

20.201 Take-Home Exam

Distributed: October 31, 2011, at 1:00 p.m.

Due: November 7, 2011, 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, Knutson or the teaching assistant. This includes help from other students (whether or not they are taking 20.201), other faculty, other 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.

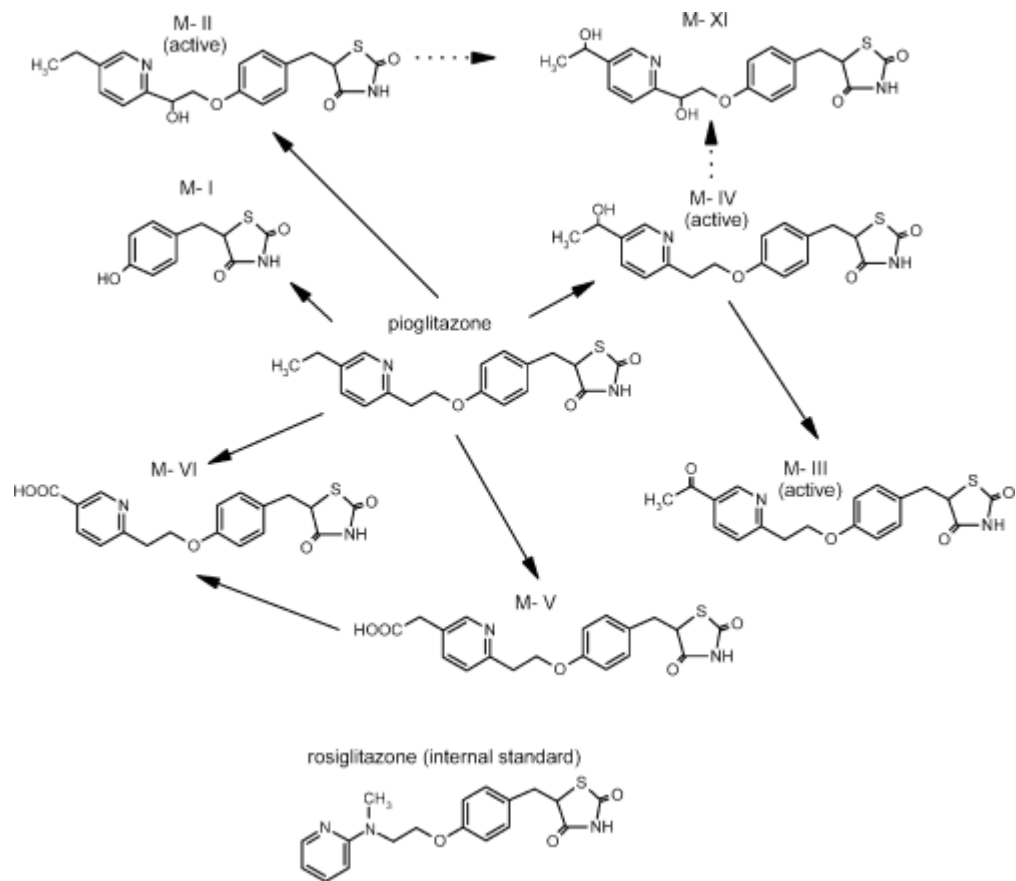
Please prepare your answers to each question as a separate document - labeled with your name and the question number. Please prepare your answers using word processing software and insert either digitized hand-drawn graphics or computer-generated graphics. The answers should not exceed the stipulated length per question. Please submit your answers by email to both Drs. Dedon and Tannenbaum before class starts at 1:30p on November 7. Late exams, without prior approval, will not be accepted.

Question 1 - prepare your answers to this question as a separate document

10 pts. A. Drugs are designed to interact with specific targets. The targets may be receptors, active sites of enzymes, and/or allosteric sites on proteins. From a pharmacodynamic point of view, comment on the different safety issues for these targets (<100 words) and suggest how you would counter-screen to eliminate off-target toxicity (<100 words).

10 pts. B. A study has been conducted to compare the efficacy profile of drugs on the market that have one target vs. drugs that have several targets. It turns out that drugs with more targets are more effective. Someone has now proposed to take advantage of this finding with a new strategy in which more than one drug is formulated into a single pill so that one dose will hit multiple targets. The FDA has announced that it will consider this proposal. An FBI database search has discovered that you once took 20.201, so you are brought in to advise the agency. Your first job is to list the advantages and disadvantages of this approach from both a PK and PD perspective. You are also asked whether this approach would have any new safety issues, since both drugs will have been shown to be safe when taken alone, one in the morning and one at night. (<200 words).

