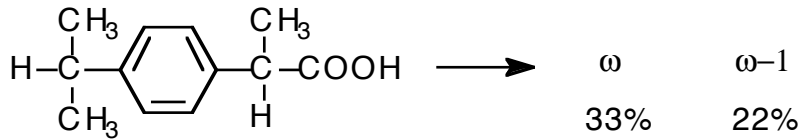


Med Chem 570 Problem Set 1 Drug Metabolism (2016)

1. This problem set covers the first 6 lectures.
2. The terms in the table below are a collection of items from the lectures that you should be able to define and relate in a sentence or two to drug metabolism as set out in the notes. If a drug is named you should know what it is used for (ie carbamazepine is used in the treatment of epilepsy) and it's relationship to drug metabolism (it is an inducer to CYP3A4, a substrate of CYP3A4 and can increase the clearance of coadministered CYP3A4 substrates). I will select from this list for the definitions section of the exam.
3. Also note that you should be able to answer the questions given in the first old exam on the website so treat that as a problem set to test yourself.

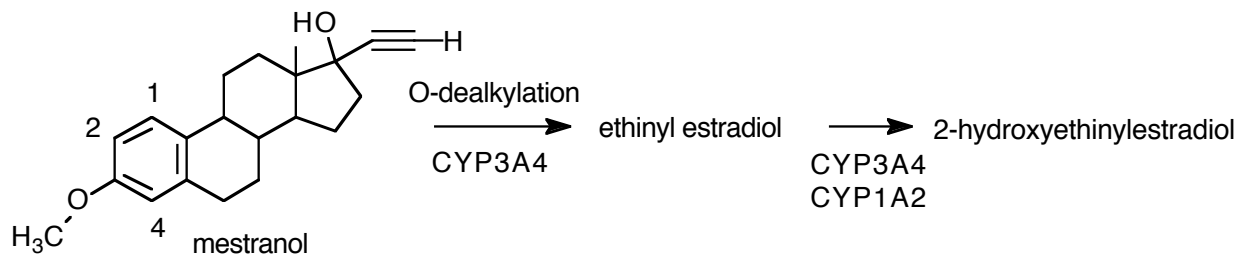
Arene Oxide	Glucuronyl Transferase	PAPS
Acetaminophen	Glucuronic acid	Perferryl oxygen
Aromatic hydroxylation	Glutathione	Phase I metabolism
AH Receptor	Glutathione-S-Transferase	Phase II metabolism
AUC	Half-life	Phenotypic Variation
Bile	hERG channel	PI boosting
Bioavailability	Hepatic Vein	Plavix
Carbinolamine	Hepatocyte	Polymorphism
Carbamazepine	Heme	Polytherapy
Catalytic Efficiency	Hemiacetal	Portal Vein
(V _{max} /K _m)	Hydroxylation	Product Profile
Clearance	Induction	Prothrombin Time
Clozapine	Interactant Drug	PXR Receptor
Codeine	Irreversible Inhibitor	Reversible Inhibitor
Compound 1	LD50	Ritonavir
Conjugation	MBI (Mechanism based inhibitor)	Rifampacin
Cytochrome P450 Reductase	Mercapturate	sp ² carbon
CYP1A2	Metabolic Ratio	sp ³ carbon
CYP3A4	Metabolic Specificity	Substrate selectivity
CYP2D6	Microsomes	Terfenadine
CYP2C9	Microsomal P450's	Therapeutic Index
CYP2C19	Midazolam	Tosardes de Pointe
Cytosol	Mitochondrial P450's	UDP glucuronyl transferase
DDI	Mixed function oxidase	UDPGA
ED50	Morphine	Warfarin
Endoplasmic Reticulum	N-dealkylation	Wild Type
EM, IM, PM	NAPQI	Xenobiotics
Epoxidation	NIH Shift	
Epoxide Hydrolase	Nortryptiline	
First Pass Effect	Object Drug	
Genotypic Variation	O-dealkylation	

