Pathology 438 Midterm Examination due: by 1:00 PM, 6 May 2015
Spring 2015
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The electronic responses to this examination are due at 1:00 PM on Wednesday, 6 May 2015. Submit them to shalloran@lifewest.edu.

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

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

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

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

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

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

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

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 underdoing 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 it's effects are variable and P450 isoform-dependent. And while the effects of the enzymes CYP2D6, CYP2C9, and CYP34A have been well studies; 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 (go figure), fluconazole (antifungal), miconazole (antifungal), amentoflavone, sulfaphenozole, apigenin, amiodarone, antihistamines, chloramphenicol, fenofibrate, flavones, flucastatin, isoniazoids, 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.

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

a) Find at least one report/article that discusses the differences in how men and women respond to toxicants or drugs. Your search for an article may focus on one particular toxicant/drug or you may summarize an article that treats these

differences in a broad survey. In any article you obtain, be sure to indicate at least three significant points, but list all of them if there are more.

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

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

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 it's 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 IMI
Gastric pH	acidity M >F> preg F	Altered absorption of acid/bases depending on specific dru 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 X

Some 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 wom therapeutic and potential side effects.
Aspirin	Clearance, half-life	Aspirin is cleared more rapidly from women th
Benzylamine		Following transdermal absorption women exc
Beta-Blockers; metoprolol,	Oral clearance lower in women, lowe volume of distribution in women resulting in higher systemic exposure	The greater reduction in blood pressure in wor pharmacodynamic differences.
Cefazolin	Clearance, volume of distribution, half-life	**Clearance increases during pregnancy as a decreases. There is no change in volume of**
Cefotaxime	Clearance	Clearance is decreased in women.
Ciprofloxacin	Clearance	Clearance is lower in women than men
IM Cephradine		Slower rate of absorption and lower bioavailal

Overall great study, very interesting material.

Bibliography

1. Gilbert, S. G. (2004). *A small dose of toxicology: The health effects of common chemicals* (2nd ed.). Boca Raton: CRC Press.

2. Howland, R. D., Mycek, M. J., Harvey, R. A., Champe, P. C., & Mycek, M. J. (2012). *Pharmacology* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

3. Hodgson, E. (2004). *A textbook of modern toxicology* (3rd ed.). Hoboken, NJ: Wiley-Interscience.

4. Thorp, C. (2008). *Pharmacology for the health care professions*. Oxford: Wiley-Blackwell.

5. Valproic acid (PIM 551). (n.d.). Retrieved from http://www.inchem.org/documents/pims/pharm/pim551.htm#SectionTitle:6.5%20%20Elimination%20and%20excretion

6. Crump, K. S., Andersen, M. E., & Bellinger, D. (1996). Biologically Based, Quantitative Risk Assessment of Neurotoxicants. *Fundamental and Applied Toxicology*. doi:10.1006/faat.1996.0002

7. Cytochrome P450 2C9-CYP2C9. *Pharmacogenetics and genomics.* 2010. Van Booven Derek, Marsh Sharon, McLeod Howard, Carrillo Michelle Whirl, Sangkuhl Katrin, Klein Teri E, Altman Russ B.

8. Soldin O, Mattison D. Sex Differences in Pharmacokinetics and Pharmacodynamics. *Clinical pharmacokinetics*. 2009;48(3):143-157. doi:10.2165/00003088-200948030-00001.