Eur J Med Res (2006) 11: 236-244

PHARMACOKINETICS AND TOLERABILITY OF A COMBINATION OF INDINAVIR, LOPINAVIR AND RITONAVIR IN MULTIPLY PRETREATED HIV-1 INFECTED ADULTS

N. von Hentig[1], A. Müller[2], C. Rottmann[2], T. Lutz[3], S. Klauke[4], M. Kurowski[5], S. Staszewski[2], S. Harder[1]

[1]Institute for Clinical Pharmacology, Frankfurt,
[2]HIV-Treatment and Research Unit at the J.W.Goethe University Hospital Frankfurt,
[3]Private Practice Grue-neburgweg,
[4]ifs Stresemannallee Frankfurt, 5Therapia GmbH Berlin, Germany

Abstract

Objectives: The authors evaluated the pharmacokinetics and tolerability of indinavir/lopinavir/ritonavir in a protease inhibitor only combination.

Methods: Plasma drug levels of patients taking indinavir/lopinavir/ritonavir 800/400/100mg twice daily (n = 24, group 1) were compared to patients taking either lopinavir/ritonavir 400/100mg (n = 35, group2) or indinavir/ritonavir 800/100mg (n = 33, group3) twice daily plus nucleos(t)ide reverse transcriptase inhibitors (NRTI). Steady-state drug concentrations were measured by LC/MS/MS. Minimum and maximum concentrations (C_{min}, C_{max}), area under the concentration-time curve ($AUC_{0\text{-}12h}$), total clearance (CL_{tot}) and half-life (t1/2) were calculated. HIV viral load, CD4 cell count and adverse events causing early termination of therapy were correlated over a period of 48 weeks.

Results: Plasma levels of lopinavir/ritonavir were significantly enhanced when combined with indinavir compared to a regimen of lopinavir/ritonavir+NRTI: Mean lopinavir $AUC_{(0\text{-}12h)}$ 80912 ng*h/mL vs. 60548 ng*h/mL; C_{min} 4633 ng/mL vs. 3258 ng/mL; C_{max} 8023 ng/mL vs. 6710 ng/mL. Mean ritonavir $AUC_{(0\text{-}12h)}$ 6907 ng*h/mL vs. 3467 ng*h/mL; C_{min} 220 ng/mL vs. 125 ng/mL; C_{max} 1059 ng/mL vs. 522 ng/mL. Indinavir levels were comparable for both indinavir containing regimen. A significantly smaller number of patients stopped indinavir/lopinavir/ritonavir therapy (group1: 16.7%) than indinavir/ritonavir + NRTI treatment (group3: 45.5%) due to adverse events. Virological failure was the main reason for early termination of treatment with indinavir/lopinavir/ritonavir before week 48 (group1: 50%)

Conclusions: indinavir/lopinavir/ritonavir 800/400/100mg twice daily represents a therapy option with an adequate safety but only short term efficacy for extensively pretreated patients.

Key words: Double protease inhibitor, pharmacokinetics, indinavir, lopinavir, ritonavir

INTRODUCTION

Antiretroviral therapy options for patients who no longer have treatment alternatives with reverse transcriptase inhibitors due to toxicity or viral resistance are limited. Some boosted double protease inhibitor therapy regimens have been evaluated and described as therapy alternatives for these patients. Lopinavir/saquinavir [1-4] and atazanavir/saquinavir [5, 6] used low-dose ritonavir as pharmacoenhancer. Nevertheless, potential pharmacokinetic interactions might limit their use, as most protease inhibitors are substrates of the same cytochrome P450 liver pathways. They are metabolized by these enzymes and inhibit or induce them to varying degrees [7, 8]. Also autoinduction of cytochromes plays a role in exhibiting different plasma exposure over the first weeks of treatment, as it was demonstrated for ritonavir [9-11]. Combinations of lopinavir/ritonavir 400/100mg and amprenavir 600mg [12-15] or fosamprenavir 700mg twice daily [16, 17] demonstrated complex two-way pharmacokinetic interactions. If more than two protease inhibtors are coadministered it is difficult to predict drug-drug interactions in such combinations. Furthermore therapeutic options of boosted dual-PI regimen are often limited by resistance or toxicity [18, 19].

The goal of this observational study was to evaluate the steady state pharmacokinetic interactions of a combination of lopinavir/ritonavir 400/100mg and indinavir 800mg twice daily as a protease inhibitor therapy in the absence of either nucleos(t)ide or non-nucleoside reverse transcriptase inhibitors. It was conducted in patients, who were treatment-experienced and had lost therapy options with nucleoside or non-nucleoside reverse transcriptase inhibitors due to toxicy or viral resistance. All compounds are established protease inhibitors with well described pharmacokinetics, safety profile and efficacy for different dosing regimens. Indinavir has been approved unboosted for a thrice daily intake of 800mg and boosted for a twice daily dosage of indinavir/ritonavir 800/100mg [20] and ritonavir

boosted lopinavir has been approved in a single formulation for the twice daily dosage of lopinavir/ritonavir 400/100mg [21]. Pharmacokinetic interactions of lopinavir/ritonavir with indinavir 400 or 600mg [22, 23] have been studied as well as a combination of lopinavir/ritonavir 400/100mg with 800mg indinavir in a small number of patients [24].

Methods

Patients

The observational study was performed in adult HIV-infected outpatients. Patients in group 1 (n = 24) were treated with lopinavir/ritonavir 400/100mg and indinavir 800mg twice daily without the addition of nucleoside/nucleotide or non-nucleoside reverse transcriptase inhibitors. Two groups of patients who were predominantly antiretroviral treatment-naïve or less therapy experienced served as controls. Group 2 (n = 35) consisted of patients, taking lopinavir/ritonavir 400/100mg plus 2-3 nucleoside/nucleotide reverse transcriptase inhibitors and patients in group 3 (n = 33) received indinavir/ritonavir 800/100mg twice daily also in combination with 2-3 nucleoside/nucleotide reverse transcriptase inhibitors.

