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DUAL PROTEASE INHIBITOR THERAPY IN HIV-INFECTED PATIENTS: Pharmacologic Rationale and Clinical Benefits

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■ **Abstract** HIV protease inhibitors, as components of combination antiretroviral drug regimens, have substantially reduced the morbidity and mortality associated with HIV infection. They selectively block the action of the virus-encoded protease and stop the virus from replicating. In general, these drugs have poor systemic bioavailability and must be dosed with respect to meals for optimal absorption. Protease inhibitor–containing regimens require ingestion of a large number of capsules, are costly, and produce or are susceptible to metabolic drug interactions. Simultaneous administration of two protease inhibitors takes advantage of beneficial pharmacokinetic interactions and may circumvent many of the drugs' undesirable pharmacologic properties. For example, ritonavir increases saquinavir concentrations at steady state by up to 30-fold, allowing reduction of saquinavir dose and dosing frequency. Ritonavir decreases the systemic clearance of indinavir and overcomes the deleterious effect of food on indinavir bioavailability. These benefits reflect inhibition of presystemic clearance and first-pass metabolism, as well as inhibition of systemic clearance mediated by hepatic cytochrome P450 3A4. Several dual protease inhibitor combination regimens have shown great promise in clinical trials and are now recommended as components of salvage therapy for HIV-infected patients.

HIV PROTEASE INHIBITORS

Peptidic inhibitors of the HIV-encoded protease have had a major impact on the AIDS epidemic, by increasing patient survival and decreasing disease progression (1). Unfortunately, these agents must be given in combination with other active antiretroviral drugs, and regimens associated with clinical benefit have a number of disadvantages and much room for improvement.

The history of the development of these drugs is instructive. The protease gene was recognized within the sequence of the first HIV-1 genome, published in 1985

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(2), prior to identification of a functional protein. The biological activity of this enzyme was elucidated the following year (3). The HIV protease is encoded in the 5′ end of the *pol* gene and is expressed as part of the *gag-pol* polyprotein. This gene encodes a 99–amino acid aspartyl protease, which functions as a homodimer and is typical of retroviral proteases (4). The enzyme targets HIV-specific amino acid sequences in the *gag* and *gag/pol* polyproteins whose cleavage is essential for the maturation of the nascent virion (5). *Gag* polyprotein cleavage by protease produces four smaller functional proteins (p17, p24, p9, p6), which contribute to virion structure and RNA packaging (6). Although mammalian cells contain a number of aspartyl proteases, none appear to efficiently cleave the *gag* polyprotein, and conversely the HIV-encoded protease is not known to cleave any host cell–encoded proteins (7).

HIV protease was crystallized and its structure resolved at the atomic level in 1987 by two groups working independently at Merck Laboratories and the National Cancer Institute (8, 9). The first reports of inhibitors of this enzyme appeared in 1988 (10); the X-ray crystal structure of HIV protease complexed with peptidic inhibitors was resolved at about the same time (11). Although these initial inhibitors were of low potency, the first selective and potent inhibitors appeared within a few months (12).

One of these compounds, saquinavir, became the first protease inhibitor approved for prescription use in the United States. Saquinavir entered phase I trials in 1992, and just 3 years later, in December of 1995, this drug received accelerated approval from the US Food and Drug Administration (FDA) for use in combination with antiretroviral nucleosides (13). This was one of the most rapidly developed and approved drugs in modern times. However, an even shorter timeline was completed just a few months later, in March of 1996, when ritonavir received full approval for the treatment of patients with advanced AIDS; phase I studies of this agent had begun in late 1993. Indinavir, whose clinical development paralleled that of ritonavir, received FDA approval a few weeks later. A fourth peptidic protease inhibitor, nelfinavir, was approved in 1997, and a fifth, amprenavir, was approved in 1999.

The currently approved HIV protease inhibitors are based on modifications of virus-specific substrate peptides, for example the phenylalanine-proline scissile bond at position 167–168 of the *gag-pol* precursor (1). These compounds contain three or more chiral centers, which must be preserved to retain activity (Figure 1). Available drugs are active against clinical and laboratory isolates of both HIV-1 and HIV-2, with in vitro IC_{50}s (the concentration required to inhibit virus production by 50%) ranging from 2 to 60 nM (1). Antiviral activity parallels the K_i (drug concentration required to reduce enzyme activity by 50%) for purified HIV-1 encoded protease enzyme, which ranges from 0.1 to 2.0 nM (1). These compounds are inactive or weakly active against other human aspartyl proteases, with K_is of $>$10,000 nM for human renin, pepsin, and gastricin, and have little or no toxicity in tissue culture cell lines (minimal toxic concentrations $>$10,000 nM) (1).

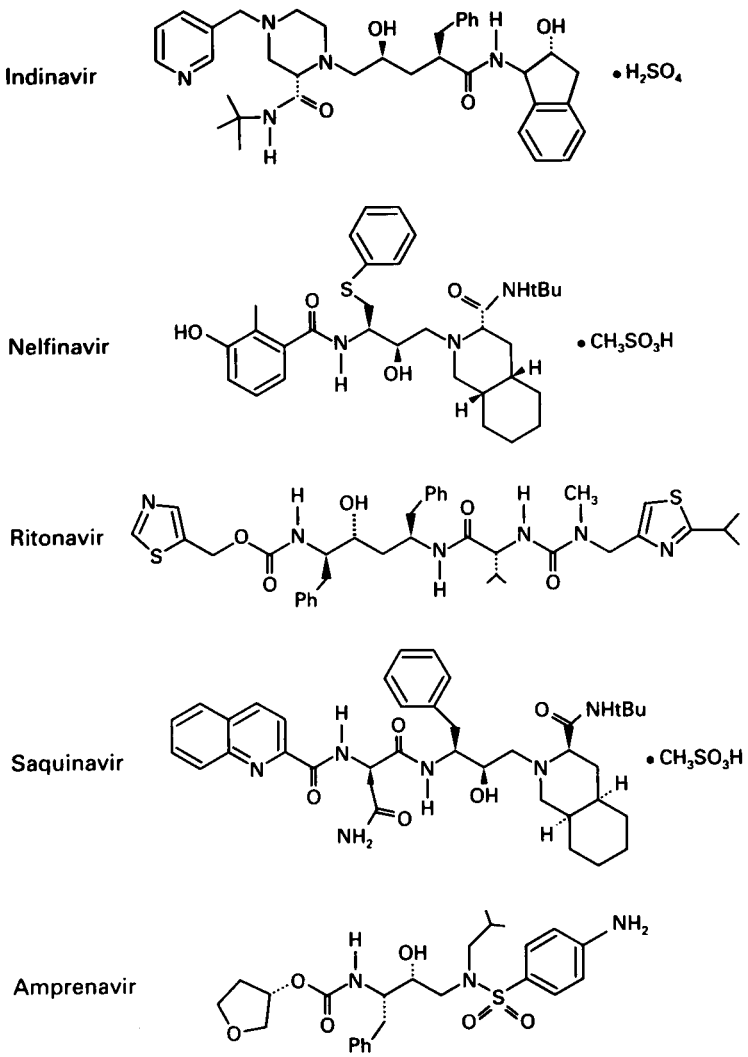

Figure 1 Structures of the five HIV protease inhibitors approved by the US Food and Drug Administration. (Reprinted from Reference 1, with permission.)

