Atazanavir and lopinavir/ritonavir: pharmacokinetics, safety and efficacy of a promising double-boosted protease inhibitor regimen

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Objective: To assess the pharmacokinetics and tolerability of lopinavir (LPV), ritonavir (RTV) and atazanavir (ATV) as a double-boosted protease inhibitor regimen in HIV-infected adults.

Methods: Sixteen patients who started LPV/RTV (400/100 mg b.i.d.) and ATV (300 mg q.d.) were enrolled in the study group (arm A). LPV pharmacokinetics were compared to those of two historical groups: arm B, 15 patients who received LPV/RTV (400/100 mg b.i.d.); and arm C, 25 patients who received LPV/RTV/saquinavir (SQV) (400/100/1000 mg b.i.d.). ATV pharmacokinetics were compared to those of 15 consecutive patients who received ATV and RTV (300/100 mg q.d.) (arm D). Drug concentrations were measured by HPLC.

Results: LPV concentrations were significantly higher in arm A than in arms B and C. Median (interquartile range) LPV area under the curve $(AUC)_{0-12}$ values were 115.7 (99.8–136.5), 85.2 (68.3–109.2) and 85.1 (60.6–110.1) μg/h/ml, respectively. C_{max} values were 12.2 (10.7–14.5), 9.5 (6.8–13.9) and 10.0 (6.9–13.6) μg/ml, respectively. C_{min} values were 9.1 (7.1–10.4), 5.6 (4.7–8.2) and 5.5 (4.2–7.5) μg/ml, respectively. No difference was observed for ATV AUC_{0-24} or C_{max} between arms A and D. ATV C_{min} values were 1.07 (0.61–1.79) in arm A and 0.58 (0.32–0.83) in arm D ($P = 0.001$). Treatment was not discontinued in any patient because of adverse effects. At 24 weeks, viral load was < 50 copies/ml in 13 of 16 patients.

Conclusions: The combination of ATV and LPV/RTV provided high plasma concentrations of both PI, which seemed to be appropriate for patients with multiple prior therapeutic failures, yielding good tolerability and substantial antiviral efficacy.

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Introduction

Developing safe and effective therapies for treatment-experienced patients on a failing regimen is an important objective for the management of patients with HIV infection [1,2]. The use of low-dose ritonavir (RTV) as a pharmacokinetic (PK) enhancer of other protease inhibitors (PI) has changed the management of HIV infection [3].

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Recently, triple PI regimens, including two active PI combined with RTV as an enhancing agent, have been examined in treatment-experienced patients [4–6]. Some of the potential pharmacological reasons for using double-boosted PI regimens are to provide additive or synergistic antiviral activity against HIV [7,8], to utilize PI combinations with non-overlapping or limited resistance profiles for patients with few treatment options, and to achieve high plasma levels of two PI for patients with extensive treatment histories and different resistance patterns among viral quasispecies, in the hope that each PI will retain activity against isolates that are resistant to the other.

Administration of two PI boosted with low RTV doses can produce complex drug interactions with unexpected results [9]. PK studies are thus required to ensure that therapeutic drug concentrations are being achieved in plasma. A huge decrease in the concentration of both PI has been described when lopinavir (LPV)/RTV is administered with amprenavir (APV) or fosamprenavir (FPV) [10–14]. This unfavourable reaction does not occur when LPV/RTV is administered with saquinavir (SQV) [15,16] or when SQV and RTV are administered with atazanavir (ATV) [17].

The fixed combination of LPV and low-dose RTV (Kaletra) facilitates simultaneous boosting of another PI, whereas the antiviral activity of LPV/RTV is often advantageous in the salvage setting; both factors make LPV/RTV an attractive agent for use in double-boosted PI combination regimens [18]. Given its low daily pill burden, modest CYP3A4 inhibition, different resistance profile and limited effects on lipid profiles, ATV appears to be a viable candidate to combine with LPV/RTV in double-PI regimens [19,20].

This study reports the safety and steady-state pharmacokinetics of LPV, RTV and ATV administered as a double-boosted PI combination in HIV-infected adults with multiple prior therapeutic failures. We also investigated whether the plasma pharmacokinetics of LPV and RTV are affected by co-administration of ATV and whether plasma pharmacokinetics of ATV are affected by co-administration of LPV/RTV.

Methods

Subjects and design

Non-randomized, open-label pilot study in which co-administration of LPV/RTV and ATV was evaluated in HIV-infected patients with few therapy options. Sixteen consecutive patients who started ARV therapy including a combination of LPV/RTV and ATV plus other ARV drugs were enrolled in the study (arm A: study group). All ARV drugs were chosen according to previous ARV treatments and viral susceptibility as determined by genotype. None of the patients were allowed to take any CYP450 inhibitors or inducers, or any gastric acid-reducing drugs within 14 days of enrolment in the study. LPV/RTV was administered orally at a dose of 400/100 mg twice daily. ATV was administered orally at a dose of 300 mg once daily. All subjects provided informed consent before any study procedures were undertaken.