All three groups included patients at any CD4 cell count or viral load at baseline of therapy. Protease inhibitor therapy-experienced patients were elected for the indinavir/lopinavir/ritonavir therapy regimen after interpretation of a genotypic resistance testing, showing sufficient sensitivity of the HIV to the therapy compounds [25]. Patients completed a 12h-pharmacokinetic measurement following a standardized pharmacokinetic protocol [1]. Routine liver function tests assured that patients with hepatic impairment were excluded from the analyses as well as subjects with comedication expected to inhibit or induce CYP3A4 metabolism. All data were obtained as part of the therapeutic drug monitoring (TDM) which is regularly performed in the medical HIV treatment and research unit. Verbal consent for the TDM procedure was obtained from patients and documented in the patients records. This study design is observational, no additional intervention was performed and ethics approval was not obtained according to the National Medical Act and the advice of the responsible ethics committee.

Study Protocol

After at least two weeks on the regimen (median 4 weeks for group 1 and 2, 12 weeks for group 3 respectively) patients underwent a pharmacokinetic assessment following a standardized protocol at steady state conditions. The schedule of drug intake was documented by the patients for 6 half-lifes prior to the pharmacokinetic assessment. In addition all concomitant drug intake had to be documented by the patient and physician, including daily intake of herbal agents or nutritive supplements. At the day of the pharmacokinetic assessment fasting trough levels were obtained immediately before drug intake, followed by a standardized continental breakfast. Plasma samples were then collected at 1; 2; 4; 6; 9; 12 hours after the drug intake, while patients remained in-house.

Pharmacokinetic Assay

Lopinavir, indinavir and ritonavir plasma concentrations were determined by liquid chromatography-tandem mass spectrometry methods (equipment from Merck-Hitachi, Germany at Therapia GmbH Berlin, Germany). The lower limit of quantification (LLQ) was 20ng/mL, linearity was proven up to 20000 ng/mL [26].

Pharmacokinetic Evaluation

Pharmacokinetic calculations based on plasma concentrations which were above the LLQ. C_{min} and C_{max} values were read directly from the plasma concentration-time curves of lopinavir, indinavir and ritonavir within the standard dosing interval (τ = 12 hours). The following pharmacokinetic parameters were obtained by using a non-compartmental analysis model: The AUC (τ) = AUC_{ss} (0,12) is the area under the concentration-time curve at steady state conditions from time zero (trough) over the time span of the dosing interval t = 12h, obtained with the logarithmic trapezoidal rule. The total clearance of lopinavir, indinavir and ritonavir was determined by CL tot = D/AUC(t) assuming complete bioavailability. This implies that changes in the bioavailability by interaction between the compounds could not be differentiated from a reduction in the clearance by other causes. The elimination half-life $t_{1/2}$ is calculated from the elimination constant λ_z with the equation

$$t_{1/2} = \frac{\ln^2}{\lambda_z} = \frac{0.69315}{\lambda_z}$$

(time) at steady state conditions. All pharmacokinetic analyses were performed with TOPFIT2.0® [27].

The pharmacokinetic evaluation followed two major questions: First of all we calculated the difference of steady state pharmacokinetic parameters between the three therapy regimens in order to evaluate potential interactions between the applicated drugs. Then we compared the pharmacokinetic parameters of responders and non-responders to therapy and patients who stopped therapy due to the experience of drug related adverse events before week 48. Response to therapy was defined as a sustained increase in CD4 cell count and a minimum decrease in viral load of $>2\log_{10}$ over 48 weeks.

Statistical Methods

Pharmacokinetic parameters were subject to descriptive and exploratory statistics (mean, standard deviation). Primary target variables were the AUC_{ss} (τ), C_{max}, C_{min}, CL_{tot} and $t_{1/2}$ of lopinavir, indinavir and ritonavir. The statistical analysis was based on the comparison of the primary target variables with the T-test for the absolute difference between the groups, including the Levene variance ratio test for the equality of group means. Absence of a significant difference between lopinavir, indinavir and ritonavir exposure be-

tween groups was suggested when no significant difference according the T-test, together with a 95% confindence interval including 0, was determined. SPSS 11.5 for Windows was used for the statistical analyses [28, 29].

Results

Disposition of Patients

The demographics and characteristics of patients at therapy baseline were comparable for all groups, except parameters correlated with pretreatment history (see Table 1). Mean age was between 41 and 42 years. The majority was male and caucasian with a comparable bodyweight and CDC-status. Baseline viral load and CD4 cell count differed between the groups according to the variability of length and history of pretreatment: Baseline CD4 cell count exhibited a mean of 129/µl, 215/µl and 196/µl for the groups 1, 2 and 3. Baseline viral load showed a reciprocal relation with a mean $\log_{10}$ of 4.9,4.3 and 4.76 respectively. Clinical and laboratory tests did neither detect markedly elevated liver enzymes nor signs of cirrhosis in any patient included in the analyses.

In contrast to the control groups, most of the patients of the indinavir/lopinavir/ritonavir group 1 were multiply pretreated patients. Patients in group 1 showed a mean of 10 previous treatments versus 3 previous treatments in group 2 and a majority of treatment naïve patients in group 3. The majority of patients in group 1 experienced prior treatment failure and no patient was treatment naïve at the beginning of double protease inhibitor treatment. In contrast, 45.7 % of patients in group 2 and 75.8 % in group 3 received their first-line therapy in this study.

The concomitant reverse transcriptase inhibitors which have been taken in both control groups include zidovudin (group 2 n = 18; group 3 n = 19), lamivudin (group 2 n = 31; group 3 n = 27), didanosin (group 2 n = 2;group 3 n = 3), abacavir (group 2 n = 4; group 3 n = 4), tenofovir-DF (group 2 n = 11; group 3 n = 7) and stavudin (group 2 n = 4; group 3 n = 3).

Pharmacokinetics

Table 2 presents the pharmacokinetic parameters of lopinavir, indinavir and ritonavir for the different therapy regimens (groups 1, 2 and 3). The plasma exposure of lopinavir exhibits significant differences between groups 1 and 2 over the entire dosing interval, when coadministered either with indinavir/ritonavir or with ritonavir and nucleoside/nucleotide reverse transcriptase inhibitors (Table 2, Fig. 1a). The mean lopinavir AUC_{ss} was 80912 ng*h/mL in group 1 versus 57395 ng*h/mL in group 2 (difference = +41.0%; p = 0.001). Also mean C_{max} (8023 ng/mL for group 1 versus 6451 ng/mL for group 2, +24.4%, p = 0.030), C_{min} (4633 ng/mL for group 1 versus 3056 ng/mL for group 2, +51.6%, p = 0.004), $T_{1/2}$ (12.95 h in group 1 versus 8.9 h in group 2, +45.5, p = 0.010) and CL tot (94 mL/min for group 1 versus 132 mL/min for the group 2, -28.8%, p = 0.002) were significantly different in both groups.