15 pts. C. Pioglitazone is metabolised by CYP2C8 and CYP3A4 as shown below. It is designed as an inhibitor of PPAR α . It has recently been identified as a potential risk for bladder cancer. Your job is to find out whether it is the metabolic or pharmacologic component that might be responsible for the epidemiological findings.

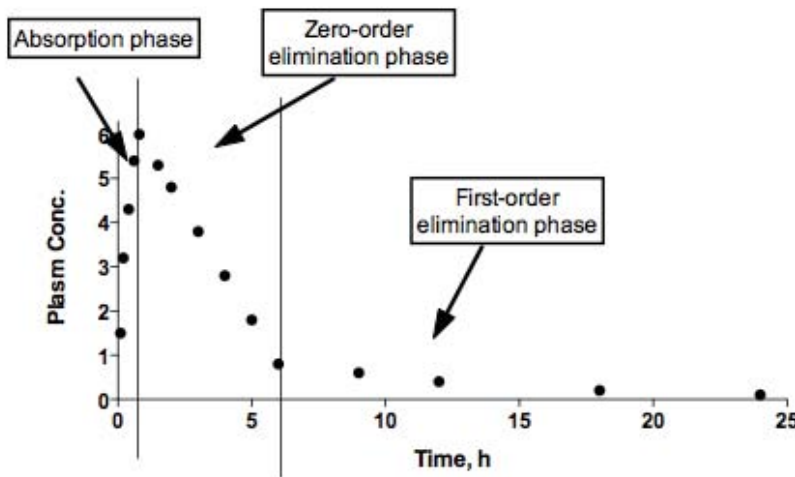


Question 2 - prepare your answers to this question as a separate document

15 pts. A. You have been hired as the Chief of ADME at ACME Pharmaceuticals. Your predecessor characterized the pharmacokinetics of a new anti-hypertension agent, Gosolow, 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 10 mg/kg and obtained a V_d of 0.08 L/kg, which is similar to the rat and mouse studies. However, this dose of the drug produced the plasma pharmacokinetics shown in the table on the right. Use this data to answer the following questions.
1.5 PAGE MAX FOR ALL ANSWERS.

Time, hr	C_p , $\mu\text{g/mL}$
0.1	1.5
0.2	3.2
0.4	4.3
0.6	5.4
0.8	6
1.5	5.3
2	4.8
3	3.8
4	2.8
5	1.8
6	0.8
9	0.6
12	0.4
18	0.2
24	0.1

A.1) There are three distinct phases apparent in the plasma pharmacokinetics of this drug. Draw a graph and designate the three phases. Explain what is happening during the first hour of the pharmacokinetic time-course and why the curve behaves as it does.



A plot of the data is shown above. While the labeling requires information obtained in other parts of this question, you should have at least designated the three phases. The data in the first hour show a non-linear increase in plasma concentration that is clearly

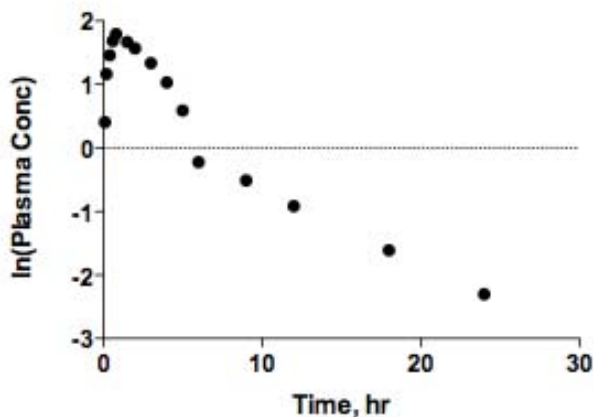
not due to an intravenous injection or unlikely due to intravenous infusion, even if one did not assume instantaneous distribution following an I.V. injection. So the drug was most likely administered by another route such as oral, dermal or other non-venous route. Plots of both plasma concentration and $\ln(\text{plasma concentration})$ versus time up to 1 hour after drug administration are nonlinear

and we really do not know why. There are many possible explanations here and creative but sound explanations were rewarded.