LPV/RTV pharmacokinetics were compared with those of two recently published historical control groups of patients receiving LPV/RTV (400/100 mg b.i.d.): arm B included 15 consecutive patients participating in the ABT-378–ritonavir expanded access trial [21], receiving LPV/RTV (400/100 mg b.i.d.) and two nucleosides, arm C included 25 patients participating in the Retrogen study [15,22], who received LPV/RTV (400/100 mg b.i.d.) and SQV (1000 mg b.i.d.) and three nucleosides (didanosine EC, lamivudine and abacavir).

A concurrent comparison group of 15 consecutive patients who received ATV (300 mg q.d.) and RTV (100 mg q.d.) and two nucleosides were also enrolled (arm D).

Baseline data, including demographic data, prior AIDS-defining diseases according to 1993 Centers for Disease Control and Prevention classification, previous antiretroviral treatments, and genotyping data (in patients with a viral load > 1000 copies/ml), were obtained before starting the present salvage treatment. The safety and tolerability of the study medications was assessed throughout the study on the basis of clinical adverse events. World Health Organization toxicity grading scales were used to characterize abnormal analytical results (liver and kidney function tests, fasting blood lipids, haematology), and physical examination [23]. Study visits were scheduled at baseline and at weeks 4, 12, and 24. At each visit, CD4 cell count, HIV RNA and routine clinical examination and laboratory tests were performed.

Blood collection and drug concentration assays

Blood samples for the measurement of LPV, RTV, and ATV concentrations were collected at steady state, after at least 1 month of antiretroviral therapy. All subjects were instructed to take LPV/RTV and ATV at 9:00 a.m., and LPV/RTV at 9:00 p.m. with breakfast and dinner, respectively, during the week before the day of intensive pharmacokinetic assessment of drug concentrations. To assure that the dose was taken 24 h before the pre-dose analysis, patients filled a form with the exact time they took the preceding dose. On that day, patients came to the hospital between 8:15 and 8:45 a.m. after overnight fasting. Both study drugs were administered at the hospital at 9:00 a.m. with a standard breakfast. Blood samples were drawn before dosing and at 1, 2, 3, 4, 6, 8, and 12 h post-dosing. All samples were centrifuged at $1500 \times g$ for 20 min, and serum was stored at $-80^{\circ}C$ until assay.

Serum concentration-time data were analysed by non-compartmental methods. The area under the serum

concentration–time curve from 0 to 12 h (AUC_{0-12}) (LPV and RTV given b.i.d) or 0 to 24 h (AUC_{0-24}) (ATV and RTV given q.d.) was calculated by using the trapezoidal rule in the Abbottbase Pharmacokinetic Systems (Abbott Laboratories, Abbott Park, Illinois, USA). The highest serum concentration of the drugs (C_{max}), with the corresponding sampling time (T_{max}), the concentration before the morning dose (C_{trough}), and the lowest concentration (C_{min}) were determined directly from the concentration–time data. Since pre-dose ATV concentration was determined 24 h after the preceding dose, the value obtained at this time was also used for the 24 h post-dosing value in pharmacokinetic analysis ($C_{24} = C_0 = C_{trough}$).

Serum concentrations of ATV, LPV, and RTV were simultaneously measured with a sensitive, validated method developed in our laboratory [15], consisting of linear gradient reverse-phase ion-paired high performance liquid chromatography with ultraviolet detection at 220 nm (ATV, LPV) and 240 nm (RTV). Within-day and between-day variation of protease inhibitors (ATV, LPV and RTV) quality control samples in serum were 2.95% to 4.86% and 3.63% to 8.84%, respectively. Mean accuracy was 105.4, 108.8 and 106.7 for ATV, LPV and RTV respectively. The lower limit of quantification was 25 ng/ml for ATV, LPV and RTV. The assay was linear up to concentrations of at least 10 μg/ml. Our laboratory participates in an international external quality assurance program from the Netherlands [24].

Statistical analysis

SPSS software for Windows (version 12.0; SPSS, Chicago, Ill.) was used for statistical analyses. For quantitative variables, the medians and interquartile ranges (25th to 75th percentiles; IQR) were used as measures of central tendency and dispersion. The number of patients in each category and the corresponding percentages are given for qualitative variables. The between-group characteristics were compared by the Mann–Whitney or Wilcoxon tests for quantitative variables and the chi-square test for qualitative variables, with the continuity correction for the chi-squared when a subgroup included five or fewer subjects. Correlations were analysed by Spearman's rank test. All statistical tests were two-tailed and performed at a level of statistical significance of 0.05.