1. Often the removal of an inducing agent B from a therapeutic regimen of drugs A and B can be a problem. Why? Consider what would happen if the warfarin dose (Drug A) was increased to compensate for the inducing effect of rifampin (Drug B) in the original polytherapy. Now take away the rifampin. What happens to the enzymes. What happens to the warfarin levels? What is the toxicity?
2. You are head of a drug firm. Research has brought to you two drugs for the treatment of a disease. The major difference between the drugs is that one is significantly metabolized by two P450 enzymes while the other is metabolized by only one P450 enzyme. Based on this data decide which drug to take to clinical trials (a \$100,000,000 decision) and defend your choice. List three questions you might ask your researchers. Explain why they are important criteria for making a decision.
3. Epilepsy is often treated with a combination of drugs such as carbamazepine and phenytoin (polytherapy). CYP3A4 is induced by phenytoin and carbamazepine. Carbamazepine by not phenytoin is cleared by CYP3A4. What problems might you expect if (a) carbamazepine is removed or (b) phenytoin is removed from this two-drug regimen.
4. Codeine is often given in combination with tylenol (APC) for relief of pain. What is the major advantage of using tylenol in this combination?
5. Discuss why it would be useful know the CYP2D6 genotype for a patient and to make it part of the patient's medical record. What problems might this present?
6. The idea that P450 reactions can be used to convert an inactive pro-drug to the pharmacologically-active occurs with drugs like Plavix. What would be two major disadvantages of this approach to drug delivery?
7. What evolutionary advantage is conferred by the facts that (a) P450 enzymes often produce multiple metabolites (b) xenobiotics are often metabolized by more than one P450 (c) most of the P450 enzymes are located in the liver.
8. What are the roles of P450 reductase and NADPH in the P450 cycle?
9. Major metabolites of ibuprofen are the ω and ω -1 hydroxy metabolites. What are their structures? Why is this question ambiguous?



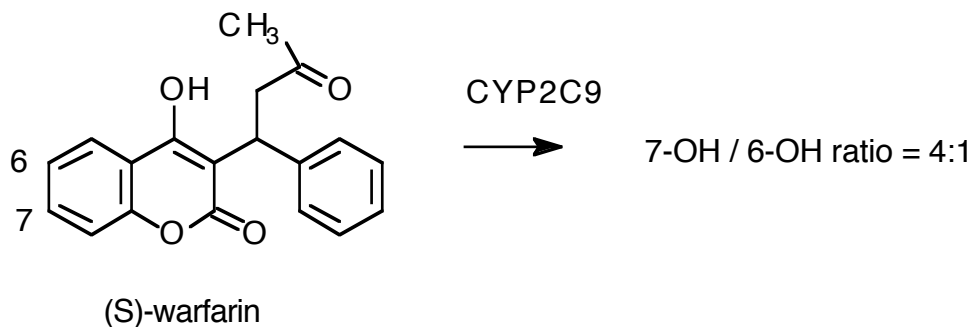
Ibuprofen

10. Ethinyl estradiol is an oral contraceptive that is metabolized to the inactive 2-hydroxyethinyl estradiol. Mestranol is an inactive contraceptive but is often used as a component of birth control pills in combination with ethinylestradiol.



- What is the structure of ethinyl estradiol.
- What do we call drugs such as is mestranol that are converted to active forms by enzymes in the body?
- Speculate as to why mestranol (which is inactive) is given in combination with ethinyl estradiol?

11. (S)-warfarin is converted to a 4:1 mixture of its 7 and 6 hydroxylated metabolites by CYP2C9. These metabolites account for 85% of the clearance of (S) warfarin from the body. Show a metabolic scheme that demonstrates how a single metabolic intermediate can lead to the formation of both metabolites.

