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Pharmacokinetic Enhancement of Inhibitors of the Human Immunodeficiency Virus Protease by Coadministration with Ritonavir

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Coadministration with the human immunodeficiency virus (HIV) protease inhibitor ritonavir was investigated as a method for enhancing the levels of other peptidomimetic HIV protease inhibitors in plasma. In rat and human liver microsomes, ritonavir potently inhibited the cytochrome P450 (CYP)-mediated metabolism of saquinavir, indinavir, nelfinavir, and VX-478. The structural features of ritonavir responsible for CYP binding and inhibition were examined. Coadministration of other protease inhibitors with ritonavir in rats and dogs produced elevated and sustained plasma drug levels 8 to 12 h after a single dose. Drug exposure in rats was elevated by 8- to 46-fold. A >50-fold enhancement of the concentrations of saquinavir in plasma was observed in humans following a single codose of ritonavir (600 mg) and saquinavir (200 mg). These results indicate that ritonavir can favorably alter the pharmacokinetic profiles of other protease inhibitors. Combination regimens of ritonavir and other protease inhibitors may thus play a role in the treatment of HIV infection. Because of potentially substantial drug level increases, however, such combinations require further investigation to establish safe regimens for clinical use.

Inhibitors of the aspartic proteinase from the human immunodeficiency virus type 1 (HIV-1) protease are promising new agents for the chemotherapy of AIDS. Treatment regimens employing a protease inhibitor can produce a rapid and sustained decline of plasma viral RNA and a concomitant elevation of CD4 cells in HIV-infected individuals (4, 8, 9, 21, 29, 30). Because of the peptidomimetic structures of many HIV protease inhibitors, their utility is compromised by modest oral bioavailability resulting from poor absorption and/or rapid hepatobiliary elimination. Unlike that of nucleoside analogs, which undergo phosphorylation resulting in sustained intracellular concentrations of their active triphosphate derivatives, the activity of protease inhibitors is dependent on the continuous maintenance of circulating concentrations that suppress viral maturation. Consequently, maximal viral suppression is achieved with frequent, high doses of protease inhibitors in combination with nucleoside analogs (8, 29). Sustained, highly suppressive concentrations may be critical for impeding the emergence of resistant variants. HIV displays both a high mutation rate and rapid replication kinetics in vivo (2, 11, 24, 33). Treatment regimens producing trough concentrations of inhibitor in plasma that allow persistent low-level viral replication therefore favor the accumulation of multiple mutations required for significant resistance (3, 22). By contrast, the continuous maintenance of high levels of inhibitor in plasma is associated with a delay in resistance development (22).

Recently, we reported the identification of ritonavir (ABT-538), a potent and selective inhibitor of HIV protease with high oral bioavailability (14). Repeated oral dosing (600 mg twice a day) of ritonavir in humans produced high (maximum concentration [C_{max}] = 11.2 μg/ml) and sustained (half-life [$t_{1/2}$] = 3 to 5 h) steady state plasma drug concentrations (4). A phase I and II clinical trial with 600 mg of ritonavir twice daily as monotherapy demonstrated a >0.8 log decline in mean HIV plasma RNA and a median increase in CD4 levels of >100 cells/μl after 32 weeks (4). In phase III studies, this regimen, either as monotherapy or in combination with one or two concomitant nucleosides, significantly delayed the progression of HIV-related disease and prolonged survival in patients with advanced AIDS (1). Previously we reported that the sustained plasma ritonavir concentrations relative to those of other inhibitors of its class correlated with the replacement of labile chemical functionalities with groups less susceptible to oxidative metabolism (14). Subsequent studies demonstrated that the metabolism of ritonavir in human liver microsomes is mediated predominantly by members of the cytochrome P-450 3A (CYP3A) and CYP2D subfamilies (20). Because the metabolism of ritonavir is characterized by a low K_m (<1 μM), typical substrates and inhibitors of CYP3A (e.g., ketoconazole and troleandomycin) only poorly inhibited ritonavir metabolism even at high concentrations. In contrast, ritonavir was a potent, reversible inhibitor of the oxidative metabolism of the known CYP3A substrates nifedipine, 17α-ethynylestradiol, and terfenadine (20).

The HIV protease inhibitors saquinavir (26) and indinavir (31) are also metabolized primarily by CYP3A (6, 10). Their pharmacokinetic characteristics dictate three-times-daily dosing in humans. Because of the importance of plasma drug concentrations for antiviral effect with protease inhibitors, and

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in view of the potent CYP3A inhibition by ritonavir, we initiated investigations of the interactions between ritonavir and other peptidomimetic protease inhibitors in rat and human liver microsomes. To model potential pharmacokinetic interactions between ritonavir and those inhibitors, we also coadministered each agent with ritonavir in animals. We now report that concentrations of various protease inhibitors in plasma are substantially (8- to 46-fold) enhanced in rat and dog following coadministration with ritonavir. Preliminary data indicate that codosing ritonavir and saquinavir in humans produces a profound enhancement of plasma saquinavir concentrations. This pharmacokinetic enhancement may have implications for the therapy of HIV infection with combinations of ritonavir and other protease inhibitors.