Indinavir plasma levels did not exhibit considerable differences between groups 1 and 3. Mean AUC_{ss} was 37617 ng*h/mL for group 1 versus 38607 ng*h/mL for group 3 (difference = +2.6%), C_{max} was 5777 ng/mL for group 1 versus 5754 ng/mL for group 3 (+0.4%), C_{min} was 839 ng/mL for group 1 versus 931 ng/mL for group 3 (-9.9%) Also $t_{1/2}$ and CL_{tot}. were comparable between both groups (Table 2, Fig. 1b).

Table 1. Patients baseline characteristics.

	IDV/LPV/RTV group 1 (n = 24)	LPV/RTV + NRTI group 2 (n = 35)	IDV/RTV + NRTI group 3 (n = 33)
parameter	mean (±SD) [%]	mean (±SD) [%]	mean (±SD) [%]
male / female	21/ 3 [87.5 /12.5]	29/6 [82.9/17.1]	23/10 [69.7/20.3]
caucasian/other	21/ 3 [86.7/14.3]	30/5 [85.7/14.3]	30/3 [90.9/9.1]
age	41 (±7)	42 (±7)	41 (±9)
CDC[1] C3	13 [54.2]	18 [51.4]	11 [33.3]
B3	6 [25.0]	5 [14.3]	9 [27.3]
A3	3 [12.5]	8 [22.9]	9 [27.3]
A2 or B2	2 [8.3]	4 [11.4]	4 [12.1]
bodyweight	72 (±10)	71 (±13)	73 (±13)
CD 4	129 (±124)	215 (±190)	196 (±168)
log 10 HI-Viral Load	4.90 (±1.06)	4.30 (±1.71)	4.76 (±1.44)
previous treatments	10.3 (±7.5)	2.6 (±3.7)	1.0 (±2.0)
previously taken PI	2.5 (±2.08)	0.7 (±0.8)	0.3 (±0.6)
treatment naive	0	16 [45.7]	25 [75.8]

[1]Classification according to the Center for Disease Control and Prevention

Table 2. Summary of the mean (±SD) steady state pharmacokinetic parameters for lopinavir, indinavir and ritonavir including mean ratio (MR) and p-value for the difference between the groups.

	Lopinavir			**Indinavir**			**Ritonavir**					
	group 1 (+IDV/RTV)	group 2 (+RTV/NRTI)	difference gr 1 vs 2	group 1 (+LPV/RTV)	group 3 (+RTV)	difference gr 1 vs 3	group 1 (+LPV/IDV)	group 2 (+LPV)	group 3 (+IDV)	difference gr 1 vs 2	difference gr 1 vs 3
Parameter	mean (±SD)	mean (±SD)	% p-value[a]	mean (±SD)	mean (±SD)	% p-value[a]	mean (±SD)	mean (±SD)	mean (±SD)	% p-value[a]	% p-value[a]
C_{min} (ng/mL)	4633 (±2326)	3056 (±1769)	51.6 .004	839 (±531)	931 (±838)	-9.9 n.s.	220 (±143)	118 (±84)	517 (±433)	86.4 .004	-57.5 .001
C_{max} (ng/mL)	8023 (±2688)	6451 (±2636)	24.4 .030	5777 (±2378)	5754 (±1940)	0.4 n.s.	1059 (±1061)	519 (±426)	1735 (±862)	104.0 .025	39.0 .010
AUC_{ss} (ng*h/mL)	80912 (±29164)	57395 (±23179)	41.0 .001	37617 (±15140)	38607 (±15739)	2.6 n.s.	6907 (±5055)	3394 (±2447)	14707 (±8212)	103.5 .004	-53.0 <.001
CL_{tot} (mL/min)	94 (±36)	132 (±57)	-28.8 .002	376 (±164)	420 (±211)	-10.5 n.s.	352 (±222)	919 (±928)	162 (±93)	-61.7 .001	117.3 <.001
$T_{1/2}$ (h)	12.95 (±6.54)	8.90 (±3.80)	45.5 .010	3.52 (±0.99)	3.16 (±1.34)	11.4 n.s.	4.32 (±1.54)	5.24 (±2.15)	5.04 (±4.37)	-17.6 .079	14.3 n.s.

Fig. 1. (a) Mean ± SD plasma concentration-time profiles for lopinavir (LPV) 400mg BID in the presence of indinavir/ritonavir (IDV/RTV) 1000/100mg BID or ritonavir plus nucleoside/nucleotide reverse transcriptase inhibitor (NRTI)
(b) Mean ± SD plasma concentration-time profiles for indinavir (IDV) 1000mg BID in the presence of lopinavir/ritonavir (LPV/RTV) 400/100mg BID or ritonavir 100mg BID plus nucleoside/nucleotide reverse transcriptase inhibitor (NRTI).
(c) Mean ± SD plasma concentration-time profiles for ritonavir (RTV) 100mg BID in the presence of lopinavir/indinavir (LPV/IDV) 400/1000mg BID or either lopinavir 400mg BID or indinavir 1000mg BID plus nucleoside/nucleotide reverse transcriptase inhibitors (NRTI).

In contrast, ritonavir plasma levels (Table 2, Fig. 1c) showed significant differences between all groups. The ritonavir AUC_{ss} of group 1 was 6907 ng*h/mL versus 3394 ng*h/mL for group 2 (difference = +103.5%, $p<0.001$) and 14707 ng*h/mL for group 3 (+53.0%, $p<0.001$). Ritonavir C_{min} was 220ng/mL for group 1 versus 118 ng/mL for group 2 (+86.4%, p = 0.004) and 517 ng/mL for group 3 (-57.5, p = .001). C_{max} reflected a comparable correlation and was 1059 ng/mL for group 1 versus 519 ng/mL for group 2 (+104.0%, p = 0.025) and 1735 ng/mL for group 3 (-39.0%, $p<0.010$).

