4. P450 Drug Metabolism DDIs: Inhibition (2013)

Factor 4: Inhibition of Metabolism:

When a P450 enzyme that is responsible for metabolizing an object drug (R) is inhibited by an interactant drug (I) then the capacity of the liver to clear the object drug from the system is decreased and, absent a dose correction, the blood levels of the object drug will increase. The following points are generally true with respect to inhibition.

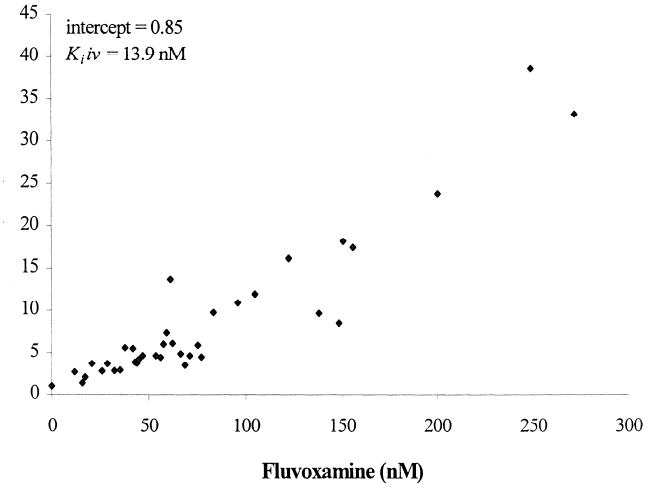
1) The magnitude of the inhibition will be dose dependent. Higher inhibitor doses provide higher blood levels leading higher inhibitor concentrations at the site of inhibition (the enzyme). We normally assume that the concentration of the inhibitor that the enzyme is exposed to is equal to the unbound concentration in plasma.
   a) <u>The extent of inhibition will be a function of the inhibitor levels in the blood so inhibition of object drug metabolic clearance depends on the dose and blood levels of [I]</u>.

      i) Below we see an example where the extent of inhibition, given as the fold decrease in metabolic clearance of an object drug (Cl(control)/Cl(inhibitor) is dependent upon the plasma concentration of the inhibitor (fluvoxamine). In this plot the Y-axis shows the extent of inhibition (bigger numbers=more inhibition).

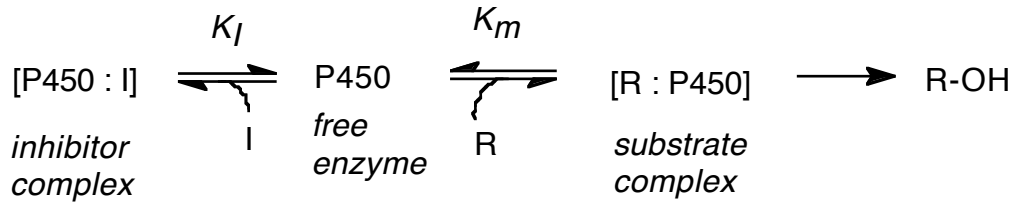
2) Mechanisms of Inhibition: Reversible vs Irreversible Inhibition Mechanisms.

Cl(control)/Cl (inhib)

(Value of 25 is 96% Reduction in clearance)



   i) <u>Reversible Inhibition: The inhibitor "competes" for enzyme with the object drug.</u> (Increasing the concentration of inhibitor shifts equilibrium to the left)



(1) The affinity of the enzyme for the inhibitor is given by the constant K_i. The lower the K_i the more potent the inhibitor. The K_i is equal to the concentration of inhibitor that is required to bind up half of the available enzyme.

(2) Each enzyme inhibitor pair has a characteristic and constant K_i. This means that we can apply the inhibition information obtained about one inhibitor-enzyme pair to predict the effect that will be observed on all object drugs metabolised by that enzyme.

(3) Graphs like that shown above of in vivo data allow us to "calibrate" in vivo effect of an interactant drug.

(4) We can determine a Ki for an inhibitor enzyme pair by doing in vitro experiments in microsomes. If we know the plasma levels of the inhibitor we can predict the magnitude of an interaction from the I/K_i ratio and specific information about the percent contribution of the enzyme to the clearance of the object drug.