MATERIALS AND METHODS

Inhibition of HIV protease inhibitor metabolism by ritonavir. HIV protease inhibitors were synthesized as described in literature reports (5, 13, 18). Saquinavir was obtained from Roche Laboratories. Human liver microsomes were prepared as previously described (20). Substrate (saquinavir [25 μM], indinavir [50 μM], nelfinavir [25 μM], or VX-478 [25 μM]) was coincubated in phosphate buffer (pH 7.4) with various concentrations of ritonavir, 1 mg of liver microsomal protein/ml, and an NADPH-generating system containing the following: $MgCl_2$ (15 mM), $NADP^+$ (4.0 mM), glucose 6-phosphate (10 mM), and glucose 6-phosphate dehydrogenase (2.0 U/ml). The sample workup included stopping the reaction with two volumes of acetonitrile, evaporation of protein-free supernatant under nitrogen, and reconstitution of the residue in mobile phase for high-pressure liquid chromatography (HPLC) analysis. The disappearance of substrate was quantitated by reversed-phase HPLC. Fifty percent inhibitory (IC_{50}) values were calculated by the graphical method.

The CYP isoforms involved in the rat liver microsomal metabolism of saquinavir and indinavir were identified with isoform-specific immunoinhibitory antibodies. Anti-rat CYP3A1 antibodies (Human Biologics Inc., Phoenix, Ariz.) were combined with microsomal protein (antibody-to-protein ratio, 10:1) and incubated for 30 min at room temperature. The substrate and buffer were added, and the reaction was started by adding the NADPH-generating system. The incubation was conducted for 30 min at 37°C. Preimmune serum was substituted for antibodies in control incubations. The sample workup and analysis were as described above.

CYP binding studies. The synthesis of ritonavir analogs will be described elsewhere. Details of the syntheses can be obtained from D. J. Kempf. All synthetic analogs were characterized by nuclear magnetic resonance and mass spectral analysis and were shown to be of ≥95% purity. Binding spectrum studies were carried out with human liver microsomes; a model UV-2101 PC UV/visible scanning spectrophotometer (Shimadzu Scientific Instruments Inc., Wood Dale, Ill.) was used. Washed microsomes were diluted with 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA, so that the final concentration of CYP was 1.0 nM (1.53 mg of protein/ml). Compounds (1 to 5 μM) were added singly in spectral grade dimethyl sulfoxide (DMSO; 1.0% [vol/vol], and difference spectra (350 to 500 nm) were recorded under aerobic conditions at room temperature (27). The magnitude of the spectral perturbation was calculated as ΔA/nanomole of CYP, where ΔA equals the absorbance at the peak wavelength minus the absorbance at the minimum wavelength.

Inhibition of terfenadine hydroxylase activity. The effect of ritonavir and its analogs on CYP3A-dependent terfenadine hydroxylase activity was determined as previously described (28). Briefly, the assay mixture (0.5 ml) consisted of the following at the indicated final concentrations: 0.1 M potassium phosphate buffer (pH 7.4), EDTA (0.1 mM), $MgCl_2$ (15 mM), [^{3}H]terfenadine (10 μM, $\sim K_m$), and 1 mg of liver microsomal protein per ml. All compounds were dissolved in ethanol and diluted into the assay mixture so that the proportion of ethanol never exceeded 1.0% (vol/vol). After a 3-min equilibration period, the reaction was initiated with a NADPH-generating system containing the following: $NADP^+$ (4.0 mM), glucose 6-phosphate (10 mM), and glucose 6-phosphate dehydrogenase (2.0 U/ml). At the appropriate time, the reaction was terminated with an equal volume of chilled methanol. Thereafter, the samples were allowed to stand on ice for 5 min. After the incubates were centrifuged (16,000 × *g*, 10 min), an aliquot of the supernatant was directly analyzed by radio-HPLC. Where applicable, the IC_{50} for inhibition of hydroxylase activity was obtained from a plot of percentage activity remaining (relative to control) versus $\log_{10}$ drug concentration (1 to 10 μM). Assays run in the presence of ethanol alone served as controls.

