov/pubmed/25776729>

"Catapres ® (Clonidine hydrochloride, USP)." Boehringer Ingelheim. October 2011. Web 03 May 2015.

http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/017407s037lbl.pdf

Norberto F, Moreira J, Rosa E, et al. "Kinetics and mechanism of nitrosation of clonidine – a bridge between nitrosation of amines and ureas." *Journal od Chemical Society Perkin*. University of Santiago, Chile. 09, 1993. Web. 04 May 2015.

http://www.researchgate.net/publication/225029255_KINETICS_AND_MECHANISM_OF_NITROSATION_OF_CLONIDINE_-_A_BRIDGE_BETWEEN_NITROSATION_OF_AMINES_AND_UREAS

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.
Metabolizes xenobiotics (ex. ibuprofen, warfarin, NSAIDS, oral anti-diabetic agents (hypoglycemics), angiotensin II receptor blockers).
- ii. Explain the mechanism of catalysis (you can even draw the steps)
Hydroxylation.
- iii. Provide the names of any substances known to inhibit the cytochrome, if any.
Phenytoin (substrate, inhibitor, and inducer), fluconazole, miconazole, amentoflavone, sulfaphenazole, valproic acid, apigenin.
- 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.
Found on chromosome 10q24. N-terminal sequence MALLAVF.
- v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc.
Acidity.

"CYP2C9 cytochrome P450, family 2, subfamily C, polypeptide 9." *National Center for Biotechnology Information*. U.S. National Library of Medicine, April 26 2015. Web. 03 May 2015.

<http://www.ncbi.nlm.nih.gov/gene/1559>

Kumar V, Rock D, Warren C, Tracy T, Wahlstrom J. "Enzyme Source Effects on CYP2C9 Kinetics and Inhibition." *National Center for Biotechnology Information*. U.S. National Library of Medicine, April 23 2006. Web. 03 May 2015.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2377028/>

Horn J, Hansten P. "Get to Know an Enzyme: CYP2C9" *Pharmacy Times*. March 1 2008. Web. 03 May 2015.

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Locuson C.W., Wienkers L.C., Jones J.P., Tracy T.S. "CYP2C9 protein interactions with cytochrome b5: Effects on the coupling of catalysis." *Drug Metab Dispos*. 2007 Jul;35(7): 1174-1181. Web 04 May 2015.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2386961/>

3. 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?

Cyclosporine

Vasoconstriction of afferent arterioles through altered release of vasoactive substances (such as angiotensin II, endothelin, prostaglandins, and nitric oxide) causes nephrotoxicity. Also stimulates genes to produce growth-factor beta, osteopontin, and collagen I and IV. Biopsies have shown interstitial fibrosis, tubular atrophy, glomerulosclerosis, vascular damage (smooth muscle).

NSAIDS + cyclosporine may increase risk of renal toxicity, especially in patients with rheumatoid arthritis.

6-10mg/kg/day associated with toxic manifestations in the kidneys.

Detoxified in the liver by CYP450 enzymes.

Busauschina A, Schnuelle P, van der Woude F.J. "Cyclosporine nephrotoxicity." *Transplant Proceedings*. March 2004. Vol 36, Issue 2, Supplement. P S229-S233. Web. 03 May 2015.

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The Proceedings From The 13Th International Symposium Of, and The Institute For Functional Medicine. "Managing Biotransformation: The Metabolic, Genomic, and Detoxification Balance Points." *Managing Biotransformation: The Metabolic*, (n.d.): n. pag. Web.

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Two Diagrams present

Monounsaturated Fatty Acids

1) Valproic Acid is a short chain fatty acid, used in psychiatric disorders.

It acts on GABA levels in the brain, blocks Histone deacetylase inhibitor, and blocks Voltage gated ion channels.

GABA is formed from α -Ketoglutarate in the TCA cycle. Valproic Acid inhibits GABA transaminase and succinate semialdehyde Dehydrogenase, which degrades GABA.

Valproic Acid also has anti-epileptic properties by blocking Voltage gated Sodium & Ca^{2+} channels, decreasing the frequency of firing of neurons. It has also been shown to inhibit HDAC1 which increases the expression of Apoptosis and anti-tumour action.

knutson VPA is a highly bound protein resulting in low clearance.

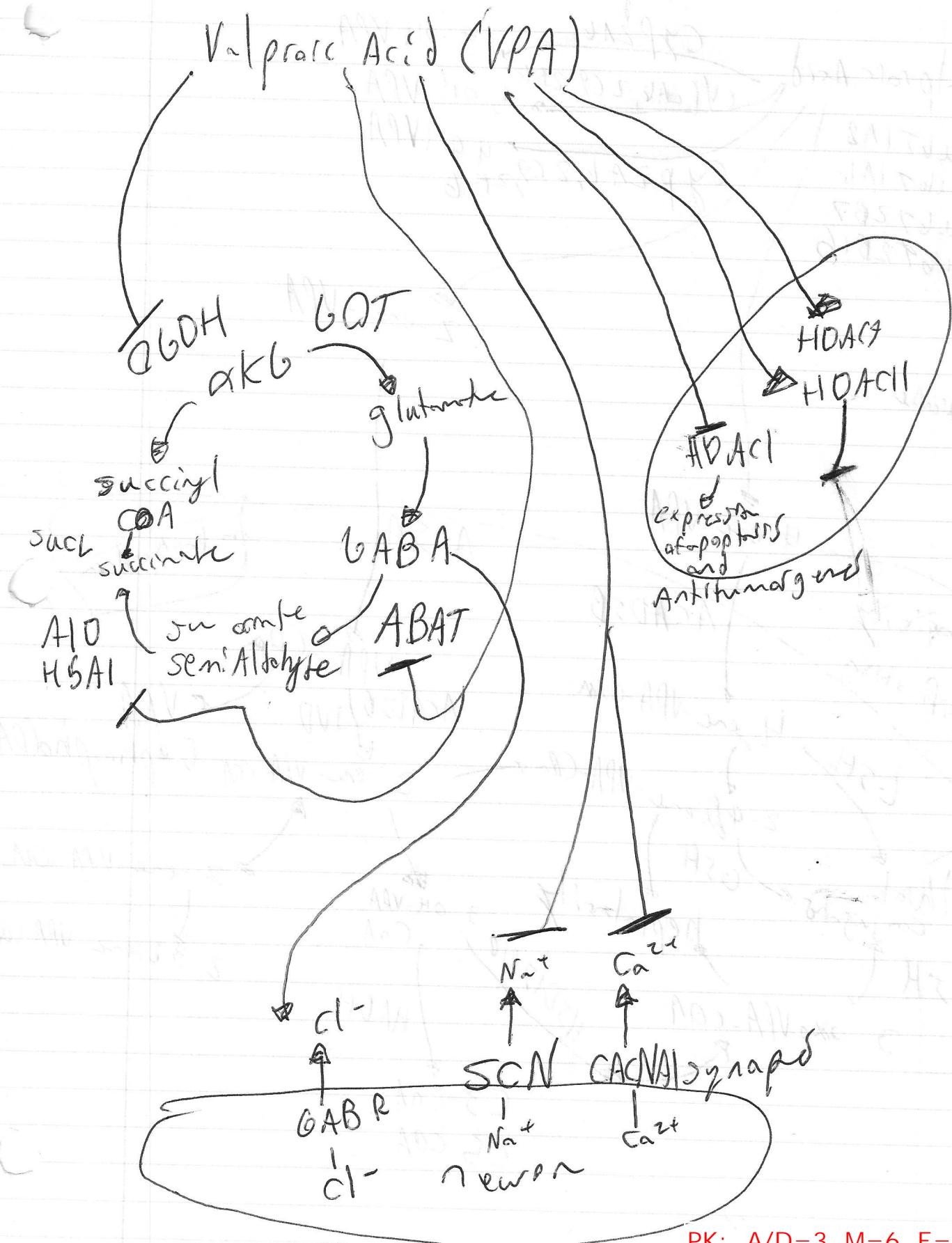
3 mechanisms for metabolism, glucuronidation, Beta oxidation in the mitochondria and Cyp450 mediated oxidation (this is minor role however).

The Valproate Glucuronide is the major metabolite 30-50% excreted in the urine.

It also can be brought into the liver in a carnitine shuttle. Then through Beta oxidation it is broken down to several metabolites.

A slow hydrolysis by an Acyl CoA thioesterase creates 3-Oxo-VPA which is the ~~end product~~ turned over by a thioester which turns it into a thio ester.

Pharmacodynamics



PK: A/D=3 M=6 E=4

PD: 10

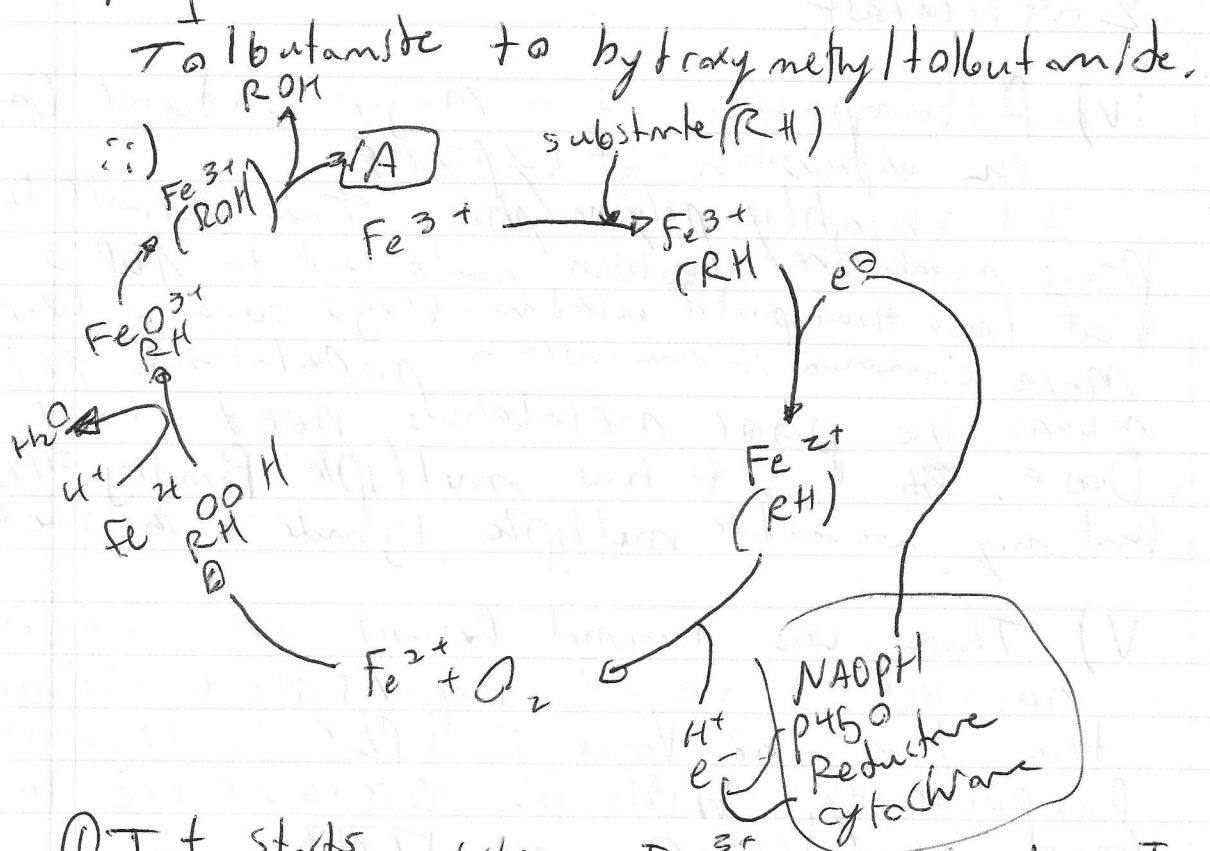
REF=5

T=28

Cyp 2C9

(2)

i) It is a phase I drug metabolizing Cytochrome P450 enzyme isoform that is primarily expressed in the liver and has the 2nd highest expression of all CYP isoforms. It is responsible for 15-20% of all drugs in the phase I of detoxification. It is known to metabolize



- ① It starts when Iron $\overset{\text{3+}}{\text{Fe}}$ binds to the Iron. The Iron becomes reduced to Fe^{2+} .
- ② O_2 then binds to the enzyme.
- ③ O_2 is then activated in a 1-electron reduction. A single O_2 atom is dissociated with an oxidation of Iron and protonation of complex involves H_2O leaving.

The remaining O_2 atom is highly energetic and reacts with substrate, which then leaves

3) Amanglycosides and Nephrotoxicity.

The dose over several days of 10 to 20mg/kg of body weight is for the amanglycoside dose.

This change the lysosomes of the proximal tubule. This effects the reabsorption of many

minerals such as Mg^{2+} , Ca^{2+} , K^+ and also

Creatinine, Phosphorus, and increase in excretion.

If continued over time, it can lead to acute renal failure.

If high doses of 40mg/kg are achieved from cortical necrosis and renal dysfunction occur rapidly.

While tubular necrosis is evident the mechanism of how it happens is unknown though several mechanisms could potentially be the source.

The damage is usually reported after it has been done, as toxicity is absorbed rapidly.

As stated it would greatly decrease the reabsorption in the proximal tubule leading to dehydration, polyuria, hypotension, nausea, fatigue, weakness. This is because the blood volume is decreasing dramatically and you are losing sugar cells, blood, and tissue. These are all caused for function ~~loss~~. Due to 90% of reabsorption occurring at the proximal convoluted tubule, it will cause large losses.

It is largely eliminated by glomerular filtration.

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Marisa Sum
Toxicology MT
Spring, 2015

Question #1: Describe pharmacokinetics and pharmacodynamics of (c) Valproic acid?

