Using Pharmacokinetics to Optimize Antiretroviral Drug-Drug Interactions in the Treatment of Human Immunodeficiency Virus Infection

John G. Gerber

From the Division of Clinical Pharmacology and Toxicology and Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver

Better understanding of the pharmacokinetics of antiretroviral drugs has resulted in the design of combination therapies for the treatment of human immunodeficiency virus (HIV) infection. This has improved the bioavailability and prolonged the plasma half-life of some of the drugs, resulting in enhanced antiviral activity. However, antiviral combination therapy can also result in adverse drug-drug interactions and diminished antiretroviral activity. In this review, we examine drug interactions involving combinations of protease inhibitors, combinations of protease inhibitors with nonnucleoside reverse transcriptase inhibitors, and combinations of nucleoside analogues for the treatment of patients with HIV infection. We discuss examples and mechanisms of pharmacokinetic interactions that improve or decrease antiviral efficacy.

The use of multidrug therapy for HIV infection makes it imperative to understand how antiretroviral drugs interact with one another. Antiretroviral drug combinations can result in pharmacokinetics that are favorable, unchanged, or adverse.

There are presently 14 antiretroviral drugs on the market. Six of them are nucleoside reverse transcriptase inhibitors (NRTIs): zidovudine, didanosine, stavudine, zalcitabine, lamivudine, and abacavir. Three are nonnucleoside reverse transcriptase inhibitors (NNRTIs): nevirapine, delavirdine, and efavirenz. Five are protease inhibitors (PIs): saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir.

To understand how antiretroviral drugs interact with each other, a knowledge of their pharmacokinetics is an absolute necessity. Pharmacokinetics are simply what the body does to a drug when it is administered by any route. Pharmacokinetics for an orally administered drug include its absorption, first-pass metabolism, distribution, metabolism (either activation or inactivation), and elimination. Bioavailability is the term that defines the fraction of the drug that reaches the systemic circulation after oral administration. Low bioavailability can be the result of poor absorption as well as extensive first-pass metabolism.

For a drug to traverse the intestinal epithelial membrane, it must be in solution in the aqueous milieu of the gastrointestinal tract and be sufficiently lipophilic to pass through lipid membranes. The vast majority of antiretroviral drugs undergo passive diffusion through the gastrointestinal lining, driven by a concentration gradient. Once in the intestinal epithelial cells, lipophilic drugs can be transported back to the luminal surface by the P-glycoprotein [1], a multidrug-resistance–transport protein, and/or metabolized by the intestinal cytochrome P450 (CYP) 3A isoenzymes [2]. The exact mechanisms by which the CYP enzymes interact with P-glycoprotein to adversely affect the bioavailability of drugs are currently unclear. It has been noted, however, that many drugs with a high affinity for CYP3A are also substrates for the P-glycoprotein [3]. Once in the portal circulation, drugs can be further metabolized by the liver before they reach the systemic circulation.

In the systemic circulation, drugs are distributed into tissues on the basis of their relative affinity for tissue components versus plasma components. Drugs with high affinity for tissue proteins have a large volume of distribution because of their preferential partitioning into tissues. Most lipophilic drugs are attracted to plasma proteins; therefore, plasma concentrations of drugs are a composite of both bound and free drugs. At steady state, the free drug is in equilibrium with the intracellular compartment as long as the drug enters the cells by simple diffusion and is not actively pumped out of the cytoplasm.

Therefore, total plasma concentration of a drug may be an underestimation of the actual concentration necessary for an effect. This has certainly been observed with the lipophilic HIV PIs. For example, the HIV PI SC-52151 becomes highly protein-bound, such that effective free concentrations are not achievable despite seemingly high total plasma concentrations [4]. It has been proposed that in the CNS, P-glycoprotein–mediated efflux in brain capillary endothelial cells inhibits the accumulation of HIV PIs at this site [5, 6].