Pharmacokinetic analysis. All protease inhibitors were formulated as 5-mg/ml solutions in a mixture of ethanol-propylene glycol-D5W with appropriate equivalents of methanesulfonic acid. Sprague-Dawley-derived rats (male; 0.25 to 0.35 kg; $n = 2$ to 6) and beagle dogs (male and female; 8 to 12 kg; $n = 3$ to 6) received 10- and a 5-mg/kg of body weight doses, respectively, by oral gavage with and without an equal ritonavir dose. Plasma samples, obtained as a function of time after dosing (rat, 10 time points over 8 h; dog, 12 time points over 12 h), were extracted into mixtures of ethyl acetate and hexane, concentrated, and analyzed by reversed-phase HPLC with an internal standard (21a). The plasma drug concentration of each sample was calculated by a least-squares linear regression analysis (unweighted) of the peak area ratio (parent/internal standard) of the spiked plasma standards versus concentration. C_{max} and the time to reach C_{max} (T_{max}) were read directly from the observed plasma concentration versus time data. The plasma elimination half-life was estimated from the log-linear regression of the terminal plasma concentrations as a function of time after dosing. The area under the plasma concentration versus time curve was calculated by using the linear trapezoidal rule over a single-hour dosing interval. In a two-way crossover study, healthy human volunteers (male and female; fasted; $n = 8$) were given three 200-mg capsules of saquinavir with or without two 100-mg capsules of the semisolid formulation of ritonavir.

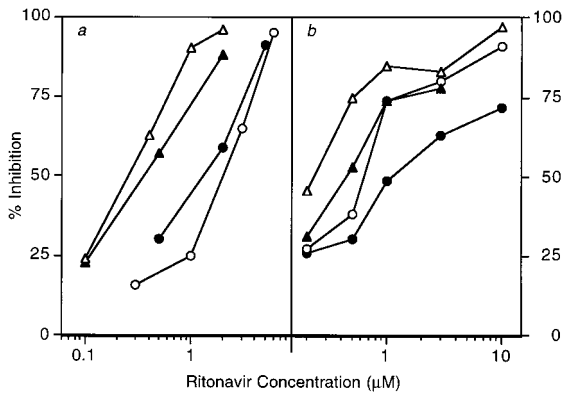


FIG. 1. Inhibition of the oxidative metabolism of HIV protease inhibitors by ritonavir in rat and human liver microsomes. (a) Saquinavir (human, open triangles; rat, solid triangles) and indinavir (human, open circles; rat, solid circles); (b) nelfinavir (human, open circles; rat, solid circles) and VX-478 (human, open triangles; rat, solid triangles).

RESULTS

Inhibition of the CYP-mediated metabolism of HIV protease inhibitors by ritonavir. In order to understand and predict the pharmacokinetic interactions between ritonavir and the other peptidomimetic HIV protease inhibitors under clinical investigation, we examined the in vitro metabolism of saquinavir (Ro 31-8959) (26), indinavir (L-735,524) (31), nelfinavir (AG 1343) (13), and VX-478 (18) in the presence of ritonavir. Each agent was coincubated with various concentrations of ritonavir in human liver microsomal preparations (20). In vitro metabolism rates for both ritonavir and the protease inhibitor substrates were measured by monitoring the disappearance of parent. Ritonavir potently inhibited the metabolism of saquinavir and indinavir in human liver microsomes, with IC_{50} values of 0.25 and 2.2 μM, respectively (Fig. 1a). By comparison, the IC_{50} of the known CYP3A inhibitor ketoconazole (17), which also inhibited the metabolism of saquinavir, was 1.8 μM. In a similar manner, the in vitro metabolism of nelfinavir and VX-478 was inhibited by ritonavir (IC_{50} = 0.62 and 0.10 μM, respectively; Fig. 1b). In contrast, the rate of ritonavir metabolism was not affected by high concentrations (50 μM) of either saquinavir or indinavir. In order to confidently model human pharmacokinetic interactions between ritonavir and the above-described HIV protease inhibitors by using animal studies (see below), we also examined the rates of metabolism of those agents in rat liver microsomes in the presence of ritonavir (Fig. 1a and b). Potent inhibition of saquinavir, indinavir, nelfinavir, and VX-478 metabolism by ritonavir (IC_{50} = 0.42, 1.5, 1.05, and 0.40 μM, respectively) was noted. Immunoinhibitory anti-rat CYP3A1 antibodies, which cross-react with constitutive rat CYP3A2, inhibited 91 and 67% of saquinavir and indinavir male rat liver microsomal metabolism, respectively.

