Clinical pharmacology of HIV protease inhibitors: focus on saquinavir, indinavir, and ritonavir

• R.M.W. Hoetelmans, P.L. Meenhorst, J.W. Mulder, D.M. Burger, C.H.W. Koks and J.H. Beijnen

Introduction

The Acquired Immune Deficiency Syndrome (AIDS) as a disease was first noted in 1981 when the Center for Disease Control reported rare forms of pneumonia and skin cancer in young gay men in California and New York [1 2]. The identification and isolation of the Human Immunodeficiency Virus (HIV) as the causative agent of the disease in 1983 led to the initiation of drug development efforts. These efforts yielded the first effective drug, zidovudine, a reverse transcriptase inhibitor, in 1987 [3]. In the following years other reverse transcriptase inhibitors were introduced (didanosine, zalcitabine, lamivudine, stavudine) and until recently, monotherapy or combination therapy regimens with these drugs were the mainstay of antiretroviral therapy.

However, the beneficial effects of (combinations of) these drugs are only temporarily [4 5]. Recently, two new classes of potent antiretroviral drugs, the non-nucleoside reverse transcriptase inhibitors and the protease inhibitors, were introduced and tested in clinical trials. The non-nucleoside reverse transcriptase inhibitors will not be discussed here.

The protease inhibitors show the potential of suppression of HIV not seen hitherto and this has led to discussion whether there is now the opportunity to change AIDS from a fatal to a chronic disease, or even to eradicate the virus.

The protease inhibitors comprise a heterogeneous class of compounds that target another stage of the HIV life cycle than the reverse transcriptase inhibitors. Therefore, combination of protease inhibitors and reverse transcriptase inhibitors is an attractive option to suppress HIV replication.

Protease inhibitors are in general well tolerated drugs that do not show overlapping toxicities with reverse transcriptase inhibitors. Protease inhibitors are extensively metabolised by cytochrome P450 enzymes. Thus, the potential of drug-drug interactions is evident [6]. Package inserts for protease inhibitors identify numerous potential drug-drug interactions.

Cross-resistance between protease inhibitors may raise problems in the near future.

In this review we describe the pharmacology of HIV protease inhibitors, and focus on saquinavir, indinavir, and ritonavir. Clinical results with these compounds are evaluated. Furthermore, adverse effects, resistance, dosage and administration, clinical pharmacokinetics, pharmacokinetic-pharmacodynamic relationships, and drug-drug interactions are discussed.

HIV protease function

Human immunodeficiency virus (HIV) carries most of its genetic information in three genes: *gag*, *pol* and *env*. *Gag* encodes the proteins of the core and nucleocapsid; *pol* encodes the enzymes involved in viral

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Abstract
In this review the clinical pharmacology of HIV protease inhibitors, a new class of antiretroviral drugs, is discussed. After considering HIV protease function and structure, the development of inhibitors of HIV protease is presented. Three protease inhibitors are reviewed in more detail: saquinavir, indinavir, and ritonavir. Clinical trial results with these agents are evaluated. Furthermore, adverse effects, resistance, dosage and administration, clinical pharmacokinetics, pharmacokinetic-pharmacodynamic relationships, and drug interactions are discussed.

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replication: reverse transcriptase, ribonuclease H, integrase and protease, and *env* encodes the envelope glycoproteins [7]. These genes are expressed as large precursor proteins, which undergo posttranslational processing. The *env* polyprotein is cleaved by host cellular enzymes. The polyproteins encoded by the *gag* and *pol* genes are proteolytically cleaved by an HIV-encoded protease. The *gag* precursor protein is translated directly from the *gag* gene and is cleaved by HIV protease into four proteins of the interior virion. The *pol* gene, however, is not directly translated into a precursor *pol* protein. The replicative enzymes encoded by the *pol* gene are cleaved from a large fusion protein of *gag-pol*. Translation of this fusion protein occurs less frequently than translation of the *gag* gene. The replicative enzymes can only be formed from this fusion protein, since an overlap of genetic information between the *gag* and *pol* genes exists. The protease first cleaves itself out of the *gag-pol* fusion protein. Afterwards, the protease processes precursor proteins to yield structural proteins and replicative enzymes (Figure 1) [7].

Inhibition of HIV protease activity leads to production of non-infectious virions, which have the morphological features of immature particles [8].

In vitro studies have shown that other substrates may serve as substrates of HIV protease, including cytoskeletal and sarcomeric proteins, calmodulin, NFkB and fibronectin [9]. The significance of protease activity on the cytopathic effects of HIV infection *in vivo* with respect to these observations is not clear yet.

HIV protease structure

Generally, proteases can be classified into four groups based on the structure of the active site: the aspartic, cysteine, serine and metallo proteases [10]. HIV protease is an aspartic protease. The enzyme is a bilobal protein, comprising two identical 99-amino acid monomers. The HIV protease is a symmetric C_2 dimer. Each monomer contributes a highly conserved part, Asp-Thr-Gly, to the active site. The active site is located where the two lobes of the monomers are joined [11].

Mammalian proteases of the aspartic group (like renin and pepsin) are also bilobal proteins. However, these are asymmetric enzymes. The symmetric nature of the HIV protease and the lack of symmetry of mammalian proteases have been utilized in the search for selective inhibitors of HIV protease.

Five non-contiguous regions of the HIV protease are highly conserved across isolates of HIV-1. These regions are associated with important characteristics of the enzyme: the substrate-binding region and the catalytic site [9]. At the amino acid level, HIV-2 and SIV (simian immunodeficiency virus) vary ≈ 50% from HIV-1. However, most of the amino acid variations occur outside the catalytic site [12]. Therefore, it may be not surprising that protease inhibitors show activity against HIV-2 *in vitro* [13].

Development of HIV protease inhibitors

Early inhibitors of HIV protease were peptidyl analogues that were used as inhibitors of renin. These early peptidyl analogues showed poor stability (owing to the vulnerability to degradative enzymes), low oral bioavailability (due to poor solubility in both water and lipids) and rapid hepatic metabolism [7]. Next step was to design agents that mimic the transition state of the amide bond hydrolysis, resulting in increased stability. Furthermore, it was tried to reduce these agents in size to increase bioavailability. Computer-aided drug design has been used to search for inhibitors once the crystal structure of the HIV protease was elucidated [14 15]. Structure-based search has provided several C_2 symmetric inhibitors. These agents show more affinity to the viral protease than to their physiological substrates. Furthermore, these symmetric compounds show less affinity to the asymmetric mammalian proteases, thus increasing specificity. In general, efforts to improve bioavailability (by increasing water solubility) resulted in decreased antiviral activity. Thus, a balance between acceptable bioavailability and sufficient inhibitory activity had to be pursued [7].

At this moment, approximately 30 protease inhibitors are under (pre)clinical investigation. Three pro-

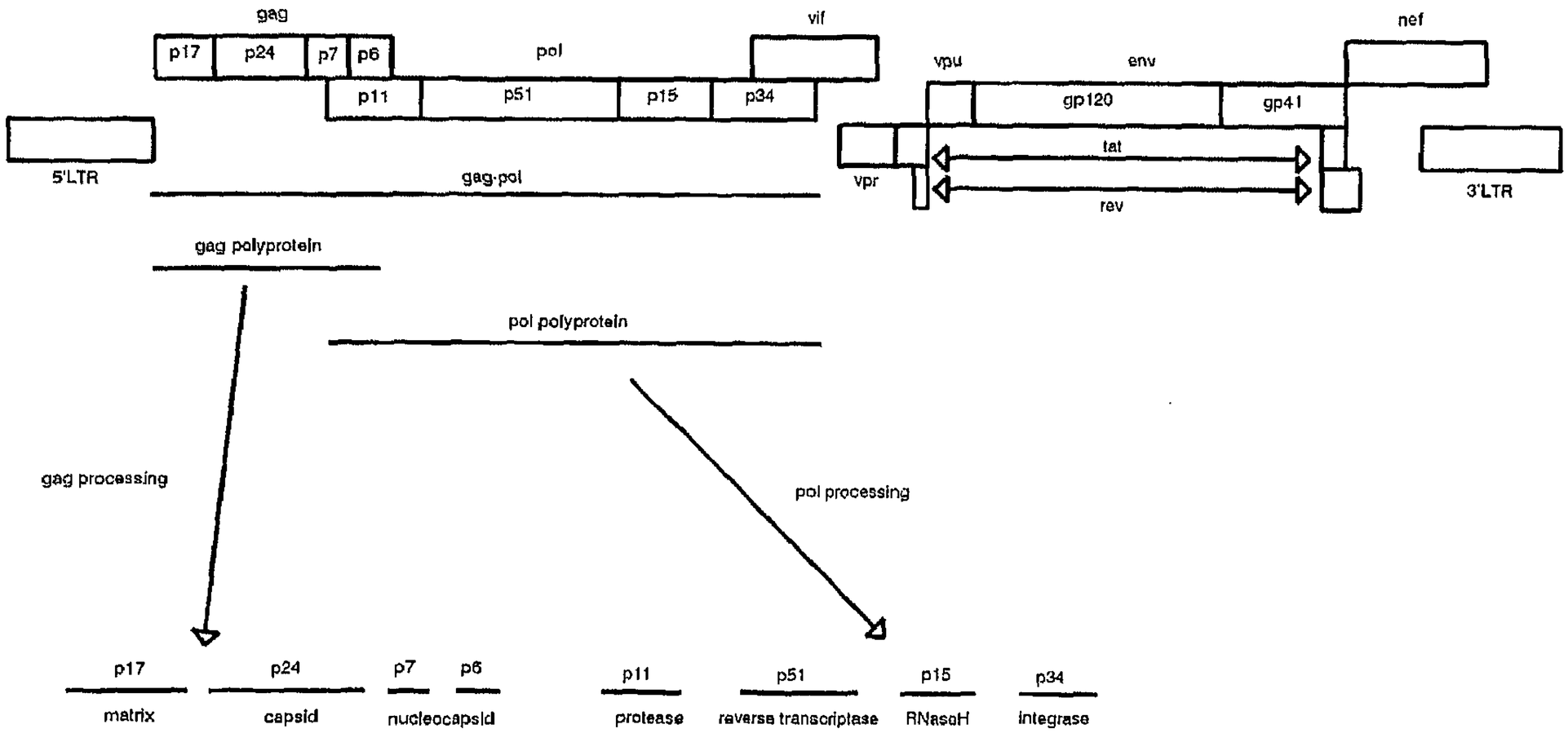

Figure 1
HIV-1 genome and the processing of the gag and pol genes.