All approved HIV protease inhibitors cause a rapid and profound decline in plasma HIV viral loads in patients, as measured by quantitative polymerse chain reaction or branched-chain DNA assays of HIV RNA copies per milliliter of plasma. Protease inhibitor monotherapy produces a 100- to 1000-fold decrease in plasma HIV RNA, with peak effects 4–12 weeks after starting therapy (14, 15). In clinical trials, reductions in viral loads are paralleled by increases in CD4 lymphocyte counts, which average 100–150 cells/mm^3 (14–16). The addition of

other antiretroviral drugs has little effect on the magnitude of initial viral load decline, but it improves the durability of response by preventing drug resistance (1).

The magnitude and duration of suppression of viral loads are directly related to drug dose and dosing regimen. With indinavir, regimens employing <2400 mg per day of drug were associated with a rebound in viral loads within 3 months of starting monotherapy (17). With ritonavir, regimens of 300, 400, 500, or 600 mg every 12 h produced in the first weeks of therapy equivalent reductions in viral loads, but sustained reductions in plasma HIV and a sustained increase in CD4 count were associated only with the 600-mg/12-h regimen (14, 15). A similar dose-response has been reported for nelfinavir (18) and amprenavir (19).

In three large randomized and blinded clinical trials, HIV protease inhibitor therapy increased patient survival and decreased morbidity. In a randomized, double-blind, placebo-controlled clinical trial involving 1090 patients, ritonavir, added to existing nucleoside analog therapy in patients with baseline CD4 counts of $<100/mm^3$, produced a 53% reduction in disease progression or death compared with placebo, and a 43% reduction in mortality (20). In a randomized, placebo-controlled trial involving 978 patients, combination therapy with saquinavir and zalcitabine (ddC) produced a 40% reduction in all clinical endpoints (death or disease progression) and a 68% reduction in death compared with monotherapy arms (21). A three-drug regimen of indinavir plus zidovudine and lamivudine reduced clinical progression and death by 50% compared with the two-drug regimen of zidovudine and lamivudine, and it reduced mortality by 57% (22).

Although combination chemotherapy with protease inhibitors has had a major impact on the morbidity and mortality of HIV infection, these new drug regimens are associated with a number of problems. First, many patients cannot manage the large number of pills and strict dietary requirements and have difficulty adhering to the prescribed regimen. For this reason, in some settings, treatment failure with initial combination regimens may be as high as 50% (23).

The use of HIV protease inhibitors has been associated with a significant number of clinical toxicities. Prominent drug-specific toxicities include circumoral and peripheral parasthesias with ritonavir, and hyperbilirubinemia and nephrolithiasis with indinavir. Class-wide toxicities include nausea, vomiting, diarrhea (with all drugs except indinavir), glucose intolerance, elevated lipids, and fat redistribution (1).

The latter three side effects constitute a cluster of symptoms know as HIV lipodystrophy syndrome. The mechanism of these toxicities is poorly understood. Because HIV infection per se is associated with a number of metabolic abnormalities (24), this may be the consequence of reversing underlying problems mediated by the virus. Proposed drug-specific mechanisms include down-regulation of insulin receptor expression, inhibition of adipogenesis, accelerated lipolysis, and interference with retinoic acid receptor pathways (25). Because this syndrome has been reported in individuals taking classes of antiretrovirals other

than protease inhibitors (26), it could be induced by or associated with other classes of drugs.

Improving the convenience, tolerability, and cost of available regimens and promoting long-term adherence to effective regimens are priorities for clinical and preclinical drug development.

PHARMACOKINETIC PROPERTIES OF HIV PROTEASE INHIBITORS

The pharmacokinetic properties of five approved protease inhibitors are summarized in Table 1. All of these compounds are primarily metabolized by cytochrome P450 enzymes, four by the 3A4 isoform and one (nelfinavir) by 2C19 (1, 27). Data from in vitro studies suggest that both intestinal and hepatic enzymes contribute to metabolism of orally administered drugs; saquinavir is metabolized as extensively by CYP3A4 from human intestine as by the corresponding enzyme in liver (28). First-pass metabolism accounts for the limited oral bioavailability of several of these drugs. For example, saquinavir's fractional bioavailability ranges from $<$4% to $\sim$12% (see Table 1). Peak absorption for all these drugs occurs within 3 h of oral administration, and elimination half-lives range from 1.8 to 10 h.

Interindividual variability in pharmacokinetics is large, as indicated by a coefficient of variation for the mean area under the concentration-time curve (AUC) of $>$30% in all cases (Table 1). Several factors contribute to pharmacokinetic variability, including the effects of first-past metabolism and food (29). A high-fat meal substantially increases the bioavailability of saquinavir and nelfinavir but reduces the bioavailability of indinavir and amprenavir (Table 1). The same high-fat meal increases the bioavailability of ritonavir capsules but decreases the bioavailability of ritonavir liquid formulation (Table 1). It is currently recommended that nelfinavir and saquinavir be given with a moderate-fat meal, and that indinavir be given in the fasted state or with a light, low-fat snack. Amprenavir and ritonavir may be taken with or without food, but amprenavir should not be given with a high-fat meal.

All the approved HIV protease inhibitors are highly protein bound, with the exception of indinavir (Table 1). Amprenavir, nelfinavir, and ritonavir bind extensively to alpha$_1$-acid glycoprotein (AAG); the estimated association constant (K_a) for nelfinavir and saquinavir is close to 1 μM (30). The addition of physiologic concentrations of AAG increases the IC_{90} (that is, reduces the anti-HIV potency) of several peptidic protease inhibitors by a factor of 10 or more (31, 32). Binding affinities for albumin are generally much lower than for AAG, and this protein has less effect on drug activity in vitro (33).

Fractional penetration of HIV protease inhibitors into the central nervous system is low. However, this may be an artifact of protein binding. The reported

TABLE 1 Pharmacokinetics of approved HIV protease inhibitors[a]

Drug	Dose (mg)	% Bioavailability (approx. oral F)	Food effect (%)	C_{max} (μg/ml)	T_{max} (h)	$T_{1/2}$ (h)	Variability (CV%, AUC)	Protein binding (%)	V_d (L/kg)	CSF (%)	Clearance route	P450 induction	P450 inhibition
Amprenavir	1200 BID	NR	−21	5.4	1.9	7.1–10.6	63	90	6.1	2	Hepatic (75%) 3A4	No	Yes (3A4)
Indinavir	800 Q8H	60–65	−77	7.7	0.8	1.8	22–47	60–65	NR	2.2–76	Hepatic (88%–90%)	No	Yes (3A4)
Nelfinavir	750 TID	>78	+200–300	3.0–4.0	2.0–4.0	3.5–5.0	NR	>98	2.0–7.0	<1	Hepatic (>78%) 2C19	Yes	Yes (3A4)
Ritonavir	600 BID	66–75	−7/+15	11.2	2.0–4.0	3.0–5.0	30–36	98–99	0.4	1	Hepatic (>95%) 3A4	Yes	Yes (3A4>>2D6)
Saquinavir	600–1200 TID[b]	<4–12	+670	0.2	NR	NR	46–84	98	10.0	<1	Hepatic (>97%) 3A4	No	Yes (3A4)

