20.201 Take-Home Exam

Distributed: October 19, 2012, at 2:30 p.m.

Due: October 24, 2012, at 1:30 p.m.

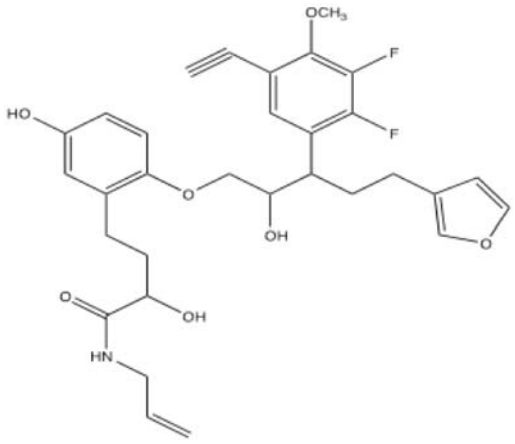
Directions: This take-home exam is to be completed without help from any other individual except Drs. Dedon, Tannenbaum, Hoffmaster or Lim. This includes help from other students (whether or not they are taking 20.201), faculty, people via the Internet, and so on. You are permitted to use your books, class notes, library resources, internet information resources, and other inanimate materials.

Please prepare your answers to all questions as a single PDF document - labeled with your name. Please prepare your answers using word processing software and insert either digitized hand-drawn graphics or computer-generated graphics. The answers should not exceed the stipulated length per question. Please submit your answers by email to all of the following email addresses before class starts at 1:30p on November 7˙ Late exams, without prior approval, will not be accepted.

Profs. Dedon, Tannenbaum and Hoffmaster considered the depth and breadth of the answers in addition to the correctness of the answers. This was a take-home exam with a significant time element for writing thoughtful, well-argued answers. If you did not rationalize your answer, then we subtracted points.

Question 1 - 45 points total

A. Hypoamericillin 2 has just been developed (structure below). It is more potent than Hypoamericillin (structure in your lecture notes) in terms of the concentration in vitro that gives



a 50% inhibition of its target, but you have been given the job of predicting solely on the basis of its structure whether it would be a safer drug. Since the original Hypoamericillin was isolated from soil in the Brazilian rain forest, you are not certain of its pharmacophore, but both molecules have obvious toxicophores. Speculate on what you think is the pharmacophore and the toxicophores in this potential drug and back up your analysis with specific ideas.

B. For the drug you have been assigned you have already been asked to work out the metabolic pathways. Now you are to describe the following:

1. What are the safety considerations for your drug?
2. Examine the metabolic pathways to determine whether there are one or more reactive intermediates that could lead to toxicity. Are there other potential mechanisms for consideration of safety?

Question 2 – 35 points total

A. You have been hired as the Chief of ADME at ACME Pharmaceuticals. Your predecessor characterized the pharmacokinetics of a new antibiotic, Hypoamericillin (Ha), in mice and rats and obtained a plasma elimination half-life of 14 hr and a volume of distribution (V_d) of 0.08 L/kg with a dose of 0.1 mg/kg in both rodent models. Your job is to define the pharmacokinetics in humans in the first phase I clinical trial for this drug candidate. In your first human studies, you administered 25 mg doses and obtained a V_d of 0.08 L/kg, which is similar to the rat and mouse studies. In addition, you performed comparative bioavailability studies with Ha alone and in combination with ibuprofen (Ib; Ha causes headaches in 90% of patients) and another experimental compound (X) that was designed to prevent Ha metabolism (Table shown above). Use these data to answer the following questions. ***1.5 PAGE MAX FOR ALL ANSWERS.***

Ha Dose, mg	Plasma elimination $t_{1/2}$, hr	Route	AUC, µg/l*h
25	10	IV	750
	10.2	Oral	700
	9.9	IM	100
25 + 250 mg Ibuprofen	14.2	IV	1070
	14.5	Oral	1000
25 + 250 mg Compound X	10	IV	740
	10	Oral	220

A.1) Calculate the bioavailability of Ha for the Oral and IM routes of administration. Describe possible mechanisms that could explain the different AUC values for Ha administered alone. What additional information would you need to establish the mechanisms?