Table 1 *Effects of saquinavir monotherapy on CD4+ lymphocyte counts and HIV RNA load*

Reference			change in CD_4^+ lymphocyte count from baseline (cells/μL)				change in HIV RNA load from baseline (^{10}log units)			
	dose (mg tid)	patients (N)	maximum	week of maximum	change 16	at week 24	maximum	week of maximum	change 16	at week 24
17	25	10	+23	4	-29		-0.10	12	-0.07	
(O 13328)	75	12	+30	3	-31		-0.07	4	+0.15	
	200	12	+68	3	-20		-0.40	4	-0.30	
	600	10	+104	6	+36		-0.70	8	-0.05	
18	75	14				-36				
(V 13329)	200	17				-22				
	600	14				+6				
19	3600 mg/day	20	+72	4	+31		-1.06	2		-0.48
	7200 mg/day	20	+121	20	+82		-1.34	4		-0.85

tease inhibitors are now available and will be discussed in more detail: saquinavir, indinavir, and ritonavir.

Saquinavir

Saquinavir (Ro 31-8959, Invirase®) was the first member of its class to be approved in 1995 in the USA by the FDA under its accelerated approval regulations for use in combination with approved nucleoside reverse transcriptase inhibitors in patients with advanced HIV infection. Saquinavir is marketed by Roche. Saquinavir has been approved in the European Community in October 1996. Saquinavir (Figure 2) is a peptide derivative, which is a transition-state mimetic of the Phe-Pro peptide bond.

Efficacy of saquinavir monotherapy

Dosages of saquinavir ranging from 25 to 600 mg *tid* were investigated in two randomised, double-blind phase I/II studies. The O13328 study was performed during 16 weeks in 49 antiretroviral naive patients with CD_4^+ lymphocyte counts <500 cells/μL [17]. Effects on CD_4^+ lymphocyte counts and HIV RNA load are summarized in Table 1. The highest dose group showed the largest increase in CD_4^+ lymphocytes and the largest reduction in HIV RNA load.

The V13329 study was performed in 45 zidovudine-experienced patients with CD_4^+ lymphocyte counts ranging from 50-250 cells/μL (Table 1). The highest dose regimen (600 mg *tid*) was the most effective in terms of increase in CD_4^+ lymphocytes and decrease in HIV RNA load [18].

A third study compared high dosages of saquinavir monotherapy, 3600 and 7200 mg/day, during 24 weeks [19]. Patients had CD_4^+ lymphocyte counts ranging from 200-500 cells/μL (Table 1). The high-dose regimen produced a greater reduction in plasma HIV RNA and PBMC HIV cultures, and a greater increase in CD_4^+ lymphocytes.

Thus, these studies with saquinavir monotherapy showed greater and more sustained efficacy with higher dosages. The maximum decrease in HIV RNA load with saquinavir monotherapy in currently recommended doses (600 mg *tid*) is comparable to that seen with monotherapy with nucleoside reverse transcriptase inhibitors. Saquinavir monotherapy does not lead to a sustained decline in HIV RNA load and increase in CD_4^+ lymphocytes.

Efficacy of saquinavir in combination therapy

In several clinical studies the antiviral effect of saquinavir in combination therapy has been investigated (Table 2). In a double-blind, randomised trial (ACTG 229), triple therapy (saquinavir/zidovudine/zalcitabine) was compared with two double therapies (saquinavir/zidovudine, and zidovudine/zalcitabine) in 297 zidovudine-experienced patients with CD_4^+ lymphocyte counts ranging from 50-300 cells/μL [20]. The median increase in CD_4^+ lymphocyte count at week 24 and 48 was higher in the triple therapy group (Table 2). Suppression of HIV in cultures of PBMCs and of HIV RNA load was also greater and more sustained in the triple therapy group.

The V13330 study compared saquinavir and zidovudine monotherapy with combination therapy of saquinavir/zidovudine in 71 previously untreated patients with CD_4^+ lymphocyte counts <300 cells/μL [21]. Patients received saquinavir monotherapy 600 mg *tid*, zidovudine monotherapy 200 mg *tid*, saquinavir 75 mg *tid* plus zidovudine 200 mg *tid*, saquina-

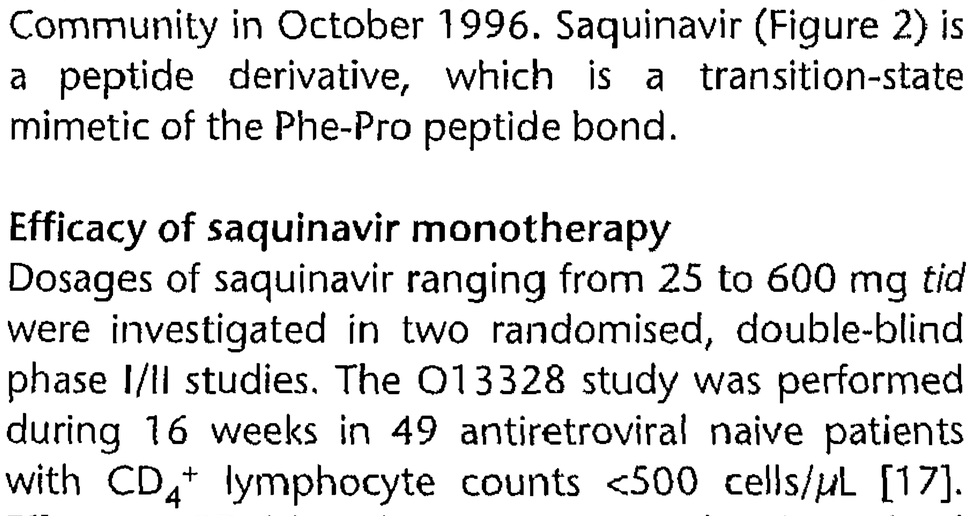

Figure 2
Molecular structure of saquinavir (Invirase®).

Table 2 *Effects of saquinavir in combination with nucleoside analogues on CD4+ lymphocyte counts and HIV RNA load*

Reference			*change in CD_4^+ lymphocyte counts from baseline (cells/μL)*					*change in HIV RNA load (^{10}log units) from baseline*					
	drug regimen (mg tid)	*patients (N)*	*change at week* 4	8	16	24	48	*change at week* 4	8	12	16	20	24
20 (ACTG 229)	SAQ 600+AZT 200+DDC 0.75	98				+31	+22						
	SAQ 600+AZT 200	99				+18	+5						
	AZT 200+DDC 0.75	100				+6	+3						
21 (V 13330)	SAQ 600	15				+33							
	AZT 200	13				+22							
	SAQ 75+AZT 200	14				+21							
	SAQ 200+AZT 200	14				+34							
	SAQ 600+AZT 200	15				+50							-1.09
22,23 (NV 14256)	SAQ 600+DDC 0.75	308			+26[a]						-0.6[a]		
	SAQ 600	318			+10[a]						-0.1[a]		
	DDC 0.75	314			+3[a]						-0.3[a]		
24	SAQ 600+AZT 200+3TC 150[b]	33	+108				-1.96						
25	SAQ 600+D4T 40[b]	14	+32	+57			-1.6	-0.9					
26	SAQ 400[b]+RIT 400[b]	140										-3.21	
	SAQ 400[b]+RIT 600[b]											-3.17	
	SAQ 400 +RIT 400									-2.68			
	SAQ 600[b]+RIT 600[b]									-2.73			
27	SAQ 600[b]+RIT 600[b]	7	+39	+59			-1.44	-1.03					

[a] determined in a subset of 451 patients [23]
[b] *bid*
[c] total of both study arms
[d] mean of both study arms
Abbreviations: 3TC = lamivudine, AZT = zidovudine, D4T = stavudine, DDC = zalcitabine, RIT = ritonavir, SAQ = saquinavir.

vir 200 mg *tid* plus zidovudine 200 mg *tid*, or saquinavir 600 mg *tid* plus zidovudine 200 mg *tid*. The greatest increase in CD_4^+ lymphocyte count was observed in patients who used saquinavir 600 mg *tid* plus zidovudine 200 mg *tid* (Table 2). Furthermore, the largest reduction in HIV RNA load (1.6 ^{10}log units at week 2) was also observed in this arm.

The NV14256 trial compared the combination therapy saquinavir/zalcitabine with saquinavir and zalcitabine monotherapies in 940 patients with CD_4^+ lymphocyte counts between 50-300 cells/μL, and who had received zidovudine for at least 16 weeks [22]. Treatment with the combination of saquinavir/zalcitabine significantly increased the time to the first AIDS-defining event and death, or death alone compared with zalcitabine monotherapy. The relative risk of developing a first AIDS-defining event or death in the combination group was 0.47 (95% confidence interval 0.33 to 0.67) compared to zalcitabine monotherapy; the relative risk of death was 0.28 (95% confidence interval 0.13 to 0.60) in favour of the combination therapy. No differences in morbidity or mortality were observed between the monotherapy arms. In a subset of 451 patients changes in CD_4^+ lymphocytes and HIV RNA at week 16 have been presented [23]. The combination therapy was associated with a greater increase in CD_4^+ lymphocyte count and a greater reduction in HIV RNA load compared with the monotherapy arms (Table 2). The difference in CD_4^+ lymphocyte count was not statistically significant between the two monotherapy arms, although the reduction in HIV RNA load was higher in the zalcitabine monotherapy arm compared to the saquinavir monotherapy arm.