A.2) The pharmacokinetic behavior of the drug from 1 hr onward is unusual. This is clearly not a case of rapid distribution of the drug into another compartment followed by slower plasma elimination, as we discussed for a pharmacokinetic model with two or more compartments. Why not? What information suggests that the drug is limited to a single compartment?

The volume of distribution of the drug is ~4-7 L (depending on the size of the patient), so it is likely limited to the vascular compartment. Thus, the two different phases apparent after 1 hr likely represent elimination of the drug by metabolism or excretion. If there were a rapid distribution phase, then one would not expect to see the rather prolonged linear portion of the curve (i.e., zero-order kinetics) between 1 and 6 hr.

A.3) What is the pharmacokinetic behavior of the drug in each of the second and third phases? That is, what types of kinetics governs the plasma elimination of the drug after 0.8 hr? Show your reasoning.



The second phase between 1 and 6 hr is zero-order, as demonstrated by the very linear plot shown above ($r^2 = 1$, $k_{elim} = 1$). The third phase appears to be first-order, as shown in the ln-plot of the same data ($k_{elim} = 0.119$, $r^2 = 0.9996$).

A.4) Describe two mechanisms that could explain the plasma pharmacokinetics in the second and third phases of the plasma elimination time course. How would you establish which mechanism is actually responsible for the pharmacokinetics?

Early zero-order elimination kinetics suggests saturation of a transport protein or metabolic enzyme, with the plasma concentration eventually dropping below the K_m of the enzyme or transporter and a resulting change to first-order kinetics.

There are many possible experiments that you could perform to distinguish the two mechanisms: measure the kinetics of metabolite formation, measure the transport

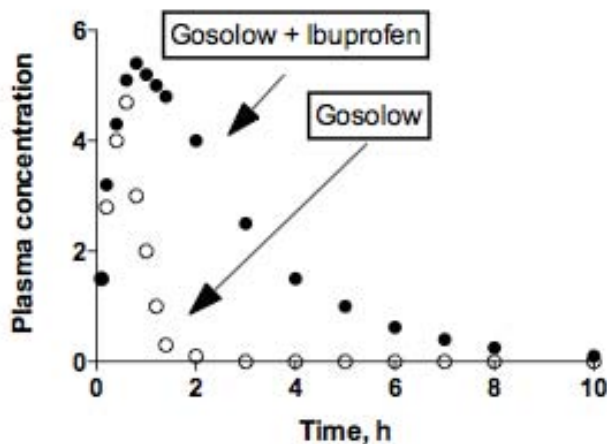
kinetics with an in vitro assay such as those described by Keith Hoffmaster, measure the kinetics of excretion in the urine for the drug and its metabolites.

10 pts. B. In another study, 40 mg of Gosolow was administered and you obtained the plasma pharmacokinetic data shown in the third column of the adjacent table (plasma concentrations of Gosolow as a function of time). However, you noticed that in patients taking both Gosolow (40 mg) and Ibuprofen (400 mg), the latter for the terrible headaches caused by Gosolow, the pharmacokinetic profile for Gosolow changed, as shown in the second column in the table below. Use the data to answer the following questions.

ONE PAGE MAXIMUM FOR ALL ANSWERS.

Time, hr	p, $\mu\text{g/mL}$	
	Gosolow + Ibuprofen	Gosolow
0.1	1.5	1.5
0.2	3.2	2.8
0.4	4.3	4
0.6	5.1	4.7
0.8	5.4	3
1	5.2	2
1.2	5	1
1.4	4.8	0.3
2	4	0.1
3	2.5	0
4	1.5	0
5	1	0
6	0.62	0
7	0.4	0
8	0.25	0
10	0.1	0

B.1) Create a graph of the two data sets and explain the pharmacokinetic behavior of Gosolow in the first hour. What is the effect of Ibuprofen on the area under the plasma concentration versus time curve (AUC) for Gosolow?



As shown in the graph above, the presence of Ibuprofen did not affect the uptake kinetics but it prolonged the elimination of Gosolow and thus increased its AUC.

B.2) Provide two explanations for the effect of ibuprofen on Gosolow pharmacokinetics.