Definitions:

Pharmacodynamics is often summarized as the study of what a drug does to the body

Pharmacokinetics is the study of what the body does to a drug.

Valproic acid (VPA) is a branched short-chain fatty acid derived from naturally occurring valeric acid. VPA is used in the treatment of epilepsy and seizures but also migraine, bipolar, mood, anxiety and psychiatric disorders. It is widely used in pediatric epilepsy because of its multiple mechanisms of action and acceptable safety profile [Article:10319910]. The dose requirements for VPA are highly variable that are age specific to the patient. [Article:10594867] Interactions with other drugs are common which is why therapeutic drug monitoring is commonly used. Life-threatening adverse drug reactions include hepatotoxicity [Articles:21038416, 21544075], teratogenicity [Article:21521026] and pancreatitis [Article:15526953]. Children appear to be at increased risk for severe hepatotoxic reactions to VPA. The risk of fatal hepatotoxicity is highest (approximately 1:600) in children less than two years of age receiving concurrent anticonvulsant therapy. (Valproic Acid Pathway, Pharmacokinetics, 2015)

Pharmacokinetics

Valproic Acid binds to protein (87-95%) which means that it stays in the body longer and has a low clearance rate of 60 mL/hr/kg [Article:20146700]. VPA can be eliminated via three metabolic processes: glucuronidation, beta oxidation in the mitochondria (which are the major pathways accounting for 50% and 40% of dose respectively), and cytochrome P450 mediated oxidation [Articles:2112956, 18838507, 20089352]. VPA is a fatty acid which can be broken down via in the mitochondria. Breaking down VPA into its metabolites can be hepatotoxic. A protein metabolic cycle called carnitine facilitates and brings VPA across liver mitochondria membrane. (Valproic Acid Pathway, Pharmacokinetics, 2015)

The pathways inside the mitochondria are as follows: (Valproic Acid Pathway, Pharmacokinetics, 2015)

1. Oxidation: medium-chain acyl-CoA synthase catalyzes the formation of valproyl-CoA (VPA-CoA)
2. 2-methyl-branched chain acyl-CoA dehydrogenase converts VPA-CoA to 2-propyl-valproyl-CoA (2-ene-VPA-CoA) through (ACADSB)[Article:2112956]. Isovaleryl-CoA dehydrogenase (IVD) catalyzes this step [Article:21430231]. VPA-CoA also gets converted in to VPA-dephospho-CoA, though the exact phosphatase mediating this reaction has not been identified [Article:15483197].
3. 2-ene-VPA-CoA is further converted to 3-hydroxyl-valproyl-VPA (3-OH-VPA-CoA) by an enoyl-CoA hydratase and crotonase (ECSH1)
4. 3-OH-VPA-CoA is metabolized to 3-keto-valproyl-CoA (3-oxo-VPA-CoA) through the action of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (HSD17B10)[Articles:1988037, 21843514].

It is still not conclusive whether the beta-oxidation of VPA is complete in mitochondria. In CYP-mediated oxidation of VPA, CYP2C9 and CYP2A6 are the main enzymes. CYP2B6 has been shown to form metabolites in vitro but at a very small level. The key CYP-mediated branch of the VPA pathway is the generation of the metabolite 4-ene-VPA by CYP2C9, CYP2A6 and CYP2B6 [Articles:9353388, 16945988]. In addition these metabolizing enzymes also mediate the metabolism of VPA to the inactive 4-OH-VPA and 5-OH-VPA [Article:14597963]. CYP2A6 also contributes partially to the formation of 3-OH-VPA [Article:16945988]. Combination therapy of the DNA methyltransferase inhibitor 5-azacytidine (5-AZA) and VPA as treatment for myelodysplastic syndromes (MDS) demonstrated that carriers of the CYP2C19 variant, CYP2C19*2 required higher VPA doses to achieve the target therapeutic plasma concentration, indicating that CYP2C19 is also involved in the VPA pathway [Article:19638460]. (Valproic Acid Pathway, Pharmacokinetics, 2015)

VPA Elimination:

VPA is eliminated through urine as valproate-glucuronide which accounts for approximately 30-50% [Article:18838507]. One study shows that “in vitro studies of human liver microsomes and purified recombinant proteins have reported glucuronidation of VPA by UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7 and UGT2B15 [Articles:15761113, 18838507, 17687269.]” Other studies have disputed the role of UGT2B15, suggesting that VPA inhibits UGT2B15 but is not glucuronidated by it [Article:12732356]. UGT1A1 does not have activity against VPA in vitro [Articles:12732356, 18838507]. (Valproic Acid Pathway, Pharmacokinetics, 2015)

Pharmacokinetics

VPA and Seizures

VPA acts on γ amino butyric acid (GABA) levels in the brain, blocks voltage-gated ion channels, and also inhibits HDAC. These pathways can help decrease the number of convulsions a person experiences.

Antiepileptic drugs like VPA aim to control GABA pathways because impairment of GABAergic inhibitory activity can lead to convulsions. GABA is formed from α -ketoglutarate through the tricarboxylic acid cycle and metabolized to succinate semialdehyde by GABA transa-minase (*ABAT*) and then to succinate by succinate semialdehyde dehydrogenase (*ALDH5A1*). α -Ketoglutarate can also be converted to succinyl CoA through the action of α -ketoglutarate dehydrogenase (*OGDH*), shunting it away from the formation of GABA. Ex-vivo and in-vitro studies have shown that VPA inhibits *ABAT* and *ALDH5A1*, both of which are involved in the GABA degradation pathway. One in-vitro study also showed that *OGDH* was inhibited by high concentrations of VPA [24].

Besides increasing GABA levels, VPA may also have antiepileptic activity by reducing the high-frequency firing of neurons by blocking voltage-gated sodium, potassium, and calcium channels (including those coded for by *CACNA1C*, *CACNA1D*, *CACNA1N*, and *CACNA1F* and the *SCN* gene family) [24,25]. (Ghadke-Puranik, 2014 Apr)

VPA and Cancer

VPA was recently shown to inhibit HDAC1 as well as other HDACs that may increase the expression of genes involved in apoptosis and antitumor action. Therefore, VPA is now under

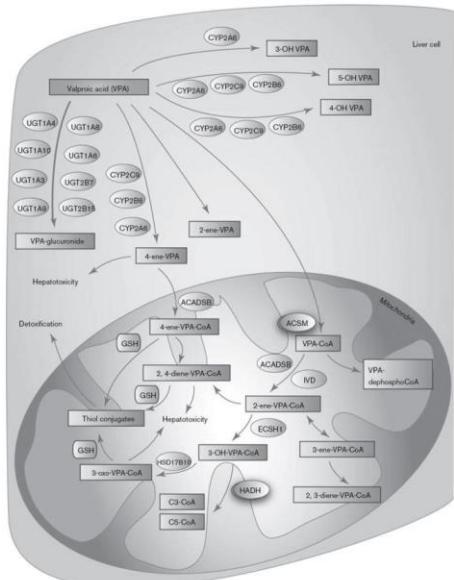
PK: AD=3 M=6 E=3

PD: 10

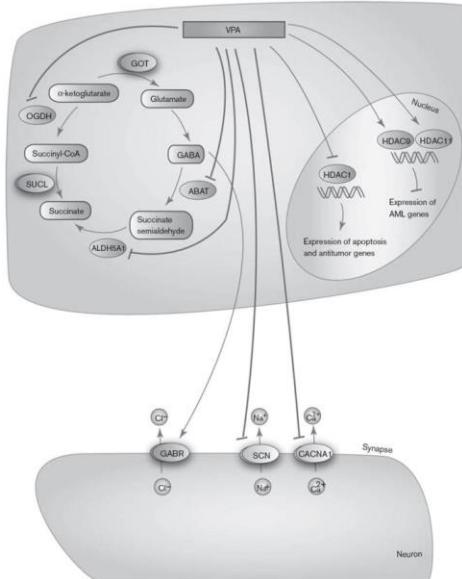
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T=27

consideration to be a potential antitumor agent. It is being considered for other ways it can help cancer patients too. (Valproic Acid Pathway, Pharmacokinetics, 2015)



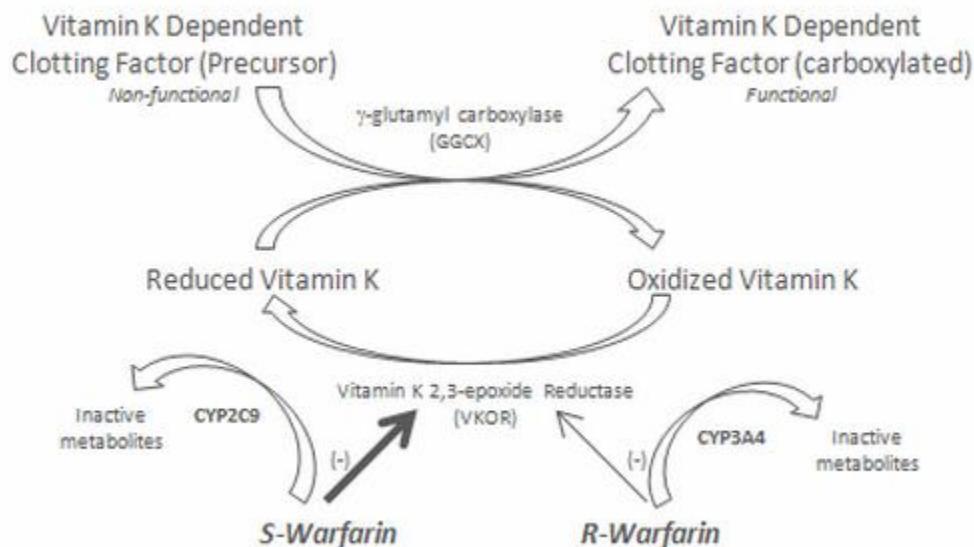
Graphic representation of the candidate genes involved in valproic acid (VPA) pharmacokinetics. A fully interactive version of this pathway is available online at PharmGKB at <http://www.pharmgkb.org/pathway/PA165964265>. CYP, cytochrome P450.



Graphic representation of the candidate genes involved in valproic acid (VPA) pharmacodynamics. A fully interactive version of this pathway is available online at PharmGKB at <http://www.pharmgkb.org/pathway/PA165959313>.

Question #2: (b) CyP2C9

- A. Enzyme cytochrome P-2C9 is largely used to help oxidize xenobiotic and endogenous compounds. It metabolizes any drug that undergoes “Phase I metabolism.” (Booven, 2010 Apr) CyP2C9 metabolizes substrates like NSAIDs. An article from PharmGKB says that this enzyme metabolizes the S-Isomer in warfarin, which is the anti-coagulative part of the drug.
- B. The mechanism of catalysis: “The structure showed unanticipated interactions between CYP2C9 and warfarin, revealing a new binding pocket, suggesting that CYP2C9 may simultaneously accommodate multiple ligands during its biologic function [Article:[12861225](#)]. Structural analysis suggested that CYP2C9 may undergo an allosteric change when binding warfarin.” (Gene: CYP2C9, 2015)
 - a. Example: (How is warfarin (Coumadin, Jantoven) use influenced by genetic polymorphisms to CYP450 2C9?, 2014)



- C. There are competitive inhibitors and noncompetitive inhibitors of CyP2C9. Strong active inhibitors are antifungal drugs such as fluconazole, miconazole and antibacterial drugs such as sulfaphenazole and anticonvulsants like Valproic Acid (VPA). Noncompetitive inhibitors are nifedipine, phenethyl isothiocyanate and medroxyprogesterone acetate. (CYP2C9, 2013)
- D. Gene and/or protein structure unknown.
- E. No known enzyme kinematic parameters.

(i)6 (ii)4 (iii)4 (iv)0 (v)4
REF=5 T=23

Question #3: (a)

Drug and blockers different in men v. women (Beer, 2013)

1. This study suggests that men and women metabolize steroids like DHEA-S and DHEA in response to insulin may be regulated differently.
2. Reducing circulating insulin with a Ca²⁺ channel blocker is associated with a rise in serum DHEA-S concentration in women and in men. This is not different between the two sexes.
3. A reduction of fasting serum insulin levels in men also showed a concurrent rise in serum DHEA and DHEA-S levels.

Report=20

Ref=5

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Pathology 438

Spring 2015

NAME Mary Whalen

Midterm Examination

due: by 1:00 PM, 6 May 2015

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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 binding of an agonist at a receptor thus inhibiting the signal that would be produced by the receptor-agonist binding. 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

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>

PK: AD=4 M=4 E=4

PD: 6

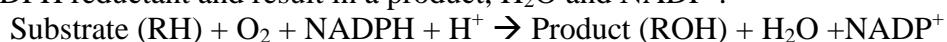
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T=23

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

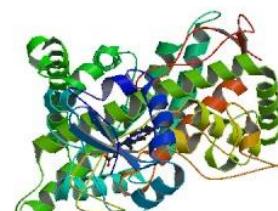
- i. Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize.
 - ii. Explain the mechanism of catalysis (you can even draw the steps)
 - iii. Provide the names of any substances known to inhibit the cytochrome, if any
 - iv. If its 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 O₂ molecule, receives electrons from another enzyme handling NADPH reductant and result in a product, H₂O and 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 anticoagulant 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, bishydroxycoumarin, chloramphenicol, cimetidine, fluconazole, fluvastatin, miconazole, phenylbutazone, sulphipyrazone, sulphadiazine, sulphamethizole, sulphamethoxazole, sulphaphenazole, trimethoprim, and zafirlukast.⁸

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 Williams & 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/>

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.