An exploratory, single arm, open-label study of combination therapy with saquinavir (600 mg *tid*) plus lamivudine (150 mg *bid*) plus zidovudine (200 mg *tid*) has been conducted [24]. 33 HIV infected, antiretroviral naive patients with CD_4^+ lymphocyte counts ranging from 150-500 cells/μL were included (Table 2). The peak increase in CD_4^+ lymphocyte count was 153 cells/μL; the peak decline in HIV RNA load was 2.10 ^{10}log units. After week 4, 34% of the patients had HIV RNA load below the limit of detection (200 copies/mL).

Saquinavir (600 mg *tid*) was added to 14 patients with advanced HIV infection who were previously treated with stavudine (40 mg *bid*) [25]. A decrease in HIV RNA load of 1.6 ^{10}log units was observed at week 4; after 8 weeks a decrease of 0.9 ^{10}log units was

reported. The increase in CD_4^+ lymphocyte count was 32 cells/μL at week 4, and 57 at week 8. However, the changes in CD_4^+ lymphocytes were not statistically significant.

Combination therapy of saquinavir and ritonavir (see further) has been elaborated. Rationales for combining these protease inhibitors are: both drugs demonstrated improvement in survival and disease progression in large clinical trials, they show divergent resistance patterns, and ritonavir enhances and sustains saquinavir plasma concentrations.

In a randomized, open-label study the efficacy of the combination of saquinavir and ritonavir in four dose schedules was investigated in 140 patients with CD_4^+ lymphocyte counts between 100-500 cells/μL [26]. Administration of reverse transcriptase inhibitors was stopped. Preliminary data up to 20 weeks of therapy have been presented [26]. Combination therapy shows a strong decline in HIV RNA load (Table 2).

In a pilot study the combination of saquinavir and ritonavir was studied in 7 patients with advanced HIV disease (median CD_4^+ lymphocyte count 10 cells/μL) [27]. From day 1 to 7 patients were treated with ritonavir 600 mg *bid*. From day 8 to 14 saquinavir 200 mg *bid* was added, and increased to 600 mg *bid* after day 15. After 4 weeks of combination therapy, the decrease in HIV RNA load was 1.44 ^{10}log units, and 1.03 ^{10}log units after 8 weeks of combination therapy. CD_4^+ lymphocyte counts increased 39 cells/μL after 4 weeks, and 59 cells/μL after 8 weeks of combination therapy, respectively (Table 2).

The comparative trials indicate that saquinavir is more effective when administered in combination with nucleoside reverse transcriptase inhibitors compared to monotherapy. Furthermore, the decline in HIV RNA load ranges from approximately 1 to 2 ^{10}log units when saquinavir is combined with stavudine, ritonavir, or zidovudine plus lamivudine. The duration of response in combination therapy regimens appears also to be more sustained. In conclusion, the efficacy of saquinavir is larger in combination therapy regimens compared to monotherapy.

Saquinavir in primary HIV infection

Three patients were treated with saquinavir (7200 mg/day), zidovudine (500 mg/day), and lamivudine (600 mg/day) after primary infection with HIV [28]. Pretreatment HIV RNA loads were 6.5, 4.4, and 4.0 ^{10}log units. HIV RNA load fell below the limit of detection (200 copies/mL) after 15, 3.5, and 2 weeks, respectively. Duration of treatment for at least 5, 1.5, and 0.5 months, respectively, still shows HIV RNA load below the limit of detection with no signs of clinical progression. Treatment was well tolerated and required no drug discontinuation.

Adverse effects

In general, saquinavir is well tolerated. In the large NV14256 trial the most common adverse effects of saquinavir given in a dose of 1,800 mg/day included diarrhoea, abdominal discomfort, and nausea [18]. Diarrhoea was the most common single adverse event occurring in 3.8% of all patients. The most common laboratory abnormality was the increase in creatine phosphokinase level in 4% of the patients.

In a study with high dosages of saquinavir monotherapy (3600 and 7200 mg/day), adverse reactions (most commonly gastrointestinal problems and elevated serum aminotransferase levels) were more common in patients receiving the highest dose regimen, but most adverse effects were mild, tolerable and all were reversible [19].

In the ACTG 229 study (comparing saquinavir/zidovudine/zalcitabine, saquinavir/zidovudine, and zidovudine/zalcitabine) no differences in adverse effects or laboratory abnormalities were reported for the treatment groups [20]. The combination of saquinavir plus ritonavir is also well tolerated [26 27].

Resistance to saquinavir

In vitro studies have revealed saquinavir-resistant HIV strains, with IC_{90} values up to more than 50 times those for wild-type HIV [29-34]. Two common mutations are found: the G48V, and the L90M mutation [31 32 34-36]. The G48V mutation is the first to appear *in vitro* [35]. Recently, the *in vitro* susceptibility of various HIV-1 clinical isolates for the combination of saquinavir and indinavir was determined [37]. Against a pan-susceptible clinical isolate the interactive effects of saquinavir and indinavir ranged from synergistic at low doses to antagonistic at high doses. Against a zidovudine-resistant strain the combination showed antagonism at all doses. The clinical consequences of these *in vitro* observations is not clear yet. Significant cross-resistance between saquinavir and other protease inhibitors has not been observed *in vitro* so far [18 36 38].

The G48V and L90M mutations have been detected in viral isolates from patients treated with saquinavir monotherapy, with the L90M mutation being most frequently observed (up to 45%) [18]. The frequency of this mutation in patients who also used zidovudine and zalcitabine was lower. The mutations were not observed in virus from untreated patients. The presence of both mutations was detected in 2.4% of the patients using saquinavir monotherapy [36]. Patients treated with higher dosages of saquinavir (up to 7200 mg/day) showed lower frequencies of saquinavir-resistant mutations [19 36]. In general, genotypic and phenotypic resistance to saquinavir appears to develop relatively slow. High dose therapy with saquinavir or combination therapy with nucleoside analogues appears to reduce the risk of the development of resistant virus. Cross-resistance with other protease inhibitors *in vivo* has been reported, but its incidence appears to be low [18 39 40].

Dosage and administration

The formal dosage of saquinavir is 600 mg *tid* in combination with nucleoside analogues. However, in some countries a dosage of 1200 mg *tid* is now common practice. Saquinavir should be taken within 2 hours of a meal [18]. Saquinavir (as mesylate) is available as 200 mg capsules. A new formulation of saquinavir is in development (soft gelatin capsule) and is expected to increase oral availability from 4 to 12%. A formulation for paediatric use is also being developed [18].

Pharmacokinetics

As a result of limited absorption and extensive first-pass metabolism the bioavailability of a single 600 mg oral saquinavir dose taken with food is 4%. In the fasting state the bioavailability is 18 times lower [18 41].

In healthy volunteers the maximum plasma concentration (C_{max}) after a single 600 mg oral dose taken with food is 66.1 ng/mL [18 41 42]. After multiple doses the C_{max} was 90.4 ng/mL [18]. Time to C_{max} (T_{max}) is 3-4 hours after administration of saquinavir capsules with food [18 41]. Administration of saquinavir suspension in a fasted state yielded a T_{max} value of 0.77 hours [43]. Steady-state plasma concentrations of saquinavir in HIV infected patients appear to be higher than in healthy volunteers with a C_{max} of 242.3 ng/mL after multiple oral doses of 600 mg [18]. A 1-hour infusion of 12 mg saquinavir in healthy volunteers yielded a steady-state volume of distribution of 703 L, thus suggesting extensive tissue binding. Saquinavir is highly bound to plasma proteins (>98%) [16]. The value for total plasma clearance was 98.8 L/h, and the terminal plasma half-life was 13.2 h [41]. Elimination of saquinavir is predominantly non-renal; after a 600 mg oral dose 88% was detected in the faeces, whilst 1% was excreted in the urine [18]. Saquinavir pharmacokinetics appear to be non-linear, with higher dosages leading to a more than proportional increase in the area under the concentration versus time curve (AUC) and C_{max} [18]. Saquinavir is rapidly metabolised by the cytochrome P450-3A4 isoenzyme to a number of inactive mono- and dihydroxylated metabolites [18 44].

The pharmacokinetics of saquinavir in HIV infected patients with severe diarrhoea or wasting syndrome have been investigated [45]. Preliminary results indicate that plasma concentrations of saquinavir are at least equal to those achieved in healthy volunteers. Saquinavir pharmacokinetics have not been studied in patients with hepatic or renal insufficiency.

Pharmacokinetic-pharmacodynamic relationships

In contrast to nucleoside reverse transcriptase inhibitors, pharmacokinetic-pharmacodynamic relationships are more easily found with protease inhibitors. Most likely this can be explained by the need for intracellular phosphorylation to the active triphosphate anabolites in case of the nucleoside analogues. In the O13328 study, a positive linear relationship was found between saquinavir AUC and the change in CD_4^+ lymphocytes after 2 weeks of treatment [17]. In a 24-week study, two high doses of saquinavir monotherapy (3600 and 7200 mg/day) were compared in 40 HIV infected patients. In a subset of 16 patients the pharmacokinetics of saquinavir were studied and a strong correlation between saquinavir AUC and decrease in viral load at week 4 was observed in both arms [19].