7.5 points

Bioavailability = $F = \frac{AUC_{ev}}{AUC_{iv}}$ so $F = \frac{AUC_{oral}}{AUC_{iv}}$ = 700/750 = 0.93 and $F = \frac{AUC_{im}}{AUC_{iv}}$ = 100/750 = 0.13

The oral route is nearly as efficient as the IV route for uptake of Ha into the circulation – 93% of the drug enters the general circulation from the oral route. However, only 13% of Ha enters the general circulation following an IM injection. This could mean that Ha is unstable when injected into muscle (e.g., metabolism; similar to first-pass metabolism in the liver – the drug never makes if out of the muscle due to rapid metabolism) or that it does not enter the blood capillaries well when injected into muscle tissue, with a depot effect. It is not possible that the rate of removal of Ha from muscle is so slow that the time frame of our PK analysis missed the bulk of the drug movement into the circulation, since the plasma half-life is the same for all routes of administration. If the rate of movement of Ha from muscle to tissue was slower than the routes of elimination from plasma, then the plasma half-life would equal the rate of loss of drug from the muscle. We would need to (1) rate of metabolism of Ha in the muscle and (2) kinetics and extent of urinary or fecal excretion of the drug and its metabolites.

A.2) Calculate the plasma elimination rate constants for the IV and Oral routes in the presence of ibuprofen. Describe possible mechanisms that would account for the changes in both plasma elimination rates and the AUC values caused by co-administration of ibuprofen. What additional information would you need to establish the mechanisms?

7.5 points

$k_{elim} = 0.693/t_{1/2}$, so for oral route: k_{elim} = 0.693/14.2 hr = 0.049 hr^{-1}; and for IV route: k_{elim} = 0.693/14.5 hr = 0.048 hr^{-1}. The co-administration of ibuprofen has decreased the rate of elimination of Ha from plasma, which results in a proportional increase in the AUC – the area under the plasma concentration-time curve. Several mechanisms could account for this behavior, including ibuprofen-mediated inhibition of Ha metabolism (competition for enzymes

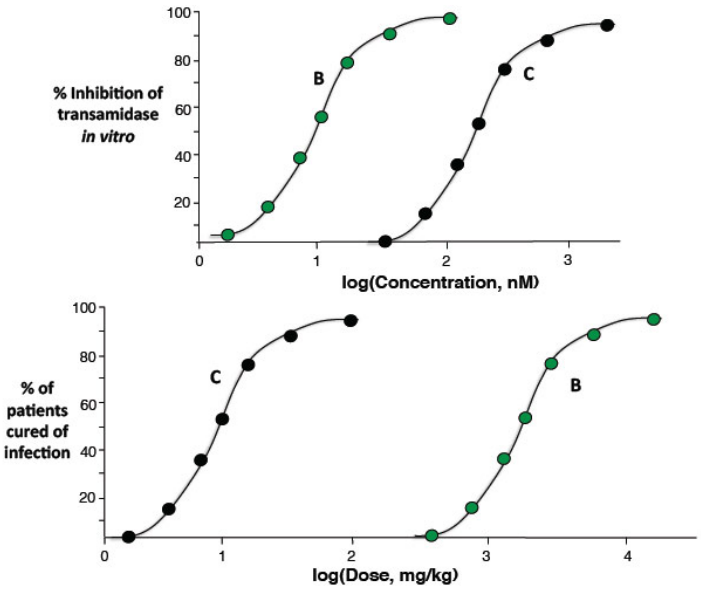
or ibuprofen-induced downregulation of metabolic enzymes), competition for elimination transporters in liver or kidney (or ibuprofen-induced downregulation of transporters), and enhanced reuptake from the kidney nephron following filtration at the glomerulous. Competition for plasma protein binding sites, with increased free Ha concentration in plasma, would not account for the changes in elimination kinetics since the rate of elimination of Ha would not change in this case. Information needed includes evidence for transporters and drug metabolic pathways, along with the routes of elimination.

A.3) Describe possible mechanisms that would account for the changes in the AUC values caused by co-administration of compound X in light of the lack of change in the plasma elimination kinetics. What additional information would you need to establish the mechanisms?

7 points

The statement that Compound X was designed to interfere with Ha metabolism is a "red herring" – something to mislead you. If this was the case, then the design did not work. Co-administration of Compound X by either route does not affect the plasma elimination rate of Ha. However, while the AUC for Ha does not change when it is co-administered with Compound X by the IV route, there is a significant decrease in the AUC when both drugs are administered by the oral route. Since the rate of plasma elimination is not affected by Compound X or route of administration, then we must assume that hepatic metabolism and other elimination mechanisms are unaffected by Compound X. The fact that Compound X reduces the AUC of Ha when administered by the oral route suggests that Compound X interferes with the uptake of Ha from the intestines, it enhances first-pass metabolism of Ha (but not overall hepatic metabolism since K_{elim} has not changed; this would be highly unlikely), or that it enhances the metabolism of Ha in intestinal epithelial cells following uptake from the gut lumen. We could test the transport interference hypothesis using the in vitro models presented by Prolf. Hoffmaster.

