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

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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

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]

Pharmacokinetics: Doxorubicin is inactivated in the GI tract. Within a dose range of 20-60 mg/m^2, 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]

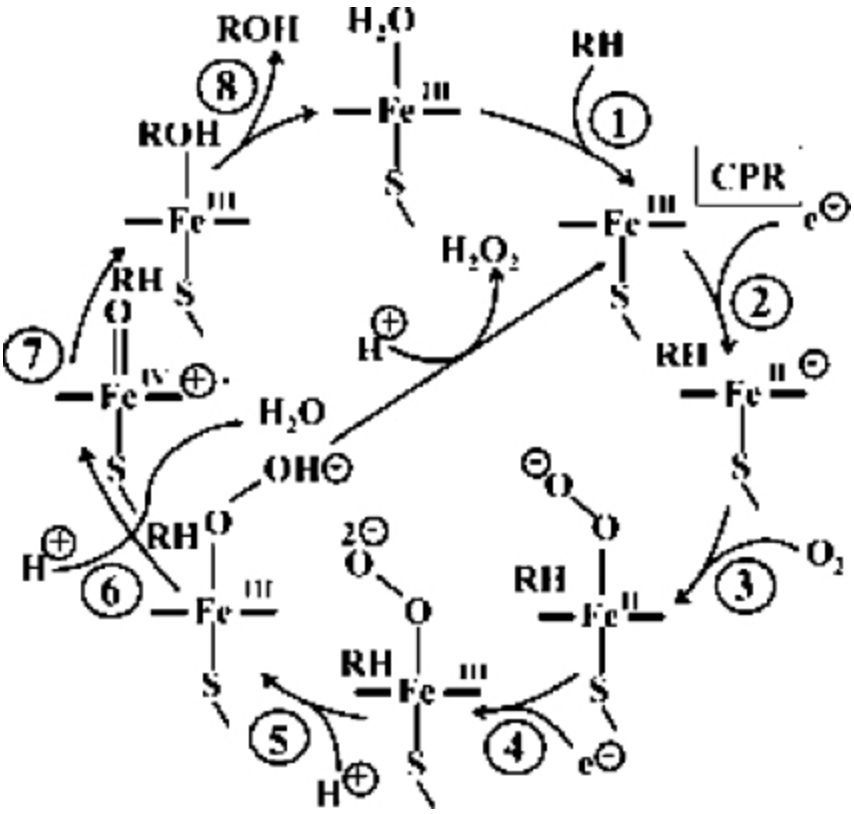
2. Select <u>one</u> of the cytochrome P450 enzymes below in the (a) through (d) list, and describe it as thoroughly as possible in points (i) through (v) below. You should cite at least one reference to a peer-reviewed publication or to a monograph for the course. For any information you put in your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

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 use 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 Fe-O_2 adduct to give a short-lived peroxo state. 4) Two proton bind to the peroxo 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
Azithromzcin
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 server as the site of substrate oxidation, so it is catalytically essential. The second ligand, erythromycin, is one of the largest substrates for CYP3AR, 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.

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?

Aspirin is one of nephrotoxic substance. It affect to 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]

Reference

1. Finkel,R.,& Clark, M. (2009). Pharmacology (4th ed.). Philadelphia: Lippincott Williams & Wilkins.

2. Nina E. Kontny, Gudrun Wurthwein, Boos Joachim, Alan V. Boddy, Miriam Krischke, Uwe Fuhr, Patrick A. Thompson, Markus Jorger, Jan H. M. Schellens, Georg Hempel. (2012). Population pharmacokinetic of doxorubicin: establishment of a NONMEM model for adults and children older than 3 years. Cancer Chemother Pharmacol 71(3):749-63

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7. Bluemle LW Jr, Goldberg M, (1969). Renal accumulation of salicylate and phenacetin: possible mechanisms in the nephropathy of analgesic abuse. J Clin Invest 47(11):2507-14

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