[a]Published mean values and ranges from studies in adults without hepatic or renal dysfunction from References 1 and 77. AUC, Area under the concentration-time curve during an average dosing interval; BID, twice daily; C_{max}, maximal concentration during a dosing interval; CSF, cerebrospinal fluid; CV, coefficient of variation; F, bioavailability; L, liters; NR, not reported; P450, cytochrome P450 drug-metabolizing enzymes; Q8H, every 8 h; TID, thrice daily; T_{max}, time to maximal concentration; $T_{1/2}$ half-life of the principal elimination (β) phase; V_2, volume of distribution.
[b]Dose of saquinvir depends on formulation: 600 mg TID for the hard-gel and 1200 mg TID for the soft-gel formation.

ratio of cerebrospinal fluid to plasma concentrations is ≤1% for amprenavir, nelfinavir, ritonavir, and saquinavir, whereas the comparable ratio for indinavir is at least 12%. This roughly parallels the free fraction of each drug available in human plasma (Table 1). Whether this has a bearing on clinical drug activity is controversial; in most studies, reductions in HIV RNA in the plasma are accompanied by similar reductions in the cerebrospinal fluid (34).

METABOLIC DRUG INTERACTIONS AND HIV PROTEASE INHIBITORS

Because available peptidic protease inhibitors are all substrates for cytochrome P450 (CYP450) metabolism, they are susceptible to drug interactions involving P450 inhibitors or inducers (35). All five approved protease inhibitors can inhibit the metabolism of CYP450 3A at clinically achieved concentrations; ritonavir is also a weak inhibitor of CYP 2D6 (36). Nelfinavir and ritonavir are moderately potent inducers of hepatic drug metabolizing enzymes, including various CYP450 isoforms and glucuronyl transferases(1, 35).

Ritonavir is by far the most potent inhibitor of cytochrome P450. Although ritonavir's P450 inhibition has mixed competitive and noncompetitive features in vitro (36), it can be considered a reversible inhibitor in vivo because of the rapid turnover of these enzymes in the liver. Amprenavir, indinavir, and nelfinavir are less potent inhibitors (35), and saquinavir is the least potent (1). For example, the K_i for inhibition of terfenadine metabolism in vitro is 0.017 μM for ritonavir (36) but 0.7 μM for saquinavir (28), a 40-fold difference in potency.

Ritonavir induces its own metabolism; during the first 2 weeks of monotherapy using a fixed dose, steady state trough concentrations fall two- to threefold (37). Ritonavir and nelfinavir can accelerate the clearance of other metabolized drugs through enzyme induction. For example, concurrent nelfinavir reduces the zidovudine area under the concentration time curve (AUC) by 35%, and ritonavir by 25%, presumably as a consequence of induction of glucuronyl transferases (1). Nelfinavir decreases the ethinyl estradiol AUC by 47% and ritonavir by 40%; these protease inhibitors are contraindicated in women taking oral contraceptives that contain the combination of norethindrone and ethinyl estradiol (1).

The p-glycoprotein drug transporter (P-gp), which is the product of the multidrug resistance *mdr1* gene originally described in cells resistant to certain cancer chemotherapies, has recently been shown to play a role in the cellular transport of several antiretroviral drugs. HIV protease inhibitors were shown to be substrates (38, 39) and, in some cases, inhibitors of this transporter (40, 41). Transgenic mice deficient in P-gp had cerebrospinal fluid concentrations of these drugs up to 30-fold higher than those in control animals (42). However, the in vitro anti-HIV activity of indinavir, nelfinavir, saquinavir, and ritonavir was not affected by P-gp expression (43). Drug transport mediated by P-gp may represent

an additional pathway for drug interactions, especially those occurring in the intestinal tract. Selective P-gp blockade could reduce first-pass metabolism of drugs like saquinavir and could also be used to increase central nervous system penetration of these drugs.

DRUG RESISTANCE AND THE NEED FOR COMBINATION THERAPY

Drug resistance is the major obstacle to successful long-term suppression of HIV with protease inhibitor–containing regimens. Resistance is associated with specific, well-characterized mutations in the HIV protease gene (44). Resistance is a staged process wherein the virus acquires a single primary amino acid change that produces only a slight (generally less than fivefold) change in drug sensitivity. Thereafter, additional secondary mutations accumulate that confer ever-increasing resistance. Amino acid changes associated with primary resistance generally reside in the enzyme's catalytic site, whereas secondary mutations may be distant from the catalytic site. It is thought that many secondary mutations are compensatory, allowing improved proteolytic activity in the presence of primary active site mutations (44).

HIV protease tolerates a substantial amount of mutation, and catalysis is robust in the presence of altered amino acid sequences. One third or more of the 99 amino acids in the enzyme can deviate from wild-type consensus sequences without altering enzyme function (1, 44). Accumulation of protease inhibitor resistance mutations may reduce virulence in vitro and in animal models (45), but the clinical significance of this is debated.

Cross-resistance among peptidic protease inhibitors is substantial, especially once a virus acquires a number of secondary resistance mutations. Exposure to one protease inhibitor may select virus that is resistant to all other drugs in the class, even those the patient has not yet received. Indinavir monotherapy for a year, for example, can select virus resistant not only to indinavir, but also to all other approved and to several investigational protease inhibitors (46).

Once a patient develops resistant virus, that virus appears to be retained for long periods of time even after treatment stops. Patients with protease inhibitor–resistant virus who are taken off therapy may show initial response when that drug is reintroduced, but fail therapy within a few weeks with a rapid rebound in viral loads (47).

Risk of developing drug resistance is related quantitatively to plasma drug concentrations. Higher doses of drug produce higher plasma concentrations and are associated with greater duration of antiviral response and a decreased risk of genotypic or phenotypic resistance (1). Data from a study of ritonavir monotherapy suggest that the rate of accumulation of resistance mutations is inversely proportional to trough concentration of drug (C_{min}) during an average dosing

interval (48). Dosing regimens maintaining plasma drug concentrations above some resistance threshold might therefore suppress emergence of resistant virus.

The current recommended dosing regimens for amprenavir, nelfinavir, indinavir, and ritonavir produce plasma drug concentrations that are equal to or greater than the in vitro IC_{90} throughout an average dosing interval (1, 29). This seems a reasonable target, although the beneficial clinical activity of saquinavir in combination with zalcitabine came with a dosing regimen that produced drug concentrations far below the IC_{90} (21).

Noncompliance appears to play an important role in the development of drug resistance. Compliance monitoring in patients taking high-dose saquinavir suggests that an increased frequency of genotypic resistance is associated with sporadic drug-taking behavior (49). Under the assumptions of current models, combination therapy with three or more drugs should suppress resistance as long as the drugs are properly taken. These models assume that single mutations are common, but that any virus resistant to one drug in a regimen is suppressed by other drugs in the regimen. Selected, highly motivated patient populations can maintain suppression of HIV replication to below detectable limits for more than 3 years with indinavir-lamivudine-zidovudine triple therapy (50).