Drugs in the systemic circulation are usually metabolized or excreted unchanged. Lipophilic drugs, such as PIs and NNRTIs, are oxidatively metabolized by the CYP enzymes to more polar forms for subsequent biliary or renal excretion. CYPs are a group of heme-containing membrane-bound en-

Reprints or correspondence: Dr. John G. Gerber, Professor of Medicine and Pharmacology, Divisions of Clinical Pharmacology and Infectious Diseases, University of Colorado Health Sciences Center, Box C-237, 4200 9th Ave., Denver, CO 80262.

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Table 1. Drug interactions of protease inhibitors used in combination.

Drug combination	Pharmacokinetic effect	Virological outcome
Ritonavir and saquinavir	Enhanced saquinavir exposure (20- to 50-fold); prolonged half-life of saquinavir; markedly lower dose of saquinavir to achieve high concentrations	Enhanced and durable antiviral activity with twice-a-day dosing of both drugs
Ritonavir and indinavir	Enhanced indinavir exposure (3- to 5-fold); prolonged half-life of indinavir; lower indinavir dose with higher C_{min}; no dietary restriction with indinavir	Short-term antiviral effect is excellent with twice-a-day dosing of both drugs
Ritonavir and nelfinavir	Enhanced nelfinavir exposure (~2-fold); increased generation of active metabolite, AG1402	Excessive diarrhea; inconclusive virological data

NOTE. C_{min}, minimal concentration of drug.

zymes that are involved in a host of mono-oxygenase reactions. This family of enzymes is involved in steroid and fatty acid oxidation. Only a handful of CYP isozymes are involved in drug metabolism. They catalyze reactions to increase the water solubility of lipophilic drugs to facilitate elimination. The liver contains the highest quantity and the most diverse isoforms of CYP enzymes. Other cells, including small-bowel epithelial cells and renal tubular cells, also contain CYPs that contribute to drug metabolism [7, 8].

The main drug-metabolizing enzymes are CYP3A4, 2C9, 2C19, 2D6, 1A2, 2E1, 2B6, and 2A6. In both the liver and small intestine, CYP3A4 is the most abundant CYP isozyme present. Frequently, drugs are metabolized by multiple CYP isozymes, but usually 1 isozyme predominates in their biotransformation. PIs are large lipophilic molecules that appear to have an affinity for CYP3A4, which mediates their metabolism. The PIs can also inhibit CYP3A4 activity, impeding the biotransformation of other drugs that use this isozyme for metabolism [9]. If the concomitant drug has a low therapeutic index, excessive accumulation can produce severe toxicity.

Of the PIs in clinical use, ritonavir is the most potent inhibitor of CYP3A4; indinavir, nelfinavir, and amprenavir are less potent by an order of magnitude, and saquinavir is the least potent [10]. In addition to inhibition of CYP3A4, both ritonavir and nelfinavir induce the activity of CYP3A4 and other microsomal enzymes, resulting in rather complex drug-drug interactions. Ritonavir is partly metabolized by CYP2D6, and it has been demonstrated to inhibit this isozyme as well [11]. Partial metabolism of nelfinavir by the CYP2C19 isozyme results in the formation of its active metabolite, referred to as M8 or AG-1402 [12].

Delavirdine, an NNRTI, is metabolized mainly by CYP3A4 and is an inhibitor of this isozyme as well. Both nevirapine and efavirenz are inducers of CYP3A4 activity, but most of their metabolism appears to be mediated by another CYP isozyme, CYP2B6 [13].

NRTIs are water-soluble and, with the exception of zidovudine, are mostly eliminated by renal excretion. Zidovudine is conjugated by glucuronidation, and the conjugate is renally eliminated. NRTIs are prodrugs that require intracellular phosphorylation for activity, and drug-drug interactions that affect phosphorylation can affect drug activity.