(5) Most inhibitory DDI's are the result of reversible competitive inhibition of a P450 enzyme by an interactant drug.

$$E_{free} + I \underset{}{\overset{K_i}{\rightleftharpoons}} E\text{-}I \qquad \text{when } [I]=K_i \;\; [E_{free}] = [E\text{-}I]$$

The higher the $[I]/K_i$ ratio the greater the inhibition

$$Cl_{inhibited} = \frac{Cl_{control}}{1 + \frac{[I]}{K_i}}$$

ii) Irreversible Inhibition (Mechanism based enzyme inactivation (MBI)):

(1) The enzyme attempts to metabolize the inhibitor and produces a reactive intermediate that binds covalently to the active site amino acids or the heme group to produce an irreversible enzyme adduct that is no longer active.

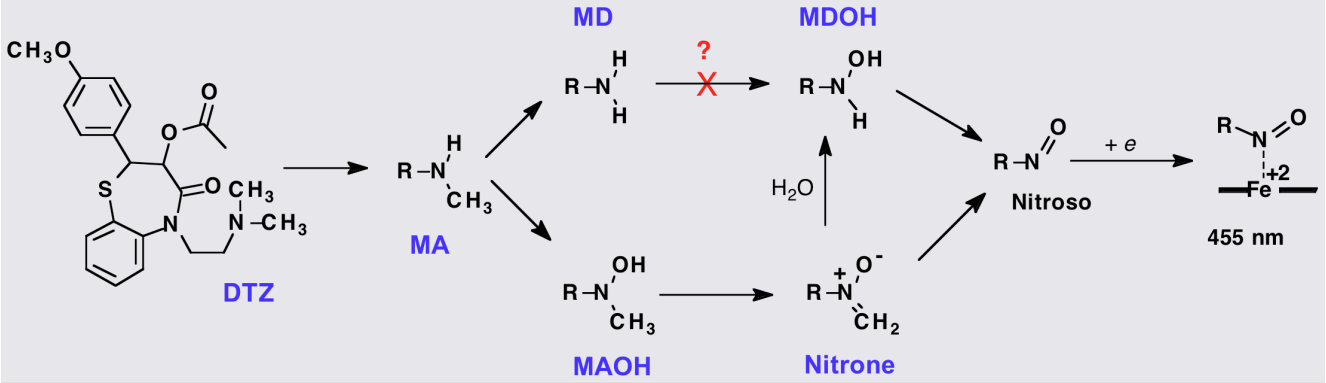
$$E_{free} + I \rightleftharpoons E\text{-}I \xrightarrow{Fe=O} EI_{inactive}$$

irreversible covalent bond between enzyme and inhibitor
Enzyme is dead forever.
Must make new enzyme to get activity back

(2) The body must produce new enzyme molecules before drug metabolism activity can be restored.

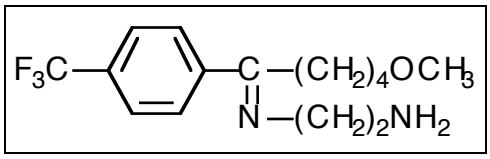
(3) The inhibition of the enzyme requires time (hours to days) to occur since it depends on a catalytic rate. Mechanism based inhibition is is time dependent.

(4) Many alkylamine containing drugs undergo sequential metabolism to produce reactive intermediates the bind directly to the heme iron of P450. These stable adducts are called metabolite intermediate complexes or **MICs**. Drugs that do this and cause DDIs include **troleandomycin (see notes pages 36 and 37),** erythromycin and diltiazem. Here a metabolite of a drug causes irreversible inhibition.



3) Principle: **Effect of an interactant drug will be observed on all object drugs metabolised by that enzyme.** The magnitude of the effect at an equivalent dose of the inhibitor will depend upon how much of the object drug clearance is due to that enzyme.

a) Here fluvoxamine is an inhibitor of CYP1A2. The data are taken from individual clinical studies.

