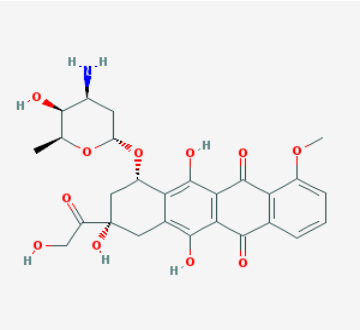
Pathology 438 Midterm Examination due: by 1:00 PM, 6 May 2015
Spring 2015
NAME _____

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

---

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.

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 aldoketoreductases 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 doxirubicin-semiquinone radical. These enzymes include mitochondrial and cystolic NADPH dehydrogenates, 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. Woman 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).

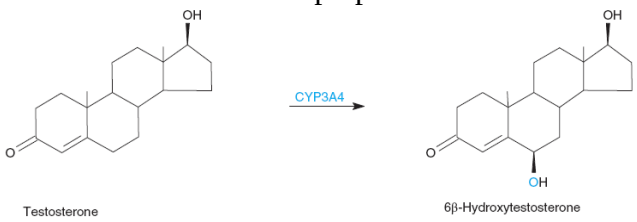
2. Select <u>one</u> of the cytochrome P450 enzymes below in the (a) through (d) list, and describe it as thoroughly as possible in points (i) through (v) below. You should cite at least one reference to a peer-reviewed publication or to a monograph for the course. For any information you put in your 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

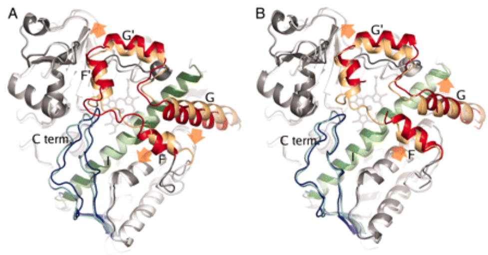
I. This protein localizes to the endoplasmic reticulum and is activated by glucocorticoids and some pharmacological agents. This enzyme is involved in the metabolism of approximately half the drugs in use today, including acetaminophen, codeine, cyclosporin A, diazepam and erythromycin. The enzyme also metabolizes some steroids and carcinogens.

II. 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.



Testosterone 6β-Hydroxytestosterone

III. 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 must 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. Retreived from http://www.eurekaselect.com/66808/article#sthash.r2hYveRW.dpuf

Fahmi, Odette. (2010). Cytochrome P4503A4 mRNA is a More Reliable Marker than CYP3A4 Activity for Detecting Pregnane 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

3. Select (a) <u>or</u> (b) <u>or</u> (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 Biotechology*. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3051160/