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

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 <u>not</u> 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 <u>may use</u> 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 <u>one</u> 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

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

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.

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 toposiomerase 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 apopotosis 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 by 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, dexrazoxane, 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].

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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 at 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 P_{450} 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

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 then 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)