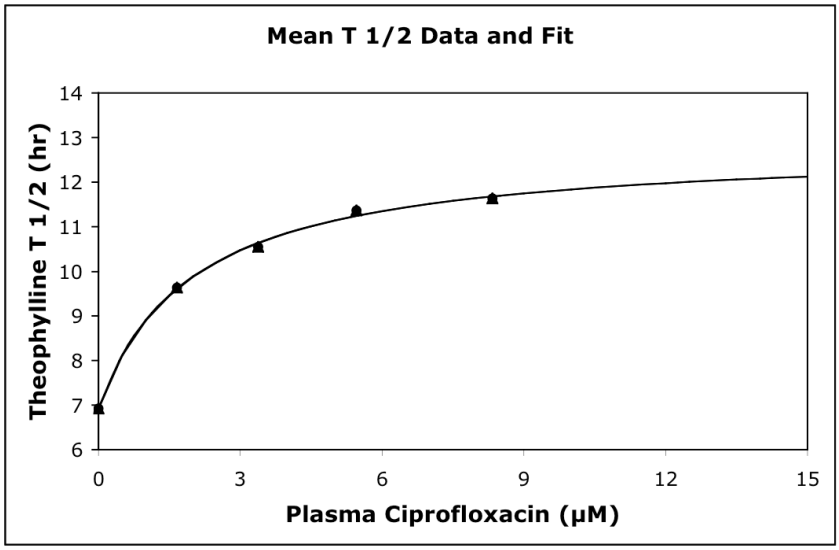


Fluvoxamine SS Dose	Object Drug	AUC	Half-life	Clearance
50 mg x 2	Clozapine	↑ 2.5 fold		
100 x 1	Caffeine		↑ 6.1 fold	↓ 5 fold
75 x 1	Theophylline	↑ 2.4 fold	↑ 2.5 fold	
50 mg x 1	Tacrine			↓ 6 fold
100 x 1	Imipramine	↑ 2.5 fold	↑ 1.8 fold	

b). Here we see some other examples abstracted from individual clinical studies where we look at different CYP1A2 inhibitors and various substrates.

CYP1A2 Substrate	**Interactant Drug or Condition** **Arrow indicate a significant change in object drug half-life**				
Object Drug	Quinolones	Fluvoxamine	Mexiletine	Smoking	Cimetidine
Caffeine	↑	↑	↑	↓	↑
Theophylline	↑	↑	↑	↓	↑
Tacrine		↑		↓	
Imipramine		↑	↑	↓	↑
Clozapine	↑	↑		↓	
Mexiletine	↑	↑		↓	
Propranolol				↓	

4) The magnitude of the inhibitory effect depends upon the dose of the inhibitor and the fraction of the drug that is metabolised by the enzyme that is inhibited. Below we see the effect of ciprofloxacin (a quinolone) on the half-life of theophylline. Maximum 2 fold effect is observed. About 60% of theophylline clearance is due to CYP1A2 so a maximum effect is observed even as the plasma levels of ciprofloxacin continue to climb. If the clearance of theophylline was totally due to CYP1A2 we would predict a rising straight line from this plot.

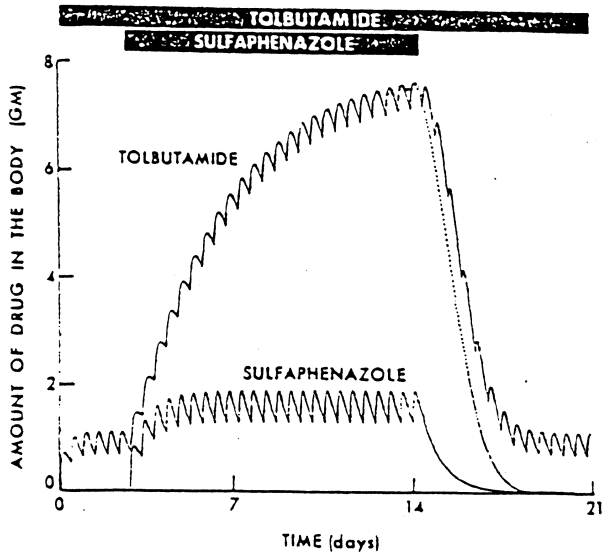


Generally I/Ki ratios and the fraction of the object drug clearance that is due to the inhibited enzyme (f_m) determines the magnitude of the effect observed.