The most likely scenario for selecting resistant viruses, then, is one in which the patient does not have three or more active drugs present all of the time, because of either inadequate pharmacokinetics or inadequate adherence to the prescribed regimen. Noncompliance, then, becomes the major cause of treatment failure and resistance, as is the case with other chronic infectious diseases, such as tuberculosis (51). These models, as well as clinical experience, dictate that the best way to reduce the risk of resistance and treatment failure is to develop regimens that are simpler to take, better tolerated, and more forgiving of individual problems with drug absorption, drug metabolism, or schedule adherence.

RITONAVIR-SAQUINAVIR PHARMACOKINETIC INTERACTIONS

In April, 1995, investigators at Abbott Laboratories discovered that ritonavir was an extraordinarily potent inhibitor of the in vitro metabolism of saquinavir and other peptidic HIV protease inhibitors. In hepatic microsomes, ritonavir inhibited the metabolism of 3.8 μg/ml of saquinavir with an IC_{50} of 0.029 μg/ml (52). At the same time, saquinavir had no effect on the in vitro metabolism of ritonavir. Animal studies showed that 10 mg/kg of ritonavir increased the saquinavir AUC by up to 38-fold (53).

Further impetus to develop this combination includes the fact that the primary resistance mutations seen in patients treated with either of these drugs (Val→Phe at position 82 of the HIV protease for ritonavir; mutations at position 48 and 90 for saquinavir) do not overlap (44). This suggests that one drug may be used to

suppress the emergence of resistance to the other. A complete list of possible benefits from dual protease inhibitor regimens is provided in Table 2.

In a single-dose crossover study using healthy volunteers, ritonavir increased the saquinavir AUC by 50- to 132-fold and increased the saquinavir C_{max} by 23- to 35-fold (52) (Figure 2). For a fixed dose of ritonavir, saquinavir concentrations were proportional to saquinavir dose. However, when the saquinavir dose was held fixed, the relationship between ritonavir dose and saquinavir pharmacokinetics was nonlinear; saquinavir AUC increased in proportion to ritonavir AUC until the ritonavir AUC exceeded 100 μg-hr/ml, at which point the increase in saquinavir AUC became less than proportional (52). Saquinavir had a small but statistically significant effect on the ritonavir AUC (6.4% mean increase) in this study (52).

The authors point out that systemic clearance of saquinavir may be at least 10 times higher than hepatic blood flow. This could be attributed to its administration with food, or to significant prehepatic clearance via intestinal cytochrome P450 or P-glycoprotein. These results suggest that the poor oral bioavailability of saquinavir (1%–12%, depending on formulation and conditions) reflects extensive first-pass metabolism rather than poor absorption. The increase in saquinavir concentrations with ritonavir is the result of improved bioavailability, perhaps to as much as 100%, with little effect on postabsorptive systemic clearance. Estimates that ritonavir reduces saquinavir's first-pass metabolism by 33-fold (52) correspond remarkably well with the increase in saquinavir C_{max} seen in single-dose studies. The fact that the saquinavir AUC ratio, with or without ritonavir,

TABLE 2 Potential clinical advantages of dual protease inhibitor therapy[a]

Pharamacokinetic effects	Clinical consequences	Other potential benefits
Increased bioavailability	Reduced dose	Decreased pill burden
Decreased systemic clearance	Reduced cost of therapy	Decrease cost of therapy
Increased AUC	Increased antiretroviral activity	Improved convenience
Increased trough (C_{min})	Less likelihood of resistance	Dual agents lacking cross-resistance
Decreased peak (C_{max})	Reduced drug toxicity	Improved adherence
Reduced pharmacokinetic variability	More predictable drug concentrations	
Increased formation of active metabolites		
Decreased clearance of active metabolites		

[a]AUC, Area under the concentration-time curve; Cmax, peak concentration; Cmin, trough concentration.

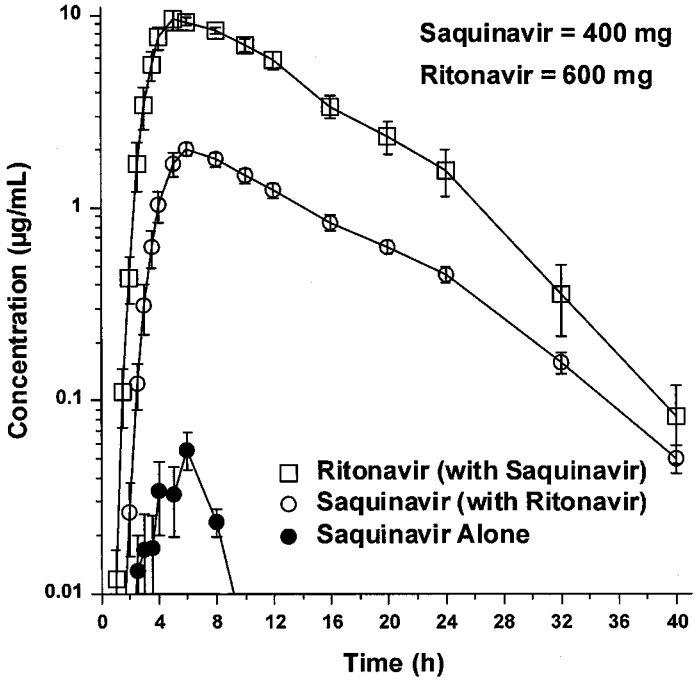

Figure 2 Impact of ritonavir on the pharmacokinetics of saquinavir. Shown are plasma concentration-time profiles in human subjects (mean + standard error of the mean) for oral saquinavir at 400 mg alone (*closed circles*), 400 mg of saquinavir plus 600 mg of ritonavir (*open circles*), and 600 mg of ritonavir alone (*open squares*). (Reprinted from Reference 52, with permission.)

was 50–400 suggests that the postabsorptive contribution of ritonavir (presumably due to inhibition of P450 3A4) was only a four- to fivefold further increase in the AUC.

Because a high-fat meal increases saquinavir plasma concentrations by three- to fourfold, and because these studies imply that saquinavir's poor bioavailability is a consequence of presystemic clearance rather than poor absorption, one must wonder how a fatty meal enhances saquinavir bioavailability. These data suggest that a high-fat meal may contain substances that (*a*) specifically interfere with saquinavir metabolism by CYP3A, (*b*) block intestinal drug transporters such as P-glycoprotein, or (*c*) do both.

In 1997, the manufacturer of saquinavir made available a new oral formulation that had a two- to threefold improvement in bioavailability. This decreases the relative pharmacokinetic benefit of ritonavir but does not alter the pharmacoki-

netic profile produced per dose of saquinavir. That is because the presystemic effect of ritonavir is the same regardless of formulation, making the apparent bioavailability of a given oral dose of saquinavir 100% whether its inherent bioavailability is 4% or 12%.

An added pharmacokinetic benefit of combining ritonavir with saquinavir is a reduction in intersubject variance. Ritonavir reduced the percent coefficient of variability for saquinavir pharmacokinetic parameters from about 70% to about 30% (52). Differential expression of intestinal CYP 3A contributes to high intersubject variability in the pharmacokinetics of drugs like saquinavir that undergo extensive first-pass metabolism (54). Eliminating this pathway as a significant contributor to saquinavir clearance would be expected to reduce pharmacokinetic variance, as is the case. This makes drug concentrations more predictable in the clinical setting.