B. You compared dose-response behavior for Ha analogs B and C, as shown in the figure below. The upper panel shows the concentration-dependence of inhibition of the activity of the drug target: a transamidase responsible for cell wall cross-linking in the bacteria. The lower panel shows the in vivo dose-response behavior for oral administration of the drug, with complete cure of the infection as the response end-point.



B.1) What class(es) of antibiotics has the same mechanism of action as Ha and its analogs B and C?

3 points

Penicillins, cephalosporins and other cell wall disruptors.

B.2) Explain the basis for the observed differences between the enzyme inhibition dose-response curve and the patient cure rate dose-response curve.

7 points

At the molecular level, drug B is more

potent inhibitor of the transamidase, perhaps by virtue of a higher binding affinity of B for the enzyme. However, B is less potent as an antibiotic than drug C following oral administration. This could be due to poorer bioavailability, more rapid metabolism, poorer entrance into infected tissues of drug B compared to drug C. Drug C is thus less effective at inhibiting the enzyme and more effective at curing the infection compared to drug B.

B.3) Which drug is more potent in terms of curing patients of infection? Which drug is more efficacious? Why?

3 points

See the answer to B.2 above. Both drugs are equally efficacious since their maximal responses in both graphs are equal.

Question 3 - 20 points total

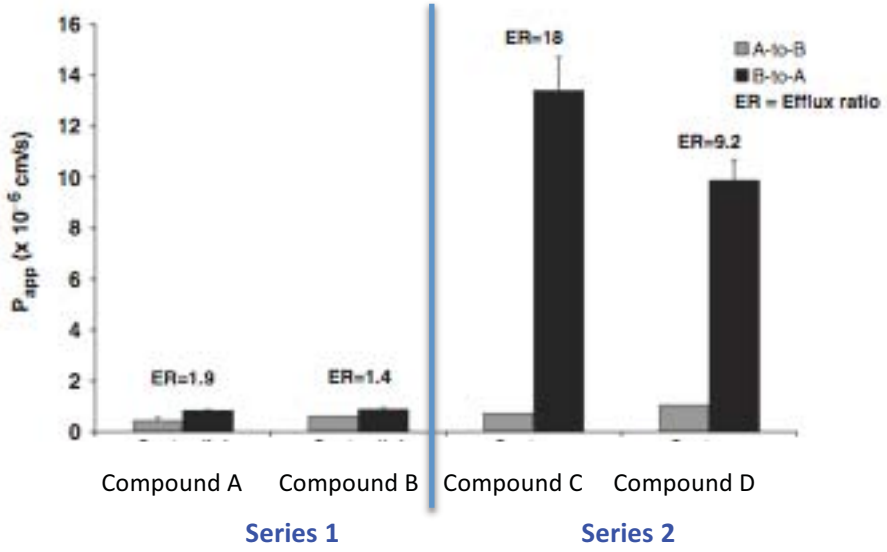
A recent startup, MIT pharmaceuticals, is developing therapies to treat renal dysfunction and renal failure in patients with diabetes. The mechanism of action involves a protein target selectively localized to the kidney tissue (e.g., not expressed elsewhere in the body), although two other closely related protein homologs are expressed in the liver and the brain. They have just hired you as the head of the ADME laboratory and are looking to your expertise to help them address several concerns around drug transporters. Several lead compounds are in early development and two candidates have been into Phase I clinical trials in healthy volunteers.

A. Data from the current lead molecule suggests that renal clearance is 0.9 L/min in early clinical trials. Do you suspect active transport to be involved in the clearance? Why or why not?

B. The lead clinician on the team is proposing to go into phase II trials with patients that will take metformin concomitantly throughout the trial. An alternate clinical design would be to select a patient population that would be on rosuvastatin (Crestor), injectable insulin, and sitagliptin (Januvia). Which patient population would you recommend the team pursue and why?

C. One patient from the phase I trial had clinical signs of acute renal toxicity. Further investigation of this patient's medical history showed that the patient should have been excluded from the trial since they were also taking cimetidine. Provide a hypothesis as to what may have contributed to this patient's nephrotoxicity – be specific and support with 1-2 relevant publications (citations only please).

D. The lead chemist on the discovery team is worried that the two lead backup series of compounds are very potent against one of the closely related targets (expressed in the brain). She favors Series 1 because these compounds are 10x more potent against the desired target. Below are representative data from two compounds in each series in Caco-2 cells. Interpret these data and provide guidance to the chemist.



20.201 Mechanisms of Drug Actions

Fall 2013