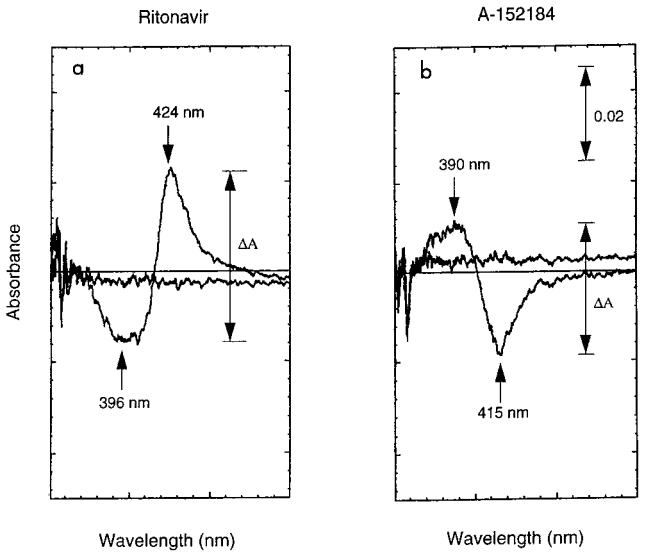


FIG. 2. Spectral perturbation elicited by ritonavir and A-152184 with human liver microsomes.

This indicated that both saquinavir and indinavir metabolism is mediated primarily by CYP3A2 (the major constitutive isoform of the CYP3A subfamily in male rat liver [23]) in rat liver microsomes.

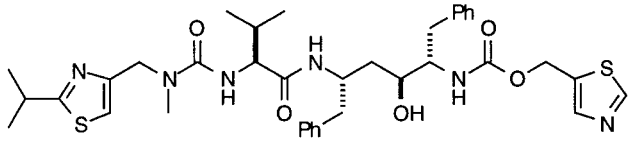
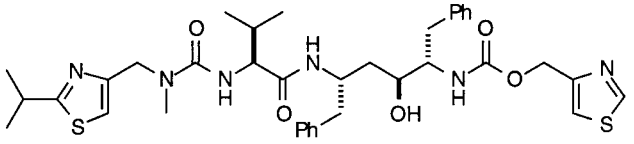
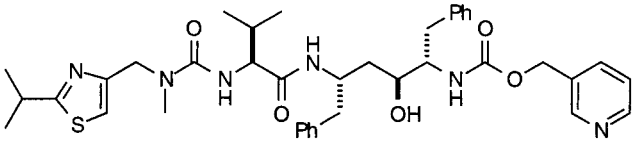
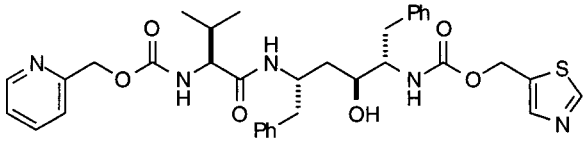
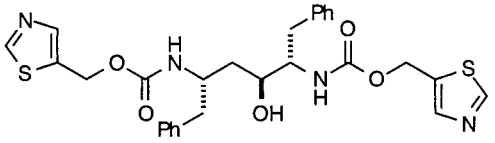
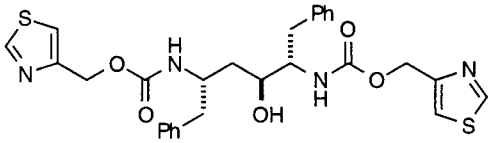
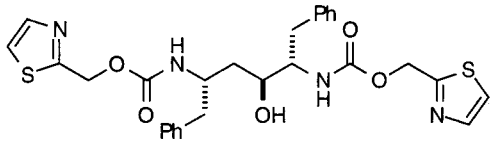
Characterization of CYP binding and inhibition by ritonavir. To understand the basis for the above-described inhibition of CYP-mediated metabolism, we examined the binding of CYP by ritonavir in a spectroscopic assay (27). Human liver microsomal preparations were treated with either a solution of ritonavir in DMSO or pure DMSO. Visible difference spectra were recorded from 350 to 500 nm (Fig. 2a). The presence of a broad trough (390 to 410 nm), with peak (λ_{max}) and minimum (λ_{min}) wavelengths at 424 nm and 395 nm, respectively, is indicative of a Type II spectral perturbation, suggesting a direct, reversible interaction of ritonavir with the oxidized (ferric) heme iron of CYP (27). To probe the structural requirements for CYP binding by ritonavir, we next screened the CYP binding of related analogs (Table 1). The isomeric compound A-152184, which differs from ritonavir only in the position of attachment on the unsubstituted thiazolyl group, elicited a Type I spectrum (λ_{max}, 386 nm; λ_{min}, 416 nm; Fig. 2b), indicating a lack of direct heme binding. The extent of binding to CYP was estimated from the difference spectra by the magnitude of ΔA per nanomole of CYP. By this measure, ritonavir was determined to bind ~40% more extensively to CYP than the regioisomeric analog A-152184. In contrast to A-152184, the pyridyl analogs A-81525 and A-83962 displayed Type II binding in magnitudes similar to that of ritonavir (Table 1). Inhibition of terfenadine hydroxylase (CYP3A) activity in liver microsomes by ritonavir and its structural analogs was also examined. The IC_{50} value for ritonavir (0.38 μM) was significantly (4- to 10-fold) lower than those of the three analogs. In a similar manner, three truncated compounds were examined for CYP binding and inhibition (Table 1). Although each exhibited Type II (heme) binding, the magnitude of binding by the 5-thiazolyl isomer A-81272 was substantially higher than those of the 4-thiazolyl (A-80969) and 2-thiazolyl (A-80931) isomers. Furthermore, of the three truncated structures, only A-81272 inhibited terfenadine hydroxylation, and at a sixfold higher concentration than that of ritonavir.