$$Cl_{inhibited} = \frac{Cl_{control}}{1 + \frac{[I]}{K_i}} + (1 - f_m) \quad \text{for } (0 < f_m < 1)$$

	Magnitude of Effect on Object Drug Clearance	
Inhibitor Concentration	f_m (% of Object Drug Metabolized by the Inhibited Enzyme)	
[I]/Ki	Low	High
Low	↓	↓↓
High	↓↓	↓↓↓↓

a) Sulfaphenazole and tolbutamide Below is shown the effect of sulfaphenazole (interactant drug) on tolbutamide (object drug) levels in the body. Note that the amount of tolbutamide (cleared mostly by CYP2C9) increases substantially when sulfaphenazole is added to the dosing regimen. Note also that the interaction takes time to develop and dissappear.



Corollary: Any drug that is substantially cleared by CYP2C9 will experience a drug/drug interaction with sulfaphenazole. Thus since warfarin is substantially cleared by CYP2C9 it also is subject to an interaction with sulfaphenazole.

b) Another drug that inhibits CYP2C9 drug metabolizing capacity is cimetidine. As expected from the above corollary the half-lives of (S)-warfarin and tolbutamide are both increased by cimetidine. (Prothrombin times greater than 30-are associated with uncontrolled bleeding).

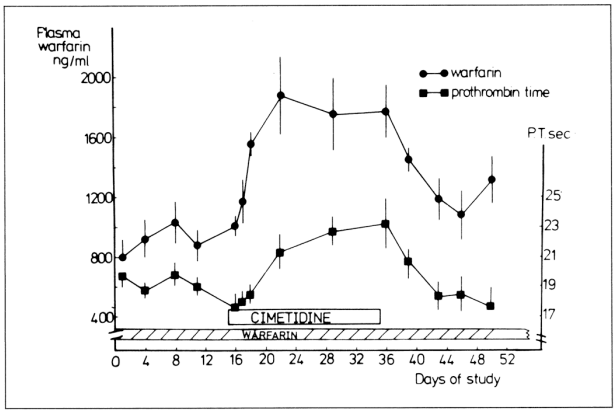


FIGURE 6-9. Mean plasma warfarin concentration and prothrombin-time in seven patients who received a constant anticoagulant dose followed by warfarin plus cimetidine 1000 mg per day. Adapted with permission from reference 221.

c) <u>A more complex example of a different kind (the parent drug is toxic and a metabolite is active)</u>

(1) Terfenadine (Seldane) was an extremely popular (15 million scrips a year in 1990) non-sedating H1 antagonist used to treat allergies.

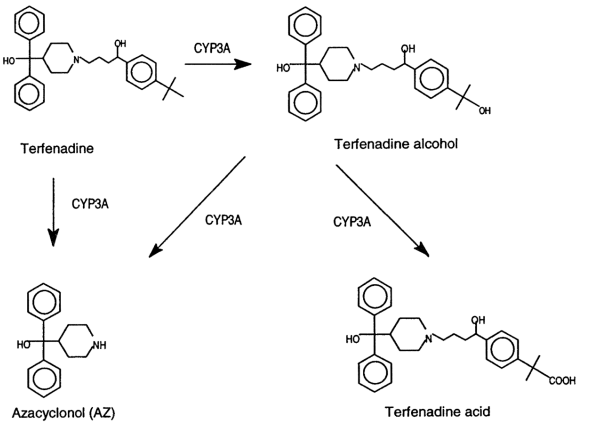


FIG. 32-1. Metabolic pathways for terfenadine oxidation.

(2) When marketed, nothing was known about what enzymes produced it's metabolites. Interestingly it was known that the <u>levels of terfenadine in the blood were very low</u> and sometimes undetectable <u>relative to levels of its metabolites.</u> This suggested that the antihistamine effect may be due to a metabolite.

(3) As it turns out, CYP3A4 converts almost all of the dose (>99%) to metabolites before the drug (oral administration) reaches the systemic circulation (a massive first pass effect) The acid metabolite was the major circulating species.