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/>

Report=20 Ref=5 T=25

Midterm Examination

1. Describe toxico/pharmacokinetics and toxico/pharmacodynamics of **Clonidine**

Clonidine is a drug most commonly used to treat high blood pressure and is also alone or in conjunction with other drugs used to treat ADHD. Common brand names for this anti hypertensive drug include Catapres, Kapvay, and Nexiclon XR. It has been used clinically for over 40 years and unfortunately, in the case of treating high blood pressure, is commonly communicated as needed "for the rest of your life."

It's classified as a centrally acting alpha adrenergic agonist. It stimulates alpha2 receptors in the brain which decreases peripheral vascular resistance therefore lowering blood pressure. It specifically targets presynaptic alpha2 receptors in the brainstem, binding decreases presynaptic Calcium levels leading to a net effect of a decrease in sympathetic tone. Clonidine is most often prescribed orally but because of the adverse side effects is being both studied and prescribed transdermally.

When taken orally the drug is absorbed rapidly after an initial lag time of 19-22 minutes and peak levels of plasma concentration (Cmax) is reached between 2.4 and 2.9 hours. Sampling over 48 hours is necessary for accurate pharmacokinetic action. The half life of the elimination phase ranged from 9.0 to 15.1 hours. Cmax increased proportionally with increased doses. Clonidine causes a marked reduction in pulse rate and a dose dependent decrease in blood pressure.

Pharmacodynamics of the drug vary greatly. The most common adverse affects, >10% frequency, include dizziness, drowsiness, dry mouth, headache, and skin lesions if taken transdermally. Other affects with 1-10% frequency include anxiety, constipation, sedation, nausea, and erectile dysfunction.

S.N Anakevar, B. Jarnott, et al. *Pharmacokinetic and Pharmacodynamic studies of oral Clonidine in normotensive subjects*. European Journal of Clinical Pharmacology. 1982. Volume 23, Issue 1. Pp 1-5. link.springer.com/article/10.1007%2FBF01061368

en.m.wikipedia.org/wiki/Clonidine

PK: AD=4 M=3 E=3

PD: 9

Ref: 5

T=24

2. Cytochrome P450 enzyme: CYP3A4

- i. Cytochrome P450 is a family of oxidizing enzymes and CYP3A4 is specifically involved in oxidation of small foreign organic molecules or xenobiotics. Acetaminophen and erythromycin are examples of drugs metabolized by CYP3A4.

- ii. CYP3A4 is mainly found in the liver and the gut, namely the intestines. It's involved not only with drug metabolism but also with the synthesis of cholesterol, steroids, and other lipids. Cytochrome P450 enzymes have a large active site and can bind more than one substrate at a time to perform complex metabolism including hydroxylation, epoxidation, oxidation, and dehydrogenation reactions.
 - iii. Fruit ingestion is known to inhibit the action of CYP3A4. Primarily grapefruit and grapefruit juice however, Noni fruit and pomegranates can exhibit the same effects. Ingestion of these can increase the bioavailability of some drugs and in other cases, the reaction can be fatal, astemizole and terfenadine are examples.
 - iv. All members of the cytochrome P450 family, including CYP3A4, are hemoproteins, a protein containing a heme group with an iron atom. In humans the CYP3A4 protein is encoded by CYP3A4 gene which is on chromosome 7g21.1. Although the CYP3A4 gene has 28 single nucleotide polymorphisms (SNPs), none have been found to contribute to inter individual variability *in vivo*.
 - v. In the particular study that I reviewed kinetic parameters of Km, Vmax , and Vmax/Km of 215 CYP3A4 mediated reactions of 113 drugs in human liver microsomes and lipophilicity values of the 113 drugs were calculated. Overall, Km decreases but Vmax/Km increases with increasing substrate lipophilicity, and Vmax appears to be independent of substrate lipophilicity. Another way of putting it would be that a low Km generally produces a highVmax/Km ratio for a substrate.

A literature review of enzymatic parameters for CYP3A4 mediated metabolic reactions of 113 drugs in human liver microsomes. www.ncbi.nlm.nih.gov/pubmed/16611019

en.wikipedia.org/wiki/CYP3A4

(i)6 (ii)4 (iii)4 (iv)3 (v)4
REF=5 T=26

3. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women

This study compared the effects of delta-9-tetrahydrocannabinol (delta9-THC) both intravenously and orally in men and women. (1) No differences in dynamic activity, metabolism, excretion, and kinetics were observed. Delta9-THC is converted by microsomes hydroxylation into an intermediate which is a potent psychoactive metabolite. (2) Major differences in ratio of concentration of this psychoactive metabolite to delta9-THC were found after intravenous dosing compared with oral administration. However, no differences across male and female. (3) For delta9-THC the terminal phase or half life for both sexes irrespective of the route, ranged from 25 to 36 hours.

ME Wall, BM Sadler, et al. *Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women*. Clinical Pharmacology and Therapeutics. September, 1983. Volume 34, Issue 3. P 179. onlinelibrary.wiley.com

Report= 20
Ref=5

MIDTERM

1. **Valproic Acid** (VPA) is a branched short-chain fatty acid derived from naturally occurring valeric acid. VPA is used in the treatment of epilepsy and seizures but also migraine, bipolar, mood, anxiety and psychiatric disorders. Recent work has explored its use as an adjuvant agent in cancer, HIV therapy, CLL and neurodegenerative disease because of its action as histone deacetylase (HDAC) inhibitor. VPA is available in oral, rectal and injectable dosage forms.

The drug label carries a black box warning for life-threatening adverse drug reactions (ADR) including hepatotoxicity, teratogenicity and pancreatitis. Although VPA hepatotoxicity may occur at any age, the risk of fatal hepatotoxicity is greatest in children less than two years of age receiving concurrent anticonvulsant therapy. Hyperammonemia is also a documented ADR of VPA.

There are 3 routes of VPA metabolism in humans: glucuronidation, beta oxidation in the mitochondria, and cytochrome P450 (CYP) mediated oxidation. Valproate-glucuronide is the major urinary metabolite of VPA (approximately 30-50%) VPA can be metabolized via endogenous pathways in the mitochondria.

“VPA crosses the membrane of liver mitochondria via the facilitation of carnitine. Inside the mitochondria, the first step of oxidation is the formation of valproyl-CoA (VPA-CoA) catalyzed by medium-chain acyl-CoA synthase (coded for by the genes ACSM1-5) [Article:[21843514](#)]. VPA-CoA is then converted to 2-propyl-valproyl-CoA (2-ene-VPA-CoA) through 2-methyl-branched chain acyl-CoA dehydrogenase (ACADSB)[Article:[2112956](#)]. Isovaleryl-CoA dehydrogenase (IVD) was also recently reported to catalyze this step [Article:[21430231](#)]. VPA-CoA also gets converted in to VPA-dephospho-CoA , though the exact phosphatase mediating this reaction has not been identified [Article:[15483197](#)]. 2-ene-VPA-CoA is further converted to 3-hydroxyl-valproyl-VPA (3-OH-VPA-CoA) by an enoyl-CoA hydratase, crotonase (ECSH1) and then 3-OH-VPA-CoA is metabolized to 3-keto-valproyl-CoA (3-oxo-VPA-CoA) through the action of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (HSD17B10)[Articles:[1988037](#), [21843514](#)]”

REFERENCES

Cytotoxic activity of valproic Acid on primary chronic lymphocytic leukemia cells. - PubMed - NCBI. (2015). Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/25923087>

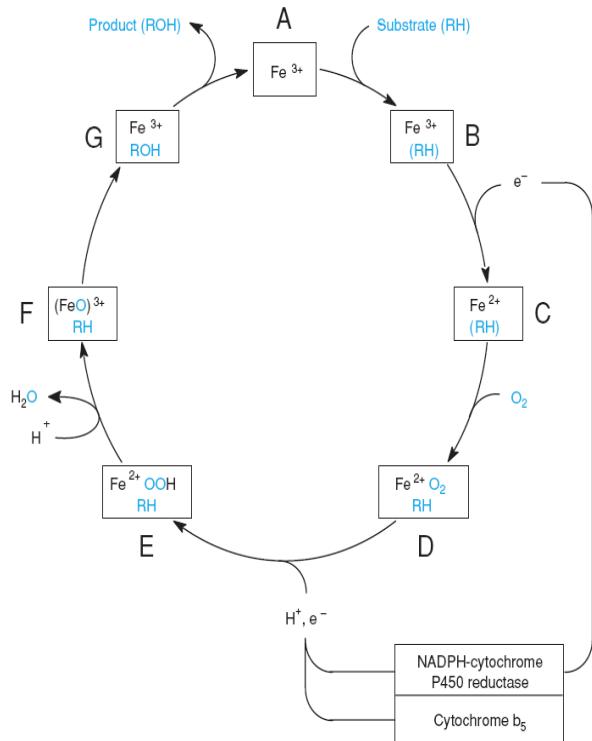
Ghodke-Puranik Yogita, Thorn Caroline F, Lamba Jatinder K, Leeder J Steven, Song Wen, Birnbaum Angela K, Altman Russ B, Klein Teri E. "[Valproic acid pathway: pharmacokinetics and pharmacodynamics](#)" *Pharmacogenetics and genomics* (2013).

PK: AD=1 M=6 E=1

PD: 8

Ref: 5 T=21

2. Over 100 therapeutic drugs are metabolized by CYP2C9, including drugs with a narrow therapeutic index such as [warfarin](#) and [phenytoin](#) and other routinely prescribed drugs such as [acenocoumarol](#), [tolbutamide](#), [losartan](#), [glipizide](#), and some nonsteroidal anti-inflammatory drugs. By contrast, the known extrahepatic CYP2C9 often metabolizes important endogenous compound such as [arachidonic acid](#), [5-hydroxytryptamine](#), and [linoleic acid](#).



Substrates are largely NSAIDS, and strong inhibitors include St. Johns wort and Valproic Acid

REFERENCES

CYP2C9 - Wikipedia, the free encyclopedia. (n.d.). Retrieved May 6, 2015, from <http://en.wikipedia.org/wiki/CYP2C9>

(i)4 (ii)4 (iii)4 (iv)0 (v)0
REF=3 T=15

3. The article, Women and Prescription Drugs: The Gender Gap Tightens by Dr. David Sack, M.D. focuses on the history of addiction being a mans disease, but now women are caught up and surpassing them with the use of prescription drugs, but also with the revelation that drugs affect men and women differently.

“Today, prescription painkillers are a drug of choice among women, in part because women are more likely to suffer from chronic pain...Women are more often prescribed painkillers and for longer periods of time than men. In fact women are 50% more likely to leave their doctors office with a prescription even if they have the same condition.”

“Drugs’ negative effects strike women harder and faster than men. For example, alcohol does as much damage to womens’s bodies in four years as it does to men’s bodies in 14 years.”

“...because of physiological differences such as women’s slower metabolism and ratio of fat to water in the body. These differences cause women’s bodies to hold onto drugs and alcohol longer...”

REFERENCES

Sack, M.D., D. (n.d.). Women and Prescription Drugs: The Gender Gap Tightens | David Sack, M.D. Retrieved from http://www.huffingtonpost.com/david-sack-md/prescription-drug-abuse_b_3756121.html

Report=20 Ref=5

Sarah Merritt

Pathology 438

Spring 2015

NAME _____

Midterm Examination

due: by 1:00 PM, 6 May 2015

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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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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

(Ghodke-Puranik)

(Loscher)

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

- 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

(Horn JR PharmD)

(Various)

(Kumar V)

3. Select (a) (b) (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 that 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?

(<http://jpet.aspectjournals.org/content/316/3/1195.full>)

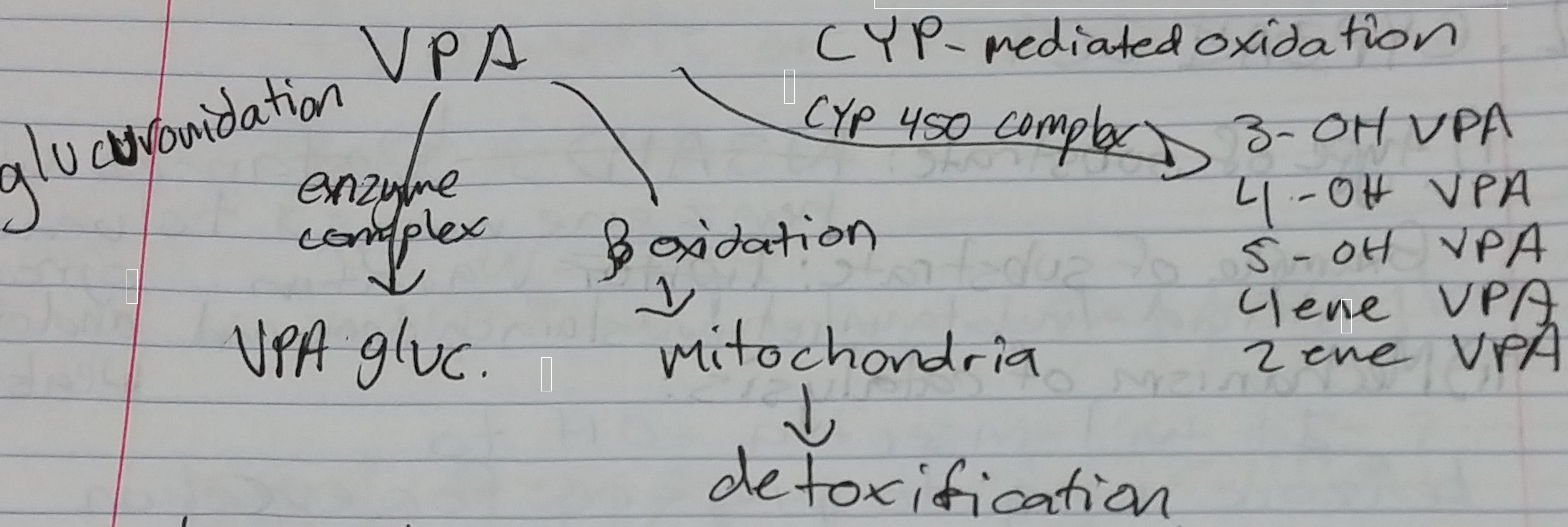
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PD: 9