Efficacy of the Indinavir/Lopinavir/Ritonavir Therapy regimen

Regarding response, non-response and early termination of therapy, we found the following results: The majority of patients in the lopinavir/indinavir/ritonavir group (66.7%) did not complete the 48 week period of treatment. Reasons for discontinuation of treatment were virological failure (50%) and the experience of adverse events (16.7%). In the lopinavir/ritonavir and NRTI group the majority of patients (71.4%) completed 48 weeks of treatment. Five patients who were protease inhibitor pretreated (14.3%) discontinued treatment before week 48 due to virological failure and two patients (5.7%) experienced adverse events leading to early termination of therapy. Two patients discontinued therapy due to non-compliance (at week 12 and 24 respectively) and one patient died of a non- disease related reason (at week 44). In contrast to these results 17 patients (51.5%) of the indinavir/ritonavir plus NRTI group did not complete a 48-week period of therapy and in 15 cases (45.5%) adverse events were the reason for an early termination of treatment.

We could not find a correlation between drug plasma exposure of any of the measured drugs and virological failure of therapy in our study groups. The analysis of the lopinavir/indinavir plasma exposure of group 1 showed no differences between responders (n = 8, 33.3%) and non-responders (n = 12, 50%): Mean lopinavir AUC_{ss} was 83503 vs. 78319 ng*h/mL (-6.2%), C_{min} 4549 vs. 4718ng/mL (+3.6%) and C_{max} were 8209 vs. 7836 ng/mL (+4.8%). Mean indinavir AUC_{ss} was 38810 vs. 36423 ng*h/mL (-6.2%), C_{min} 828 vs. 851ng/mL (+2.8%) and C_{max} were 6041 vs. 5513ng/mL (-9.6 %). Despite high protease inhibitor plasma levels, most of the non-responders emerged in group 1. The evolution of CD4 cell count and viral load is described by Figures 2a and 2b in the as-treated analysis.

Safety of the Indinavir/Lopinavir/Ritonavir Therapy regimen

Although the plasma levels of indinavir were comparable, the number of patients discontinuating therapy before week 48 due to therapy-related adverse events

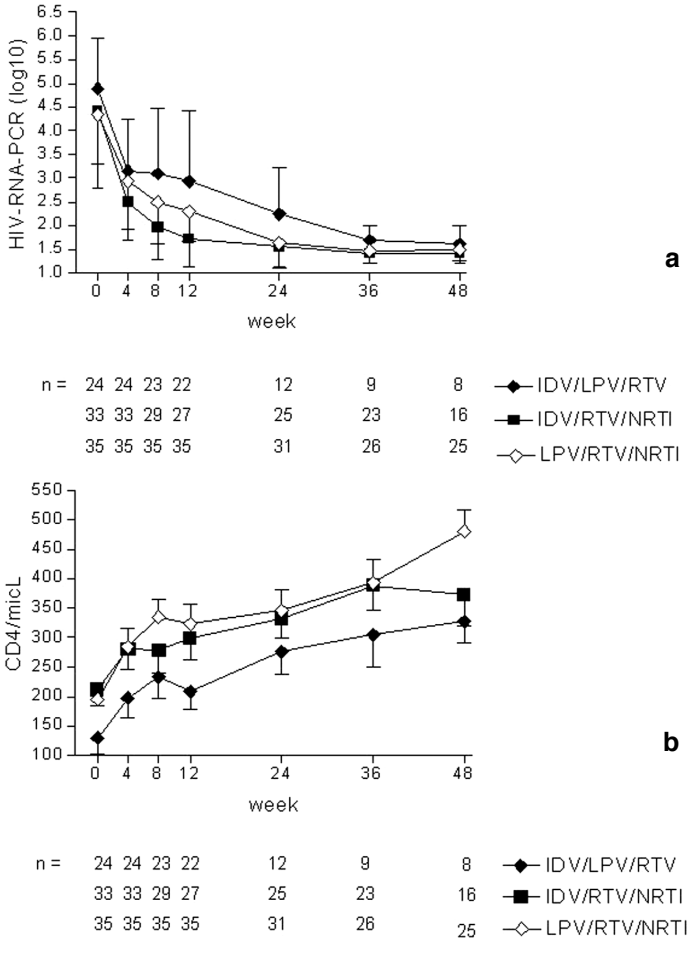

Fig. 2. (a) Mean (±SD) viral load decline in the as-treated analysis.
(b) Mean (±SD) CD4 cell count in the as-treated analysis.

differed markedly between group 1 (indinavir/lopinavir/ritonavir: n = 3, 12.6%) and group 3 (indinavir/ritonavir plus NRTI; n = 15, 45.5%). Differences were found between the indinavir plasma drug levels of patients who discontinuated treatment due to emerging adverse events and patients completing 48 weeks of teatment in group 3 only: Mean indinavir AUC_{ss} was 44766ng*h/mL vs. 32721ng*h/mL (difference = +36.8%; p = 0.036) and C_{max} 6597ng/mL vs. 4912ng/mL (+34.3%; p = 0.014). Differences in group 1 concerning the plasma exposure inpatients discontinuating treatment were non-significant: Mean indinavir AUC_{ss} was 42724ng*h/mL vs.36595 ng*h/mL (difference = +16.7%) and C_{max} 6888ng/mL vs. 5554ng/mL (+24%). Also ritonavir plasma concentrations were not significantly different between patients who discontinuated treatment due to adverse events or those who completed 48 weeks of treatment (mean AUC_{ss} 16369ng*h/mL vs. 13649ng*h/mL, difference = +20.0%; mean C_{max} 1974ng/mL vs. 1550ng/mL, +27.3%; mean C_{min} 678ng/mL vs. 399ng/mL, +70.0%, p = 0.08). In addition there was no correlation detected between lopinavir levels and premature discontinuation of therapy.