Other known inhibitors of cytochrome P450 3A4 increase the steady state AUC of saquinavir by no more than fivefold, and inhibitors of intestinal cytochrome P450, such as grapefruit juice, increase the saquinavir AUC by no more than threefold (1, 52). Ritonavir does not affect the pharmacokinetics of other P450 substrates, even those with extensive first-pass metabolism—increasing the AUC by no more than fivefold—to nearly the same extent as it affects saquinavir (1). This suggests a unique chemical specificity for the interaction between ritonavir and saquinavir. It is likely that ritonavir inhibits intestinal P450 3A4, and recent data suggest that ritonavir may also be a potent inhibitor of P-glycoprotein (40, 41). Selective interaction with one or both of these pathways may account for the surprising magnitude of ritonavir's effect on saquinavir oral bioavailability.

Because ritonavir is also a P450 inducer and undergoes autoinduction during the first 10–14 days of therapy (37), steady state concentrations of saquinavir should be lower when these two drugs are combined. Multiple-dose pharmacokinetic interaction studies found that the steady state saquinavir AUC was increased only 20- to 30-fold (55). This is still a substantial increase, but lower than that seen in single-dose studies.

The current clinical recommendation is to combine 400 mg of ritonavir with 400 mg of saquinavir twice daily. Although ritonavir's approved dose is 600 mg twice daily (BID), the lower dose was chosen to account for the plateau in ritonavir's pharmacokinetic benefit with increasing doses, and to compensate for gastrointestinal toxicity seen with the 600-mg dose. This regimen has proven to be well tolerated and highly effective in long-term clinical trials.

An interesting question is whether lower doses of ritonavir will have as much pharmacokinetic benefit as the 400-mg dose. One single-dose study using healthy volunteers found that combining 200 mg of ritonavir with 600 mg of saquinavir increased the saquinavir AUC by an average of 74-fold (56), an effect similar to that seen with 400 mg of ritonavir. Combining 100 mg of ritonavir with 600 mg of saquinavir increased the saquinavir AUC by an average of nearly 30-fold (56). Thus, lower doses of ritonavir may provide as much, or nearly as much, pharmacokinetic benefit.

RITONAVIR-INDINAVIR PHARMACOKINETIC INTERACTIONS

The magnitude of the pharmacokinetic interaction between ritonavir and indinavir is not as great as that seen with ritonavir and saquinavir. The estimated K_i for inhibition of indinavir metabolism in human hepatic microsomes is 0.085 μg/ml (57). When rats were given a single dose of 10 mg of each drug per kg, ritonavir increased the indinavir AUC by eightfold (53). Still, there are several features of indinavir pharmacokinetics that would benefit from ritonavir coadministration. These include indinavir's rapid hepatic metabolism with a half-life of 1.8 h, a dosing regimen of every 8 h, food restrictions, hydration requirements, and large interindividual pharmacokinetic variability (see Table 1).

In a steady-state pharmacokinetic interaction study using healthy volunteers on ritonavir for 14 days, the combination of 200 or 400 mg of ritonavir with 400 or 600 mg of indinavir increased the indinavir AUC by three- to sixfold compared with 800 mg of indinavir alone (57). Ritonavir increased the indinavir C_{max} up to twofold and increased the indinavir concentration 8 h after dosing by 11- to 33-fold (see Figure 3). The estimated K_i for inhibition of indinavir metabolism in vivo, 0.10 μg/ml, was very close to the in vitro K_i in human hepatic microsomes (57). Because ritonavir is a P450 inducer, and baseline indinavir pharmacokinetics were assessed under noninduced conditions, the actual magnitude of metabolic inhibition would be underestimated under these circumstances. In this study indinavir did not appear to have a significant effect on ritonavir pharmacokinetics compared with historical control subjects (57).

Ritonavir coadministration significantly reduced the pharmacokinetic variability of indinavir. The coefficient of variation for indinavir AUC fell from 30% to 16%, and for C_{min} (concentration after 8 h) from 50% to 39% (57).

Unlike saquinavir, the oral bioavailability of indinavir is at least 60%. The estimated contribution of intestinal CYP450 3A4 to indinavir metabolism is less than 4% (57). Therefore, the pharmacokinetic benefit of ritonavir should be due mainly to decreased systemic clearance rather than to increased bioavailability. Ritonavir decreased the postabsorptive clearance of indinavir by at least two- to threefold compared with baseline, noninduced kinetics (57). This suggests that inhibition of hepatic CYP 3A4 is the main source for pharmacokinetic enhancement of indinavir by ritonavir, with reduced first-pass metabolism making a minor contribution.

For a fixed indinavir dose, increasing ritonavir from 200 to 400 mg produced relatively little increase in the indinavir AUC (57). This could be due to the increasing importance of clearance mechanisms other than CYP3A4 for indinavir as the ritonavir dose increases. As the ritonavir AUC increased, indinavir clearance asymptotically approached the non-CYP3A4 clearance, which was thought to represent the combined contributions of renal clearance, glucuronidation, and

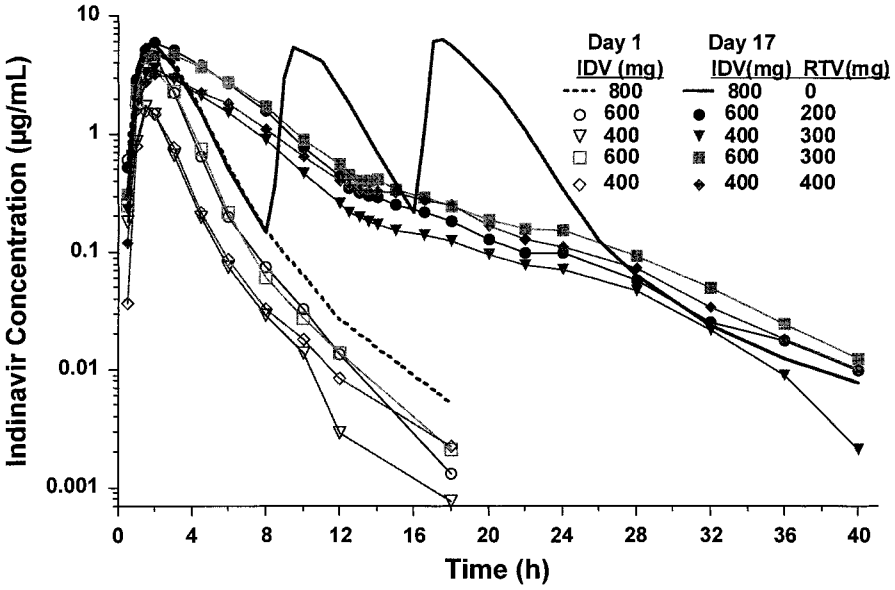

Figure 3 Impact of ritonavir (RTV) on the pharmacokinetics of indinavir (IDV). Shown are mean plasma concentration-time profiles in human subjects at day 1 for oral indinavir alone at a dose of 800 mg (*dashed line*), 600 mg (*open circles* and *open squares*), and 400 mg (*open triangles* and *open diamonds*), and at day 17 for 800 mg of indinavir alone (*solid line*), 600 mg of indinavir plus 200 mg of ritonavir (*closed circles*), 400 mg of indinavir plus 300 mg of ritonavir (*closed triangles*), 600 mg of indinavir plus 300 mg of ritonavir (*closed squares*), and 400 mg of indinavir plus 400 mg of ritonavir (*closed diamonds*). (Reprinted from Reference 57, with permission.)