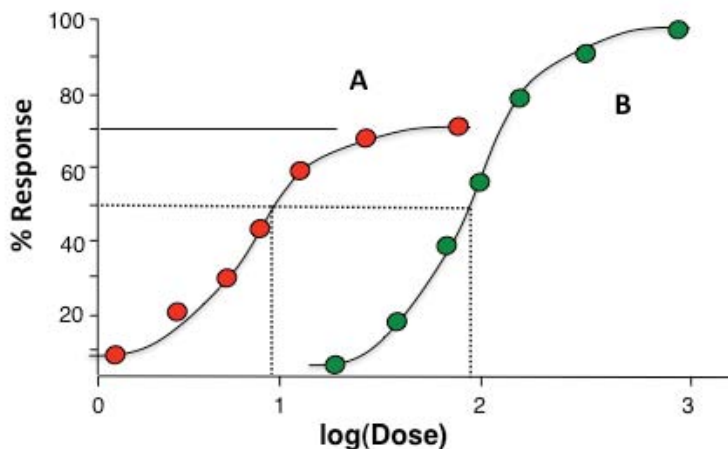
There are many options here. The most likely is that Ibuprofen either blocks the metabolism of Gosolow, perhaps by directly inhibiting a metabolic enzyme, or it competes for a transport protein involved with elimination of Gosolow from the blood. Other possible explanations include induction of Gosolow metabolism (if Ibuprofen had been given prior to Gosolow with enough time to allow expression

of metabolic enzymes) and blocking transporters involved with distribution of Gosolow to tissues.

B.3) Estimate the plasma elimination rate constants for Gosolow in the presence and absence of Ibuprofen. What must you assume in these calculations?

We must assume that the elimination kinetics are first-order. This is borne out by a \ln -plot of the non-zero post-absorption plasma concentration data ($t = 1-10$ hr for Gosolow + Ibuprofen; and $t = 0.6-2$ hr for Gosolow alone). The elimination rate constants are $2.5-3 \text{ hr}^{-1}$ (variation allowed for choice of time points to plot) for Gosolow alone and 0.44 hr^{-1} for Gosolow + Ibuprofen. The fit for the Gosolow alone data is not perfectly linear, so perhaps we are not completely correct about in assuming first-order elimination (or whoever wrote the problem didn't choose the data points well).

10 pts. C. The following questions relate to dose-response and pharmacokinetic relationships for two lead anti-arrhythmic agents being developed at ACME Pharmaceuticals. Both are administered orally. The response index is a complete remission of a cardiac arrhythmia.



C.1) Drug A is 10-times more potent than Drug B, but Drug B is 100% efficacious while Drug A only elicits a response in 70% of the test group. Draw (sketch) dose-response graphs that illustrate these differences in potency and efficacy.

The graph shows that the EC_{50} for drug A is 10-times lower than the EC_{50} for drug B, while the maximal efficacy of drug A is only 70%.

C.2) The area under the plasma concentration versus time curve (AUC) for Drug A is 10-times larger for Drug B when both are administered orally. Further, the AUC value for intravenous administration of Drug A is also 10-times larger than that for Drug B. Can you determine the bioavailability of the drugs from this data? Explain your reasoning. Describe two factors that could account for the larger AUC values for Drug A compared to Drug B.

Given: $AUC_{\text{oral}} A = 10 \times AUC_{\text{oral}} B$ and $AUC_{\text{IV}} A = 10 \times AUC_{\text{IV}} B$

Therefore: $(AUC_{\text{oral}} A)/(AUC_{\text{IV}} A) = (10/10) \times (AUC_{\text{oral}} B)/(AUC_{\text{IV}} B)$

No, we cannot determine the bioavailability for the drugs since we only know the relative AUC values for A and B. Both drugs must be absorbed orally to some extent, since we were able to measure their plasma concentrations at enough time points to generate an AUC value, and the bioavailability ($AUC_{\text{oral}}/AUC_{\text{IV}}$) for drug A could be any value from >0 to 1. However, we do know that the bioavailability of drug A is identical to that for drug B, as demonstrated in the above equations.

The larger AUC values for drug A could be caused by any of several mechanisms, all of which account for 10-fold differences in both oral and IV AUC values.