The following paragraphs will describe some of the important drug-drug interactions that may occur with the use of combinations of PIs, combinations of PIs and NNRTIs, and combinations of NRTIs. The description of the interactions through the inhibition or induction of CYP isozymes is based on mean changes in the pharmacokinetic parameters. The organ-specific expression of the CYP isozymes across the population is large. Therefore, the usual dosing of these drugs can result in variable concentrations at steady state, with some subjects having high and potentially toxic levels and others having low levels that are potentially inadequate to suppress the virus. The issue of therapeutic drug monitoring of antiretroviral agents is reviewed in this supplement by Acosta et al. [14]. Pharmacodynamic interactions of these drug combinations that enhance or diminish their activity is outside the scope of this review.

Drug-Drug Interactions of PIs Used in Combination

Saquinavir was the first PI marketed in the United States and was formulated in a hard-gel capsule as a mesylate salt (Invirase; Hoffman–La Roche, Nutley, NJ). This drug has very unfavorable pharmacokinetics: even in combination therapy with NRTIs, its efficacy has been very limited because of the low and variable plasma concentrations that have been achieved. Administration of high doses of Invirase [15] and the new soft-gel formulation [16] has resulted in enhanced antiretroviral activity. The pharmacokinetics of saquinavir were also markedly improved when it was combined with ritonavir [17] (table 1). Saquinavir has very low oral bioavailability, which is probably secondary to its metabolism by intestinal CYP3A4 and its affinity for the P-glycoprotein [18]. In addition, the drug has a very short half-life because of high systemic clearance.

Administration of ritonavir was found to enhance the bioavailability and prolong the elimination half-life of saquinavir, such that the plasma-concentration time/area under the curve (AUC) of saquinavir increased as much as 30- to 50-fold compared with that of saquinavir alone [19]. This combination reduces the pill burden and the cost of antiretroviral therapy.

Ritonavir may inhibit both intestinal and hepatic CYP3A4 and thus improve the bioavailability of saquinavir by decreasing its systemic clearance. The combination of ritonavir and saquinavir (400 mg/400 mg) appears to have extremely potent antiretroviral activity, judged on the basis of the documented durable responses observed in patients [20]. It is unclear how much ritonavir contributes to the antiviral effect of the high concentration of saquinavir. This is an important question, because ritonavir is poorly tolerated at high doses. However, there are only limited pharmacokinetic data on single low doses of ritonavir with saquinavir.

In healthy volunteers, ritonavir increases saquinavir exposure in a dose-dependent manner [17]. More important, there are no efficacy data on low-dose ritonavir combined with saquinavir. Lower and better-tolerated doses of ritonavir may make this combination more attractive as therapy. However, before a lower dose of ritonavir can be recommended for use with saquinavir, we need comparative efficacy data from studies with adequately large sample sizes.

Indinavir is a potent and relatively well-tolerated PI. The drug has some pharmacokinetic advantages over the other PIs but many disadvantages as well. The main advantage is that only 60% of indinavir is protein-bound in the circulation. At steady state, the total plasma concentration of the drug therefore may more closely reflect the diffused intracellular concentration, which is important for its antiretroviral effects [21]. However, the drug has numerous pharmacokinetic disadvantages. Indinavir has to be taken on an empty stomach or with a low-fat snack [22]. The drug has a very short half-life because of the high systemic clearance; therefore the present recommended dose is 800 mg every 8 h.

Because of the large variability in both bioavailability and systemic clearance, the trough concentration of indinavir can vary widely across a patient population and sometimes can fall significantly below the 95% inhibitory concentration [23]. In addition, because of the rigid dosing schedule and the dietary requirement for maximal absorption, drug adherence can be difficult. Inadequate plasma concentrations of indinavir may promote the evolution of PI-resistant strains of HIV. Indinavir is also the only PI with significant renal excretion [24]. Since the drug has limited water solubility, especially at higher urinary pH, a significant proportion of patients who take indinavir may develop nephrolithiasis. It has been shown that the urinary concentration of indinavir directly correlates with its plasma concentration; thus, during the first 3–4 h after drug administration, the urine becomes supersaturated with indinavir [22].