A linear relationship between AUC and the change in CD_4^+ lymphocyte count is predicted over the dose range 75-600 mg *tid* from a model based on data from 61 patients with advanced HIV disease who were treated with saquinavir for 16 weeks. An $AUC_{0\text{-}8\ hours}$ value of 700 μg/L*h was predictive of a peak increase in CD_4^+ lymphocytes of 26 cells/μL on day 19, and of CD_4^+ lymphocytes remaining above baseline during 16 weeks of treatment [18].

Thus, though limited data on saquinavir pharmacokinetics and efficacy are available, relationships have been found between saquinavir pharmacokinetics (AUC) and efficacy in terms of decrease in HIV RNA load and increase in CD_4^+ lymphocytes. Regarding the low and variable bioavailability of saquinavir [41], monitoring drug concentrations in patients to ensure drug efficacy and to prevent the risk of drug resistance, appears to be warranted.

Drug interactions

Co-administration of rifampin 600 mg/day and saquinavir 600 mg *tid* decreased the steady-state AUC and C_{max} values of saquinavir with 80% in healthy volunteers [18]. Co-administration of rifabutin 300 mg/day and saquinavir 600 mg *tid* decreased the AUC and C_{max} of saquinavir with 40% [46]. The maximum effect had been reached after 1 week. Presumably, these important observations are caused by the induction of cytochrome P450 enzymes by rifampin and rifabutin. Concomitant administration of saquinavir and drugs that are metabolised by cytochrome P450-3A4 isoenzymes may result in increased plasma concentrations of the latter drugs. Therefore, it is advised not to prescribe astemizole and terfenadine to patients who use saquinavir, since this combination may lead to an increased risk of cardiac arrhythmia [18]. The AUC and C_{max} of saquinavir were increased three-fold when saquinavir (600 mg *tid*) was combined with ketoconazole 200 mg/day in healthy volunteers in a multiple-dose study [18]. Single-dose administration of saquinavir plus ranitidine increased the bioavailability of saquinavir by 67% [18].

Co-administration of saquinavir with ritonavir increased the AUC and C_{max} of saquinavir by >290- and 18-fold in rats, respectively [47]. Co-administration of saquinavir and ritonavir in healthy volunteers also revealed an increase in saquinavir exposure [48]. The pharmacokinetics of saquinavir and ritonavir were investigated after single- and multiple-dose administration in several regimens. The single-dose study revealed that combining saquinavir 600 mg and ritonavir 600 mg increased saquinavir C_{max} values nearly 30-fold; saquinavir AUC values increased 90-fold. Ritonavir pharmacokinetics were not affected. The multiple-dose study showed similar results.

Combination of saquinavir 600 mg *tid* and ritonavir 300 mg *bid* showed a 16-fold increase in saquinavir C_{max} and a 21-fold increase in AUC [49].

Plasma concentrations of saquinavir may be decreased if phenobarbital, phenytoin, dexamethasone, or carbamazepine are co-administered, although no data are currently available from studies. Concurrent administration of saquinavir and cisapride, calcium-channel blocking agents, clindamycin, dapsone, quinidine, triazolam, or midazolam may theoretically lead to increased concentrations of the co-administered drugs due to inhibition of their metabolism [50].

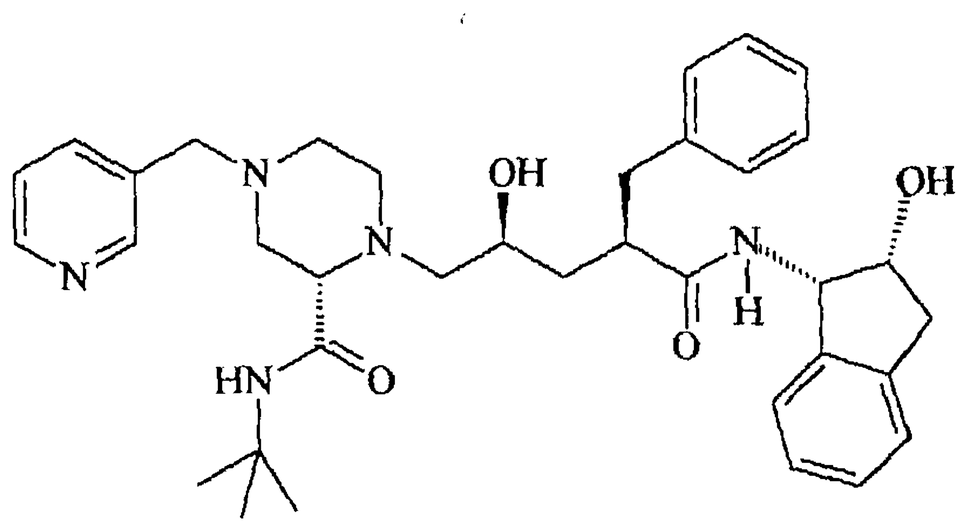

Figure 3
Molecular structure of indinavir (Crixivan®).

Table 3 *Effects of indinavir monotherapy on CD4+ lymphocyte counts and HIV RNA load*

Reference			*change in CD_4^+ lymphocyte count from baseline (cells/μL)*						*change in HIV RNA load from baseline (^{10}log units)*			
	dose	*patients (N)*	*change at week*						*change at week*			
			8	*12*	*14*	*24*	*36*	*48*	*6*	*8*	*24*	*48*
57	1.6-1.8 g/day	38	+89			+82		+72			-0.3	-0.3
16	600 mg *qid*	16		+104		+105	+126			-1.98		
59	2.4 g/day 3.0 g/day 3.2 g/day	70[a]				+80 to +145[b]		+85			-2.3 to -2.6[b]	-2.63
56	600 mg *qid*	5				+143					-1.55	-0.64

[a] total of three study arms
[b] not specified

Indinavir

Indinavir (L-735,524, MK-639, Crixivan®, Figure 3) is marketed by Merck. In the USA indinavir is approved by the FDA under its accelerated approval regulations for use as monotherapy or in combination with approved nucleoside analogues in patients with HIV infection. Indinavir has been approved in the European Community in October 1996.

Initially starting from a peptide renin lead compound, a series of hydroxyethylene dipeptide inhibitors of HIV protease were developed [51 52]. Though highly potent, these compounds lack aqueous solubility and desirable pharmacokinetic properties. The incorporation of an amine resulted in hydroxyaminopentane amides, in which potent antiviral activity and acceptable bioavailability are combined [53 54].

Efficacy of indinavir monotherapy

Results from phase I/II trials in zidovudine-experienced patients with <500 CD_4^+ lymphocytes/μL and >25 pg/mL of p24 antigen have been presented [57]. Doses of indinavir ranging from 1.6 to 1.8 g/day resulted in an initial decline in HIV RNA load of 1.1 ^{10}log units (Table 3).

In a double-blind phase II trial in 74 patients with ≥500 CD_4^+ lymphocytes/μL, indinavir at two dose levels (200 or 400 mg *qid*) was compared with zidovudine monotherapy (200 mg *tid*) [58]. Increases in CD_4^+ lymphocytes and decreases in HIV RNA load and p24 antigen levels were observed. The largest initial decrease in HIV RNA load was observed in the highest indinavir dose group (> 1 ^{10}log reduction). HIV RNA load returned to baseline between 12 and 24 weeks and viral resistance developed. After 24 weeks of therapy the indinavir dosage was increased to 600 mg *qid*. After 52 weeks of therapy CD_4^+ lymphocytes remained above baseline in patients treated with indinavir. No further decrease in HIV RNA load, however, was observed after dose escalation.

An open-label monotherapy study at a dosage of 600 mg *qid* in 16 zidovudine-experienced patients with CD_4^+ lymphocyte cell counts <500 cells/μL resulted in significant declines in HIV RNA load and increases in CD_4^+ lymphocytes (Table 3) [16].

In one study high doses of indinavir monotherapy were compared with respect to increase in CD_4^+ lymphocyte cell count and decrease in viral load [59]. A total of 70 patients with a median entry CD_4^+ lymphocyte cell count of 250 cells/μL and a median plasma HIV RNA load of 70,795 copies/mL were included (Table 3). No differences between the treatment arms were observed in immunological or viral markers of disease progression. At week 24 approximately 40% of all patients had plasma HIV RNA levels below the limit of detection (200 copies/mL). After 48 weeks of treatment with 2,400 mg/day 54% of the patients had plasma HIV RNA levels below the limit of detection. No differences were observed at this time point, nor compared to the higher dose groups, nor compared to the results at week 24.

An open-label, 24-week phase I/II study was conducted in 5 patients with CD_4^+ lymphocyte counts <300 cells/μL, and ≥20,000 HIV RNA copies/mL [56]. Patients were extensively pretreated with nucleoside analogues and received indinavir 600 mg *qid*. Effects on HIV RNA load and CD_4^+ lymphocytes are summarised in Table 3.

The safety and efficacy of indinavir has also been investigated in HIV infected children. A phase I/II study in 26 HIV infected children with indinavir monotherapy has been presented [60]. Patients were divided into two groups according to their age (< 12 or ≥ 12 years). Three dose groups were created: 250, 350, and 500 mg/m^2 *tid*. Formulations of indinavir used were a suspension and capsules. After 12 weeks of therapy the suspension was replaced by capsules at a fixed dose of 250 mg/m^2 *tid*; 5 children used capsules throughout the study. Zidovudine plus lamivudine were added to the regimen after week 16. The median increase in CD_4^+ lymphocytes in the 250 and 350 mg/m^2 *tid* dose groups at week 16 was 99 cells/μL. HIV RNA levels in patients receiving indinavir suspension in the two lowest dose groups showed a median maximum decrease of 0.7 ^{10}log units.