Pharmacokinetic enhancement of HIV protease inhibitors by ritonavir coadministration. The above results indicate that the CYP-mediated metabolism of HIV protease inhibitors is potently inhibited by ritonavir in both rat and human liver microsomes. We next investigated the pharmacokinetic interaction between ritonavir and six peptidomimetic protease inhibitors (saquinavir, indinavir, nelfinavir, VX-478, A-80987 [15], and A-77003 [16]) in rats. Each agent was administered orally to rats at 10 mg/kg, either as a single agent or in combination with 10 mg of ritonavir/kg. Plasma concentrations (0 to 8 h) are shown in Fig. 3, and pharmacokinetic parameters are provided in Table 2. Within experimental error, the levels of ritonavir were not affected by coadministration with other agents (data not shown). In contrast, the levels of saquinavir, indinavir, nelfinavir, VX-478, and A-80987 in plasma were enhanced by coadministration with ritonavir to concentrations which equaled or exceeded that of ritonavir itself. High levels of each inhibitor in plasma were sustained, so that values for the area under the 0- to 8-h plasma concentration curve (AUC) were enhanced by 8- to 36-fold (Table 2). The highest C_{max} and AUC values were observed with A-80987, which is structurally the most similar to ritonavir. T_{max} was extended by 4- to 14-fold. When the agents were administered alone, their levels were unquantifiable (<0.01 μg/ml) by ≤6 h. In contrast, coadministration with ritonavir provided concentrations of these HIV protease inhibitors in plasma that were substantially in excess of their respective in vitro anti-HIV 50% effective concentration values for >8 h. Only A-77003, a compound with exceedingly high clearance in humans (25), did not display comparable levels despite a 46-fold AUC enhancement by ritonavir.

In preparation for human interaction studies, we further characterized the interaction of ritonavir and saquinavir by coadministering various doses in rats (Table 3). At a 10-mg/kg codose of ritonavir, 1-, 5-, and 10-mg/kg doses of saquinavir produced a dose-dependent increase in the 0- to 8-h AUC of saquinavir. The levels of ritonavir in plasma were unchanged except for a slight elevation at the highest (10-mg/kg) dose of saquinavir. At a constant dose of saquinavir (10 mg/kg), 1- and 0.1-mg/kg doses of ritonavir produced a significant (4- and 2.6-fold, respectively) enhancement of saquinavir exposure even though the concentrations of ritonavir in plasma were below the limit of quantitation (0.01 μg/ml). Interestingly, a 5-mg/kg dose of saquinavir with either a 5- or 10-mg/kg ritonavir dose provided similar levels of saquinavir in spite of higher ritonavir exposure with the higher dose. This lack of proportionality is presumably a result of the first-pass metabolism of saquinavir being largely saturated in the presence of ritonavir doses that are >5 mg/kg. Finally, we administered ritonavir and saquinavir separately and together (5 mg/kg each) to dogs (Table 2). In a manner comparable to that observed in rat, the C_{max} and 0- to 12-h AUC of saquinavir were enhanced by 6- and 38-fold, respectively, by codosing with ritonavir. Although not determined, it is presumed that the locus of the interaction is canine CYP3A.

Single-dose interaction of ritonavir and saquinavir in humans. Plasma saquinavir concentrations were similarly elevated by ritonavir coadministration in humans. In a two-way crossover study of healthy volunteers (n = 8), a single 600-mg dose of saquinavir was administered either alone or in combination with a single 200-mg dose of ritonavir. Neither regimen produced any evidence of toxicity. Plasma concentrations from typical subjects (one male and one female) are shown in Fig. 4. When saquinavir was given alone, its maximal concentration in both subjects reached only ca. 0.1 μg/ml. In contrast, coadministration with ritonavir produced a C_{max} for saquinavir of ca. 3