Emergence of Severe Adverse Events

The emergence of severe adverse events leading to the early termination of treatment differed markedly between the compared regimens. 4 patients (16.7%) of the indinavir/lopinavir/ritonavir group discontinued treatment due to skin-related adverse events such as xerodermia (n = 2; 8.3%), panaris (n = 1; 4.2%) and alopecia (n = 1; 4.2%). Only 2 patients (6.0%) taking lopinavir/ritonavir plus NRTI discontinued therapy showing up with a skin rash (n = 1; 3.0%) and continuous diarrhea (n = 1; 3.0%). Whereas 15 patients (45.5%) taking indinavir/ritonavir plus NRTI stopped the treatment due to skin rash (n = 3; 8.6%), xerodermia (n = 2; 5.7%), hyperbilirubinemia (n = 1; 2.9%), increase of creatinine (n = 1; 2.9%), continuous vomiting (n = 4; 11.4%), kidney stone or renal colic (n = 2; 5.7%), lipodystrophy (n = 1; 2.9%) or sexual dysfunction (n = 1; 2.9%).

Discussion

To date, a number of pharmacokinetic studies on different combinations of boosted protease inhibitors have been performed, either with or without the addition of reverse transcriptase inhibitors. Therapy regimens combining amprenavir and lopinavir/ritonavir have resulted in complex pharmacokinetic interactions, so that dose adjustments were recommended [14, 21, 23, 30, 31]. The ACTG study A5143 was even closed to enrolement because pharmacokinetic data showed a significant decrease of plasma levels of lopinavir and amprenavir in a combination of lopinavir/ritonavir 400/100mg and fosamprenavir 700mg twice daily [16]. Also the new protease inhibitor tipranavir decreased plasma levels of lopinavir, ritonavir, saquinavir and amprenavir significantly [32]. In contrast, a boosted double protease inhibitor combination of saquinavir/lopinavir/ritonavir 1000/400/100mg exhibited no influence of either saquinavir on lopinavir and vice versa [1, 4]. And therapies containing atazanavir, saquinavir and ritonavir turned out with pharmacokinetic data, suggesting their benefit for patients who need to achieve high plasma drug levels [5, 6]. We also took into acount, that the influence of indinavir on CYP3A4 has been described, exhibiting a considerable inhibitory potency (K_i for CYP 3A4 = 0.17 ± 0.01 microM) which may be combined in a double protease inhibitor therapy with the low-dose ritonavir boosting effect [7, 33]. As a result, our observational study investigated the pharmacokinetic and pharmacodynamic interactions within a boosted double protease inhibitor therapy of lopinavir/ritonavir 400/100mg plus indinavir 800mg twice daily in the absence of reverse transcriptase inhibitors in therapy experienced patients.

Lopinavir/Ritonavir Levels Are Enhanced when Combined with Indinavir

The key finding of the pharmacokinetic analysis was the significant enhancement of plasma levels of lopinavir and ritonavir, when coadministered with indinavir, while the overall plasma concentrations of indinavir showed no differences when administered with or without lopinavir. Kilby et al. have described the correlation between ritonavir doses of 100, 200 or 400mg and protease inhibitor boosting effect in volunteer pharmacokinetic drug interaction studies. A strong effect of any dose of ritonavir on C_{max} and C_{min} of saquinavir but no greater effect of higher versus lower ritonavir dosages on either parameter was found [34]. This agrees with previously published data, underscoring the dissociation between plasma exposure of ritonavir and pharmacoenhancing effect on saquinavir and lopinavir. Even very low ritonavir plasma concentrations sufficiently enhanced saquinavir and lopinavir plasma concentrations [1, 35]. Therefore we can assume that higher lopinavir levels in combination with indinavir and ritonavir (800/100mg twice daily) are due to the fact that indinavir itself works as a second booster [34] for lopinavir (1,37–fold plasma exposure in the presence of indinavir) rather than the elevated ritonavir plasma exposure. As the twice daily intake of indinavir 400mg in combination with lopinavir/ritonavir 400/100mg described by Isaak et al. did not significantly alter the median lopinavir PK parameters [22], the higher indinavir dose of 800mg may have had an impact on lopinavir plasma exposure in our study population.

Also ritonavir plasma exposure exhibited significant differences between all three groups. The comparably low ritonavir levels in combination with lopinavir (group 2) are well described [1, 15, 16, 35-38]. As expected ritonavir plasma levels were significantly higher when combined with lopinavir/indinavir or indinavir alone compared to a combination with lopinavir and NRTI. This leads to the assumption, that low ritonavir levels result from a CYP 3A4 enzyme induction by lopinavir which is partially superseded by the inhibiton of the same enzyme by indinavir. Even so low ritonavir levels sufficiently enhance indinavir plasma exposure in patients.

Boosted Double Protease Inhibitor Therapies

Focusing the discussion on boosted double protease inhibitor therapies it should be taken into account, that the interactions of more than two protease inhibitors are hard to predict. This is not only due to the fact that all protease inhibitors are substrates of CYP450. Also yet unknown mechanisms of interaction of the cytochromes or drug transporters in the gastrointestinal mucosa or the target tissues may play a role, possibly influencing resorption and distribution of lopinavir and indinavir in human compartments. A modulation of P-glycoprotein function by protease inhibitors has allready been shown in human lymphocytes and cell-monolayers [39] and the inhibition of P-glycoprotein or multidrug resistance-associated proteins increased the saquinavir brain uptake in mice significantly [40]. And it has been described that plasma concentrations of some protease inhibitors as substrate of P-gp are influenced by either genotypes encoding P-gp expression in human cells or P-gp inhibition by comedication [39-46]. As mentioned before there are various examples for drug-drug interactions in protease inhibitor combinations, which were described but finally not yet explained.

Correlations between Indinavir/Ritonavir Plasma Exposure and Adverse Events

Concerning possible pharmacodynamic effects of the pk-interactions, the increased levels of lopinavir, which could cause a higher rate of gatrointestinal side effects or elevated plasma lipids [12, 47-49] were expected to be a limitation of the treatment in combination with indinavir. This concern has indeed not been confirmed in this study. Regarding the markedly higher plasma exposure of lopinavir, it was not related to a significant occurrence of severe drug related adverse events: All four adverse events resulting in early termination of indinavir/lopinavir/ritonavir treatment were consistent with commonly reported indinavir-related adverse events (xerodermia, panaris and alopecia).