In contrast to saquinavir, no larger antiretroviral efficacy of indinavir (measured as virological or immunological response) was obtained with higher dosages than currently recommended, e.g. 800 mg *tid*

Table 4 *Effects of indinavir in combination with nucleoside analogues on CD4+ lymphocyte counts and HIV RNA load.*

Reference		*change in CD_4^+ lymphocyte counts from baseline (cells/μL)*		*change in HIV RNA load (^{10}log units) from baseline*		*patients with HIV RNA below detection limit (%)*		
	drug regimen (mg tid)	*change at week* 24	44	*change at week* 24	44	*week* 24	32	44
63	IDV 600 *qid*+AZT 200			-2.5				
	IDV 600 *qid*			-1.5				
	AZT 200			-0.3				
62	IDV 800+AZT 200+3TC 150[a]	+126	+218	-2.2	-2.2	92	83	83
(protocol 035)	IDV 800	+105	+158	-0.7	-0.9	38	36	22
	AZT 200+3TC 150[a]	+14	+14	-0.6	-0.2	0	0	0
64	IDV 800+AZT 200	+121		-1.09		36		
(protocol 028)	IDV 800	+125		-0.86		37		
	AZT 200	+16		-0.27		7		
65	IDV 800+AZT 200	+95		-1.19		56		
(protocol 033)	IDV 800	+109		-1.03		37		
	AZT 200	+14		-0.26		2		

[a] 3TC was administered twice daily.
Abbreviations: 3TC = lamivudine, AZT = zidovudine, IDV = indinavir.

[59]. The maximal decline in HIV RNA load observed with indinavir monotherapy in currently recommended doses (2.4 g/day) is generally more than 2 ^{10}log units. This indicates that indinavir monotherapy may result in larger and more sustained suppression of viral load compared to monotherapy with either saquinavir or reverse transcriptase inhibitors.

Efficacy of indinavir in combination therapy

A summary of three phase II trials with indinavir has recently been presented [61]. In two studies, patients were randomised to indinavir monotherapy (800 mg *tid*, n=67), zidovudine or zidovudine plus didanosine (n=47), or indinavir plus a reverse transcriptase inhibitor (n=53). After week 24 the median increase in CD_4^+ lymphocytes ranged from 26-80 cells/μL in the indinavir monotherapy arms. The median maximal decline in viral load ranged from 2.0 to 2.3 ^{10}log units in this group; 9 to 40% of these patients had plasma HIV RNA levels below the limit of detection (200 copies/mL). The changes in viral load and CD_4^+ lymphocyte counts were not different in patients receiving combination therapy with indinavir plus a nucleoside analogue, though the percentage of patients with viral load below the limit of detection tended to be greater in the combination therapy arm.

In a randomized, double-blind study indinavir monotherapy was compared with combination therapy of zidovudine/lamivudine, and indinavir/zidovudine/lamivudine in 97 HIV-infected patients (protocol 035) [62]. Patients had 50-400 CD_4^+ lymphocyte cells/μL and ≥20,000 copies of HIV RNA/mL, and had received ≥6 months of zidovudine therapy. Results of the effects on HIV RNA load and CD_4^+ lymphocyte counts up to week 44 have been presented (Table 4). The triple combination therapy was the most effective regimen with respect to the decrease in HIV RNA load (maximum -2.2 ^{10}log units at 24 weeks), increase in CD_4^+ lymphocytes (maximum +218 at 44 weeks), and the percentage of patients with HIV RNA load below the detection limit, 500 copies/mL (92% at week 24).

Preliminary results of a double-blind, randomised trial of indinavir monotherapy, zidovudine monotherapy, and indinavir/zidovudine combination therapy in 73 zidovudine-naive patients with CD_4^+ lymphocyte cell counts <500 cells/μL and a HIV RNA load >20,000 copies/mL have been presented (Table 4) [63]. The maximal decrease in HIV RNA load was -2.6, -2.3, and -0.6 ^{10}log units for the combination, the indinavir monotherapy, and the zidovudine monotherapy arms, respectively. The indinavir-containing arms showed increases of 50 CD_4^+ lymphocyte cells/μL compared to the zidovudine monotherapy arm after 24 weeks of therapy. The results on HIV RNA load indicate that the addition of zidovudine to indinavir does not have a profound effect on the initial decrease in HIV RNA load, but that the effect might be more sustained.

In a double-blind, randomized trial (protocol 028), indinavir monotherapy was compared with zidovudine monotherapy, and combination therapy of indinavir/zidovudine in 224 patients with CD_4^+ lymphocyte counts ranging from 50-250 cells/μL (Table 4) [64]. Results up to week 24 revealed a larger increase in CD_4^+ lymphocytes, and a larger decrease in HIV RNA load in the indinavir containing arms. No differences between the indinavir monotherapy arm and the combination arm were detected. In the indinavir-containing arms the percentages of patients with undetectable HIV RNA load were higher than in the zidovudine monotherapy group.

A double-blind, randomized trial (protocol 033)

was conducted in 266 patients with CD_4^+ lymphocyte counts ranging from 50-500 cells/μL, who were naive to zidovudine and protease inhibitors. Indinavir monotherapy was compared with zidovudine monotherapy, and combination therapy of indinavir/zidovudine (Table 4) [65]. Results up to week 24 reveal a larger increase in CD_4^+ lymphocytes, and larger decrease in HIV RNA load in the indinavir-containing arms. No differences between the indinavir monotherapy arm and the combination arm were detected. The percentages of patients with undetectable HIV RNA load were higher in the indinavir-containing arms than in the zidovudine monotherapy arm.

An interim analysis of protocols 028 and 033 in 490 patients showed that addition of zidovudine to indinavir therapy causes no additional benefit on CD_4^+ lymphocyte cell count, but adds small, though statistically significant effect on the decrease in HIV RNA load, especially in patients with high baseline viral load [66].

A pilot study was conducted to investigate the effect of indinavir monotherapy with intermittent interleukin-2 in HIV-infected patients with CD_4^+ lymphocyte cell counts <300 cells/μL [67 68]. Three treatment arms were created: in the first arm patients received interleukin-2 for 5 days every 2 months in a dose of ≥12 MIU/day plus indinavir 600 mg *qid* (A). In the second arm patients received indinavir 600 mg *qid* for 10 days during a similar interleukin-2 cycle (B), and in the third arm patients received indinavir monotherapy 600 mg *qid* (C). After 14 weeks the following results were obtained. In the first group the increase in CD_4^+ lymphocyte count was 196 cells/μL, and the increase in HIV RNA load was 0.03 ^{10}log units. In the second group the increase in CD_4^+ lymphocyte count was 85 cells/μL, and the HIV RNA load increased with 0.4 ^{10}log units. In the third group the CD_4^+ lymphocyte count increased with 113 cells/μL, while the HIV RNA load decreased with 0.57 ^{10}log units. These results indicate that the combination of indinavir and interleukin-2 leads to increases in CD_4^+ lymphocyte cell count, but does not lead to changes in HIV RNA load.

Phase III protocols are currently underway. ACTG 320 will be a randomized, double-blind study of indinavir with open-label zidovudine and lamivudine in patients with <200 CD_4^+ lymphocytes/μL. Disease progression and death are the primary endpoints of this study.

Adverse effects

Adverse effects of indinavir include nephrolithiasis in up to 5% of the patients [16]. The frequency of nephrolithiasis increases with doses exceeding 2.4 g/day. Nephrolithiasis may be caused by crystallisation of indinavir in the urine. Therefore, it is recommended that patients drink at least 1.5 L of fluid extra per day. Furthermore, asymptomatic hyperbilirubinaemia (primarily as elevated indirect bilirubin) is reported in up to 15% of the patients.

Resistance to indinavir

The *in vitro* susceptibility of HIV-1 clinical isolates for the combination of saquinavir and indinavir has been described before [37]. Complete *in vitro* cross-resistance between indinavir and ritonavir has been reported [69]. Approximately two-thirds of indinavir resistant viral strains studied by Merck are cross-resistant to saquinavir, but all saquinavir-resistant strains studied thus far are (at least initially) fully sensitive to indinavir [69].

In protocol 019 patients were treated with indinavir monotherapy, zidovudine monotherapy, or combination therapy with indinavir/zidovudine. The emergence of resistance to indinavir was reduced (but not significant) in patients treated with the combination therapy compared to the indinavir monotherapy arm [70]. The resistance to zidovudine was significantly reduced in patients receiving indinavir plus zidovudine compared to zidovudine monotherapy.

In protocol 020, patients received either a combination of indinavir/zidovudine/didanosine, or zidovudine/didanosine, or indinavir monotherapy [70]. A highly significant reduction in indinavir resistance was observed in patients receiving the triple combination. Similarly, a significant reduction in resistance to zidovudine or didanosine was observed in patients of this group.

Dosage and administration

The currently recommended dosage of indinavir is 800 mg *tid*, either alone or in combination with nucleoside analogues. Indinavir should be taken with a light meal or on an empty stomach (one hour before, or two hours after a meal). Indinavir (as the sulphate) is available as 200 and 400 mg capsules. It is recommended to reduce the dosage of indinavir to 600 mg *tid* in patients with liver insufficiency and in patients with repeated nephrolithiasis despite adequate intake of fluids [71].

Pharmacokinetics

The pharmacokinetics of indinavir were investigated in asymptomatic patients at 100, 200, and 400 mg *qid* in a multiple dose study [72]. The mean T_{max} was <1 hour and the plasma elimination half-life was 1-2 hours. After 10 days of administration the plasma C_{max} exceeded the target concentration of 100 nM in the highest dose group (the IC_{95} for virus inhibition is <100 nM). The plasma trough concentration was 199 ± 139 nM.