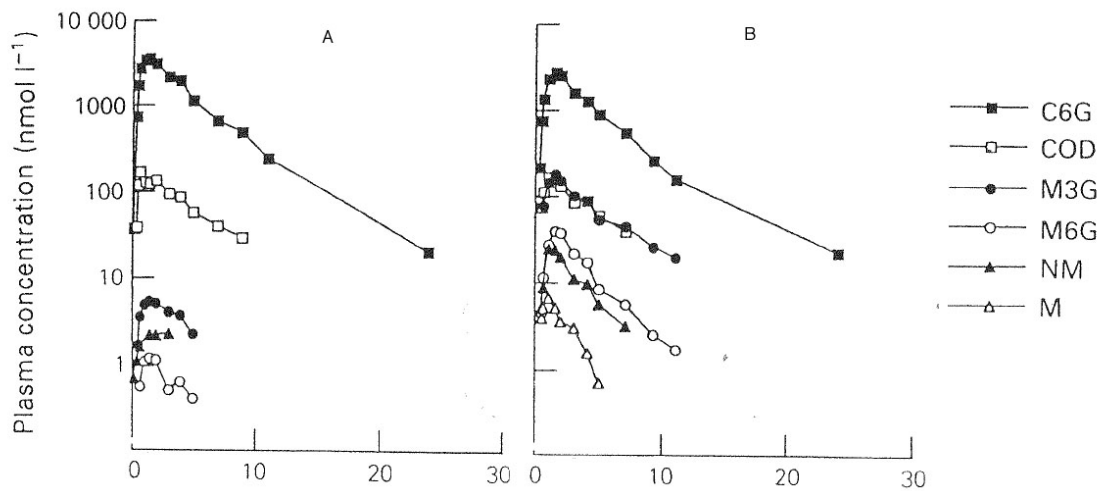
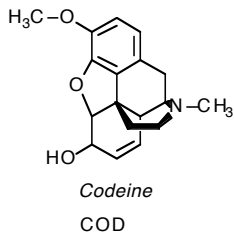


A small amount of the 8-hydroxymetabolite is also formed by CYP2C9. What does this tell you about the oxidation of (S)-warfarin by CYP2C9?

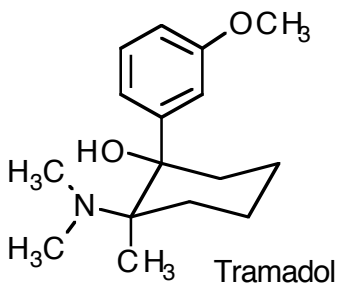
12. The plasma profiles of codeine and metabolites for 2 individuals (labeled A and B) are shown below. The X-axis is time in hours after an oral dose of codeine. [M=morphine;

C6G=COD-6-glucuronide; M3G = morphine-3-glucuronide; NM (ignore)]. Note the data is shown on a log scale on the Y-axis.

- Which individual is the poor metabolizer? Explain how you know this from the profiles?
- Is this a problem for cough suppression? Explain.



13. (20 points) Tramadol is an orally administered active analgesic. The drug has a blood half-life of 5 hours. Its pharmacologically-active O-dealkylated metabolite has a blood half-life of 8 hours and reaches peak levels that are 20% of parent. Answer the following questions.



Found in urine

- N-dealkylation (CYP3A4 10% of dose)
- O-dealkylation (CYP2D6 15% of dose)
- Glucuronide and sulfate (50-75% of dose)
- Tramadol (5% of dose)

- Show the mechanism of formation and structure of the O-dealkylated metabolite.

- b. Show the structure of the sulfate metabolite of parent drug. Is this a phase one or a phase two reaction?
- c. Would you expect a significant drug drug interaction between tramadol and a CYP3A4 inducer? Why or why not. Name a drug that is an inducer of CYP3A4.
- d. Do you think that CYP2D6 poor metabolizers that take this drug are at risk? Why or why not?
- e. Are you surprised that the O-dealkylated metabolite has a longer half-life than the parent drug? Explain using general principles learned in class.

14. The mean dose requirement for nortriptyline in CYP2D6 PMs is 15 mg (notes page 30) whereas the requirement in EMs is around 110 mg. Estimate the percent of nortriptyline clearance that is due to CYP2D6 in the normal (EM's: homozygous for the wild type allele) population. State any assumptions in your calculation.

Med Chem 571 Problem Set 1 (2013) - Answers

1. If a patient was stabilized on this two drug regimen and the inducing agent (B) was removed the levels of drug A would increase as the enzyme levels fell back to normal and the clearance of A would fall to normal levels. In the case of warfarin, a drug with a narrow therapeutic index, removal of the inducing agent would lead to over anticoagulation (long prothrombin times or INR) due to the increase in warfarin levels and the patient may bleed to death.
2. I would ask how many significant metabolites are produced from each drug and what they are. The more metabolites the better since the possibility that one of them might reach toxic concentrations in the body is reduced. I would also look for metabolites that might signal the formation of a reactive intermediate. For instance metabolites formed as a consequence of an arene oxide or epoxidation producing pathway. I would also ask about the percent of dose that is converted to glutathione or mercapturate conjugates since this might indicate that a short-lived reactive intermediate is formed.