(4) As clinical experience with this drug increased there were reports of cardiotoxicity (increase in Q-T interval on an electrocardiogram: *tosardes de pointes*) when the drug was given with CYP3A4 inhibitors such as ketoconazole, itraconazole and others. This effect is now known to be due to blockage of the hERG channel, a potassium channel in the heart tissue, by terfenadine. hERG blockade can result in ventricular fibrillation and death.

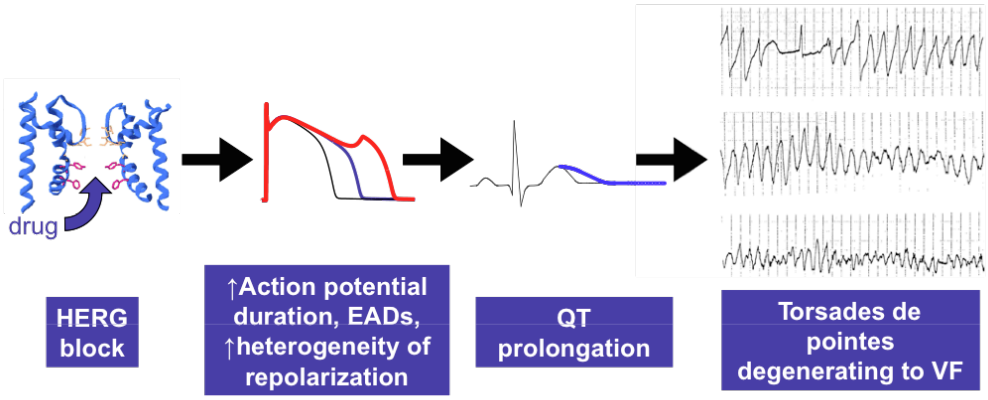


FIG. 5. Mechanisms of proarrhythmia associated with human *ether-à-go-go*-related gene (HERG) channel blockade. Blockade of the HERG channel produces prolongation of the QT interval (blue) and generates an EAD (red) in the cardiac action potential. These changes, which are heterogeneous across the ventricular wall, create a substrate for TdP. In this example, *torsades de pointes* degenerates into ventricular fibrillation.

(5) It turns out that the antihistamine effects of terfenadine were due mostly to the major circulating metabolite terfenadinic acid, while the cardiotoxic effect was due to the terfenadine itself.

(6) Those individuals that experienced the toxicity were taking other compounds that inhibited CYP3A4 such as troleadomycin, ketoconazole, erythromycin and grapefruit juice.

(7) Terfenadine was removed from the market in the United States. The acid metabolite (fexofenadine: Allegra) has replaced terfenadine.

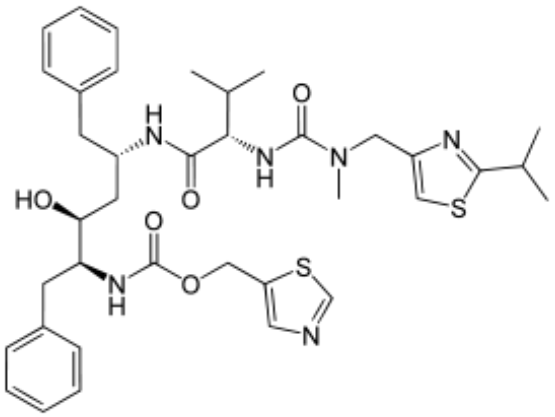
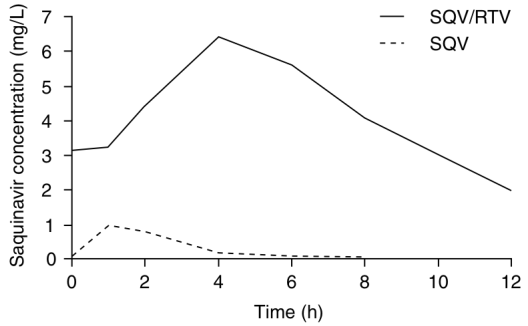
(8) Many amine containing drugs are now known to occasionally cause QT prolongation by binding to the hERG channel. Thus inhibition of their metabolism can lead to a hightened risk of this reasonably common off-target toxicity. Note the examples below (1,2,3,5,6,17,18,19) for psychiactric inpatients. The object drugs are on the left in the table.