TABLE 1. CYP binding and inhibition by analogs of ritonavir

Ritonavir or related analog	Structure	CYP binding[a] (ΔA/nmol CYP)	IC_{50} (μM)[b]
Ritonavir		0.033	0.38
A-152184		0.024	3.0
A-83962		0.032	1.5
A-81525		0.033	3.8
A-81272		0.045	2.3
A-80969		0.008	>7.5
A-80931		0.008	>7.5

[a] Spectra were recorded at a final inhibitor concentration of 5 μM. Except for A-152184, all compounds yielded a Type II spectrum (λ_{max}, ~424 nm; λ_{min}, ~395 nm). A-152184 elicited a Type I spectrum (λ_{max}, ~386 nm; λ_{min}, ~416 nm). ΔA is a measure of the extent of binding to CYP.
[b] Inhibition of terfenadine hydroxylase (CYP3A) activity in human liver microsomes.

μg/ml. Furthermore, circulating concentrations of saquinavir were detected in both subjects 40 h following a single combination dose. Ritonavir levels in the male subject tightly paralleled the saquinavir levels, while in the female subject, saquinavir concentrations were slightly higher than those of ritonavir. Comparison of the 0- to 40-h AUC values for saquinavir in the presence and absence of ritonavir indicated 63- and 83-fold enhancement for the male and female subjects, respectively, by the 200-mg dose of ritonavir. Full details will be reported elsewhere.

DISCUSSION

Chemotherapeutic regimens containing HIV protease inhibitors have produced unprecedented effects on surrogate markers (plasma viral RNA and CD4 cells) in HIV-infected individuals (8, 9). Clinical benefit (reduction in death and progression to AIDS) has been demonstrated in late-stage patients with ritonavir therapy (1). Nonetheless, the initial effect of monotherapy with protease inhibitors (4, 21) wanes in many patients due to the emergence of strains with reduced susceptibility (3, 12, 22). Recent evidence suggests that resistance development is a consequence of low-level residual viral replication during periods of time when drug levels are not completely suppressive (22). Combination therapy with a protease inhibitor and nucleoside analogs can provide greater viral suppression and a more prolonged effect on surrogate markers (8). Dual-protease-inhibitor regimens with agents of divergent resistance profiles offer an alternate strategy for increasing the benefit of these agents. The results presented here suggest that the elevation and prolongation of the levels of other protease inhibitors in plasma by ritonavir coadministration may produce a composite suppression of viral replication in excess of the sum of that observed with either agent individually. Furthermore, maintenance of high concentrations of those agents in

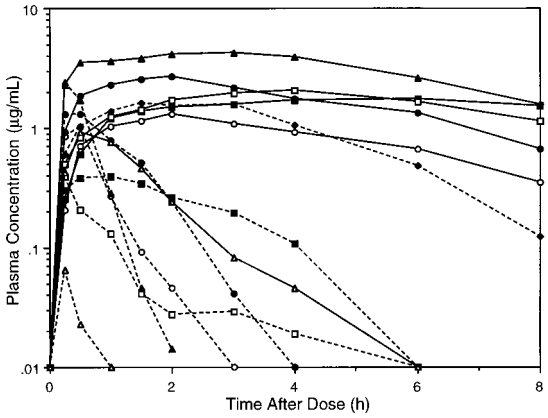


FIG. 3. Concentrations of HIV protease inhibitors in plasma following 10-mg/kg doses to rats, either as single agents or in combination with 10 mg of ritonavir per kg. Dashed lines, inhibitors given as single agents; solid lines, inhibitors coadministered with ritonavir. Open squares, saquinavir; open circles, indinavir; solid squares, nelfinavir; solid circles, VX-478; solid triangles, A-80987; open triangles, A-77003; solid diamonds, ritonavir.

combination with ritonavir in plasma may allow less frequent dosing, thereby increasing patient compliance (7).

We previously reported that ritonavir inhibits the metabolism of prototypic substrates of CYP3A (20). The current work demonstrates the potent in vitro inhibition of the metabolism of licensed and investigational HIV protease inhibitors by ritonavir. The degree of inhibition in rat liver microsomes was similar to that observed in human microsomes, suggesting that pharmacokinetic studies in rats might be useful in modeling human pharmacokinetic interactions between ritonavir and those agents. Coadministration of ritonavir with saquinavir, indinavir, nelfinavir, or VX-478 in rats produced a prolonged and substantial increase in the plasma levels of those inhibitors with little effect on the concentrations of ritonavir. The AUC values of each of the four inhibitors above, after codosing with ritonavir, were similar to or greater than that of ritonavir given separately (7.6 μg · h/ml). These results suggest that the bioavailabilities of many peptidomimetic HIV protease inhibitors are limited not by poor absorption but by efficient CYP-mediated metabolism in the intestine or liver. Coadministration with ritonavir may prove to be a powerful tool for the rapid determination of the cause of low oral bioavailability of a variety of classes of pharmaceutical agents. Furthermore, screening for selective CYP inhibition may provide a new approach to the design and development of peptidomimetics as oral therapeutics. Finally, coadministration with ritonavir might be useful for enhancing the levels in plasma (17) of rare or costly agents which are cleared predominantly via CYP3A, e.g., cyclosporine (19) and FK-506 (32).