I would ask whether either of the drugs was an inhibitor of the major P450 enzymes at projected therapeutic concentrations. Potent inhibition of one of the P450 enzymes might rule out a potential drug since physicians are reluctant to prescribe drugs that provoke drug-drug interactions.

I would ask whether either drug was significantly metabolized by CYP2D6 since it would be good to avoid the complication of treating two classes of patients (PM's and EM's).

All other concerns aside, two enzymes involved in the clearance of a drug are better than one since the clearance is likely to be less variable in the population. Also the likelihood of a drug interaction is reduced. In fact drug companies are very interested in developing candidate drugs that are cleared by multiple enzymes for this very reason.

3. If phenytoin was removed the levels of CYP3A4 might drop because phenytoin is an inducer, but not a substrate, of CYP3A4. Remember inducers of enzyme are not necessarily metabolized by those enzymes. CYP3A4 is a major enzyme involved in the clearance of carbamazepine and levels of carbamazepine might rise causing side effects.

If carbamazepine was removed I would be less concerned about phenytoin PK since carbamazepine has no effect on the enzymes that metabolize phenytoin.

4. CYP2D6 poor metabolizers who do not make morphine from codeine would still get some analgesia from the tylenol.
5. As our knowledge of the battery of drugs whose clearance is significantly dependent upon CYP2D6 is compiled, knowledge of a patient's genotype could be used to develop different dose regimens that will work for poor metabolizers and normal metabolizers and avoid

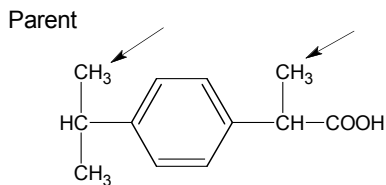
unnecessary trial and error dosing to effect. This is useful since the genotypic difference is lifelong (once a poor metabolizer always a poor metabolizer). The caveat to incorporating this type of information into the patients record relates to patient rights and privacy issues. Patients rights advocates argue that, should this type of information become available to insurance companies as well, that population based assessments of relative risk may lead discriminatory pricing or exclusion from coverage. This problem is also a major concern relative to the genetic basis of many disease states such as cancer and cardiovascular disease. Discrimination in the form of insurance pricing and availability based on relative risk of genetic subgroups is a hot issue. Imagine if poor metabolizers were found to have a higher relative risk of cancer. Should they pay more for health insurance?

6. One major disadvantage is the variability in the P450 enzyme levels in the population. Dosing to effect could be difficult if the metabolic clearance of the prodrug was highly variable since this would mean that the active metabolite levels would also be highly variable. The desired pharmacological effect would be tied to P450 metabolism, which is highly variable in the population. Thus dosing would likely have to be individualized and closely monitored as drugs are added to and removed from therapy. In essence the dose of the prodrug will likely be poorly related to the concentrations of the active metabolite due to interindividual variability in metabolism. Second, polytherapy might with this prodrug might cause inhibition of the enzyme required to create the drug from the prodrug, causing a subtherapeutic effect. Note that in the case of terfenadine this was not a problem because almost all of the drug was converted to metabolites in the first pass through the liver so the levels of the active metabolite were highly related to dose. The problem here was that the prodrug (although it was not designed as a prodrug) turned out to be toxic when inhibitors of CYP3A were co-administered.
7. Multiple metabolites means that the xenobiotic load created by administration of the parent drug is spread out among many, often more polar and readily excreted metabolites reducing risk of toxicity from metabolites.

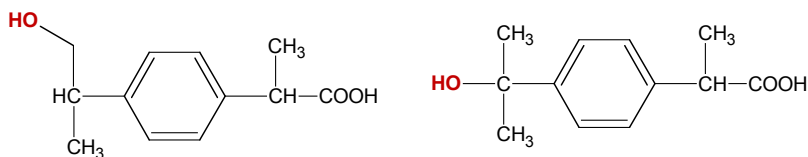
Multiple enzymes increase the likelihood of multiple metabolites and as well as lower interindividual variability in clearance of the parent drug even when the enzymes produce the same metabolite.

The location in the liver makes sense because the drug must transit the liver, where it is subject to metabolism, first. This helps to reduce systemic exposure to a potentially toxic xenobiotic.