CYP isoforms other than 3A4. Of note, ritonavir might induce glucuronidation more effectively at higher doses.

The effect of decreasing ritonavir dose and increasing indinavir dose was the subject of a separate study (58). In healthy volunteers administered ritonavir for 14 days, the 24-h indinavir AUC with a regimen of 100 mg BID of ritonavir/800 mg BID of indinavir was fourfold higher than with 800 mg every 8 h of indinavir alone (see Figure 4). In the same study, the 24-h AUC of indinavir with a regimen of 400 mg BID of both ritonavir and indinavir was 40% lower than with the BID regimen of 100 mg of ritonavir/800 mg of indinavir and 55% lower than with the BID regimen of 200 mg of ritonavir/800 mg of indinavir (58). However, the mean 12-h trough concentrations of the 400/400 regimen and the 100/800 regimen were nearly the same (Figure 4).

In two studies, coadministration of ritonavir and indinavir abolished the effect of food on indinavir bioavailability. A high-fat meal reduces the bioavailability of oral indinavir by up to 85% (57). Doses of 100, 200, or 400 mg of ritonavir BID reversed the effect of a high- or low-fat meal on indinavir pharmacokinetics,

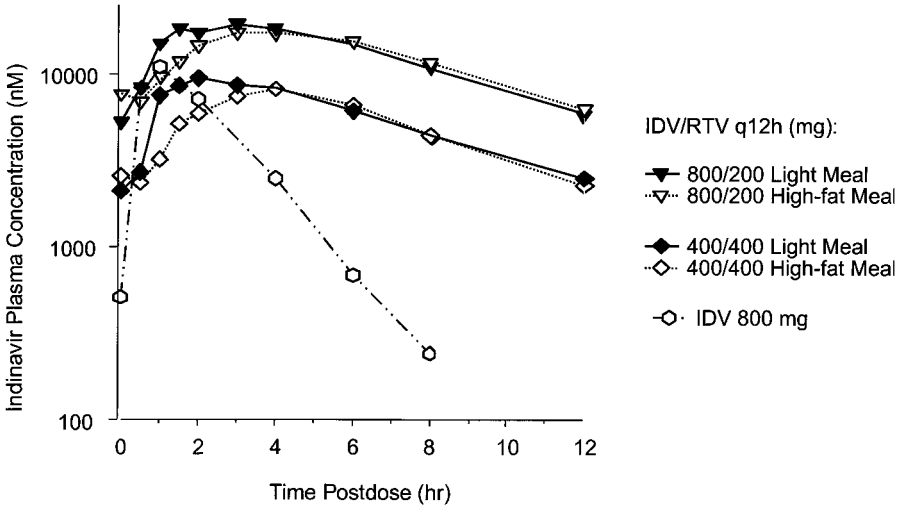

Figure 4 Effect of increasing ritonavir (RTV) dose with high- or low-fat meals on indinavir (IDV) pharmacokinetics. Shown are mean plasma concentration-time profiles from human subjects receiving oral indinavir alone at a dose of 800 mg fasting (*open hexagons*), 400 mg of indinavir plus 400 mg of ritonavir with a high-fat (*open diamonds*) or low-fat (*closed diamonds*) meal, and 800 mg of indinavir plus 200 mg of ritonavir with a high-fat (*open triangles*) or low-fat (*closed triangles*) meal. (Data taken from Reference 58; figure kindly provided by Al Saah, Merck Laboratories.)

compared with 800 mg of indinavir given in the fasted state (58, 59). Ritonavir should be enhancing indinavir oral bioavailability and pharmacokinetics through inhibition of cytochrome P450 and/or drug transporters such as P-glycoprotein. This finding suggests that the deleterious effects of food on indinavir may be mediated by interaction with intestinal epithelial drug transporters or P450 complexes, processes blocked by ritonavir.

PHARMACOKINETIC INTERACTIONS INVOLVING OTHER DUAL PROTEASE INHIBITOR COMBINATIONS

Ritonavir-Nelfinavir

Nelfinavir was originally marketed as a 750-mg thrice-daily (TID) regimen, and combination with ritonavir provided a way to reduce dose and dosing frequency. A single-dose drug interaction study using healthy volunteers showed that ritonavir increased the nelfinavir AUC by 152%, whereas nelfinavir increased ritonavir's AUC by only 9% (1). A steady state pharmacokinetic interaction study using HIV-infected volunteers evaluated the combination of 400 mg of ritonavir BID with 500 or 750 mg of nelfinavir BID. After 5 weeks of dosing, ritonavir

use was associated with a 162% median increase in the 24-h AUC after 500 mg of nelfinavir BID (dose normalized), and a 62% increase in the 24-h AUC after 750-mg BID, compared with historical control subjects taking only 750 mg of nelfinavir TID (60). At the same time, the median change in ritonavir's dose-normalized 24-h AUC was +3% with 500 mg of nelfinavir BID, and –21% with the 750-mg BID regimen (not statistically significant, see Figure 5).

This pharmacokinetic interaction is more complicated than others, because both drugs are CYP450 inducers as well as inhibitors. The fact that when the dose was increased from 500 to 750 mg BID, the AUC of nelfinavir did not increase significantly may reflect increased autoinduction with the higher dose. In addition, there was a trend for nelfinavir to reduce ritonavir's trough concentrations at the higher (750 mg) nelfinavir dose. This may have decreased the magnitude of ritonavir's beneficial impact on nelfinavir pharmacokinetics.

Nelfinavir is the only HIV protease inhibitor known to produce an active metabolite, the hydroxy-butylamide M8 (AG1402), which is the major metabolite of nelfinavir in humans and has equipotent anti-HIV activity in vitro (61). Ritonavir had a more significant impact on the pharmacokinetics of the M8 metabolite than on nelfinavir itself. After 5 weeks of dosing, ritonavir use was associated with a 430% median increase in the 24-h AUC of M8 in patients taking nelfinavir (500 mg BID), and a 370% increase in the 750-mg BID M8 AUC, compared with historical control subjects taking 750 mg of nelfinavir TID alone (60) (see Figure 5).