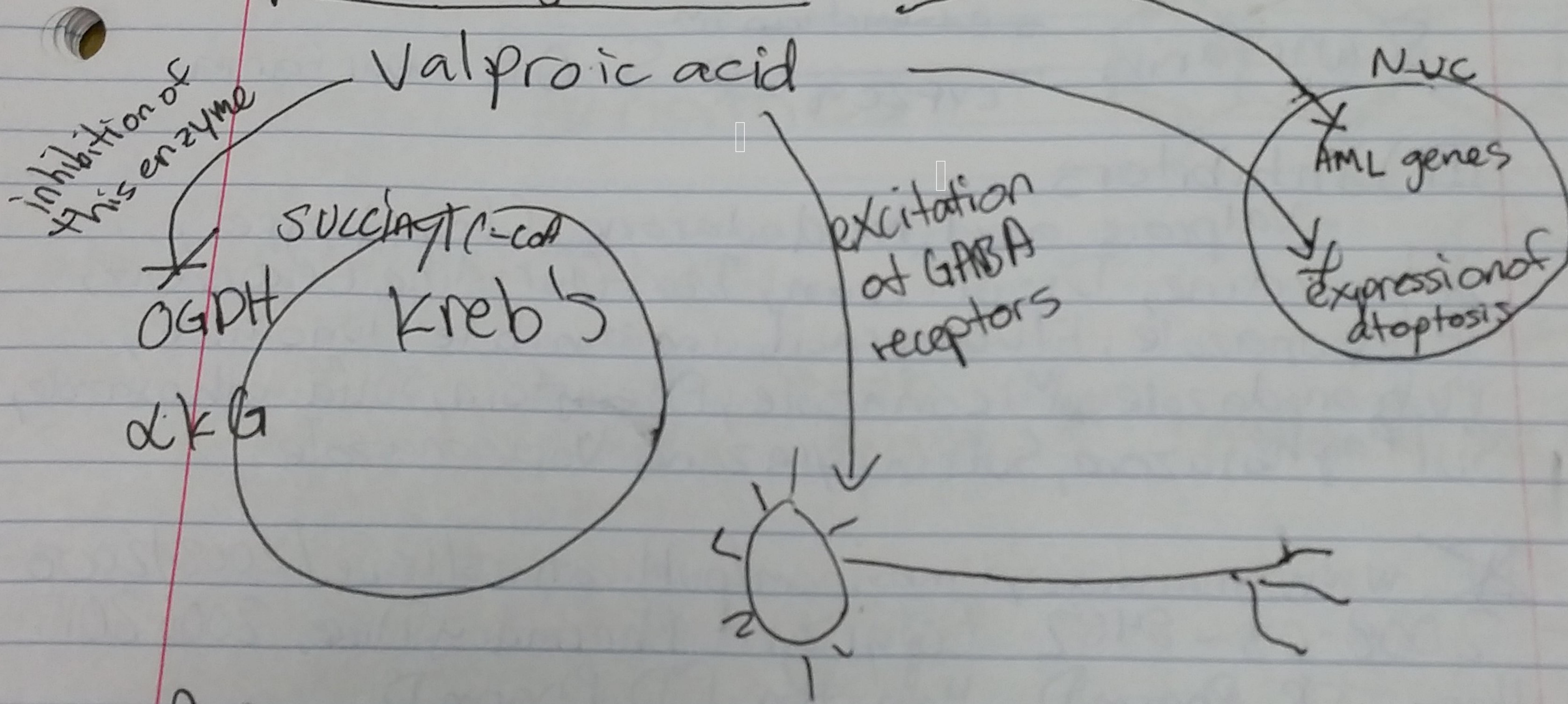
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T=21

1) Valproic acid - pharmacokinetics



pharmacodynamics



References:

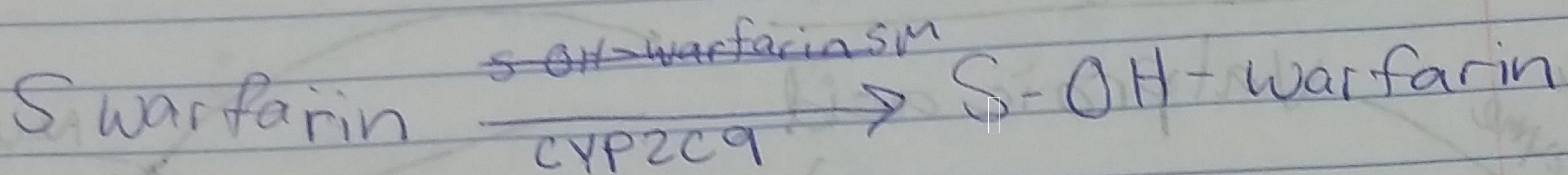
- ① Valproic acid pathway: pharmacokinetics & pharmacodynamics
- Ghodke-Puriwani, Thorn CF, Lamba JK, Leeder JS
Pharmacogenet Genomics 2013 Apr; 23(4):336-41
- ② Basic pharmacology of valproate: a review after 35 yr of clinical

use for the treatment of epilepsy. (Löscher)
CNS Drugs 16 (10): 669-694 (2002)

2. CYP3A4 2C9

(i) 5
(ii) 4
(iii) 4
(iv) 4
(v) 4
REF=5
T=26

- i) type of substrate: NSAIDs, substances w/
benzene rings & toluene
Example of substrate: ~~lipitor~~ Warfarin groups.
steroids, melanin, retinoids, arachidonic acid, and other
weak acids.
- ii) Mechanism of catalysis:
- It will insert a -OH to
benzoic ring to prepare for excretion



III) Inhibitors

- Valproic acid, Amiodarone, Clopidogrel,
Delavirdine, Disulfiram, Doxifluridine, Favipiravir,
Fluconazole, Fluorouracil, imatinib, leflunomide,
Metronidazole, Miconazole, Phenytoin, Sulfamethoxazole,
Sulfaephazone, Sulfinpyrazone, Voriconazole.

www.pharmacytimes.com/publications/issue/2008/2008-03,
2008-03-8462 Copyright Pharmacy Times 2006-2015
Horn JR PharmD, Hansten PD PharmD

IV. It's a series of α helices surrounding a heme group

www.wikipedia.org/wiki/CYP2C9

→ It's a p-glycoprotein induced enzyme so concurrent use w/
a CYP2C9 inhibitor will cause toxicity.

V. The only study I found that evaluated Km for CYP2C9 was performed ¹⁰ yrs ago. though no confirmed rate was found because of variable, they did find stand alone CYP2C9 performed sm much slower than with cofactors.

8. <http://dmd.aspetjournals.org/content/34/11/1903.full>
DMD Nov 2006 vol. 34 no. 11 1903-1908
Kumar V, Rock D, Warren C, Tracy TS, Wahlstrom J

3. Hydrocodone 131.2 M prescriptions yearly
webmd.com/news/20110420/the-10-most-prescribed-drugs.

Report=20 REF=4

- Male rats are more sensitive to opioid morphinans
- Male rats appear to have less pain tolerance.
- This sensitivity is independent of estrogen cycle.
- Non-morphinan opioid anti nociceptors opioids, fentanyl and methadone did not have the same sex based variance.
- Tip off, city does not play a role in this.
- These results were only true in vivo (vs. in vitro studies).

<http://jpet.aspetjournals.org/content/316/3/1195.full>

Shea Lindsay

Pathology 438
Spring 2015
NAME _____

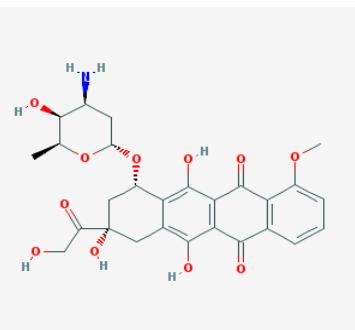
Midterm Examination

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

Doxorubicin-



Doxorubicin is an antineoplastic in the anthracycline class. General properties of drugs in this class include: interaction with DNA in a variety of different ways including intercalation (squeezing between the base pairs), DNA strand breakage and inhibition with the enzyme topoisomerase II. Most of these compounds have been isolated from natural sources and antibiotics. However, they lack the specificity of the antimicrobial antibiotics and thus produce significant toxicity. The anthracyclines are among the most important antitumor drugs available. Doxorubicin is widely used

for the treatment of several solid tumors while daunorubicin and idarubicin are used exclusively for the treatment of leukemia. Doxorubicin may also inhibit polymerase activity, affect regulation of gene expression, and produce free radical damage to DNA. Doxorubicin possesses an antitumor effect against a wide spectrum of tumors, including grafted or spontaneous. Nonencapsulated doxorubicin is metabolized by NADPH-dependent aldo-ketoreductases to the hydrophilic 13-hydroxyl metabolite doxorubicinol, which exhibits antineoplastic activity and is the major metabolite; these reductases are present in most if not all cells, but particularly in erythrocytes, liver, and kidney. Although not clearly established, doxorubicinol also appears to be the moiety responsible for the cardiotoxic effects of the drug. They have not yet determined if the nonencapsulated conventional drug is more toxic than the liposomally encapsulated form of the drug. Doxorubicin is capable of undergoing 3 metabolic routes: one-electron reduction, two-electron reduction, and deglycosidation. However, approximately half of the dose is eliminated from the body unchanged. Two-electron reduction yields doxorubicinol, a secondary alcohol. This pathway is considered the primary metabolic pathway. The one electron reduction is facilitated by several oxidoreductases to form a doxorubicin-semiquinone radical. These enzymes include mitochondrial and cytosolic NADPH dehydrogenases, xanthine oxidase, and nitric oxide synthases. Deglycosidation is a minor metabolic pathway (1-2% of the dose undergoes this pathway). The resultant metabolites are deoxyaglycone or hydroxyaglycone formed via reduction or hydrolysis respectively. Enzymes that may be involved with this pathway include xanthine oxidase, NADPH-cytochrome P450 reductase, and cytosolic NADPH dehydrogenase.

Adriamycin (Doxorubicin Hydrochloride): reasonably anticipated to be a human carcinogen. Because normal defense mechanisms may be suppressed by doxorubicin therapy, the patient's antibody response to the vaccine may be decreased. The interval between discontinuation of medications that cause immunosuppression and restoration of the patient's ability to respond to the vaccine depends on the intensity and type of immunosuppression-causing medication used,

the underlying disease, and other factors; estimates vary from 3 months to 1 year. LD₅₀=21800 ug/kg was found in subcutaneous doses in rats. DOX is metabolized to doxorubicinol (DOXol) and this metabolite has been implicated in cardiotoxicity. The metabolism has been reported to occur via aldo-keto reductase (AKR) 1C3, aldehyde reductase, and carbonyl reductases. However, others have reported that AKR1C3 did not metabolize DOX to DOXol. DOXol also appears to perturb the iron homeostatic processes that are associated with aconitase- iron regulatory protein-1 (ACO1), possibly causing cardiotoxicity. Dexrazoxane, an iron chelator, demonstrated clear cardioprotective properties in clinical studies when administrated before or with DOX. In addition, the glycosidic DOX bond can be cleaved to yield 7-deoxydoxorubicinone, again yielding ROS and hydrogen peroxide. DOX itself has also been shown to form a complex with iron that forms radicals. In addition to ROS, reactive nitrogen species (RNS) are also implicated in DOX cardiotoxicity via the disruption of nitric oxide (NO) regulation. Rodents treated with DOX showed heart dysfunction from the production of peroxynitrite formed from the rapid reaction of nitric oxide and superoxide in a mechanism involving nitric oxide synthases.

Absorption: Doxorubicin (nonencapsulated conventional) is not stable and actually goes through little absorption in the GI tract. It is also very hard on the tissues of the GI system so the drug has to be administered through an IV.

Distribution: Within 30 seconds of injection, the drug is present in the heart, liver, lungs and kidneys by binding to cellular components, specifically nucleic acids. This drug does not cross the blood-brain barrier, but it did show signs of crossing the placenta in mice and is present in breast milk. The distributive half-life was 5 minutes, which indicates that it is quickly taken up by the tissues when administered through IV.

Excretion: Nonencapsulated doxorubicin and its metabolites are excreted predominantly in bile; about 10-20% of a single dose is excreted in feces in 24 hours, and 40-50% of a dose is excreted in bile or feces within 7 days. About 50% of the drug in bile is unchanged drug, 23% is doxorubicinol, and the remainder is other metabolites including aglycones and conjugates. About 4-5% of the administered dose is excreted in urine after 5 days, principally as unchanged doxorubicin. It appears that very little further urinary excretion of the drug occurs after 5 days. Although only small urinary concentrations of the drug usually are achieved, doxorubicin often imparts a red color to the urine for the first hours to days after administration, and patients should be advised to expect this effect during therapy. Women and obese patients were seen to have a slower clearance of the drug. Children, age 2 and younger had a faster clearance than adults. The peak concentration of the drug in breast milk was at 24 hours, but still saw concentrations up to 72 hours after administering the drug. About half of the drug is excreted from the body unchanged.

Citations:

National Center for Biotechnology Information. PubChem Compound Database; CID=31703, <http://pubchem.ncbi.nlm.nih.gov/compound/31703> (accessed May 2, 2015).