On the other hand plasma drug levels of indinavir showed differences between patients experiencing or not experiencing adverse events severe enough to stop treatment. Despite comparable mean indinavir plasma levels in both indinavir containing regimens, a significantly smaller number of patients stopped therapy due to adverse events in the indinavir/lopinavir/ritonavir group. This may be either a result of the increased acceptance of drug related adverse events by patients being treatment-experienced or due to the influence of the comedication with nucleoside/nucleotide reverse transcriptase inhibitors or ritonavir. Particularly as significantly higher ritonavir plasma levels were detected when coadministered together with indinavir and nucleoside reverse transcriptase inhibitors. Although most of the reported adverse events were presumably related to the exposure of indinavir this may have had an additional effect on the cumulation of drug related adverse events in this treatment group.

We have to be aware of the limitations of this study, which was not designed to prove a direct correlation between drug plasma exposure and the occurrence of severe adverse events in single patients. The pharmacokinetic assessment was performed around week 4 while the described adverse events occurred at any time during the following 44 weeks.

No Evidence of Correlation between Plasma Drug Levels and Efficacy

We could not find a correlation between drug plasma exposure of indinavir and lopinavir and virological failure of therapy in the boosted double protease inhibitor regimen. Rather than the -comparably high- plasma drug levels other factors may play a role in developing viral resistance in extensively pretreated patients such as the number of protease inhibitor resistance mutations allready existing as a result of prior treatment [50]. Replaced by protease inhibitor-susceptible virus at time of the beginning of therapy these virus strains may be reselected under a protease inhibitor only therapy.

Pharmacokinetic Studies in Outpatients

Although studies in outpatients are not common for the investigation of drug-drug interactions or pk/pd-correlations, this sampling strategy followed a standardized protocol, which was designed to assess comparable data from patients in an ambulatory setting. The sample size as well as the normal distribution of values in the tested groups points should have limited the influence of intraindividual variability on the results, which has been reported recently [51]. Therefore the interindividual range of plasma drug levels in this study is representative and was not rejected as a bias. Furthermore it points to the necessity of therapeutic drug monitoring and its potential benefit for the single patient [52, 53]. If warranted therapeutic drug monitoring should follow a standardized protocol and be repeated frequently.

Summary and Outlook

Keeping in mind the limitations of this observational study design and the comparably small number of participants we can carefully state that lopinavir/ritonavir 400/100mg plus indinavir 800mg twice daily is a boosted double protease inhibitor combination of virologically potent compounds with an adequate saftey but only short term efficacy for protease inhibitor treatment experienced patients. As the evaluated groups are not comparable regarding pretreatment history and clinical baseline data, purely descriptive data on viral load and CD4 cell count are presented. We could not find a correlation between plasma exposure of indinavir or lopinavir and virological success of the boosted double protease inhibitor therapy regimen. Higher lopinavir levels in this combination did not admittedly improve therapy outcome: Only 33.3 percent of patients finished a 48 week period of treatment. Virological failure occurred mostly between week 12 and 24 (n = 10,

41.7%), two cases were reported after week 36 (8.3%). This boosted double protease inhibitor therapy regimen must be seen in a historic context and must be compared to other boosted double protease inhibitor combinations [54] such as lopinavir/saquinavir/ritonavir or atazanavir/saquinavir/ritonavir which are available today.