Indinavir pharmacokinetics were studied in HIV negative persons after a single gift in the dose range from 20-1,000 mg [73]. The T_{max} was <1 hour, and the plasma half-life was 1-2 hours. The sulphate salt of indinavir resulted in smaller inter-subject variability compared to the base. The AUC of indinavir was 70 to 80% lower if 400 mg was administered with a meal compared to fasted administration. In single- and multiple-dose studies the AUC increased disproportional with dose. Little accumulation in plasma occurred following multiple doses (< 30% increase in AUC).

At a dosing regimen of 800 mg *tid* AUC values were 30,691 ± 11,407 nM*h, C_{max} values reached 12,617 ± 4,037 nM, and the plasma trough concentration was 251 ± 178 nM [74].

The plasma protein binding of 60% is relatively low compared to saquinavir and ritonavir [56]. Indinavir might benefit from lower plasma protein binding; this may result in more substantial penetration into cerebrospinal fluid, or in higher tissue concentrations [16].

Seven metabolites of indinavir have been detected

in the urine of healthy volunteers [55]. The cytochrome P450-3A4 appears to be the major enzyme responsible for formation of metabolites. The metabolites comprised <0.5% of the dose in the first four hours after administration. The cumulative amount of unchanged indinavir in the urine is approximately 12% of the administered dose.

Patients with mild to moderate hepatic insufficiency and clinical evidence of cirrhosis showed a decrease in indinavir metabolism resulting in 60% higher AUC values after a single 400 mg dose [74]. The half-life of indinavir increased to 2.8 h. Indinavir pharmacokinetics have not been studied in patients with severe hepatic or renal insufficiency. Indinavir pharmacokinetics appear to be comparable in men and women, and in Caucasians and Blacks [74].

Pharmacokinetic-pharmacodynamic relationships

In an open-label phase I/II study in 5 HIV-infected men the relationship between indinavir pharmacokinetics and pharmacodynamics was studied [56]. Indinavir was administered as a monotherapy regimen. No relationship was found between C_{max} and the decrease in viral load. However, a positive relationship was found between indinavir AUC and the reduction in viral load. Furthermore, a positive relationship was found between indinavir trough plasma concentration (C_{min}) and the reduction in viral load. The relationships between AUC or C_{min} and the decrease in viral load is extremely steep. Thus, the possibility of monitoring indinavir concentrations to ensure sufficient exposure should be investigated.

Drug interactions

Pharmacokinetic drug-drug interactions with indinavir are anticipated for drugs that are substrates for cytochrome P450-3A enzymes. Pharmacokinetic interaction studies have been conducted in HIV infected patients [75]. The effect of trimethoprim, sulfamethoxazole, zidovudine, stavudine, isoniazid, clarithromycin, rifabutin, and fluconazole on indinavir AUC, and the effect of indinavir on the AUC of these drugs has been investigated. No clinically significant interaction occurred with trimethoprim, sulfamethoxazole, zidovudine, stavudine, isoniazide, and fluconazole. The plasma concentrations of clarithromycin and rifabutin, however, increased after co-administration with indinavir; the interaction with rifabutin was considered to be clinically significant. The rifabutin plasma AUC and C_{max} increased with 173 and 135%, respectively. Rifabutin decreased the plasma AUC and C_{max} of indinavir with 34 and 25%, respectively. Therefore, a dose adjustment to half the standard dose of rifabutin is recommended if coadministered with indinavir [71 75].

The absence of a clinically significant pharmacokinetic interaction between indinavir and fluconazole was confirmed in 13 patients [76]. In this study an unexpected, significant decrease (24%) in indinavir plasma AUC was observed if fluconazole was coadministered.

Concurrent administration of ketoconazole 400 mg plus indinavir 400 mg resulted in an increase in indinavir AUC and C_{max} of 62 and 14%, respectively. Therefore, it is recommended to reduce the dose of indinavir to 600 mg *tid* if coadministered with ketoconazole [71].

No pharmacokinetic interaction study with rifampin has been performed yet. Since rifampin is a strong inducer of cytochrome P450-3A4 enzymes it is anticipated that concurrent administration of indinavir and rifampin may lead to decreased indinavir concentrations.

Other drugs that induce cytochrome P450-3A4 enzymes include phenobarbital, phenytoin, dexamethasone, and carbamazepine. Concurrent administration of one of these drugs and indinavir may lead to decreased indinavir concentrations [71].

Coadministration of indinavir and itraconazole may lead to increased indinavir concentrations due to the inhibitory effect of itraconazole on the cytochrome P450-3A4 enzyme. This potential interaction has, however, not been investigated yet.

Up to now no study has been performed in which the effect of concurrently administered didanosine on indinavir absorption is investigated. Since didanosine is an acid-labile compound, its formulation contains compounds to increase gastric pH. Adequate absorption of indinavir, however, requires a low gastric pH value. Therefore, it is recommended that didanosine and indinavir should be administered with a minimum time interval of 1 hour [71].

It is anticipated that concurrent administration of indinavir and ritonavir will lead to an increase in indinavir plasma concentrations due to inhibition of cytochrome P450 enzymes [71]. Concurrent administration of indinavir and astemizole, terfenadine, quinidine, cisapride, alprazolam, midazolam or triazolam may lead to increased concentrations of the coadministered drugs due to inhibition of their metabolism by indinavir [71].

Ritonavir

Ritonavir (ABT-538, Norvir®, Figure 4) is marketed by Abbott and was approved by the FDA in the USA for use alone or in combination with approved nucleoside analogues in patients with advanced HIV infection. Approval for patients with advanced HIV infection was based on data demonstrating delay in disease progression and reduction of mortality. Ritonavir was also approved by the FDA under its accelerated approval regulations for patients with early HIV infection based on a beneficial effect on surrogate parameters. In the European Union ritonavir was the first HIV protease inhibitor to be approved in August 1996 for use in combination with antiretroviral nucleoside analogue(s) in HIV-infected adult patients with advanced or progressive immunodeficiency [77].

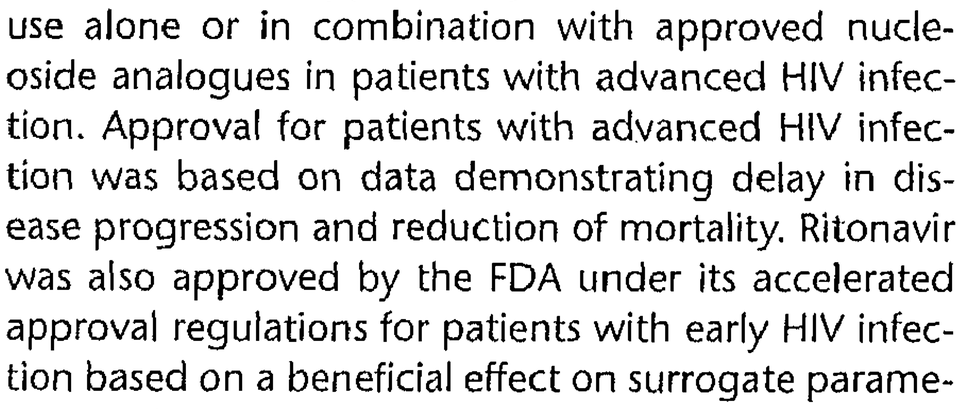

Figure4
Molecular structure of ritonavir (Norvir®).

Table 5 *Effects of indinavir in combination with nucleoside analogues on CD4+ lymphocyte counts and HIV RNA load*

Reference			change in CD_4^+ lymphocyte count from baseline (cells/μL)				change in HIV RNA load from baseline (^{10}log units)		
	dose (mg)	patients (N)	change at week 4	12	32	34	4	32	34
78	300 *bid*		+80		+25		-0.78		
	400 *bid*		+140		+70		-0.83		
	500 *bid*		+100		+25		-0.97		
	600 *bid*		+70		+230		-1.13	-0.81	
79	200 *tid*		+90	+137					
	300 *tid*		+114	+140					
	200 *qid*		+25	+60					
	300 *qid*		+63	+59					
80	400 *tid*	17	+90		+110		-1.80		-0.24
	700 *bid*	13	+110		+267		-1.82		-0.74

Efficacy of ritonavir monotherapy

A randomised, double-blind, placebo-controlled phase I/II study with ritonavir monotherapy was conducted in 84 HIV infected patients with >50 CD_4^+ lymphocyte cells/μL and ≥10 pg/mL of p24 antigen [78]. Patients were randomised to receive placebo, or ritonavir in a dosage of 300, 400, 500, or 600 mg *bid*. After 4 weeks of therapy the patients who received placebo were randomised to one of the four dosage regimens. From week 1 through 4 a decline in HIV RNA load and p24 antigen was observed in all ritonavir arms compared to placebo (Table 5). The largest decrease was seen in the highest dose-group. After 4 weeks of therapy the CD_4^+ lymphocyte count was higher in patients receiving ritonavir compared to placebo. After 16 weeks of therapy HIV RNA levels in the 300 and 400 mg *bid* dose groups returned to baseline. During the 32-week follow-up of the study no significant differences in HIV RNA load between the 300 and 400 mg dose groups were observed at any timepoint. The 500 and 600 mg dose groups, however, showed a more sustained suppression of HIV RNA load; the load in the 500 mg dose group increased after week 20 and returned to baseline at week 32. After 32 weeks of therapy, a difference in HIV RNA load was observed between the 500 and 600 mg dose groups; the decrease in HIV load after 32 weeks of therapy was -0.81 ^{10}log units in the highest dose group. The maximal decrease in HIV RNA load in the 500 and 600 mg dose groups combined (17 patients) was -1.94 ^{10}log units after 8 weeks of therapy as assessed with a more sensitive HIV RNA assay. After 24 weeks of therapy the initial increase in the CD_4^+ lymphocytes in the 300 and 400 mg dose groups was lost and counts returned to baseline. The largest median increase (230 cells/μL) after 32 weeks of therapy was observed in the highest dose groups and this was greater than in the 500 mg dose-group [78].