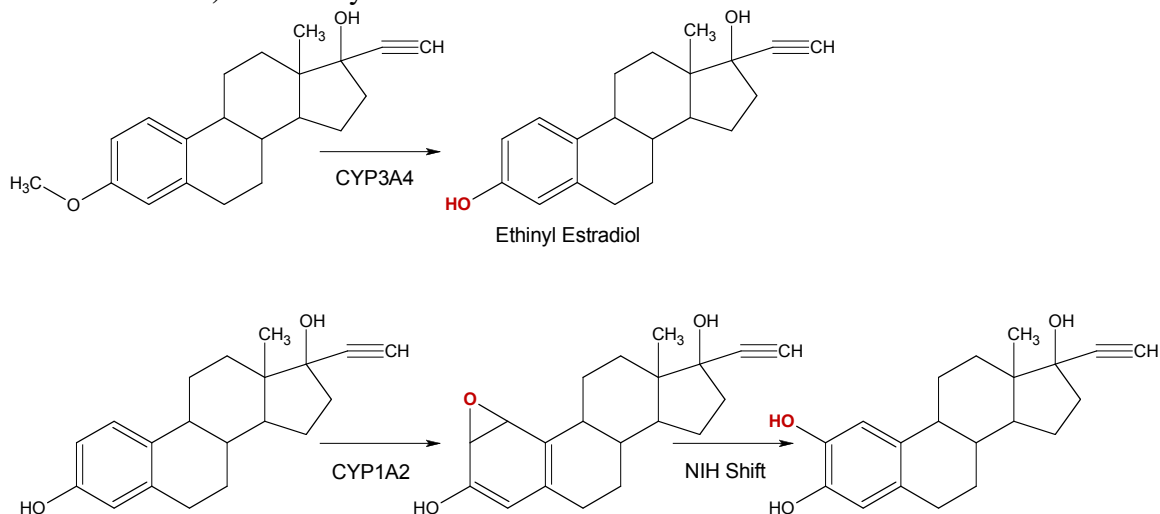
8. P450 is a membrane bound diflavin reductase that accepts electrons from NADPH and delivers them one at a time to the P450 enzymes as required during the oxygen activation steps of the P450 cycle leading to formation of the active oxidizing species, Compound I. A total of two electrons are required by the P450 enzymes in order to generate Compound I.
9. This question is ambiguous because there are two carbons that could be considered as the ω carbon (indicated by arrows on the parent compound)