The administration of ritonavir improves the bioavailability and prolongs the elimination half-life of indinavir, and it reduces the total dose necessary to achieve a potent antiretroviral plasma concentration. There are accumulating and encouraging clinical data concerning the combination of ritonavir and indinavir. The pharmacokinetic interaction study findings in normal volunteers were very positive [25]. Ritonavir (400 mg twice daily) decreases the systemic clearance and improves the oral bioavailability of indinavir to the point that a lower dose of indinavir (400 mg twice daily) results in the same drug exposure as a high dose (e.g., 800 mg every 8 h). The trough concentration of indinavir is also consistently higher with the ritonavir/indinavir combination than with indinavir alone. Most surprising is that food does not affect the bioavailability of indinavir when it is administered with ritonavir [26]. With the lower peak concentration of indinavir resulting from administration of the lower dose, the incidence of renal stone formation may be reduced.

Recently, pharmacokinetic data were presented about variable dosage combinations of indinavir and ritonavir. Burger et al. [27] treated antiretroviral-naive, HIV-infected patients with a combination of indinavir (800 mg) and ritonavir (100 mg) b.i.d. and found that the trough concentration of indinavir was 4-fold higher than with the regimen of indinavir alone, 800 mg every 8 h. This combination was well tolerated and was significantly less expensive because fewer pills were administered. Saah et al. [28] administered varying dosage combinations of indinavir and ritonavir to healthy volunteers. They found that ritonavir had a favorable and dose-dependent effect on indinavir pharmacokinetics. At a dosage combination of 800 mg of indinavir and 200 mg of ritonavir b.i.d., the trough concentration of indinavir was very high.

Efficacy and toxicity data are urgently needed concerning this unique PI dosage combination. The only clinical data on a combination of these PIs (at a dose of 400 mg/400 mg) with 2 NRTIs have been presented by Workman et al. [29] and Rockstroh et al. [30] and demonstrated excellent antiviral efficacy in PI-naive patients. These data, however, should be interpreted with caution since these were not comparative trials and indinavir with 2 NRTIs can be a very potent antiretroviral drug combination.

There are some data on the pharmacokinetics of nelfinavir in combination with ritonavir. Since both ritonavir and nelfinavir are potent inducers of microsomal drug-metabolizing enzymes, it is important they be at steady state when drug-drug interactions between these PIs are evaluated. It usually takes 10–14 days to maximally induce metabolism of these drugs. In normal volunteers, ritonavir has been shown to increase nelfinavir exposure 2-fold to 3-fold. Flexner et al. [31] examined the effect of ritonavir (400 mg twice daily) on the kinetics of 2 doses of nelfinavir and its active metabolite, M8 (AG-1402), in HIV-infected patients. Using historical controls for comparisons, they found that ritonavir increased nelfinavir exposure approximately 2-fold but increased the generation of M8 3- to 4-fold.

These data suggest that the inductive effect of ritonavir on CYP2C19 may be substantially greater than its inhibitory activity. Although clinical data were difficult to interpret because of an inadequate sample size, this combination does not appear

Table 2. Drug interactions of protease inhibitors in combination with nonnucleoside reverse transcriptase inhibitors (NNRTIs).

Drug combination	Pharmacokinetic effect	Virological outcome
Delavirdine and indinavir	Increased indinavir exposure (~50%), resulting in lower dose of indinavir	Mostly uncontrolled studies, but appeared to have some efficacy for salvage therapy after multidrug failure
Nevirapine and indinavir	Decreased indinavir exposure (~30%) but diminished variability in C_{min}; increased dose of indinavir is recommended	Excellent antiretroviral effect
Nevirapine and nelfinavir	No change in kinetics of either drug	Excellent antiretroviral effect
Efavirenz and indinavir	Decreased indinavir exposure (~30%); increased dose of indinavir is recommended	Excellent and durable antiretroviral effect

NOTE. C_{min}, minimal concentration of drug.

to be as efficacious as either ritonavir/saquinavir or ritonavir/indinavir [32].