As part of our studies, we investigated the structural features of ritonavir responsible for CYP binding and inhibition. Differences in the binding spectra of ritonavir and the regioisomeric compound A-152184 strongly suggest that the nitrogen atom within the unsubstituted 5-thiazolyl group of ritonavir binds directly to the CYP heme iron. In A-152184, that interaction is prohibited by steric hindrance; therefore, A-152184 binds to CYP only via nonheme (presumably mostly hydrophobic) interactions and inhibits CYP-mediated oxidation at 10-fold-higher concentrations. Both pyridyl-containing inhibitors (A-83962 and A-81525) exhibited direct heme binding equivalent to that of ritonavir. However, each was significantly less efficient at inhibiting CYP terfenadine hydroxylase activity than ritonavir, presumably due to greater lability toward chemical oxidation (14). Among the three truncated analogs, only A-81272, with unhindered thiazolyl nitrogen atoms, displayed significant CYP binding or inhibition. The IC_{50} of A-81272 was higher than that of ritonavir in spite of a more pronounced heme interaction, perhaps a consequence of reduced hydrophobic binding by the smaller inhibitor. Taken together, these results suggest that several structural features combine to confer high CYP-inhibitory potency upon ritonavir, including direct heme interaction via an unhindered electron-rich atom, extensive hydrophobic interactions with the CYP-active site, and stability toward CYP-mediated oxidative chemistry.

In rats, coadministration of even a small dose (0.1 mg/kg) of ritonavir significantly elevated plasma saquinavir levels. The >50-fold enhancement of saquinavir by a 600-mg ritonavir codose in humans is consistent with the powerful pharmacokinetic interaction in rats and dogs. Since the metabolism of indinavir, nelfinavir, and VX-478 is also inhibited in vitro by ritonavir and since plasma ritonavir levels in patients typically exceed 7 μM (4, 21), enhancement of the clinical levels of those agents in combination with ritonavir can be anticipated.

TABLE 2. Pharmacokinetics of HIV protease inhibitors upon coadministration with ritonavir[a]

Compound	Single agent			Coadministration				C_{max} increase (fold)	AUC increase (fold)
	C_{max} (μg/ml)	T_{max} (h)	AUC (μg · h/ml)[b]	C_{max} (μg/ml)	T_{max} (h)	C_{8h}[c] (μg/ml)	AUC (μg · h/ml)[b]		
Rats (10 mg/kg)									
Ritonavir	1.89 (0.28)	2.0	7.75 (0.58)						
Saquinavir	0.39 (0.11)	0.25	0.35 (0.04)	1.83 (0.21)	3.6	0.91 (0.17)	10.93 (1.34)	4.7	36
Indinavir	1.03 (0.38)	0.50	0.81 (0.31)	1.40 (0.24)	3.0	0.35 (0.11)	6.51 (0.84)	1.4	8
Nelfinavir	0.40 (0.17)	0.75	1.14 (0.43)	1.81 (0.19)	4.0	1.52 (0.23)	11.92 (1.00)	4.5	10
VX-478	1.61 (0.44)	0.42	1.69 (0.25)	2.88 (0.32)	1.5	0.68 (0.31)	13.50 (5.74)	1.8	8
A-80987	2.42 (0.15)	0.25	1.45 (0.48)	4.47 (0.23)	1.7	1.57 (0.23)	25.74 (2.26)	1.8	18
A-77003	0.07 (0.03)	0.25	0.03 (0.02)	0.96 (0.14)	0.67	0.00 (0.00)	1.39 (0.18)	13.7	46
Dogs (5 mg/kg)									
Ritonavir	3.58 (0.72)	1.0	8.06 (1.93)						
Saquinavir	0.63 (0.38)	0.3	0.40 (0.21)	3.87 (1.50)	1.3	0.24 (0.09)[d]	15.37 (6.72)	6.1	38

[a] Values are means (standard errors [C_{max}, C_{8h}, and AUC]).
[b] 0- to 8-h AUC for rats; 0- to 12-h AUC for dogs.
[c] C_{8h}, concentration at 8 h.
[d] Concentration at 12 h.