Thorn Caroline F, Oshiro Connie, Marsh Sharon, Hernandez-Boussard Tina, McLeod Howard, Klein Teri E, Altman Russ B. "Doxorubicin pathways: pharmacodynamics and adverse effects" *Pharmacogenetics and genomics* (2010).

PK: AD=4 M=6 E=4

PD: 10

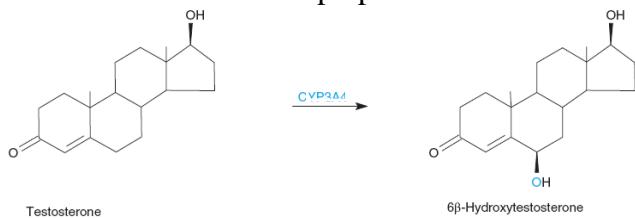
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T=29

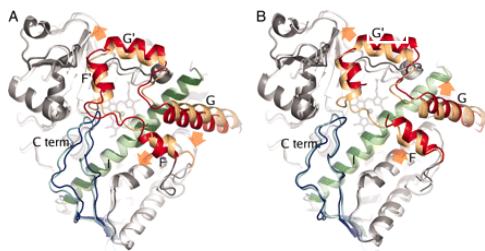
2. Select one of the cytochrome P450 enzymes below in the (a) through (d) list, and describe it as thoroughly as possible in points (i) through (v) below. You should cite at least one reference to a peer-reviewed publication or to a monograph for the course. For any information you put in your response,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

(a) CYP3A4

- This protein localizes to the endoplasmic reticulum and is activated by glucocorticoids and some pharmacological agents. This enzyme is involved in the metabolism of approximately half the drugs in use today, including acetaminophen, codeine, cyclosporin A, diazepam and erythromycin. The enzyme also metabolizes some steroids and carcinogens.
- Mechanism- Substrate (RH) + O₂ + NADPH + H⁺ → Product (ROH) + H₂O + NADP⁺, Cytochrome P450 enzymes oxidize by adding a O group on the substrate (drug) to catabolize it and prepare it for clearance.



- CYP3A4 inhibitors mainly include macrolide antibiotics (e.g., clarithromycin, and erythromycin), anti-HIV agents (ritonavir and delavirdine), antidepressants (fluoxetine and fluvoxamine), calcium channel blockers (verapamil and diltiazem), steroids and their modulators gestodene and mifepristone), and several herbal and dietary components. Many of these drugs are also mechanism-based inhibitors of CYP3A4, which involves formation of reactive metabolites, binding to CYP3A4 and irreversible enzyme inactivation.



IV.

Overall structures of CYP3A4 in complex with

ketoconazole (*A*) and erythromycin (*B*). Structures are shown in dark gray with color highlighting of helices F to G (residues 202–260) in red, helix I (residues 291–323) in green, and the C-terminal loop (residues 464–498) in blue. The complex structures are superimposed on the ligand-free structure (Protein Data Bank ID code 1TQN) shown in light colors. Orange arrows indicate the direction of coordinate shifts in the F-G region relative the ligand-free structure.

V.

CYP3A4 is the most abundant form of Cytochrome P450, making up about 30% of the total enzymes. It is also substantially expressed in the intestine and plays a dominant role in drug clearance, which is responsible for approximately 60% of P450-mediated metabolism of all marketed drugs. CYP3A4 induction causes problems in the pharmacology world due to its ability to clear drugs out the body quickly.

National Center for Biotechnology Information. (2015). CYP3A4 cytochrome P450, family 3, subfamily A, polypeptide 4 [*Homo sapiens* (human)] Gene ID: 1576, retrieved from ://www.ncbi.nlm.nih.gov/gene/1576?report=full_report#reference-sequence

Shu-Feng Zhou. (2008). Drugs Behave as Substrates, Inhibitors and Inducers of Human Cytochrome P450 3A4. *Current Drug Metabolism*. 9(4). 310-322. Retrieved from <http://www.eurekaselect.com/66808/article#sthash.r2hYveRW.dpu>

Fahmi, Odette. (2010). Cytochrome P4503A4 mRNA is a More Reliable Marker than CYP3A4 Activity for Detecting Pregnen X Receptor- Activated Induction of Drug-Metabolizing Enzymes. *The American Society of Pharmacology and Experimental Therapeutics*. 38(9). 1606. Retreived from <http://dmd.aspetjournals.org/content/38/9/1605.full.pdf+html>

Reference to class notes

(i)6 (ii)4 (iii) 4 (iv) 4 (v)2
REF=5 T=27

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

- a) **Sex Differences in Drug Disposition-** Many of the differences in men and women's ability to metabolize drugs is directly correlated with the physiological differences between genders.

For example in the GI tract and drug absorption, drug absorption occurs at different sites throughout the gastrointestinal tract, and rate of absorption is influenced by gut transit times, lipid solubility of the agent, pH at the site of absorption, and the ionization and

molecular weight of the agent. Transit times differ significantly in men and women, with mean transit times being shorter in men (44.8 hours) than in women (91.7 hours). While fiber ingestion decreases transit time, female gut transit times are consistently longer. Sex differences have also been noted in bile acid composition, which may impact the solubility of various drugs. Men have higher concentrations of cholic acid, while women have higher concentrations of chenodeoxycholic acid.

Body composition has a direct affect on the body's ability to metabolize drugs. Muscle tissue and adipose tissue can process drugs differently. Women tend to have a higher percentage of adipose tissue, while men have a higher percentage of muscle mass. Sex differences in blood distribution and regional blood flow can also impact pharmacokinetics. In general, the reference values for resting blood flow to organs and tissues for 35-year-old males and females show significant differences as a percentage of cardiac output. For example, blood flow to skeletal muscle is greater for men and to adipose tissue is greater for women. These differences may reflect sex-based differences in the percentage of total body mass represented by each tissue. Blood distribution will also impact clearance rates. Females exhibit decreased liver blood flow rates, which despite higher CYP3A4 amounts and activity, may result in lower drug clearance.

Sex hormones also play a role in the metabolism of drugs in the body. Increased levels of estrogen and progesterone alter hepatic enzyme activity, which can increase drug accumulation or decrease elimination of some drugs. Female steroid hormones and prolactin play a role in autoimmunity. Regulation of immunity and interactions between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes contribute to the 2- to 10-fold incidence and severity of autoimmune/inflammatory diseases in females compared to males. Most autoimmune diseases are detected in females of childbearing age. Metabolic changes can also depend on hormone levels that change during the menstrual cycle, with use of oral contraceptives, throughout pregnancy, or during menopause. For example, some asthmatic women have worsening symptoms before or during menstruation. An increase in oxidative stress in females has been described during intensive physical exercise, particularly in postmenopausal women. Moreover, sex hormone levels throughout the menstrual cycle are associated with the activation of specific hepatic enzymes and the rate of clearance of certain drugs. Caffeine and theophylline clearance, for example, is higher during the early follicular phase and prolonged during the mid-luteal phase.

Soldin, Offie. (2011) Sex Differences in Drug Disposition. *Journal of Biomedicine and Biotechnology*. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3051160/>

Report=20 Ref=5

Pathology 438

Midterm Examination

due: by 1:00 PM, 6 May 2015

Spring 2015

NAME STERLING PETERSEN

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 - b) clonidine
 - c) valproic acid
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
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v. Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc

- (a) CYP3A4
- (b) CYP2C9
- (c) CYP1A1
- (d) CYP2D6

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ANSWERS:

1. A.) Doxorubicin pharmacokinetics:

There are 3 main metabolic routes of anthracycline metabolism: one-electron reduction, two-electron reduction and deglycosidation. A large proportion of DOX however, approximately 50%, is eliminated from the body unchanged [Article:[19442138](#)].

Two-electron reduction of DOX to a secondary alcohol, DOXol is the major metabolic pathway [Article:[19442138](#)]. There are several enzymes that can carry out this reaction and their respective balance is different in different cell types [Article:[18635746](#)]. For example, AKR1A is considered the most important in heart tissue while CBR1 is the major contributor in liver [Articles:[18635746](#), [12963485](#), [19442138](#)]. CBR3 is also capable of forming DOXol [Article:[20007405](#)]. The role of AKR1C3 is unclear with some studies showing metabolism of DOX and others disputing it [Articles:[18616992](#), [18635746](#), [12963485](#)].

One-electron reduction of DOX is carried out by several oxidoreductases to form a DOX-semiquinone radical [Article:[2555273](#)]. These enzymes include mitochondrial NADH dehydrogenases present in the sarcoplasmic reticulum and mitochondria: NDUFS2, NDUFS3, and NDUFS7 (EC 1.6.99.3) [Articles:[12688675](#), [2850270](#), [9618942](#)] as well as cytosolic enzymes NADPH dehydrogenase (NQO1) [Article:[12688675](#)], xanthine oxidase (XDH) [Articles:[12688675](#), [1911046](#)] and nitric oxide synthases (NOS1, NOS2 and NOS3) [Articles:[9333325](#), [15054088](#)]. Re-oxidation of the DOX-semiquinone radical back to DOX leads to the formation of reactive oxygen species (ROS) and hydrogen peroxide [Article:[9576481](#)]. ROS, causing oxidative stress, can be deactivated by glutathione peroxidase (GPX1), catalase (CAT) and superoxide dismutase (SOD1) [Article:[12751786](#)]. It is the ROS released by this route of metabolism, rather than DOX-semiquinone, that some consider responsible for drug effects and adverse cardiotoxicity.

The third, minor route, deglycosidation, accounts for approximately 1-2% of DOX metabolism. This can be reductive to form the deoxyaglycone, or hydrolytic to form the hydroxyaglycone. The enzymes and their candidate genes for this process are less well characterized [Articles:[19442138](#), [10813659](#)]. In heart, no DOX hydroxyaglycone could be detected; it appears to be rapidly converted to DOXol aglycone [Article:[10813659](#)]. In heart, NADPH is required for formation of aglycones suggesting that NADPH dependent hydrolase and reductase-type glycosidases are responsible [Article:[10813659](#)]. NADPH-cytochrome P450 reductase (POR) was shown in vitro to metabolize DOX to DOX 7-deoxyaglycone [Articles:[6305277](#), [10860924](#)]. XDH and NQO1 were also implicated in this process [Article:[10860924](#)].

Citation: Thorn Caroline F, Oshiro Connie, Marsh Sharon, Hernandez-Boussard Tina, McLeod Howard, Klein Teri E, Altman Russ B. "[Doxorubicin pathways: pharmacodynamics and adverse effects](#)" *Pharmacogenetics and genomics* (2010).

Doxorubicin pharmacodynamics:

The anthracycline doxorubicin (DOX), a metabolite of *Streptomyces peucetius* var. *caesius* [Article:[5365804](#)], is a chemotherapeutic agent developed in the 1970s [Article:[17652804](#)] that is used in the treatment of a wide range of cancers, including non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, lung, ovarian, gastric, thyroid, breast, sarcoma, and pediatric cancers [Articles:[17652804](#), [1462166](#)]. This pathway shows the pharmacodynamics of doxorubicin in a stylized cancer cell and depicts candidate pharmacogenes.

Several mechanisms have been proposed to explain DOX antitumor activity [Article:[10075079](#)]. Here we describe 2 major mechanisms: the intercalation into DNA, leading to inhibition of the DNA synthesis or poisoning of topoisomerase II (TOP2A); and generation of free radicals, leading to DNA and cell membrane damage. We also show some pharmacokinetics (for more details see Doxorubicin PK Pathway) and depict transporters associated with drug resistance in cancer cell lines.

Intercalation into DNA

Anthracyclines are known to intercalate into DNA in vitro, and several crystal structures of complexes of DNA with DOX exist (see for example pdb entries 151D [Article:[8142363](#)] and 1P20 [Article:[12717724](#)]). In early in vitro studies, DOX was shown to cause DNA breaks [Article:[566561](#)] and to interfere with DNA synthesis [Articles:[1277199](#), [1089410](#)]. Other work has shown that the DNA-DOX interaction is related to the poisoning of topoisomerase II (TOP2A) [Articles:[2551497](#), [6093249](#)], but not topoisomerase I [Articles:[2164630](#), [10385686](#)]. Translocation of DOX into the nucleus is thought to occur via binding to proteasomes [Article:[11289116](#)]. Subsequent TOP2A poison-mediated cytotoxicity is postulated to involve the mismatch repair genes MSH2 and MLH1 [Article:[11477562](#)] because loss of DNA mismatch repair function results in resistance to doxorubicin [Articles:[11477562](#), [9514047](#)].

Topoisomerase II-mediated DNA damage is followed by cell death [Article:[11172690](#)]. TP53, a gene that is a major player of the DNA-damage response and apoptosis [Article:[11790556](#)], has been implicated in this DOX-apoptosis pathway. Several studies have shown an upregulation of TP53 occurs with anthracycline treatment [Articles:[12739000](#), [10914720](#)], and ERCC2 and TP53 have been shown to functionally interact in a p53-mediated apoptotic pathway with DOX treatment in lymphoblastoid cell lines [Article:[10467415](#)]. However, the actual involvement of p53 in DOX-induced apoptosis has been debated by other researchers [Article:[11172690](#)].

Generation of free radicals

DOX can undergo a one-electron reduction by several oxidoreductases to form a DOX-semiquinone radical [Article:[2555273](#)]. These enzymes include mitochondrial NADH dehydrogenases present in the sarcoplasmic reticulum and mitochondria: NDUFS2,3,7 EC 1.6.99.3 [Articles:[12688675](#), [2850270](#), [9618942](#)] as well as cytosolic enzymes NAD(P)H dehydrogenase (NQO1) [Article:[12688675](#)], xanthine oxidase (XDH EC 1.2.3.2) [Articles:[12688675](#), [1911046](#)] and endothelial nitric oxide synthase (NOS3) [Article:[9333325](#)]. Re-oxidation of the DOX-semiquinone radical back to DOX leads to the formation of reactive oxygen species (ROS) and hydrogen peroxide [Article:[9576481](#)]. ROS, causing oxidative stress, can be deactivated by glutathione peroxidase, catalase and superoxide dismutase [Article:[12751786](#)].