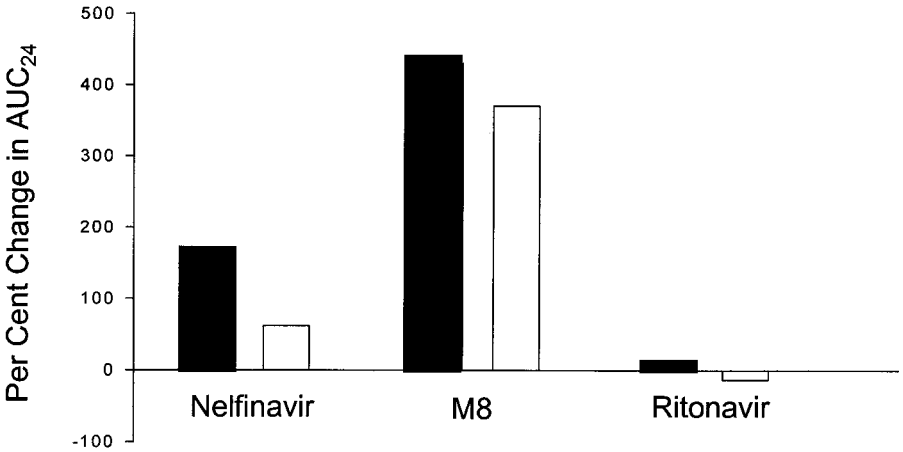

Figure 5 Pharmacokinetic interaction between nelfinavir and ritonavir. Shown is the median percent change in the 24-h AUC (area under the concentration-time curve) of nelfinavir, the nelfinavir hydroxy-butylamide metabolite M8 (AG1402), and ritonavir, normalized for drug dose in milligrams, after 5 weeks of ritonavir at a dose of 400 mg BID plus nelfinavir at 500 mg BID (*closed bars*) or 750 mg BID (*open bars*), compared with historical control subjects taking nelfinavir at 750 mg TID or ritonavir at 400 mg BID. (Data taken from Reference 60.)

M8 formation appears to be mediated mainly by CYP2C19 (27). Thus nelfinavir is the only currently approved HIV protease inhibitor whose major metabolite is not formed predominately by CYP3A4. M8 clearance, however, is mediated mainly by 3A4 (27). The discrepancy between ritonavir's effect on the pharmacokinetics of M8 and nelfinavir parent drug may reflect (*a*) induction of CYP2C19, thus increasing M8 formation, and (*b*) inhibition of CYP3A4, thus decreasing M8 clearance.

Ritonavir is an inducer of CYP2C19 activity and a potent inhibitor of 3A4, but it is a weak inhibitor of 2C19 in vitro (1, 36). Therefore, it is unlikely that the increase in nelfinavir's AUC produced by ritonavir is a consequence of inhibition of systemic clearance; this may, however, reflect improved oral bioavailability, perhaps through inhibition of P-glycoprotein, plus the inhibition of minor metabolic pathways. Alternatively, the M8 metabolite could be an inhibitor of 2C19.

Nelfinavir-Saquinavir

In single-dose pharmacokinetic interaction studies, nelfinavir increased the saquinavir AUC by up to fivefold, without affecting nelfinavir concentrations (1). However, nelfinavir is an inducer of CYP450 3A, and at steady state, the magnitude of this interaction was substantially reduced. Combining 750 mg TID of nelfinavir with 800 mg TID of the soft-gel formulation of saquinavir produced a saquinavir AUC equivalent to 1200 mg TID at steady state (62). This combination was well tolerated and was highly active against HIV in patients who were also taking two nucleoside analogs (63). However, this combination lacks many of the pharmacologic and clinical benefits of other dual protease inhibitor combinations.

Nelfinavir-Indinavir

Combining nelfinavir with indinavir produced a 50% increase in the indinavir AUC and an 80% increase in the nelfinavir AUC in single-dose studies using healthy volunteers (1). However, when these two drugs were administered to patients in a BID regimen, there was little pharmacokinetic enhancement and a disappointing anti-HIV effect, with only 10 of 21 patients suppressing their plasma HIV RNA to $<$400 copies/ml (the lower limit of quantification) after 32 weeks (64). Presumably hepatic enzyme induction by nelfinavir resulted in reduced concentrations of both drugs, and no real pharmacokinetic benefits.

Indinavir-Saquinavir

The combination of indinavir-saquinavir was reported to be antagonistic when used to inhibit HIV replication in vitro (65). Although the clinical relevance of this finding is unknown, this combination has not been pursued further in vivo, even though indinavir increased saquinavir concentrations by fivefold in single

dose studies (1). Theoretical disadvantages of dual protease inhibitor therapy (see Table 3) may discourage clinical development of some combinations.

IMPACT ON CLINICAL TREATMENT OF HIV

Ritonavir-Saquinavir

Dual protease inhibitor combinations have proven to be highly active in clinical trials. When given to antiretroviral-naïve patients as sole therapy, ritonavir plus saquinavir suppressed HIV viral loads to <400 copies/ml in most subjects after 48 weeks of treatment; overall dropout rates were 10%–15%, often due to elevated liver enzymes in subjects with preexisting hepatitis virus infections (66). Of subjects continuing on this regimen, some of whom added nucleoside analogs, 90% had viral loads suppressed to <400 copies/ml (the lower limit of quantification when this study was conducted) after 60 weeks of therapy (66). Success rates in treatment-experienced patients have not been as good (67–69), presumably because of cross-resistance from prior protease inhibitor use. However, adding ritonavir plus saquinavir to zidovudine-lamivudine therapy was associated with a durable suppression of HIV viral loads to <200 copies/ml in 10 of 16 patients taking these four drugs for 48 weeks (70). Further, 10 of 16 patients who had failed nelfinavir- or indinavir-containing regimens had viral loads suppressed to <400 copies/ml 24 weeks after switching to ritonavir plus saquinavir plus nucleoside analogs (71).

Whether ritonavir should be used as a pharmacokinetic crutch for saquinavir and other HIV protease inhibitors, or whether the drug is providing important virologic benefit in its own right, remains controversial. The pharmacokinetic benefits of lower ritonavir doses were nearly as good as those seen with the 400-mg BID regimen (56, 58). Further, a lower ritonavir dose (100 or 200 mg BID) is being used to enhance the pharmacokinetics of the investigational protease inhibitor ABT-378 (lopinavir) (72).

TABLE 3 Potential clinical disadvantages of dual protease inhibitor therapy

Increased number of agents in the regimen
Increased number of potential toxicities
Increased potential for pharmacokinetic drug interactions
Increased formation of toxic metabolites
Decreased clearance of toxic metabolites
Overlapping toxicities
Same viral target for both drugs
Cross-resistance between drugs
Pharmacologic antagonism between drugs

It is likely that 400 mg BID of ritonavir is providing virologic benefit when combined with saquinavir, because the long-term success of the 400/400 ritonavir-saquinavir BID regimen is much greater than that of high-dose saquinavir monotherapy with regimens producing similar AUCs (73). The virologic benefit of ritonavir doses lower than 400 mg BID is unknown and would have to be addressed in clinical trials. Therefore, if the patient's care provider decides that the antiretroviral regimen needs an additional active agent, the higher dose of ritonavir should probably be used. For example, a regimen of standard doses of zidovudine and lamivudine and 400 mg BID of ritonavir and saquinavir could be viewed as four active drugs; standard-dose zidovudine and lamivudine plus 400 mg BID of saquinavir and 100 mg BID of ritonavir could be viewed as three active drugs.