Other combinations of PIs have not yielded sufficient clinical or pharmacokinetic data for full interpretation. However, nelfinavir clearly has been found to increase the exposure of saquinavir, but to a lesser degree than ritonavir does [33].

Drug-Drug Interactions of PIs and NNRTIs

The combination of PIs and NNRTIs (table 2) is attractive because both groups of drugs have potent antiretroviral efficacy and they are not antagonistic. Of the NNRTIs, delavirdine is the largest by molecular weight, and it has been used extensively in clinical trials. Delavirdine is metabolized by multiple CYPs, but it inhibits CYP3A4 significantly [34]. Delavirdine inhibits the metabolism of the PIs, thereby increasing the AUC of indinavir, ritonavir, and nelfinavir by 50%–80%. It notably increases saquinavir exposure, by >400% [35, 36]. The dose of indinavir is recommended to be reduced to 600 mg every 8 h because of the inhibitory effect of delavirdine. Indinavir, saquinavir, and ritonavir do not affect the kinetics of delavirdine metabolism [37]. In contrast, both rifampin and rifabutin, potent inducers of CYP3A4 and 2C9, stimulate the metabolism of delavirdine, suggesting the involvement of CYP3A4 and CYP2C9 [38, 39]. Since ritonavir inhibits CYP3A4 and likely induces CYP2C9, the combined effect of these activities may not alter the pharmacokinetics of delavirdine.

In contrast, nelfinavir decreases the AUC of delavirdine by 50% [40]. This effect is probably secondary to the ability of nelfinavir to induce the activity of multiple microsomal enzymes. Large clinical trials with this drug combination have been limited, but the report of an uncontrolled study suggested that the combination of indinavir or nelfinavir with delavirdine has antiviral efficacy in patients for whom other antiretroviral drugs have failed [41].

Of the antiretroviral drugs, nevirapine and efavirenz have favorable pharmacokinetics because of their long plasma half-life. The weakness of all of the NNRTIs as antiretroviral drugs, however, is that HIV rapidly develops resistance to these agents. Nevirapine has a small molecular weight, predictably good bioavailability, and because of its low hepatic clearance, a long half-life [42]. Protein binding is only 60%, and the drug distributes to multiple sites, including the CNS [43]. Hepatic metabolism of nevirapine is mainly through the activity of CYP2B6 and CYP3A4. Nevirapine induces its own metabolism by activating both CYP2B6 and CYP3A4. The induction of these enzymes appears to result in an interaction of nevirapine with PIs, since the AUCs of both indinavir and saquinavir are decreased by nevirapine by ~28% [44, 45].

In the presence of nevirapine, therefore, the present recommendation is to increase the dose of indinavir to 1000 mg every 8 h. Nevirapine has no significant effect on the pharmacokinetics of either ritonavir or nelfinavir, perhaps because these drugs induce their own metabolism [46]. No PI has a significant effect on the pharmacokinetics of nevirapine, a circumstance suggesting that CYP3A4 does not have a major role in the metabolism of nevirapine. Data from clinical trials with the combination of PIs and nevirapine indicate this combination to be potent [47, 48].

Efavirenz is the latest of the NNRTIs to be approved by the US Food and Drug Administration for clinical use (September 1998). It is the most potent of the NNRTIs and has shown remarkable efficacy when combined with 2 NRTIs [49]. When efavirenz is combined with indinavir, it also causes a potent and durable antiretroviral effect [50]. Efavirenz has favorable pharmacokinetics. It is efficiently absorbed and has a very long half-life; therefore, once-a-day administration is recommended. The drug is metabolized in the liver mainly by CYP2B6 and CYP3A4.