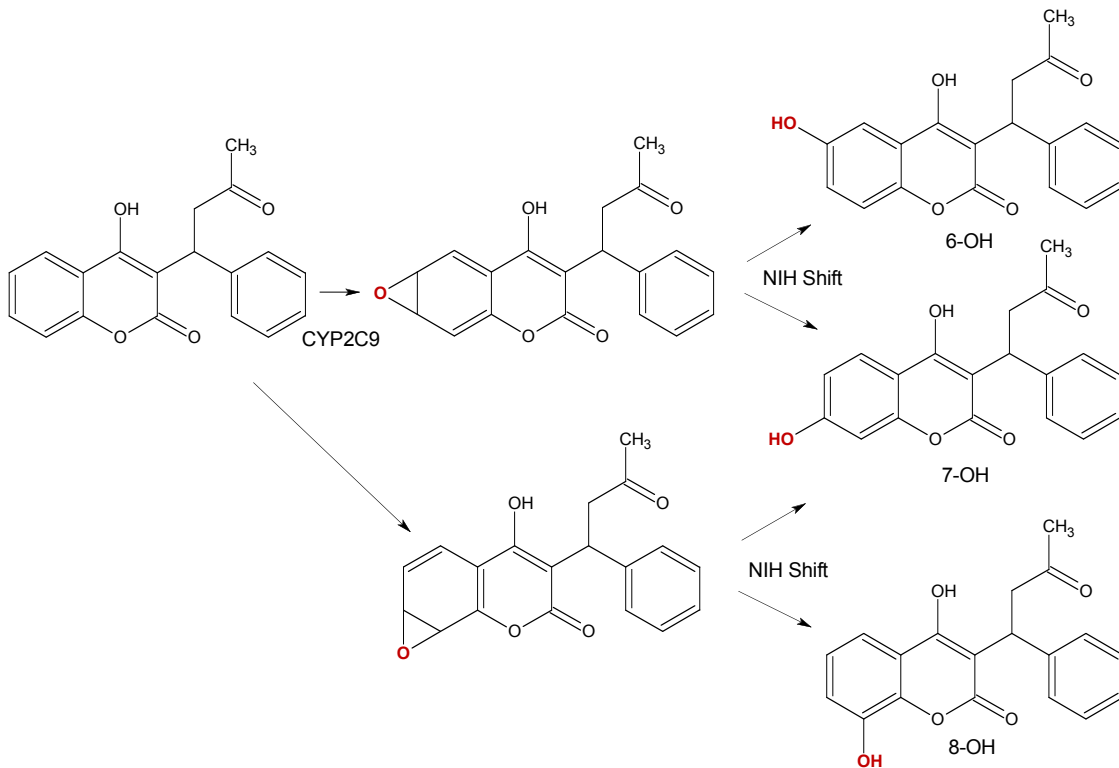
Metabolites



10. Mestranol is a prodrug of ethinyl estradiol since CYP3A4 catalyzed O-dealkylation produces the active drug. Formation of 2-hydroxyethinyl estradiol by CYP1A2 and CYP3A4 likely occurs via the intermediacy of an arene oxide (we might also expect some 1-hydroxy metabolites as well). The possibility that the arene oxide may be reactive and alkylate DNA causing mutations and cancer has been a concern for years for estradiol (not shown) and ethinyl estradiol.



11. The fact that some 8-OH warfarin is also formed indicates that the 7-8 carbon sp^2 bond is also accessible to Compound I, so two epoxides must be formed, across either the 6-7 bond or the 7-8 bond in order to generate all three phenolic metabolites. Overall this indicates that the CYP2C9 has a preference for metabolism on the exposed side of the aromatic ring.



12. The PM is Patient A. There is no morphine present in the blood of A and CYP2D6 is required to make morphine from codeine. This polymorphism is not a problem for cough suppression for the following reason. The pharmacokinetics of the cough suppressant codeine is the same in PM's and EM's since CYP2D6 metabolizes only 10 percent of the dose of codeine. Thus the AUC, which is a measure of total exposure to codeine, is not significantly different in PM's and EM's.

13.

- This is an O-dealkylation of the methyl ether to produce the phenol where you would show the partial structure of the hemiacetal and then the product phenol and formaldehyde.
- You would be making the sulphate of the alcohol present in the parent compound. This hydroxyl group would also be the only possible site for glucuronidation. Thus 50 to 75% of the clearance of tramadol is via phase II conjugation reactions.
- Only 10 percent of the dose is cleared by CYP3A4. Therefore would not expect DDI's from induction (or inhibition for that matter). Since the maximum change in parent PK (like AUC) would be only 10% or so. Carbamazepine and phenytoin induce CYP3A4.
- CYP2D6 is responsible for 15% of the clearance. The clearance in a PM would only fall to 85% of EM. Not enough to make a significant difference in tramadol PK. This is very similar to the codeine question.
- Normally we would expect the phenolic metabolite to be cleared more easily than the parent into the urine because it is more polar. Thus it is surprising but is not the only case in drug metabolism where a metabolite has a longer half-life than the parent. As you will learn in PK this is an example of elimination rate limited kinetics for a metabolite rather than the more normal formation rate limited kinetics.

14. To answer this question one has to assume that the two doses produce the same desired pharmacological effect in PM's and EM's. Therefore the AUCs in the PM and EM for nortriptyline at the two doses are the same (assume $AUC_{PM}=AUC_{EM}$). Then you use the fundamental relationship from your PK class..... $Cl_{observed} = \text{Dose Administered}/AUC_{observed}$ and solve for the ratio of clearances Cl_{PM}/Cl_{EM} which is given by $(15/AUC_{PM}) / (110/AUC_{EM})$ or 14%. Thus the clearance in PMs is about 14% of the clearance in EMs and CYP2D6 is responsible for 86% (100-14) of the clearance of nortriptyline in EMs. So to follow the logic one step further one expects that in vivo CYP2D6 inhibitors will produce a meaningful DDI with nortriptyline in EM's but not PM's.