A randomised, double-blind, placebo-controlled trial was conducted in 62 patients with CD_4^+ lymphocyte counts between 50-500 cells/μL and an HIV RNA load of ≥25,000 copies/mL [79]. Patients were randomised to receive placebo, or ritonavir in a dosage of 200 or 300 *tid*, or 200 or 300 mg *qid*. After 4 weeks of therapy patients who received placebo were randomised to one of the four regimens. At day 15 a decrease in HIV RNA load was observed in all ritonavir arms compared to placebo, with maximal decreases ranging from 0.86 to 1.18 ^{10}log units. After 4 weeks of therapy the mean decline in HIV RNA load of all therapies combined was 0.83 ^{10}log units. After 12 weeks of therapy a mean decline in HIV RNA level of 0.5 ^{10}log units was observed as determined with the branched-chain DNA assay (lower limit of quantification (LOQ) 10,000 copies/mL). A more sensitive assay (LOQ 400 copies/mL) was used to re-analyze the results in a subset of 20 patients. The mean decline in HIV RNA as measured by the latter assay at week 12 was 1.1 ^{10}log units, with a maximal decline of 1.7 ^{10}log units after 2 to 3 weeks. The duration of the antiviral effect was less sustained in the lowest (200 mg *tid*) dose-group. The results on CD_4^+ lymphocytes are summarised in Table 5.

An open-label study was performed with two potentially maximum tolerated doses of ritonavir in 30 patients with ≥50 CD_4^+ lymphocyte cells/μL and a viral load ≥25,000 copies/mL (Table 5) [80].

The efficacy of ritonavir has also been assessed in 9 HIV infected children with progressive disease or intolerance to other antiretroviral drugs in a phase I/II study [81]. Four dose levels (250 to 400 mg/m^2 *bid*) of the liquid formulation were studied. The first 12 weeks ritonavir was given as monotherapy, after this period zidovudine and/or didanosine were added. By day 28 the CD_4^+ lymphocyte count had increased with 38 cells/μL in the lowest dose group. During the time of follow-up (median 6.3 weeks) the HIV RNA load declined with 1.5 ^{10}log units.

Efficacy of ritonavir in combination therapy

Preliminary data were presented from a study in which ritonavir or placebo is added to 1090 patients with <101 CD_4^+ lymphocytes/μL receiving ongoing

Table 6 *Effects of ritonavir in combination with nucleoside analogues on CD4+ lymphocyte counts and HIV RNA load*

Reference			*change in CD_4^+ lymphocyte counts from baseline (cells/μL)*				*change in HIV RNA load (^{10}log units) from baseline*			
	drug regimen (mg bid)	*patients (N)*	*change at week* 12	16	20	28	*change at week* 12	16	20	28
77	AZT 200[a]	116		+11				-0.42		
	RIT 600	118		+62				-1.03		
	RIT 600+AZT 200[a]	120		+35				-0.80		
84	RIT 600+AZT 200[a]+DDC 0.75[a]	29			+141[b]	+140[d]			-2.36[b]	-2.00[c]
85	RIT 600+AZT 300+3TC 150[d]	17	+78				-2.5			
	RIT 600+AZT 300+3TC 150[e]	16	+130				-2.7			

[a] *tid*
[b] after 5 months of therapy
[c] after 9 months of therapy
[d] patients started immediately with triple drug therapy
[e] zidovudine and lamivudine were added after three weeks of ritonavir monotherapy
Abbreviations: NR = not reported, 3TC = lamivudine, AZT = zidovudine, DDC = zalcitabine, RIT = ritonavir.

therapy with 0, 1, or 2 nucleoside analogues [82]. A significant reduction in the risk of developing a new AIDS-defining illness or death (relative risk 0.44, 95% confidence interval 0.34 to 0.56) and death alone (relative risk 0.57, 95% confidence interval 0.35 to 0.92) was observed in patients receiving ritonavir; median follow-up time was 6.1 months. In a 16-week substudy, virologic (159 patients) and immunologic (215 patients) surrogate markers of anti-HIV activity were followed [83]. For the ritonavir group the mean maximal decrease in HIV RNA load was 1.29 ^{10}log units. The peak increase in CD_4^+ lymphocyte count was 47 cells/μL. A greater clinical benefit for patients with >5.4 ^{10}log particles of HIV RNA/mL was observed compared to patients with >5.4 ^{10}log particles/mL at baseline. The greatest effect of ritonavir therapy was seen in patients with >50 cells/μL. Thus, clinical benefit may be more pronounced with earlier therapeutic intervention with ritonavir.

356 HIV infected, antiretroviral naive patients with >200 CD_4^+ lymphocytes/μL and a mean HIV RNA level of 68,000 copies/mL were randomized to receive zidovudine monotherapy (200 mg *tid*), ritonavir monotherapy (600 mg *bid*) or a combination of both drugs (Table 6) [77]. Surprisingly, significant differences favouring ritonavir monotherapy over combination therapy were reported. However, analysis of ritonavir plasma concentrations revealed that these were higher in patients receiving monotherapy compared to combination therapy. This was attributed to poor compliance in the combination therapy arm.

Ritonavir (1200 mg) was combined with zidovudine (600 mg) and zalcitabine (2.25 mg/day) in an open-label study in 29 antiretroviral naive patients with <250 CD_4^+ lymphocytes/μL (Table 6) [84]. The maximum decline in HIV RNA load and maximum increase in CD_4^+ lymphocyte count was observed after 5 months of therapy. The ^{10}log value of infectious blood cells/10^7 PBMCs reached a maximum decline (-2.44 ^{10}log units) after 9 months of therapy.

The safety and efficacy of triple combination therapy with ritonavir (600 mg bid), zidovudine (300 mg bid) and lamivudine (150 mg bid) were examined in an open-label, randomized, two-arm study [85]. Patients started with either all three drugs simultaneously, or with ritonavir monotherapy for three weeks, followed by the addition of zidovudine and lamivudine. As the rate of mutations is theoretically dependent on the rate of viral turnover, zidovudine and lamivudine were administered after viral load had been suppressed by ritonavir in an attempt to decrease the risk of developing resistance to lamivudine. Eligible patients had to be antiretroviral naive and had CD_4^+ lymphocyte counts ≥50 cells/μL and a HIV RNA load ≥30,000 copies/mL. Tonsillar biopsies were taken to compare tissue and plasma viral load. Results after 12 weeks of follow-up are summarized in Table 6. No statistically significant difference in decrease in HIV RNA load or increase in CD_4^+ lymphocyte counts was observed. In both treatment groups viral load became undetectable in 80% of the patients after 16 weeks of therapy [86]. Preliminary results indicate that HIV RNA load in tonsillar biopsies became undetectable in 4 patients after 24 weeks of therapy. Combination therapy with ritonavir plus saquinavir has been discussed before (Table 2).

Ritonavir in primary HIV infection

Combination therapy with ritonavir (600 mg *bid*) zidovudine (200 mg *tid*) plus lamivudine (150 mg *bid*) was started in 12 patients with acute HIV infection [87]. After 28 to 240 days of therapy viral load became undetectable in 11 of 12 patients. In all patients viral load became undetectable by PBMC coculture. Therapy is now planned for a minimum of 1 year, after which lymphoid tissue will be assessed for presence of active viral replication.

Adverse effects

In a phase I/II study of ritonavir in a dosage of 300, 400, 500, or 600 mg *bid*, 85 to 100% of the patients reported at least one adverse event [78]. The most pronounced adverse events were nausea, circumoral paraesthesia, and elevated levels of hepatic enzymes.

Asymptomatic elevation of cholesterol (30 to 40%) and triglycerides (200 to 300%) persisted through the 32 weeks of this study. Elevation of ASAT and ALAT were seen at the beginning of the study.

Resistance to ritonavir

Complete *in vitro* cross-resistance between indinavir and ritonavir has been reported [69]. Cross-resistance with saquinavir, however, appears to be relatively rare. Genotypic changes with wild-type valine of codon 82 changing to alanine or phenylalanine are observed in clinical studies [78]. With higher doses of ritonavir breakthrough in HIV replication is delayed in many patients. At lower doses, however, a more rapid return to baseline HIV RNA load is observed. The loss of effect is linked to stepwise, ordered accumulation of mutations in the protease gene [88]. High plasma concentrations of ritonavir appear to delay the onset of resistance by suppressing viral replication.

Dosage and administration

The currently recommended dosage of ritonavir is 600 mg *bid*, either alone or in combination with nucleoside analogues. Due to the induction of its own metabolism [79], it is now recommended to increase ritonavir doses over several days at start of therapy. A widely accepted regimen is 300 mg *bid* (3 days), 400 mg *bid* (3 days), and 500 mg *bid* (3 days). Thereafter, ritonavir is administered in the recommended regimen of 600 mg *bid*. Ritonavir should be taken with a meal. Ritonavir is available as 100 mg capsules. It is also available as a solution (80 mg/mL). Ritonavir capsules and solution should be kept between 2-8 °C, but can be kept at room temperature for 7 days. Whether reduction of the dosage of ritonavir is necessary in patients with liver insufficiency is not clear yet.