Like nevirapine, efavirenz also induces its own metabolism. Induction of CYP3A4 by efavirenz results in enhanced metabolism of indinavir, saquinavir, and amprenavir. In clinical trials, the dose of indinavir has been increased to 1000 mg every 8 h to compensate for the ~30% decrease of the AUC when the drug is combined with efavirenz [51]. Efavirenz has a greater effect on the metabolism of saquinavir than on that of indinavir, so it is not currently recommended for use with saquinavir.

In addition to stimulating CYP3A4, efavirenz inhibits

Table 3. Drug interactions of nucleoside reverse transcriptase inhibitors used in combination.

Drug combination	Pharmacokinetic effect	Virological outcome
Stavudine and zidovudine	Decreased intracellular phosphorylation of stavudine	Loss of stavudine activity; the combination should not be used
Lamivudine and zalcitabine	Decreased intracellular phosphorylation of lamivudine	No data; the combination probably should not be used
Didanosine and hydroxyurea	Increased phosphorylation of dideoxyadenosine	Enhanced antiviral activity

CYP2C19. Therefore, the combination of efavirenz and nelfinavir may cause a small but significant increase in the AUC of nelfinavir and a significant decrease in the formation of its active metabolite, M8 [12]. Efavirenz also slightly but significantly increases ritonavir exposure. The effect of the PIs on efavirenz pharmacokinetics has generally been found to be insignificant, although recently it has been reported that the AUC of efavirenz increases slightly when it is combined with ritonavir [52].

Finally, the importance of pathways for efavirenz metabolism, other than those mediated by CYP3A4, have been suggested by data showing that rifampin decreased efavirenz exposure inconsistently and only to a minor extent [53].

Drug-Drug Interactions of NRTIs Used in Combination

For the NRTIs, adverse or favorable drug interactions occur at the level of phosphorylation. All of the NRTIs are prodrugs that require activation by 3 cellular phosphorylation steps to form a triphosphate derivative, which then competes with endogenous nucleotides for viral reverse transcriptase. With the use of multiple NRTIs in the clinical treatment of HIV infection (table 3), it is important to understand how these drugs interact at the level of intracellular phosphorylation.

Stavudine and zidovudine are thymidine analogues that share intracellular phosphorylation pathways. In addition, zidovudine monophosphate accumulates in cells in high concentrations because of its ability to inhibit thymidylate kinase, thereby slowing further phosphorylation [54]. Stavudine, in the presence of zidovudine, is very poorly phosphorylated, probably because it does not compete effectively with zidovudine at the thymidine kinase step, and further phosphorylation is slowed by the inhibition of thymidylate kinase by zidovudine monophosphate [55]. Clinical evaluation of the combination of stavudine and zidovudine administered to patients who had previously received zidovudine therapy was conducted in AIDS Clinical Trials Group (ACTG) 290. That study demonstrated that the virological outcome for patients receiving both drugs was poorer than the outcome for patients receiving stavudine alone [56].

Zalcitabine and lamivudine, both cytosine analogues, use the same phosphorylation pathways and adversely affect the phosphorylation of each other in vitro [57]. Since zalcitabine is given to patients only infrequently, clinical correlations of these in vitro adverse drug-drug interactions have not been reported. However, these 2 drugs should not be used together to treat HIV infection.

Finally, hydroxyurea, a ribonucleotide reductase inhibitor, improves the phosphorylation and the activity of numerous NRTIs, most prominently didanosine [58, 59]. The use of hydroxyurea has been shown in clinical trials to improve the efficacy of didanosine administered with stavudine [60].

In summary, with the use of multiple antiretroviral drugs for the treatment of HIV infection, it is critical to understand their pharmacokinetic interactions so that favorable effects are optimized and adverse ones avoided. This review has discussed examples of both types of interactions in the multidrug treatment of HIV infection.

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