Table 3 The top 20 drug combinations most frequently associated with high-danger interaction alerts in the study population, according to MediQ

Rank	Drug combination with high-danger interaction, according to MediQ	Frequency of interactions			Adverse event for which there is increased risk
		n	(%)	Cum. %	
	Total	2,308	(100)		
1	Clozapine–fluvoxamine	402	(17.4)	17.4	Seizures and other CNS effects, QTc/TdP and arrhythmias, bone marrow toxicity
2	Lithium–hydrochlorothiazide	190	(8.2)	25.6	Seizures and other CNS effects, QTc/TdP and arrhythmias (lithium intoxication)
3	Olanzapine–clozapine	177	(7.7)	33.3	QTc/TdP and arrhythmias, hypotension, and metabolic and endocrine disorders, including disruption in blood glucose levels
4	Metoprolol–paroxetine	114	(4.9)	38.3	Hypotension
5	Clozapine–pimozide	68	(2.9)	41.2	EPS and other CNS effects, QTc/TdP and arrhythmias
6	Torasemide–lithium	60	(2.6)	43.8	Seizures and other CNS effects, QTc/TdP and arrhythmias (lithium intoxication)
7	Levomepromazine–doxepin	58	(2.5)	46.3	Sedation, hypotension
8	Metoprolol–fluoxetine	48	(2.1)	48.4	Hypotension
9	Ramipril–spironolactone	42	(1.8)	50.2	Hypotension, hyperkalemia
10	Phenytoine–valproate	40	(1.7)	51.9	Hepatotoxicity
11	Amitriptyline–tranylcypromine	39	(1.7)	53.6	Serotonin syndrome
12	Amitriptyline–moclobemide	38	(1.6)	55.3	Serotonin syndrome
13	Moclobemide–trimipramine	38	(1.6)	56.9	Serotonin syndrome
14	Duloxetine–tramadol	35	(1.5)	58.4	Serotonin syndrome
15	Tranylcypromine–carbamazepine	31	(1.3)	59.8	Seizures and other CNS effects
16	Spironolactone–potassium	30	(1.3)	61.1	Hyperkalemia
17	Trimipramine–thioridazine	28	(1.2)	62.3	QTc/TdP and arrhythmias
18	Amitriptyline–thioridazine	22	(1.0)	63.3	QTc/TdP and arrhythmias
19	Doxepin–thioridazine	21	(0.9)	64.2	QTc/TdP and arrhythmias
20	Nefazodone–carbamazepine	21	(0.9)	65.1	Loss of nefazodone efficacy

d) An example of an intentional DDI (AIDs, ritonavir, PI boosting strategy)

i) Ritonavir (Norvir) is a first generation HIV-protease inhibitor (PI) that is now widely used as a boosting agent in the treatment of human immunodeficiency virus infection due to its potent off-target inhibitory effect on cytochrome P450 3A4 dependent metabolism of other protease inhibitors such as lopinavir and saquinavir. For example, systemic exposure (commonly measured as AUC) to saquinavir is increased 17 fold by co-administration of RTV. Currently two of the four preferred drug regimens recommended by DHHS for use in treatment-naïve AIDS patients employ RTV as an adjuvant.

Ritonavir (potent CYP3A4 MBI)	Effect of Ritonavir on Saquinavir PK.
	 **Fig. 1.** Effect of low-dosage ritonavir on the pharmacokinetics of saquinavir in two HIV-infected pregnant women during gestation. The solid line is saquinavir/ritonavir (SQV/RTV) 800/100mg twice daily and the broken line is saquinavir (SQV) 1200mg three times daily.

ii) This highly successful boosting strategy also features a co-formulated dosage form (Kaletra; (lopinavir-ritonavir)) and has been applied to the development of other co-formulations as well as an alternate boosting agent. Lopinavir/ritonavir (Kaletra) is the only PI-enhanced regimen co-formulated into a single pill. Multiple-dose pharmacokinetic studies in both healthy volunteers and HIV-infected patients suggest that the optimal combination of lopinavir/ritonavir is 400/ 100mg. (Clinical Pharmacokinetics (2004) 43 p 291-310.

iii) RTV causes time dependent loss of enzyme activity in vitro and **clinical studies have established that otherwise sub-therapeutic (for AIDs treatment) RTV doses of 100 mg/day are sufficient to "knock out" P450 3A4 activity in vivo.** Look at the table to see the effects of RTV on the PK of other coadministered drugs in HAART therapies.