Some researchers have related DOX free radical formation to cytotoxicity; these studies relate DOX cellular resistance to enzymes deactivating ROS. DOX has been shown to promote apoptosis in DOX-treated endothelial cells and myocytes through the formation of ROS and hydrogen peroxide [Article:[12139490](#)] via the activation of NF-kB (NFKB1, p50). But, activation of NF-kB blocked apoptotic cell death DOX-treated cancer cells [Article:[12139490](#)], indicating a possible different mechanism for cytotoxicity and cardiotoxicity. These researchers, and others, argue that the role of free radical formation is primarily related to cardiotoxicity and not cytotoxicity [Article:[10075079](#)], in part, because the use of an iron chelator, dextrazoxane, that binds the iron involved in the free radical formations, demonstrates cardioprotective properties without impacting clinical outcome [Articles:[15038979](#), [9777314](#), [9193324](#), [9193323](#), [18425895](#)].

Resistance

While DOX is a valuable clinical antineoplastic agent, in addition to problems with cardiotoxicity, resistance is also a problem limiting its utility [Articles:[2982511](#), [1462166](#)]. The mechanism of resistance is thought to involve, in particular, ABCB1 (MDR1, Pgp) and ABCC1 (MRP1) as well as other transporters.

In general, ABCB1 confers resistance by acting as an ATP-dependent drug efflux pump causing increased drug efflux [Article:[8763334](#)] via altered or increased expression [Articles:[8763334](#), [9073310](#)]. Cytotoxicity of DOX increases with inhibition of ABCB1 [Articles:[1352877](#), [15788683](#)]. Human ABCC1, originally cloned from a DOX-selected cancer cell line [Article:[1360704](#)] confers resistance to anthracyclines. Various studies on DOX-resistant cell lines have shown that resistance can be overcome via an inhibition of ABCB1, ABCC1 and ABCC2. [Articles:[12370750](#), [15164094](#), [7214365](#), [1352877](#), [3180056](#), [17704753](#), [11172691](#), [7954421](#)].

Studies have also shown an association between resistance and activity of other transporters. For example, RALBP1 activity was shown to be 2 times higher in a DOX-sensitive cell line versus DOX resistant cell line [Article:[12527936](#)]. In a study of a panel of lung cancer cell lines, a correlation between the DOX semiquinone levels and proteins levels for ABCC3 and ABCG2 was demonstrated [Article:[11410522](#)]. Glutathione transferase activity was found to be greater in DOX-sensitive leukemia cells than in DOX-resistant leukemia cells [Article:[2897875](#)].

Citation:

Thorn Caroline F, Oshiro Connie, Marsh Sharon, Hernandez-Boussard Tina, McLeod Howard, Klein Teri E, Altman Russ B. "Doxorubicin pathways: pharmacodynamics and adverse effects" *Pharmacogenetics and genomics* (2010).

PK: AD=2 M=6 E=1

PD: 10

Ref: 5

T=24

2D) CYP2D6 Gene:

Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize: CYP2D6 is primarily expressed in the liver and is highly expressed in the CNS. It is also one of the most important enzymes involved with the metabolism of xenobiotics in the human body, such as pollutants, dioxins, etc.(1)

Explain the mechanism of catalysis (you can even draw the steps): Catalysis works by changing the activation energy for a given reaction, which is the minimum energy needed for the reaction to occur. This is accomplished by providing a new mechanism or reaction path through which the reaction can proceed. When the new reaction path has a lower activation energy and occurs at a faster rate, that is known as catalysis.

Provide the names of any substances known to inhibit the cytochrome, if any: There may be a drug-drug interaction and be either inhibitors or inducers, which would either increase CYP2D6 activity or reduce it. Noticeable strong inhibitors are SSRIs, bupropion, quinidine, cinacalcet and ritonavir. (2)

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: I could not find any description of the exact detailed protein or gene structures.

Provide, if any, known enzyme kinetic parameters: turnover/catalysis rate, etc: It was difficult to find anything that solely talked about CYP2D6, but CYP2C9-CYP2D6 interactions can alter catalytic activity and, thus, influence in vitro-in vivo correlation predictions. (3)

Citation:

- 1) Wang B, Yang LP, Zhang XZ, Huang SQ, Bartlam M, Zhou SF (2009). "New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme". *Drug Metab. Rev.* **41** (4): 573–643. doi:10.1080/03602530903118729. PMID 19645588.
- 2) [Interactions: Cytochrome P₄₅₀ Drug Interaction Table](#). Indiana University School of Medicine. Retrieved on July 2011
- 3) Published online before print May 15, 2009, doi: 10.1124/dmd.109.026500, DMD August 2009 vol. 37 no. 8 1682-1689

(i)4 (ii)2 (iii)4 (iv)0 (v)2
REF= 5 T=17

Question 3: The differences in how men and women respond to toxicants and drugs.

In initially researching this topic, I found a lot of material on the subject that shows a lot of drugs have different reactions for men than they do for women. The higher fat percentage, difference in hormonal cycles, different digestion pathways and timing, and the fact that most drug studies in the past included men for the most part are all contributing factors.

The physiologic differences between men and women play an important role in disease prevalence and outcomes. For example, women are more likely than men to develop cataracts, depression, hepatitis, irritable bowel syndrome, migraines, multiple sclerosis, rheumatoid arthritis, and thyroid dysfunction. Men are more likely to experience myocardial infarction (MI), although women are more likely to die within a year following an MI. Despite the increased susceptibility to many diseases, women consistently live longer than men. Sex-related differences also have important implications for drug activity, including pharmacokinetics and pharmacodynamics.

Beta blockers, particularly metoprolol, produce a greater pharmacodynamic response in women. No differences in half-life have been observed between men and women; however, women taking metoprolol demonstrate a greater reduction in systolic blood pressure and heart rate while exercising. These differences are caused by a higher plasma drug concentration in women

Estrogen can influence pain pathways, alter pain perception, and affect response to certain drug classes. Because estrogen is present in substantially higher levels in women than in men, women tend to exhibit lower pain thresholds, increased pain ratings to standardized stimuli, and lower tolerance to pain. Women also demonstrate a greater analgesic response to opioids. To achieve equivalent pain relief, men require a 30 to 40 percent greater dosage of morphine. Sex differences have been attributed to dimorphism in central opioid metabolism or in opioid action at the cellular level. Women also are more likely to experience greater sedative properties and respiratory depression from opioids.

Men and women respond differently to antidepressant and antipsychotic agents. Although there appears to be no difference in depression symptom severity, women generally respond better to selective serotonin reuptake inhibitor (SSRI) therapy, especially sertraline (Zoloft), compared with tricyclic antidepressants, such as imipramine (Tofranil). This may be because women produce more tryptophan and less cortisol when exposed to SSRI therapy. Conversely, men respond better to tricyclic antidepressant medications than SSRIs.

CITATION:

Sex Based Differences in Drug Activity, Whitley Heather P., Lindsey Wesley, *Am Fam Physician*. 2009 Dec 1;80(11):1254-1258. (Whitley)

Report=20 Ref=5 T=25

Pathology 438

Midterm Examination

due: by 1:00 PM, 6 May 2015

Spring 2015

NAME – Steven Keener

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxicopharmacokinetics and toxicopharmacodynamics 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 “first generation” antiepileptic drug, thought it is also used for certain mania and bipolar disorders. The reason I chose this substance is because of its extremely narrow therapeutic to toxicity ratio. For this reason, teratogenicity is of great concern when prescribing this substance. In fact, “*in utero* exposure to valproate... is associated with an increased risk of impaired cognitive function at 3 years of age.”² The pharmacokinetics and pharmacodynamics of this substance are vast and extremely complex, however I will attempt to sort out the high points from the literature and report on them.

According to Lippincott, “Pharmacokinetics refers to what the body does to a drug.”² This process can be broken down into four basic components: Absorption, Distribution, Metabolism, and Elimination.²

Absorption: The substance is well absorbed why oral ingestion, however, considering the fact that Valproic acid is a bioavailable a free acid, it has been shown to easily upset the GI system when administered.⁵ For this reason, Divalproex Sodium is often given in place of valproic acid (Divalproex Sodium is a combination of sodium valproate and valproic acid). This combination in salt form makes the substance much more tolerable for human GI systems and thus compliance in increased.² The extent of the availability is considered to be 100%.⁵ After a meal, the substance is absorbed within 4 hours, with a peak plasma level reached within 7.5 hours after ingestion.⁵

Distribution: The substance is rapidly transported into extracellular water and is otherwise restricted to circulation.⁵ Valproic acid's mechanism of action (specifically) is not fully understood, but its distribution throughout the body is profound. The volume distribution of the free drug in plasma is 1 L/kg. It binds with albumin proteins in the blood and therefor hitches a ride throughout much of the body, including the brain. Because of the binding to albumin, Valproic acid is highly susceptible to increased effectiveness when combined with other drugs, specifically Salicylates. The protein binding rate at therapeutic concentrations is around 90%. Once protein levels drop much below this, the substance is quickly cleared and eliminated.⁵ High distribution to the liver, therefor raises in liver enzymes should be monitored.⁴ Blood levels of 50-100 mg/mL is expected at therapeutic doses.⁵

Metabolism: Primarily metabolized by the liver.² This is done by the common processes of Beta oxidation and omega oxidation. The metabolic actions of Valproic acid are also vast and widely unknown. Possible mechanisms of metabolism include the following: "sodium channel blockade, blockade of GABA transaminase, and action at the T-type calcium channels."² Valproic acid is also thought to inhibit metabolism of the CYP2C9 (discussed later in Question 2), UGT and epoxide hydrolase systems.²

Elimination: In a healthy adult, approximately 1.8% of the substance is excreted unchanged in the urine.⁵ Valproic acid is eliminated by standard first order kinetics.² This process is done at a rate of about 5-10 mL/min in healthy adults, and appears to function independent of liver blood flow.⁵ Metabolites excreted are as follows: valproic acid, glucuronide, 3-oxovalproic acid, omega oxidation products.⁵

On the other hand, pharmacodynamics refers to "what the drug does to the body."² The mechanism of this substance is truly unknown. The one majorly understood mechanism is the fact that valpoic acid increases concentrations of the neurotransmitter GABA in the brain via inhibition of the GABA-transaminase and succinic aldehyde dehydrogenase enzymes. The substance has also been shown in animal studies to inhibit neuronal activity by increasing potassium conductance.⁵

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

PK: AD=4 M=6 E=4

PD: 10

Ref: 5

T=29

- 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

I choose to comment on the enzyme CYP2C9. I must admit, this choice is out of complete randomness, however I have enjoyed learning more about this extraordinary P450 enzyme.

- i. This enzyme is a phase-1 drug-metabolizing cytochrome P450, meaning it primarily oxidizes both xenobiotics and endogenous compounds.⁷ This is obviously done so in the liver, and has been suspected of undergoing polymorphic transmission. Examples of Phase-1 drugs that this enzyme metabolizes are as follows (all lipophilic agents): Warfarin, phenytoin, acenocoumarol, tolbutamide, losartan, glipizide, and other drugs.⁶ NSAIDS are also metabolized.
 - ii. The mechanisms through which this enzyme catalyzes and exerts its effects are variable and P450 isoform-dependent. And while the effects of the enzymes CYP2D6, CYP2C9, and CYP34A have been well studied; fewer studies have been performed on CYP2C9 mechanisms. The enzyme appears to consume NADPH by Cyt P450 reductase, thus uncoupling a reaction cycle to hydrogen peroxide and water.⁷
 - iii. There are many known inhibitors of this enzyme, the list is as follows: valpoic acid (no figure), fluconazole (antifungal), miconazole (antifungal), amentoflavone, sulfaphenoazole, ibigenin, amiodarone, antihistamines, chloramphenicol, fenofibrate, flavones, flucastatin, cioniazoids, NSAIDS, probenecids.
 - iv. Not much is understood regarding this enzyme's structure, however it was first described by Williams et al. as having "an unanticipated new binding pocket, suggesting that CYP2C9 may simultaneously accommodate multiple ligands during its biological function. Structural analysis suggests that CYP2C9 may undergo an allosteric change when binding warfarin. An x-ray crystal structure of CYP2C9, in complex with the NSAID flurbiprofen, has also been described."⁷

v. None found.

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 4
REF=5 T=27

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 that 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?

This study was done in May of 2013 investigating the differences in male and female response to drugs. The authors stated that “these differences can be critical in respects to drug treatment policy,” and I would have to agree!⁸ The study was first investigated in response to the obvious difference men and women have at dealing with and “metabolizing” many of life’s other struggles, not just drugs, such as employment layoffs, occupational hazards, and so on. This was the inspiration for the study. The pharmacokinetics and pharmacodynamics are discussed in depth in this study, however I will lay out the significant points (Tables are included from the study referenced for clarity). Also to note, this study did not study specific differences in quantity, rather difference is quality (via analysis of anatomical and physiological differences between genders).⁸

It appears that the study largely followed the levels of cytochrome P450 and its many enzymes involved in its pathways of drug metabolism.

Table I
Anatomic differences between Men and Women

Parameter	Reference Adult Male	Reference Adult Female	Pregnant Female
Body Weight (kg) [*]	78	68	72.5
Body Length (cm) [*]	176	162	162
Body Surface Area (cm ²)	18,000	16,000	16,500
Total Body Water (L)	42.0	29.0	33.0
Extracellular Water (L)	18.2	11.6	15.0
Intracellular Water (L)	23.8	17.4	18.8

*CDC Advance Data No. 347 October 27, 2004

Since drugs are absorbed, distributed, metabolized, and excreted utilizing human anatomy, it makes sense that since the anatomy of males and females differs, so does the action of certain drugs/toxicants, as noted in the above Table 1.