REFERENCES

1. Stephan C, von Hentig N, Kourbeti I, Dauer B, Mösch M, Lutz T, Klauke S, Harder S, Staszewski S. Saquinavir drug exposure is not impaired by the boosted double protease inhibitor combination of lopinavir/ritonavir. Saquinavir drug exposure is not impaired by the boosted double protease inhibitor combination of lopinavir/ritonavir. AIDS 2004,18:503-508.
2. Staszewski S, Dauer B, Stephan C, Kurowski M, von Hentig N. A new strategy: boosted double protease inhibitor regimen lopinavir/r plus saquinavir without reverse transcriptase inhibitor. In: 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego, USA 2002,Abstract H-176.
3. Staszewski S, Dauer B, von Hentig N, Stephan C, Carlebach A, Mösch M, Gute P, Klauke S, Kurowski M, Stürmer M. The LopSaq Study: 24 week analysis of the double protease inhibitor salvage regimen containing lopinavir (LPV/r) plus saquinavir (SQV) without any additional antiretroviral tjherapy. In: 2nd IAS Conference on HIV Pathogenesis and Treatment, Paris, France 2003,Abstract 10290.
4. Ribera E, Lopez R, Diaz M, Pou L, Ruiz L, Falc V, Crespo M, Azuaje C, Ruiz I, Ocaxa I, Pahissa A. Steady -state pharmacokinetics of a double boosting regimen of saquinavir soft gel plus lopinavir plus mindose ritonavir in immunodefficiency virus-infected adults. Antimicrobial Agents and Chemotherapy 2004,48:4256-4262.
5. Boffito M, Kurowski M, Kruse G, Hill A, Benzie AA, Nelson MR, Moyle GJ, Gazzard BG, Pozniak AL. Atazanavir enhances saquinavir hard-gel concentrations in a ritonavir boosted once-daily regimen. AIDS 2004,18:1291-1297.
6. von Hentig NH, Mueller A, Haberl A, Lutz T, Knecht G, Kurowski M, Harder S, Staszewski S. Pharmacokinetic interactions of atazanavir (AZV) and saquinavir (SQV) in a ritonavir (RTV) boosted protase inhibitor therapy regimen. In: 15th World AIDS Conference, Bangkok, Thailand, eJAIDS 2004,6:WeOrB1235.
7. Eagling VA, Back D, Barry MG. Differential inhibition of cytochrome P450 iso-forms by the protease inhibitors ritonavir, saquinavir and indinavir. British Journal of Clinical Pharmacology 1997,44:190-194.
8. Molla A, Mo H, Vasavanonda S, Han L, Lin CT, Hsu A, Kempf DJ. In vitro interaction of lopinavir with other protease inhibitors. Antimicrobial Agents and Chemotherapy 2002,46:2249-2253.
9. Hsu A, Grannemann GR, Witt G, Locke C, Denissen J, Molla A, Valdes J, Smith J, Erdman K, Lyons N, Niu P, Decourt JP, Fourtillan JB, Girault J, Leonhard JM. Multiple-dose pharmacokinetics of ritonavir in human immunodefficiency virus-infected subjects. Antimicrobial Agents and Chemotherapy 1997,41:898-905.
10. Hsu A, Grannemann GR, Bertz RJ. Ritonavir. Clinical Pharmacokinetics and interactions with other anti-HIV agents. Clinical Pharmacokinetics 1998,35:275-291.
11. Quellet D, Hsu A, Qian J, Cavannaugh JH, Leonard JM, Grannerman GR . Effect of ritonavir on the pharmacokinetics of ethinyl oestradiol in healthy female volunteers. British Journal of Clinical Pharmacology 1998,46:111-116.
12. Raguin G, Chene G, Morand-Joubert L, Taburet AM, Droz C, Le Tiec C, Clavel F, Girard PM; Puzzle 1 Study Group. Salvage therapy with amprenavir, lopinavir and ritonavir 200mg/d or 400mg/d in hiv-infected patients in virological failure. Antiviral Therapy. 2004,9:615-625.
13. Khanlou H, Graham E, Brill M, Farthing C. Drug interaction between amprenavir and lopinavir/ritonavir in salvage therapy. AIDS 2002,16:797-798.
14. Mauss S, Scholten S, Wolf E, Berger F, Schmutz G, Jaeger H, Kurowski M, Rockstroh JK. A prospective, controlled study assessing the effect of lopinavir on amprenavir concentration boosted by ritonavir. HIV Medicine 2004,5:15-17.
15. Taburet AM, Raguin G, Le Tiec C, Droz C, Barrail A, Vincent I, Morand-Joubert L, Chene G, Clavel F, Girard PM. Interactions between amprenavir and the lopinavir-ritonavir combination in heavily pretreated patients infected with human immunodefficiency virus. Clinical Pharmacology and Therapeutics 2004,75:310-323.
16. Kashuba AD, Tierney C, Downey GF, Acosta EP, Vergis EN, Klingman K. Combining fosamprenavir with lopinavir/ritonavir substantially reduces amprenavir and lopinavir exposure : ACTG protocol A 5143 results. AIDS 2005,19:145-152.
17. Wire MB, Naderer OJ, Masterman AL, Lou Y, Stein DS. The pharmacokinetic interaction between GW433908 and lopinavir/ritonavir (APV 10011 and APV 10012). In: 11th Conference on Retroviruses and Opportunistic Infections, San Francisco, USA. 2004,Abstract 612.
18. Gallant JE. Strategies for long-term success in the treatment of HIV infection. JAMA 2000,283:1329-1334.
19. Montaner JS, Harrigan PR, Jahnke N, Raboud J, Castillo E, Hogg RS, Yip B, Harris M, Montessori V, O'Shaughnessy MV. Multiple drug rescue therapy for HIV-infected individuals with prior virologic failure to multiple regimens. AIDS 2001,15:61-69.
20. Merck&Co. Inc. Whitehouse Station U. Crixivan. Product Information. Obtained via Internet at www.crixivan.com 2002.
21. Abbott Laboratories Chicago U. Kaletra. Product information. 2003,Ref.: 03-5239-R8.
22. Isaac A, Taylor S, Cane P, Smit E, Gibbons SE, White DJ, Drake SM, Khoo S, Back DJ. Lopinavir/ritonavir combined with twice daily 400mg indinavir: pharmacokinetics and pharmacodynamics in blood, CSF and semen. Journal for Antimicrobial Chemotherapy 2004,54:498-502.
23. Harris M, Alexander C, Ting L. Rescue therapy with indinavir 600mg twice daily andf lopinavir/ritonavir: baseline resistance, virologic response and pharmacokinetics. In: 6th International Congress on Drug Therapy in HIV Infection. Glasgow, GB. 2002,Abstract P170.
24. Tseng A, Phillips E, Antoniou T. Steady-state pharmacokinetics and tolerability of indinavir when coadministered with lopinavir/r in antiretroviral experienced subjects. In: 4th International Workshop on Clinical Pharmacology of HIV Therapy. Cannes, France 2003,Abstract 8.10.
25. Sturmer M, Doerr H, Staszewski S, Preiser W. Comparison of nine resistance interpretation systems for HIV-1 genotyping. Antiviral Therapy 2003,8:239-244.
26. Kurowski M, Mueller M, Arasteh K, Moecklinghoff C. Simultaneous monitoring of five protease inhibtors in HIV-infected patients by LC-tandem mass spectrometry. In: 39th Interscience conference on Antimicrobial Agents and Chemotherapy. San Francisco, USA 1999,Abstract.
27. Heinzel G. TOPFIT 2.0 Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC,. Gustav Fischer Verlag, Stuttgart, Germany 1993.
28. Armitage P, Berry G, Matthews JNS. Statistical Methods in Medical Research. Blackwell Science Inc, Malden, USA 2002:p.105.
29. SPSS für Windows®. Version 11.5 (deutsch) 2004.