- **lower potency for drug A.** If drug A is 10-times less potent than drug B, then we would need to administer 10-times as much of drug A to achieve the same therapeutic outcome as drug B. Both drugs have the same bioavailability (same amount of each drug is absorbed from the gut and reaches the systemic circulation), so the 10-fold difference in dose would be paralleled by a 10-fold difference in AUC.
- **Decreased rate of metabolism or excretion of drug A.** These processes remove drug from the blood permanently, which will affect the amount of drug reaching the target. Distribution to tissues, unless accompanied by covalent binding or large differences in transport in one direction, will eventually be balanced by a return to blood, which would result in similar AUCs.
- **Differences in transporter activity** must be specified as excretion, distribution, absorption. Only the first one will affect IV AUC. No credit given for just stating “transporter”.
- Plasma protein binding will not affect AUC unless the binding is covalent or with thermodynamics and kinetics approaching covalent. Simply stating that there could be differences in binding does not answer the question since you need to show your reasoning.
- The only other explanation for the larger AUC values for drug A is that there was a mistake in the administered doses of the drugs (this has happened rather tragically with the death of a Boston journalist from a 10-fold overdose of an anticancer medication at Dana Farber Cancer Center a decade ago), or perhaps the analytical method used to quantify the plasma concentration of the drug was inaccurate (i.e., the measured values are not close to the true values).

Question 3 - prepare your answers to this question as a separate document

30 pts. Several transporters have been shown to be expressed in the kidney that facilitate the uptake and efflux of compounds between the blood and the urine. Since you are the lead transporter scientist at MIT pharmaceuticals, your project team is looking for guidance around potential issues with the renal transporter OATP4C1 (and other relevant renal transporters, e.g. OAT, OCT, MDR1, etc.). Someone in the biology group recently overheard that OATP4C1 might be involved in the disposition of sitagliptin (Januvia) and your team is developing a DPP4 inhibitor for the treatment of type II diabetes (same target as Januvia). Your lead compound, MIT-0320, is a potent, selective, and efficacious compound in preclinical testing but is currently being evaluated in preclinical toxicology studies. Please address the questions below and support your answers with references from the literature as needed.

- A.** The renal clearance of MIT-0320 is 3.6 ml/min in rats. GFR (glomerular filtration rate) in rats has been reported to range from 1.3 to 1.5 ml/min. Do you think that renal transporters are involved in the clearance? Why? Support your answer with fundamental pharmacokinetic equations.
- B.** What experimental systems could you use to assess if MIT-0320 is a substrate for renal transporters? What compound could you use as a positive control (e.g. a substrate for renal transporters)? If MIT-0320 is a substrate for renal transport, how might you assess if OATP4C1 was the mechanism involved (e.g. what specific experiment would you run)?
- . Your preclinical tox group decided to run a study with MIT-0320 co-dosed with probenecid to assess a potential drug interaction in rats. Their conclusion was that no drug interaction was observed. Would you recommend a drug interaction study in another species to prove a lack of interaction? Why or why not?
- D.** You are designing a transporter drug-drug interaction trial in the clinic in humans patients co-administering your compound and probenecid, how would you measure if the disposition of MIT-0320 changed?
- E.** If probenecid inhibited an MIT-0320 uptake transporter in the kidney involved in its clearance, what would the expected changes look like? To answer this question, indicate if there would be an increase, decrease or no change in each of the parameters noted in the table below:

Parameter	Anticipated Change		
Plasma Clearance of MIT-0320	↑	↓	↔
Renal Clearance of MIT-0320	↑	↓	↔
Plasma AUC of MIT-0320	↑	↓	↔
Kidney concentrations of MIT-0320	↑	↓	↔
Plasma AUC of probenecid	↑	↓	↔
Urine concentration of MIT-0320	↑	↓	↔

F. Your competitor, Januvia (sitagliptin), has shown inhibition of OAT3-mediated uptake of cimetidine ($IC_{50} = 160 \mu M$) in a human kidney cell line. Are renal drug interactions with Januvia clinically relevant? Comment specifically on whether sitagliptin would be a victim of other commonly prescribed medications (e.g., its disposition would change) or if sitagliptin itself could be the perpetrator of drug interactions with other drugs (e.g., changing another drugs disposition). Support your answer with relevant literature.

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20.201 Mechanisms of Drug Actions
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