Basic metabolic rate, concentrations of plasma in hollow organs, and presence/absence of a fetus can all alter the activity and response to a drug. For this reason, men and women can have vastly different responses to drugs.

Table IX

Sex differences in pharmacokinetics: elimination Physiological parameters which may influence differences in excretion.

PARAMETER	PHYSIOLOGIC DIFFERENCE	PHARMACOKINETIC IMPACT
Renal Blood Flow GFR	pregnant F>M>F	Increase renal elimination
Pulmonary Function	M>pregnant F>F	Increase pulmonary elimination
Plasma Proteins	decrease in pregnant F	Decreased elimination

Table III

Physiological parameters which influence absorption

PARAMETER	PHYSIOLOGIC DIFFERENCE	PHARMACOKINETIC IMPACT
Gastric pH	acidity M > F > preg F	Altered absorption of acid/bases depending on specific drug absorption of weak acid
Gastric Fluid Flow	M > F	Higher absorption in males
Intestinal Motility	M > F > pregnant F	Absorption increased in males
Gastric Emptying	M > F > pregnant F	Absorption, gastric hydrolysis increased
Dermal Hydration	Increased in pregnant F	Altered absorption in pregnant F
Dermal Thickness	M > F	Absorption decreased in males
Body Surface Area	M > pregnant F > F	Absorption increased when surface area larger

Table XSome drugs that show Sex Differences in Pharmacokinetics^{*}

Drug	Pharmacokinetic Parameter	Comments
Acebutolol	Area under the concentration-time curve	The concentration-time profile is larger in women than men. This may lead to therapeutic and potential side effects.
Aspirin	Clearance, half-life	Aspirin is cleared more rapidly from women than men.
Benzylamine		Following transdermal absorption women excrete more benzylamine than men.
Beta-Blockers; metoprolol,	Oral clearance lower in women, low volume of distribution in women resulting in higher systemic exposure	The greater reduction in blood pressure in women compared to men is due to pharmacodynamic differences.
Cefazolin	Clearance, volume of distribution, half-life	Clearance increases during pregnancy as a result of decreased body weight. There is no change in volume of distribution.
Cefotaxime	Clearance	Clearance is decreased in women.
Ciprofloxacin	Clearance	Clearance is lower in women than men.
IM Cephradine		Slower rate of absorption and lower bioavailability in women.

Overall great study, very interesting material.

Report=20 Ref=5 T=25

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Tanner
Spring 2015
Pathology 438
Midterm Exam

1. Doxorubicin

Complete description of the toxicopharmacokinetics and toxicopharmacodynamics:

There are 3 metabolic routes for doxorubicin metabolism: “one-electron reduction, two-electron reduction and deglycosidation.” About 50% of DOX is eliminated from the body without any performed change.

One electron reduction of DOX: “carried out by several oxidoreductases to form a DOX-semiquinone radical. These enzymes include mitochondrial NADH dehydrogenases present in the sarcoplasmic reticulum and mitochondria as well as cytosolic enzymes NADPH dehydrogenase, xanthine oxidase and nitric oxide synthases.”

Two-electron reduction of DOX: “to a secondary alcohol, DOX ol is the major metabolic pathway. There are several enzymes that can carry out this reaction and their respective balance is different in different cell types.”

Deglycosidation: (1-2% of DOX metabolism) “This can be reductive to form the deoxyaglycone, or hydrolytic to form the hydroxyaglycone. The enzymes and their candidate genes for this process are less well characterized. In heart, no DOX hydroxyaglycone could be detected. It appears to be rapidly converted to DOX ol aglycone. In heart, NADPH is required for formation of aglycones suggesting that NADPH dependent hydrolase and reductase-type glycosidases are responsible. NADPH-cytochrome P450 reductase was shown in vitro to metabolize DOX to DOX 7-deoxyaglycone.”

PK: AD=1 M=6 E=1

PD: 2

Ref: 5

T=15

Doxorubicin. (n.d.). Retrieved May 3, 2015, from
<https://www.pharmgkb.org/drug/PA449412#tabview=tab4&subtab=32>

Baylon, J., Lenov, I., Sligar, S., & Tajkhorshid, E. (n.d.). Characterizing the Membrane-Bound State of Cytochrome P450 3A4: Structure, Depth of Insertion, and Orientation. Retrieved May 3, 2015, from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682445/>

2. CYP3A4

- Provide a description of the type of substrates it metabolizes and give an example of one substrate it is known to metabolize:

Cytochrome P450 3A4 is typically found in the liver and intestines. The main purpose is to oxidize same foreign molecules (toxins and drugs) and remove them from the body. P450 3A4 initiates drug metabolism as well as synthesis of cholesterol, steroids, and other lipids. It is located in the endoplasmic

reticulum and metabolizes many of the drugs that are used today.
(acetaminophen, codeine, diazepam)

- b. Explain the mechanism of catalysis:

Cytochrome P450 performs many modifications on several ligands. It has a large active site, which allows it to bind substrates more readily, and also more difficult compounds such as endogenous and exogenous compounds. (hydroxylation, epoxidation of olefins, aromatase oxidation) Ex. Tamoxifen: It is hydroxylated to 4-hydroxy-tamoxifen then dehydrated to 4-hydroxy-tamoxifen quinone methide.

- c. Provide the names of any substances known to inhibit the cytochrome, if any:

Grapefruit and certain drugs (astemizole and terfenadine)

- d. If its gene and/ or protein structure is known, describe the domains (functional parts) of the enzyme, and any molecular detail that are interesting or significant to the enzyme's function

The first structure was determined around 2005 as "an active site of sufficient size and topography to accommodate either large ligands or multiple smaller ligands, as suggested by the heterotropic and homotropic cooperativity of the enzyme."

- e. Provide, if any, known enzyme kinetic parameters: turnover/ catalysis rate:

The only information I could find here was the half-life for CYP3A4 which ranges from 26-140h.

Retrieved May 3, 2015, from [http://www.cell.com/trends/biochemical-sciences/abstract/S0968-0004\(04\)00294-4?_returnURL=http://linkinghub.elsevier.com/retrieve/pii/S0968000404002944?showall=true&cc=y](http://www.cell.com/trends/biochemical-sciences/abstract/S0968-0004(04)00294-4?_returnURL=http://linkinghub.elsevier.com/retrieve/pii/S0968000404002944?showall=true&cc=y)

Retrieved May 3, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/10594474> RCSB Protein Data Bank - RCSB PDB - 2J0D Structure Summary. (n.d.). Retrieved May 3, 2015, from <http://www.rcsb.org/pdb/explore.do?structureId=2J0D>
RCSB Protein Data Bank - RCSB PDB - 2J0D Structure Summary. (n.d.). Retrieved May 3, 2015, from <http://www.rcsb.org/pdb/explore.do?structureId=2J0D> (i) 6 (ii) 4 (iii) 4 (iv) 3 (v) 3
REF = 5 T = 25

3. 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 board survey. In any article you obtain, be sure

to indicate at least three significant points, but list all of them if there are more”

“Physiologic differences between men and women affect drug activity.”

“Pharmacokinetics in women is affected by lower body weight, slower gastrointestinal motility, less intestinal enzymatic activity, and slower glomerular filtration rate.”

“Pharmacodynamic differences in women include greater sensitivity to and enhanced effectiveness of beta blockers, opioids, selective serotonin reuptake inhibitors, and typical antipsychotics.”

“Women are 50 to 75 percent more likely than men to experience an adverse drug reaction.”

Sex-Based Differences in Drug Activity. (n.d.). Retrieved May 3, 2015, from
<http://www.aafp.org/afp/2009/1201/p1254.html>

Report=20 Ref=5 T=25

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Toxicology Take-home Mid-term
By
Valerie Lyon

1. Valproic Acid pharmacokinetics and pharmacodynamics

Valproic Acid is available in tablets, rectal suppositories, syrup and injectable forms. in the pharmacy it can be found in tablet form under names like Depakote, Stavzor, Convulex, and Myproic acid. When tablets are digested and the Valproic acid (VPA) is in the blood stream this chemical is acted on by carnitine to get VPA into the mitochondria of the liver. In the mitochondria the Valproic acid is oxidized by being catalyzed by Medium-chain acyl-CoA synthase to make valproyl-CoA. This is dehydrogenated to 2-propyl-valproyl-CoA by 2-methyl-branched chain acyl-CoA dehydrogenase or isovaleryl-CoA dehydrogenase. The product from this reaction is catalyzed by enoyl-CoA hydratase to make 3-hydroxyl-valproyl-VPA. Then 2-methyl-3-hydroxybutyryl-CoA dehydrogenase catalyzes that product to make 3-hydroxyl-valproyl-VPA. Then unknown thioestrazenes metabolize 3-oxo-VPA in slow hydrolysis creating 3-oxo-VPA and Co-ASH. These products start a new pathway where 3-oxo-VPA is cleaved by 3-keto-valproyl-CoA thiolase producing propionyl-CoA and 4-ene-VPA-CoA ester which is then beta oxidized to 2,4-diene-VPA-CoA ester via ACADS. Finally the body excretes (E)-2,4-diene-VPA in the urine.

If 4-ene-VPA is reacted in a fluoridated environment 2,4,diene-VPA-S-CoA is produced which then groups with glutathione to create cytotoxic thiols that tend to take from the glutathione stores eventually stopping the beta-oxidation pathway. VPA also increases GABA levels by stopping the inhibition of its generation. It does this by preventing iGABA's degeneration by blocking ABAT and ALDH5A1 and OGDH reactions. On this. VPA blocks the voltage gated ion channels in neurons. Finally, VPA inhibits and activates HDAC and its derivatives which can inhibit apoptosis meaning that VPA is associated with antitumor activity. VPA activates HDAC 9 and 11 in cancer cell lines. This means that it might be promoted as a neural cancer fighting drug as well as an antiepileptic.

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<https://www.pharmgkb.org/pathway/PA165964265#tabview=tab0&subtab=>,
<https://www.pharmgkb.org/pathway/PA165959313>, PK: AD=1 M=6 E=2
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3696515/> PD: 8
Ref: 5 T=22

2. CYP2C9

- 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

Cytochrome P450 2C9 is known to metabolize a large number of drugs in the system. drugs it affects are things like blood pressure medications (losartan) , NSAIDs (ibuprofen) , drugs indicated for diabetic use (glipizide) , and antiepileptics (VPA). Medications like amiodarone, fluconazole, and sulfaphenazole inhibit CYP2C9's activity. CYP2C9 oxidizes and hydroxylizes drugs like Diclofenac and flurbiprofen.CYP2C9 has a crystalline structure and creates a pocket for the chemicals it catalyzes and it may have the ability to perform more than one catalization at a time. With the 4'- OH (S) - flurbiprofen, CYP2C9 consumes 2.8 nmol drug/ min/ nmol CYP2C9 and 13 nmol/ min/ nmol CYP2C9 for diclofenac.

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- Yan, Z., Li, J., Huebert, N., Caldwell, G., Du, Y., & Zhong, H. (2005, March 11). DETECTION OF A NOVEL REACTIVE METABOLITE OF DICLOFENAC: EVIDENCE FOR CYP2C9-MEDIATED BIOACTIVATION VIA ARENE OXIDES. Retrieved May 3, 2015, from <http://dmd.aspetjournals.org/content/33/6/706.full> (i) 6 (ii)4 (iii)1 (iv)0 (v)2 REF=5 T=18

- 3) 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.

It has been noted that females follow medical protocol more when their MD is also a female, and their prognosis is better over all. This may be a cultural preference because women aren't publicly known to be at as high or higher risk of heart disease. Also women don't tend to show effects of heart disease before they have problems like cardio vascular accidents. The article also suggests that the reason may be due to the fact that more seniors are female than male.

The digestion rates of the medication are different between men and women. Female's stomachs are more basic and their digestive systems take longer to process their food than men. The differences may depend on the sex hormones of each gender. Also some

generic medications are metabolized differently than the original. Franconi and Campesi give an example of polyethylene glycol that increases the availability or ranitidine in men but decreases it in women. The fillers in the medications may also have different effects between genders. Females and males present with different genetic markers and morphologies which may be a factor in how women metabolize the medications differently.

Angiotensin converting enzyme inhibitors (ACEIs) tend to have more side effects of coughs and heart swelling than in men. Eplerenone selective aldosterone antagonists showed a greater benefit in women, who tend to have more constant ratio of aldosterone to cardiac wall thickness than males after 30 days of treatment. When compared 16 months later men had done better by avoiding the hospital or mortality. only about 30% of the people in the study were female so they aren't sure if this reflects the population's truly predictable effects. Spironolactone when studied in rats only benefitted the males with high salt diets.

Part of the reason women may have a higher chance of heart rate is the fact that women tend to hold more of their sympathetic stress around their heart. Females also don't react as easily to sympathetic vasoconstriction, this may be due to the beta-adrenoreceptor density in their lymph system.

Females' heart problems when under a prescription regimen increase when estrogen is high and decrease when progesterone levels are high. problems that lead to adverse drug effects(ADEs) like multiple therapies, depression or aging affect women more than men. I wouldn't be surprised if a women's menstrual cycle including nutrient and blood loss may play a part in the increased rates of ADE occurrence. it is also suggested several times that the mass difference between males and females alter the way women react to medications.