Table I. Pharmacokinetic parameters of protease inhibitors (PIs) and various dual PI combinations. Values are listed as means ± SD or median (range), as available. For combinations, values are reported for the first PI named

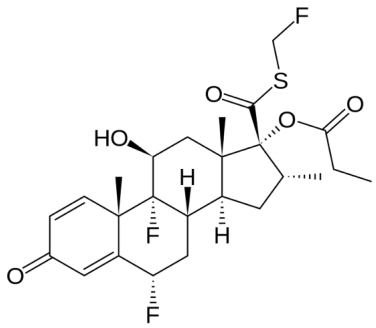
Drug or combination	Dose (mg)	C_{max} (mg/L)	C_{min} (mg/L)	AUC_τ (mg • h/L)	$t_{½\beta}$ (h)	Reference
Amprenavir	1200 bid	5.4	0.28	18.5	7.1–10.6	19
Amprenavir/ritonavir	600/100 bid	6.0	1.7	29.9		20
Amprenavir/ritonavir	1200/200 od	8.2	0.9	49.8		20
Atazanavir	400 od	3.2 ± 2.2	0.27 ± 0.3	22.3 ± 20.2	6.5 ± 2.6	21
Atazanavir/ritonavir	400/100 od	7.8 ± 1.1	1.0 ± 0.3	70.3 ± 10.5	7.0 ± 1.8	22
Indinavir	800 tid	7.7 ± 2.5	0.15 ± 0.1	18.8 ± 7.0	1.8 ± 0.4	23
Indinavir/ritonavir	800/100 bid	8.7	0.99	44	3.2	24
Indinavir/ritonavir	800/200 bid	6.3 (3.2–15.2)	0.7 (0.1–4.6)	38.2 (18.2–86.8)		25
Indinavir/ritonavir	400/400 bid	3.6 (1.5–7)	0.6 (0.1–1.6)	23 (9.3–45.7)		25
Indinavir/nelfinavir	1200/1250 bid	7.8	0.18	63.2		26
Lopinavir/ritonavir	400/100 bid	8.5	5.3	76.5		27
Nelfinavir	750 tid	3.0 ± 1.6	1.0 ± 0.5			28
Nelfinavir	1250 bid	3.5 ± 0.8	0.87 ± 0.31	24.7 ± 5.6	5.7 ± 2.7	29
Nelfinavir/saquinavir	1250/1000 bid	4.2	1.0	29.2		30
Nelfinavir/indinavir	1250/1200 bid	4.5	1.5	71[a]		26
Nelfinavir/ritonavir	1250/100 bid	4.5 ± 1.2	1.9 ± 0.8	37.7 ± 12.8	8.2 ± 2.4	29
Ritonavir	600 bid	11.2 ± 3.6	3.0 ± 2.1	60.8 ± 23.4	3.2	31
Saquinavir-HGC	600 tid	0.25		0.78		32
Saquinavir/nelfinavir	1000/1250 bid	1.5	0.1	7.3		30
Saquinavir-SGC	1200 tid	2.5		7.3 ± 6.2		32
Saquinavir/ritonavir	400/400 bid	2.5	0.5	16 ± 8		33
Saquinavir/ritonavir	1000/100 bid	3.9	0.5	23.4	3.2	34

a AUC over 24 hours.

AUC_τ = area under the concentration-time curve over the administration interval for each regimen at steady state; **bid** = twice daily; **C_{max}** = peak plasma concentration; **C_{min}** = trough plasma concentration; **h** = hours; **HGC** = hard gel capsules; **od** = once daily; **SGC** = soft gel capsules; **tid** = three times daily; **$t_{½\beta}$** = elimination half-life.