Pharmacokinetics

The pharmacokinetics of ritonavir were studied in two groups of HIV-infected patients [89]. Ritonavir was administered under fasting conditions in single oral doses of 100, 200, 400, 600, 800, and 1,000 mg. C_{max} and AUC of ritonavir increased non-linearly with the mean normalized (100 mg) C_{max} and AUC increasing from 0.416 μg/mL and 3.480 μg*h/mL at 100 mg to 1.27 μg/mL and 12.31 μg*h/mL at 1,000 mg, respectively. The mean T_{max} ranged from 3.8 hours for 100 mg to 3.1 hours for 1,000 mg. The nonlinear increase of C_{max} and AUC was attributed to saturable first-pass metabolism. The major elimination pathway of ritonavir is by cytochrome P450-3A4 and, to a lesser extent, cytochrome P450-2D6 related metabolism [77]. Four metabolites have been identified in humans and only one, the isopropylthiazole oxidation metabolite, has been found in the systemic circulation and seems to be as active as the parent compound [77]. After oral administration, 20% to 40% of unchanged ritonavir is recovered in human faeces [77]. Renal clearance of ritonavir is less than 2 mL/min. The half-life of ritonavir is approximately 3 hours. Ingestion of 600 mg ritonavir with food increases the AUC by 63%, the C_{max} by 34%, and delays the T_{max} by 1.6 hours.

The pharmacokinetics of the currently recommended dose (600 mg *bid*) in 10 HIV infected patients have been characterized [78]. A C_{max} of 11.2 μg/mL after 3.3 h, an AUC of 60.8 μg*h/mL and a trough plasma concentration of 3.03 μg/mL were reported. The half-life of ritonavir was 3.2 h with an apparent clearance of the drug of 8.9 L/h. With this recommended dose regimen ritonavir plasma concentrations were above the targeted effective concentration (based on *in vitro* data, the functional 90% effective concentration, after adjustment for binding to protein, is 2.1 μg/mL) [78].

Ritonavir is approximately 99% bound to plasma proteins [78]. Limited data in patients showed that ritonavir is present in extremely low concentrations in the cerebrospinal fluid, reflecting the free concentration in plasma [77]. No effect on relative bioavailability was detected when ritonavir oral liquid formulation was administered with either water, Advera®, Ensure®, or chocolate milk in healthy volunteers [90]. The oral bioavailability in humans has not been reported. Ritonavir pharmacokinetics have not been studied in patients with hepatic or renal insufficiency. Subgroup analyses revealed a significant reduction of the AUC of 18% in smokers versus non-smokers. Another subgroup analysis of patients with high versus low body weight revealed that AUC values did not correlate with body weight [77].

Pharmacokinetic-pharmacodynamic relationships

In a phase I/II study sustained effects on CD_4^+ lymphocyte counts and viral RNA were observed only in patients in the highest ritonavir dose groups [78]. In these patients the mean trough concentration exceeded the target concentration of 2.1 μg/mL. Thus, a relationship between ritonavir trough concentration and effect on surrogate parameters might exist.

A relationship between low ritonavir trough concentrations and the emergence of viral strains resistant to the drug has been reported [91].

As discussed before, ritonavir monotherapy resulted in improved antiviral efficacy compared with ritonavir/zidovudine combination therapy [77]. When ritonavir plasma concentrations were assessed, it appeared that patients in the monotherapy arm had higher ritonavir plasma concentrations. This might indicate, that higher ritonavir plasma concentrations are correlated with superior effects on surrogate parameters.

These indications of pharmacokinetic-pharmacodynamic relationships suggest that monitoring of ritonavir pharmacokinetics may assist in achieving optimal antiretroviral therapy.

Drug interactions

The effect of multiple doses ritonavir on the pharmacokinetics of single doses of the combination trimethoprim and sulfamethoxazole has been investigated [92]. The AUC of sulfamethoxazole decreased with 19.8% in the presence of ritonavir, the half-life decreased by 18%, whilst no significant effect was observed on C_{max}. The AUC of the N-acetyl metabolite of sulfamethoxazole was decreased with 10.4%. The AUC of trimethoprim was increased by 19.9%, the half-life was increased with 20%, and the C_{max} did not differ when coadministered with ritonavir. The increased clearance of sulfamethoxazole might be caused by induction of N-glucuronidation by ritonavir. These minor changes in pharmacokinetics were considered to be not clinically relevant.

The effect of ritonavir on the pharmacokinetics of ethinyl estradiol has been studied in 23 healthy female volunteers [93]. The ethinyl estradiol C_{max} was decreased by 32%, the AUC was decreased with 41%, and the terminal half-life was increased with 31% when ritonavir was coadministered. These changes might be caused by the induction of glucuronidation and/or cytochrome P450 hydroxylation. Use of alternative contraceptive measures or doubling the contraceptive dose should be considered when ritonavir and oral contraceptives are concurrently used.

The pharmacokinetics of rifabutin and its active metabolite 25-O-desacetyl-rifabutin were assessed with administration of ritonavir or placebo in a double-blind, parallel group study [94]. Concurrent ritonavir dosing increases the C_{min}, C_{max}, and AUC of rifabutin by 6-, 2.5-, and 4-fold, respectively. The values for C_{min}, C_{max}, and AUC of the active metabolite increased by 200-, 16-, and 35-fold, respectively. These changes are probably caused by the inhibition of metabolism of both rifabutin and its active metabolite by ritonavir. Because of these increased plasma concentrations and of the increased risk of rifabutin-associated adverse effects (arthralgia, joint stiffness, uveitis, and leucopenia) [95], an alternative to rifabutin is recommended when ritonavir and rifabutin are coadministered.

The steady-state C_{max}, C_{min}, and AUC of theophylline decreases by 32, 57, and 43%, respectively, when ritonavir is coadministered [96]. The half-life of theophylline decreases from 8.4 h to 3.6 h. These effects are time-dependent, probably due to increased cytochrome P450-1A2 activity after multiple doses of ritonavir. Thus, increasing the theophylline dose and monitoring theophylline concentrations is necessary when ritonavir is coadministered.

The effect of multiple doses of ritonavir on the pharmacokinetics of single doses of desipramine and 2-hydroxy desipramine was investigated [97]. The AUC, half-life, and C_{max} of desipramine increased 2.45-, 2-fold, and with 22.1%, respectively. The 2-hydroxy metabolite to parent ratio for AUC and C_{max} decreased by 67% in both cases. The pharmacokinetics of ritonavir were not affected. Thus, it is advised to start with lower doses desipramine (or other tricyclic antidepressants) and to monitor tricyclic antidepressant concentrations when coadministered with ritonavir.

Coadministration of zidovudine (200 mg *tid*) and ritonavir (300 mg *qid*) showed no influence on ritonavir pharmacokinetics [77]. However, zidovudine C_{max} and AUC were reduced by 27% and 25%, respectively, when ritonavir was coadministered. A similar effect on didanosine C_{max} (16% reduction) and AUC (13% reduction) has been observed [77]. Though statistically significant, no dose adjustment of didanosine is advised.

Clarithromycin exposure was increased with concomitant ritonavir administration due to inhibition of its active metabolite formation [77]. The increase of the parent drug is, however, counterbalanced by decreased active metabolite formation. Thus, no dosage reduction of clarithromycin is necessary in patients with normal renal function. When coadministered with ritonavir, a maximum dose of 1 gram of clarithromycin is recommended.

The effect of fluconazole (400 mg on day 1, 200 mg on days 2 to 5) on ritonavir (200 mg *qid*) pharmacokinetics has been assessed [98]. Ritonavir C_{max} and AUC were significantly increased with coadministration of fluconazole. However, these changes were less than 15% and were not considered to be clinically relevant. Plasma half-life and T_{max} of ritonavir were not affected.

The pharmacokinetic interaction between ritonavir and saquinavir has been discussed before.

Furthermore, an extensive list of drugs that could lead to increased concentrations of the coadministered drug due to inhibition of their metabolism by ritonavir exists [99]. These drugs include alprazolam, amiodarone, astemizole, calcium-channel blocking agents, carbamazepine, ciclosporin, cisapride, clorazepate, dexamethasone, diazepam, encainide, ergotamine, erythromycin, flecainide, fluoxetine, flurazepam, itraconazole, ketoconazole, quinidine, loratadine, mefloquine, midazolam, paroxetine, pethidine, pimozide, piroxicam, prednisolone, propafenone, propoxyphene, sertraline, terfenazine, trazodone, triazolam, warfarin, and zolpidem.

Conclusion

Enormous efforts have been made in the search for drugs to treat HIV infection for over a decade now. After the crystal structure of the HIV-encoded protease enzyme had been elucidated, computer-aided drug design played a pivotal role in the development of new compounds that inhibit this viral enzyme that is responsible for HIV maturation and infectivity. Promising representatives of these compounds have recently found their way to patients.

Protease inhibitors show a powerful sustained suppression of HIV replication, especially when used in combination therapy regimens, and have already proven to be a valuable contribution to the armamentarium of drugs to treat HIV infection. Though these agents have become available only recently, combination therapy of a protease inhibitor plus two reverse transcriptase inhibitors has already become standard antiretroviral therapy in many countries. Protease inhibitors that are now available are saquinavir, indinavir and ritonavir. In this review issues involving clinical results, adverse effects, resistance, dosage and administration, clinical pharmacokinetics, pharmacokinetic-pharmacodynamic relationships, and drug-drug interactions are discussed.

Protease inhibitors have in common that drug compliance is of enormous importance; HIV strains with resistance to these compounds will develop inevitably in patients who do not take their medication as prescribed. Indications of pharmacokinetic-pharmacodynamic relationships have been found; low exposure to protease inhibitors may lead to suboptimal or absence of immunological and virological response. Furthermore, a vast amount of (potential) drug-drug interactions has been identified, making protease inhibitors a good candidate for pharmacokinetic drug monitoring in patients.

New representatives of this class of compounds are being evaluated in clinical trials and are likely to be licensed in the following years.

The importance of protease inhibitors in clinical practice and the characteristics of the different representa-

tives is likely to become more clear in the following years.

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