Franconi, F., & Campesi, I. (2013, August 16). Pharmacogenomics, pharmacokinetics and pharmacodynamics:interaction with biological differences between men and women. Retrieved May 6, 2015, from
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1. Clonidine:

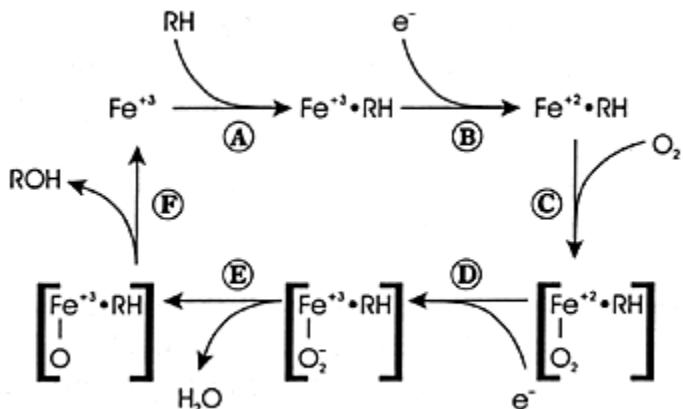
pharmacodynamics: Clonidine is an α -adrenergic agent that acts specifically on α_2 -receptors. α_2 -receptors regulate a number of signaling pathways mediated by multiple G_i proteins, G α_{i1} , G α_{i2} , and G α_i^1

Pharmacokinetics: The plasma level of clonidine peaks in approximately 3 to 5 hours and the plasma half-life ranges from 12 to 16 hours. The half-life increases up to 41 hours in patients with severe impairment of renal function. Following oral administration about 40-60% 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.²

2.CYP2C9:

i. CYP2C9 plays a major role in the metabolism of NSAIDs e.g. ibuprofen, naproxen, oral hypoglycemic, oral anticoagulants, diuretics, uricosurics, angiotensin 2 blockers, anticonvulsants and others.³

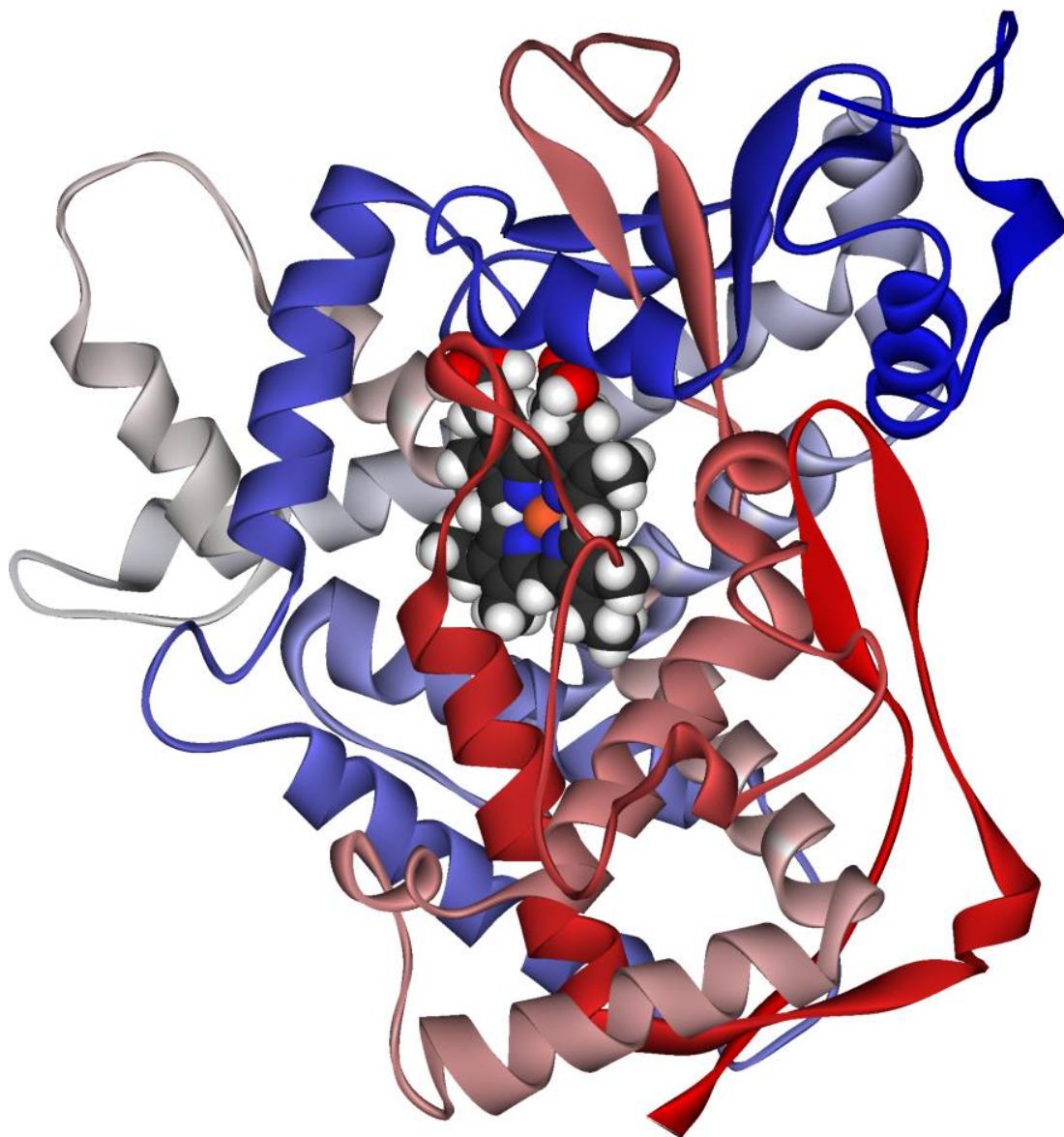
ii. Mechanism of catalysis:



4

iii. CYP2C9 inhibitors: Diclofenac, Fluoxetine, Flurbiprofin, Phenytoin, Tolbutamide, S-Warfarin₅

iv.



(i) 6 (ii) 4 (iii) 0 (iv) 0 (v) 1
REF = 2 T = 13

v. Kinetic parameters: K_m (HPLC)= 12.4 V_{max} (HPLC)= 5.22₇

3.

a. Pharmacokinetics and pharmacodynamics differ in men and women. Pharmacokinetics in women is affected by lower body weight, slower gastrointestinal motility, less intestinal enzymatic activity, and slower glomerular filtration rate.⁸ Pharmacodynamics in women include greater sensitivity to certain drugs and higher rate of adverse drug reactions.

Women tend to respond better to SSRIs whereas men respond better to cyclic antidepressant medication.

Beta blockers in women have a greater effect and lead to greater reductions in BP.

Women have a much higher likelihood of adverse drug effects (50-75% higher than men).⁹

Report=15 Ref=4 T=19

You found a good reference in Whitley
though not properly cited

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4. Clinical Pharmacology of SSRI's: Why Are CYP Enzymes Important When Considering SSRIs?. (n.d.). Retrieved from http://www.preskorn.com/books/ssri_s7.html
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Pathology 438
Spring 2015

Midterm Examination

due: by 1:00 PM, 6 May 2015

NAME _____ ZhiXuan Lawrence Jiang_____

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.

1. Select one of the substances below: (a) OR (b) OR (c). Provide as a complete a description of the toxic/pharmacokinetics and toxic/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

Doxorubicin:

Pharmacodynamics: doxorubicin has three major activities that vary in different cell.

Intercalation in the DNA: doxorubicin is able to insert to the adjacent base pairs and bind to the sugar-phosphate backbone of DNA. This binding allows the DNA uncoiling, and thus, blocks DNA and RNA synthesis.

Binding to cell membrane: This action alters the function of transport processes coupled to phosphatidylinositol activation.

Generation of oxygen radicals: Cytochrome P450 reduces the doxorubicin and oxygen to reduced metabolite and superoxide ion, which mediate single-strand scission of DNA. [1]

PK: AD=4 M=1 E=4

PD: 9 much better references; no
description of metabolism really

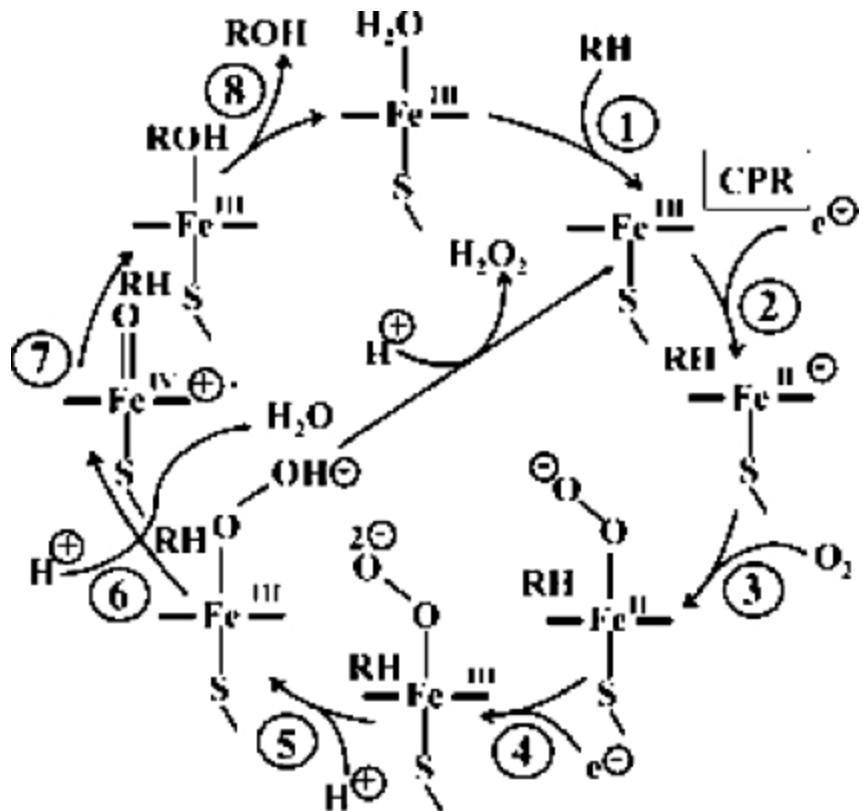
Ref: 3 T=21

Pharmacokinetics: Doxorubicin is inactivated in the GI tract. Within a dose range of 20-60 mg/m², doxorubicin binds to plasma proteins as well as to tissues, where they are widely distributed. The distribution half-life is 12 min. The half-life of the second phase is 3.3 h, and the elimination half-life is 29.6 h. The drug undergo extensive hepatic metabolism. The bile is the major route of excretion, 50% of the parent drug is excreted in bile and that 30% of doxorubicin is excreted as conjugates. 5 to 12% of the drug is excreted in renal. [1,2]

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
 - 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

CYP3A4

i) CYP3A4 is the most abundant human P450 enzyme, metabolizing a wide range of structurally diverse therapeutic agents; hence, it is the target for much drug-drug interaction. Testosterone is one of the most commonly used in vitro CYP3A4 probe. The other substrate also includes midazolam, nifedipine. [3]



ii) CYP3A4 belong to the family of cytochrome 450, so it has an active site contains a heme-iron center. 1) The substrate bind to the heme group, this also change the conformation of the active site to displace a water molecule from the distal axial coordination. This binding also change the state of the heme iron. 2) Substrate binding induce the electron from NAD(P)H via associated reductase. 3) Molecule oxygen bine to the ferrous heme center at the distal axial coordination. A second electron is transfer from the NAD(P)H via the associated reductase to reduce $\text{Fe}-\text{O}_2$ adduct to give a short-lived peroxy state. 4) Two proton bind to the peroxy state and produce water molecule. This will also produce iron (IV) oxo, which is a strong oxidizing agent, is able to catalyze variety of reactions. [4]

iii) CYP3A4 inhibitors:

- Aminodarone
- Anastrozole
- Azithromycin
- Cannabinoids
- Cimetidine
- Clarithromycin
- Clotrimazole
- Cyclosporine
- Danazol
- Delavirdine. [5]

iv) CYP3A4 is a homodimer with identical subunits. The beta-sheet rich in the N-terminal domain and a larger C-terminal domain comprised primarily of alpha-helices, and which contain the active site. The two ligand components for CYP3A4 are protoporphyrin IX containing Fe(Heme) and erythromycin. The heme serves as the site of substrate oxidation, so it is catalytically essential. The second ligand, erythromycin, is one of the largest substrates for CYP3A4, leading to greater conformational changes in the enzyme. [6]

v) The turnover rate of CYP3A4 varies widely. The half-life is found in the range of 70 to 140 hours in hepatocyte.

(i) 6 (ii) 4 (iii) 4 (iv) 4 (v) 1
REF = 5 T = 24

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 that 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?

Aspirin is one of nephrotoxic substance. It affects the distal convolute loop of the nephron. Prostaglandins act as modulators of physiologic functions in the kidney. The most important of PGs in the kidney is PGE2 and PGI2. PGI2 is to regulate the sodium reabsorption in the distal convolute loop of the nephron. Aspirin has effect to inhibit the production of PGI2. Inhibition of PGE2 synthesis can lead to increased sodium reabsorption, causing peripheral edema. Additionally, hyperkalemia is also another electrolyte disturbance that can occur as a result of inhibition of PG synthesis in the kidney. [7]

Aspirin is absorbed from the stomach and intestine by diffusion. It transformed into salicylate in the stomach, in the blood and mostly in the liver. Salicylate distributes rapidly into the body fluid, and bind to the membrane protein albumin. Salicylate has a very short half-life. In turn, it mainly metabolized by the liver. The predominance pathway is the conjugation with glycine. About 90% of salicylate is metabolized through this pathway. The last 10% of salicylate is excreted out from the urinary system. [8]

Serum salicylate is over 300 mcg/ml begin to have toxic effect. [9]

Report=15 Ref=5 T=20

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