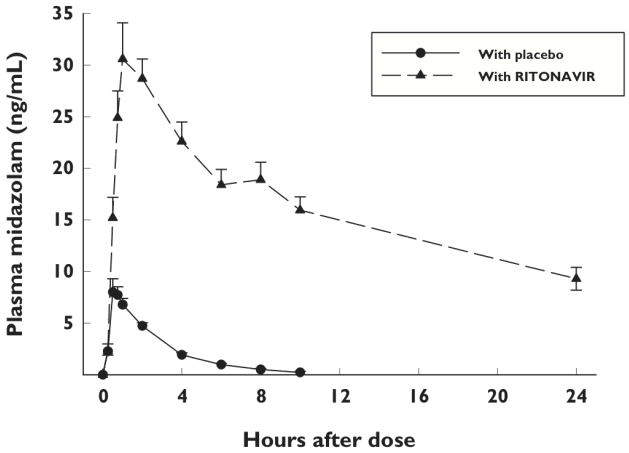
iv) P450 3A4 is responsible for the clearance of more than 50% of commonly administered drugs. Thus the large population of RTV-boosted patients (millions world wide) is at a high risk of unintended but profound drug drug interactions with object drugs significantly metabolized by CYP3A4. Let's look at a couple of them.

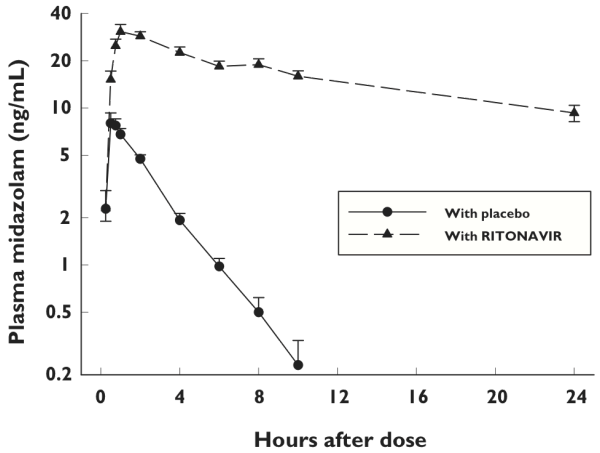
(1) Flonase Interaction:



(a) There were 25 cases (15 adult and 10 paediatric) of significant adrenal suppression secondary to an interaction between ritonavir and inhaled fluticasone, and three cases involving ritonavir and intranasal fluticasone. There has also been a pharmacokinetic interaction study performed which assessed the magnitude of the interaction. Eighteen healthy volunteers were given oral ritonavir 100 mg every 12 h for 7 days and fluticasone 200 mg daily intranasally. **The serum fluticasone area under the concentration–time curve (AUC) was increased 350-fold**. (HIV Medicine (2008) 9 pp 389-396).

(2) Midazolam Interaction (note the same data plotted on a linear plot (left) and a semilog plot (right). These results are expected why? PK parameters (ignore ALT-2074 data)





	Mean (±SE, n = 13) for treatment conditions:				Dunnett's test	
	Placebo	ALT-2074	Ritonavir	Repeated measures ANOVA	ALT-2074 *vs.* placebo	Ritonavir *vs.* placebo
C_{max} (ng ml^{-1})	9.0 (±1.0)	10.6 (±0.9)	35.6 (±2.8)	$F = 81.8$, $P < 0.001$	NS	$P < 0.05$
$t_{1/2}$ (h)	2.06 (±0.15)	2.08 (±0.17)	18.07 (±2.25)	$F = 49.4$, $P < 0.001$	NS	$P < 0.05$
Total AUC (ng ml^{-1} h^{-1})	24.65 (±1.93)	29.87 (±2.62)	651 (±77)	$F = 64.9$, $P < 0.001$	NS	$P < 0.05$
Clearance (ml min^{-1})	2157 (±142)	1844 (±167)	85.9 (±6.9)	$F = 92.2$, $P < 0.001$	NS	$P < 0.05$
Clearance (ml min^{-1} kg^{-1})	28.84 (±2.84)	24.15 (±2.38)	1.13 (±0.11)	$F = 64.1$, $P < 0.001$	NS	$P < 0.05$

*Analysis of actual values without transformation.