Ritonavir-Indinavir

Combining ritonavir with indinavir allows a significant reduction in indinavir dose. The 24-h AUC of indinavir administered as 400 mg BID with 400 mg BID of ritonavir is nearly the same as that of the standard indinavir dose of 800 mg given every 8 h (57). The reduced dosing frequency and reduced number of indinavir capsules make this regimen substantially more convenient. The indinavir trough concentration at the end of a 12-h interval with this regimen is actually higher than the trough with 800 mg of indinavir every 8 h (see Figures 3 and 4). This trough is 2.5-fold higher than the protein-corrected IC_{90} of indinavir, lengthening the duration of therapeutic coverage and possibly making the regimen more suppressive in patients who occasionally take their doses late (57).

This combination appears to be well tolerated and is highly active in the clinic. In one trial, 67 antiretroviral-naïve patients taking ritonavir and indinavir plus two nucleosides lowered mean plasma viral loads by 3.4 logs after 24 weeks of therapy, and 67% of these subjects had viral loads of $<$80 copies/ml (74).

The 400/400 ritonavir-indinavir regimen produced a lower C_{max} without affecting indinavir's renal clearance. Both of these factors could theoretically contribute to reduction of the risk for indinavir nephrolithiasis, which is thought to be both pH and concentration dependent (57, 75). Reduced indinavir peak concentrations should reduce the risk for formation of indinavir crystals in the urine, which presumably serve as the nidus for indinavir renal stones. Of 79 patients treated for a mean of 34 weeks with the 400/400 ritonavir-indinavir combination, none developed nephrolithiasis (76).

Other potential clinical benefits include elimination of the deleterious effect of food on indinavir bioavailability (58, 59), allowing the drug to be taken regardless of meals. It is also possible, though speculative, that ritonavir could reduce or eliminate the need for extra hydration with indinavir, because the C_{max} is substantially reduced (57).

One theoretical disadvantage of the ritonavir-indinavir combination is that the primary resistance mutations for these drugs (Val $\rightarrow$ Phe at position 82 of the HIV

protease) are shared (44). This could make these two agents more prone than other dual protease inhibitor combinations to select resistant mutants. Initial clinical studies in treatment-experienced patients have reported few early treatment failures (76), which suggests that this complication may be largely theoretical.

Amprenavir Combinations

Amprenavir, the most recently approved HIV protease inhibitor, has the longest elimination half-life of this drug class (7–10 h), but it has suboptimal oral bioavailability and must be dosed as eight large 150-mg capsules twice daily (77). Amprenavir is a modest P4503A4 inhibitor but is not a P450 inducer. In single-dose pharmacokinetic interaction studies, indinavir increased the amprenavir AUC by 33%, saquinavir decreased the AUC by 32%, and nelfinavir did not change the AUC compared with historical control subjects (77, 78). Amprenavir decreased the indinavir AUC by 38%, decreased the saquinavir AUC by 19%, and increased the nelfinavir AUC by 15% (77, 78).

Despite these modest pharmacokinetic interactions, combination studies were conducted with amprenavir at a dose of 800 mg TID and indinavir at 800 mg every 8 h, nelfinavir at 750 mg TID, or saquinavir at 800 mg TID. Although most patients achieved HIV viral loads $<$400 copies/ml at week 16, only 10 out of 17 achieved viral loads $<$20 copies/ml (79). Gastrointestinal toxicities such as diarrhea and nausea were common in this study.

An interesting recent observation is that ritonavir at a dose of 200 mg BID increased the amprenavir 12-h AUC up to threefold and increased the C_{min} about seven- to eightfold (S Piscitelli, personal communication). This should allow a significant reduction in the amprenavir dose (currently 1200 mg BID), but it should also make possible the exploration of once-daily dosing of amprenavir in combination with ritonavir. An additional problem with amprenavir occurs in combination with the nonnucleoside reverse transcriptase inhibitor efavirenz, which is a P450 inducer and diminishes amprenavir's AUC by up to 40% (77, 78). In the same study, low-dose ritonavir abolished the impact of efavirenz on amprenavir clearance, creating the possibility of a once-a-day antiretroviral combination including amprenavir and ritonavir and once-daily reverse transcriptase inhibitors such as efavirenz and didanosine.

Other Regimens

Other dual protease inhibitor regimens have been less widely studied, and in some cases have proven less useful, than the ritonavir-saquinavir and ritonavir-indinavir regimens. The combination of ritonavir at 400 mg BID with nelfinavir at 500 or 750 mg BID lowered viral loads by a mean of 2.8 and 2.2 logs, respectively, and increased CD4 cells counts by a mean of 236 and 120 cells/mm^3 after 48 weeks (80). However, 5 of 20 patients experienced virologic failure in this study, and all but one subject added nucleoside analogs to this regimen after 12 weeks. This regimen also produced moderate or severe diarrhea in 9 of 20 subjects.

IMPACT ON NEW DRUG DEVELOPMENT

Pharmacokinetic enhancement of one drug by another can improve the pharmacokinetic profile of investigational drugs in development. Clinical trials of ABT-378 (lopinavir), an investigational peptidic HIV protease inhibitor, have principally involved coadministration with ritonavir. Lopinavir is a peptidic analog of ritonavir with more potent anti-HIV activity in vitro (81). In drug interaction studies in hepatic microsomes and in laboratory animals, ritonavir greatly enhanced the pharmacokinetic profile of lopinavir, presumably by improving bioavailability and slowing systemic clearance. Ritonavir's effect on the lopinavir AUC was severalfold greater than ritonavir's effect on the saquinavir AUC in the same in vitro study (72, 81).

This beneficial pharmacokinetic interaction was confirmed in human volunteers; when dosed at 12-h intervals with ritonavir, mean trough concentrations of lopinavir were approximately 50-fold higher than the in vitro IC_{50} for HIV (72). In 101 HIV-infected patients taking lopinavir at 200 or 400 mg BID with ritonavir at 100 or 200 mg BID plus two nucleoside analogs for 24 weeks, HIV viral load was suppressed to $<$400 copies/ml in 93%–95% of patients and to $<$50 copies/ml in 89% (72). Mean CD4 cell counts increased by 160 cells/mm^3, a result comparable to that of other highly active antiretroviral combinations. Lopinavir-ritonavir was very well tolerated: No patients dropped out of this study because of toxicity, and mild adverse reactions were seen in only a small number of patients (72).

The availability of ritonavir to enhance the pharmacokinetic profile of lopinavir probably motivated the clinical development of this investigational protease inhibitor. A similar strategy could be employed for other promising investigational drugs in this class.

CONCLUSION

Combining drugs to take advantage of beneficial pharmacokinetic interactions dates back to coadministration of probenecid and penicillin. Additional examples include imipenem-cilastatin and cyclosporine-ketoconazole. These regimens use an inhibitor of drug clearance to allow reduced dose and reduced dosing frequency, with substantial improvement in cost and convenience for the patient. Dual protease inhibitor regimens are unique in that both drugs are active for the disease being treated and both attack the same pharmacologic target. The magnitude of the pharmacokinetic interaction between ritonavir and saquinavir is one of the largest ever described in human subjects. Combining low doses of ritonavir with ABT-378 (lopinavir) takes advantage of a similar interaction to develop a novel antiretroviral regimen. Dual protease inhibitor combinations with a lesser pharmacokinetic impact have been developed to improve concentration-time pro-

files and reduce the risk of treatment failure. Several of these regimens are now recommended as part of salvage therapy for HIV-infected patients.

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