30. Reynolds HE, Gibbons SE, Tjia JF, Khoo SH and Back DJ. The pharmacokinetic interaction of lopinavir/ritonavir and amprenavir in clinical practice. In: 14th International AIDS Conference, Barcelona, Spain 2002,Abstract TuPeB4560.
31. GlaxoSmithKline. Lexiva Prescribing Information. Research Triangle Park, USA 2004,RL-2099.
32. Leith J, Walmsley S, Katlama C, Arasteh K, Pierone G, Blick G and BI1182.51 study team. Pharmacokinetics and Safety of Tipranavir/Ritonavir (TPV/r) Alone or in Combination with Saquinavir (SQV), Amprenavir (APV), or Lopinavir (LPV): Interim analysis of BI1182.51. In: 5th International Workshop on Clinical Pharmacology of HIV Therapy, Rome, Italy. 2004.
33. Abbott Laboratories CU. Norvir product information. Ref.: 03-2337-R17-Rev 2001.
34. Kilby JM, Hill A, Buss N ,. The effect of Ritonavir on saquinavir plasma concentration is independent of Ritonavir dosage: combined analysis of pharmacokinetic data from 97 subjects. HIV Medicine 2002,3:97-104.
35. Breilh D, Pellegrin I, Rouzes A, Berthoin K, Xuereb F, Budzinski H, Munck M, Fleury HJ, Saux MC, Pellegrin JL. Virological, intracellular and plasma pharmacological parameters predicting response to lopinavir/ritonavir (KALEPHAR study). AIDS 2004,18:1305-1310.
36. Crommentuyn KM, Mulder J, Mairuhu AT, van Gorp EC, Meenhorst PL, Huitema AD, Beijnen JH. The plasma and intracellular steady-state pharmacokinetics of lopinavir/ritonavir in HIV-1-infected patients. Antiviral Therapy 2004,9:779-785.
37. Canta F, Marrone R, Bonora S, D´Avolio A, Sciandra M De Rosa FG, Di Perri G. Pharmacokinetics and hepatotoxicity of lopinavir/ritonavir in non-cirrhotic HIV and hepatitis C virus coinfected patients. The Journal of Antimicrobial Chemotherapy 2005,55:280-281.
38. Vrijens B, Tousset E, Rode R, Bertz R, Mayer S, Urquhart J. Successful projection of the time course of drug concentration in plasma during a 1-year period from electronically compiled dosing-time data used as input to individually parameterized pharmacokinetic models. Journal of Clinical Pharmacology 2005,45:461-467.
39. Profit L, Eagling V, Back DJ. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. AIDS 1999,13:1623-1627.
40. Park S, Sinko PJ. P-glycoprotein and multidrug resistance-associated proteins limit the brain uptake of saquinavir in mice. Journal of Pharmacology and Experimental Therapeutics 2005,312:1249-1256.
41. Huisman MT, SJ, Crommentuyn KM, Zelcer N, Wiltshire HR, Beijnen JH,, AH. S.
Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. AIDS 2002,16:2295-2301.
42. Huisman MT Smit JW, Wiltshire HR, Beijnen JH, Schinkel AH. Assessing safety and efficacy of directed P-glycoprotein inhibition to improve
the pharmacokinetic properties of saquinavir coadministered with ritonavir. J Pharmacol Exp Ther 2003,304:596-602.
43. Mouly SJ, Paine MF, Watkins PB. Contributions of CYP3A4, P-glycoprotein, and serum protein binding to the intestinal first-pass extraction of saquinavir. J Pharmacol Exp Ther 2004,308.:941-948.
44. Owen A, Chandler B, Bray PG, Ward SA, Hart CA, Back DJ, Khoo SH. Functional correlation of P-glycoprotein expression and genotype with expression
of the human immunodeficiency virus type 1 coreceptor CXCR4. J Virol 2004,78:12022-12029.
45. Woodahl EL, Yang Z, Bui T, Shen DD, Ho RJ. MDR1 G1199A polymorphism alters permeability of HIV protease inhibitors across P-glycoprotein-expressing epithelial cells. AIDS 2005.
46. Sinko PJ, Kunta JR, Usansky HH, Perry BA. Differentiation of gut and hepatic first pass metabolism and secretion of saquinavir in ported rabbits. J Pharmacol Exp Ther 2004,310:359-366.
47. Martinez E, Domingo P, Galindo MJ, Milinkovic A, Arroyo JA, Baldovi F, Larrousse M, Leon A, de Lazzari E, Gatell JM. Risk of metabolic anormalities in patients infected with HIV receiving antiretroviarl therapy that contains lopinavir-ritonavir. Clinical Infectious Diseases 2004,38:1017-1023.
48. Gonzalez de Requena D, Blanco F, Garcia-Benayas T, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Correlation between lopinavir plasma levels and lipid abnormalities in patients taking lopinavir/ritonavir. AIDS Patient Care and STD´s 2003,17:443-445.
49. Gutierrez F, Padilla S, Navarro A, Masia M, Hernandez I, Ramos J, Esteban A, Martin-Hidalgo A. Lopinavir plasma concentrations and changes in lipid levels during salavge therapy with lopinavir/ritonavir-conatining regimens. Journal of Aquired Immune Deficiency Syndromes 2003,33:594-600.
50. Boffito M, Arnaud I, Raiteri R, Bonora S, Sinicco A, Di Garbo A, Reynolds HE, Hoggard PG, Back DJ, Di Perri G. Clinical use of lopinavir/ritonavir in a salvage therapy setting: pharmacokinetics and pharmacodynamics. AIDS 2002,16:2081-2083.
51. Nettles R, Kieffer T, Parsons T, Johnson J, Quinn T, Jackson B, Cofranesco J, Gallant J, Carson K, Siliciano R, Flexner C. Frequent sampling in virologically suppressed patients taking HIV protease inhibitors of nonnucleoside reverse transcriptase inhibitorss defines intraindividual pharmacokinetic variability. In: 12th Conference on Retroviruses and opportunistic Infections. Boston, USA 2005,[Abstract 642].
52. Back D, Blaschke T, Boucher C, Burger D, Fletcher C, Flexner C. Optimising TDM in HIV clinical care: a practical guide to performing therapeutic drug monitoring (TDM) for antiretroviral agents. www.hivpharmacology.com. 2003, Version 1.0:Accessed 14 March 2005.
53. Van Heeswijk RP, Veldkamp A, Mulder JW, Meenhorst PL, Lange JM, Beijnen JH, Hoetelmans RM. Combination of protease inhibitors for the treatment of HIV-1-infected patients: a review of pharmacokinetics and clinical experience. Antiviral Therapy 2001,6:201-229.
54. Boffito M, Maitland D, Samarasinghe Y, Pozniak A. The pharmacokinetics of HIV protease inhibitor combinations. Current Opinion in Infectious Diseases 2005,18: 1-7.

Received: March 6, 2006 / Accepted: April 12, 2006

Address for correspondence:
Dr. Nils von Hentig, MD
Institute for Clinical Pharmacology
J.W.Goethe University Hospital, Frankfurt
Theodor-Stern-Kai 7
D-60590 Frankfurt/M., Germany
Tel. Work: +49-69-63016956
Tel. Home: +49-162-2176333
Fax: +49-69-63017636
E-mail: Hentig@em.uni-frankfurt.de