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

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.

Bibliography

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"Valproic Acid." Mayo Medical Laboratories. Mayo Foundation For Medical Education And Research. Web. 6 May 2015. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8707>.

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 homospaiens, 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)

Bibliography

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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).

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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