TABLE 3. Pharmacokinetic interaction of ritonavir and saquinavir in rats[a]

Saquinavir dose (mg/kg)	Ritonavir dose (mg/kg)	Saquinavir			Ritonavir		
		C_{max} (μg/ml)	T_{max} (h)	AUC (μg · h/ml)[b]	C_{max} (μg/ml)	T_{max} (h)	AUC (μg · h/ml)[b]
10	0	0.39 (0.11)	0.25	0.35 (0.04)			
10	0.1	0.59 (0.10)	0.4	0.91 (0.14)	0	na[c]	0
10	1	0.70 (0.20)	0.6	1.41 (0.34)	0	na	0
10	10	1.83 (0.21)	3.6	10.93 (1.34)	2.33 (0.42)	2.9	11.06 (1.97)
5	5	1.04 (0.07)	1.3	4.31 (0.10)	0.86 (0.13)	1.3	2.23 (0.30)
5	10	0.88 (0.07)	2.1	4.68 (0.54)	1.95 (0.32)	2.8	8.57 (1.30)
1	10	0.23 (0.02)	1.5	1.15 (0.10)	1.84 (0.36)	2.3	8.11 (1.45)
0	10				1.89 (0.28)	2.0	7.75 (0.58)

[a] Values are means (standard errors [C_{max} and AUC]).
[b] 0- to 8-h AUC.
[c] na, not applicable.

Because of potentially substantial drug level increases, however, such combinations should be avoided until a safe regimen can be established. Inhibition of CYP3A and, to a lesser extent, CYP2D6 by ritonavir also affects the exposure of other concomitant medications (20). These interactions dictate that certain medications having narrow therapeutic indices or steep dose-response relationships be either restricted from coadministration (e.g., terfenadine, astemizole, and midazolam) or subject to dose reduction with careful monitoring of concentrations in plasma (e.g., tricyclic antidepressants, class 1C antiarrhythmics, neuroleptics, and immunosuppressants) to avoid potentially harmful drug-drug interactions. Furthermore, the long-term consequences of CYP3A inhibition are unknown. Enhancement of pharmacokinetics via CYP inhibition will therefore require the identification of any new risks which may accompany the benefits of improved efficacy.

In confirmation of the in vitro and animal studies presented above, we observed a pronounced and sustained elevation of the plasma saquinavir levels in humans after coadministration with ritonavir. Following a 600-mg dose of saquinavir in combination with 200 mg of ritonavir, concentrations of both drugs that were ≥1 μg/ml were maintained for 12 h, well in excess of the respective in vitro 50% effective concentration values (14, 26). The AUC of saquinavir (>20 μg · h/ml) in that dose group exceeded the steady-state AUC observed with saquinavir alone at 7,200 mg/day (29). That dose of saquinavir was associated with a reduced incidence of L90M and G48V mutations, which confer resistance to saquinavir, compared with that associated with lower doses (29). Similarly, clinical studies employing ritonavir monotherapy have demonstrated that higher trough levels are inversely related to the rate of emergence of resistance in vivo (22). Our results suggest that combination therapy with ritonavir and other protease inhibitors may produce greater clinical benefit and more durable suppression of resistant mutants through higher, sustained plasma drug levels. Dual-protease-inhibitor regimens with ritonavir and saquinavir may be particularly useful, since the mutation patterns that develop during monotherapy with each agent are distinct, and cross-resistance between the two drugs is limited (22). The complete results of single- and multiple-dose interaction studies will be presented separately. Studies of HIV-infected individuals are currently under way to establish a safe and efficacious combination regimen of ritonavir and saquinavir.

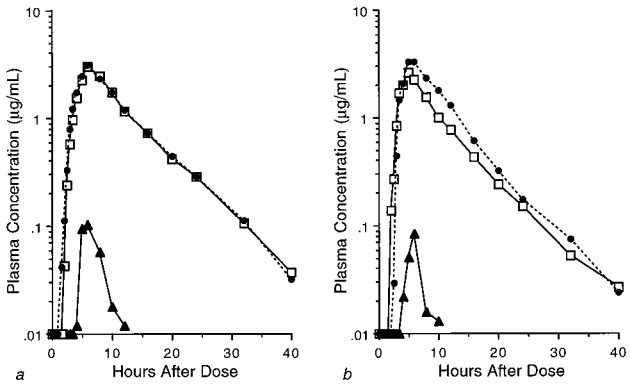


FIG. 4. Plasma drug concentrations in human volunteers following a single dose of saquinavir alone or saquinavir and ritonavir in combination. (a) Male subject; (b) female subject. Solid lines, saquinavir levels; dashed lines, ritonavir levels; filled triangles, saquinavir (600 mg) given alone; open squares, saquinavir (600 mg) given with 200 mg of ritonavir; solid circles, ritonavir (200 mg) given with 600 mg of saquinavir.

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