Results

Study population, disposition of patients, and virological data

Sixteen HIV-infected patients were enrolled in the study group and underwent pharmacokinetic study. Their baseline characteristics are shown in Table 1. All 16 patients had been heavily pre-treated with a median of six antiretroviral treatments and a median of four highly active antiretroviral treatments before the current therapy, all having failed the three main ARV drug classes. Nine patients had received LPV/RTV and three were taking LPV/RTV when the current therapy was initiated. None of the patients had received ATV before the current treatment. Two patients started the current therapy because of intolerance to their previous medication; baseline HIV RNA count was less than 50 copies/ml in these cases. The other 14 patients started the current therapy because of virological failure. There were no significant differences in baseline characteristics between the four arms (Table 1). Table 2 shows the antiretroviral agents in addition to

Table 1. Subject demographic data and baseline characteristics at the time of pharmacokinetic study.[a]

	Treatment arm			
	Arm A (n = 16) ATV/LPV/RTV	Arm B (n = 15) LPV/RTV	Arm C (n = 25) SQV/LPV/RTV	Arm D (n = 15) ATV/RTV
Age (years)	39 (34–42)	40 (36–44)	37 (33–41)	40 (37–43)
Men [n (%)]	13 (81)	12 (80)	18 (72)	12 (80)
HIV risk factor [n (%)]				
Intravenous drug user	7 (44)	6 (40)	8 (32)	5 (33)
Homosexual male	3 (19)	5 (33)	6 (24)	8 (53)
Heterosexual	3 (19)	3 (20)	10 (40)	2 (13)
Hemophiliac	3 (19)	1 (7)	1 (4)	0
Hepatitis C virus positive [n (%)]	10 (64)	7 (47)	14 (56)	7 (47)
Weight (kg) [median (IQR)]	67.5 (57.2–80.5)	69.6 (59.6–75.5)	71.0 (61.2–80.1)	67.0 (56.5–80.1)
Body mass index [median (IQR)]	23.2(20.8–26.0)	23.7 (20.8–25.3)	24.5 (22.2–27.9)	24.2 (21.8–26.7)
CD4 count (cells/μl) [median (IQR)]	299 (212–368)	438 (337–661)	325 (224–551)	323 (162–530)
HIV RNA (log_{10} copies/ml) [median (IQR)]	3.49 (2.70–4.11)	3.07 (1.40–4.41)	2.73 (1.40–3.47)	3.38 (1.40–4.38)
Haemoglobin (mmol/l) [median (IQR)]	14.1 (12.9–15.4)	14.3 (13.1–15.5)	14.2 (13.4–15.3)	14.3 (13.0–15.6)
Alanine aminotransferase (U/l) [median (IQR)]	39 (31–56)	32 (19–65)	37 (24–68)	31 (24–36)
Aspartate aminotransferase (U/l) [median (IQR)]	34 (25–85)	32 (18–58)	40 (25–71)	41 (26–50)
Creatinine (μmol/l) [median (IQR)]	0.9 (0.7–1.1)	0.9 (0.8–1.1)	1.0 (0.9–1.1)	0.9 (0.8–1.2)

ATV, atazanavir; LPV, lopinavir; RTV, ritonavir; IQR, interquartile range.

Table 2. Antiretroviral agents used in combination with atazanavir (ATV) and lopinavir (LPV)/ritonavir (RTV), baseline resistance patterns and patient follow-up.

Patient	ART[a]	Reverse transcriptase mutations	Protease mutations	HIV RNA baseline (copies/ml)	HIV RNA 6 months (copies/ml)
1	TDF	D67N, K70R, V75M, F77L, K101E, V118I, M184V, K219Q	L10V, L63P, A71V, G73S, L90M	68 000	< 50
2	TDF	L74V, M184V, T215Y, K103N, M230L	M36I, L63P	10 000	< 50
3	TDF	M41L, E44D, S68G, L74V, M184V, L210W, K101E, Y181C, G190A	M36I, L63P	4600	< 50
4	TDF	M184V, T215Y	L10F, L63P, I93L	4500	< 50
5	DDI	A62V, K65R, T69D, M184V, L100I,K103N	L10I, L63P, A71T, I84V, L90M	250 000	< 50
6	DDI	D67N, K70R, M184V, R211K, T215Y, K219E, K103N, Y181C	L63P	92 000	7600[b]
7	T20	M41L, D67N, L74V,L210W,T215Y,K103N,V118I,Y181I	L10I, M46L, G48V, I54V, L63P, A71T	150 000	310 000
8	T20	M41L, D67N, K70R, L74V, M184V, T215F, K219Q	L10I, L63S, V82A, I93L	31 000	< 50
9	T20	M41L, E44A, T69A, L74V, V118I, L210W, R211K, L100I, K103N	L10I, L33I, L63P, A71V, I84V, I93L	150 000	< 50
10	T20	D67S, T69SG, K70T, M184V, T215Y, K103N, Y181C	L63P, A71V, V77I, V82A, I93L	8700	< 50
11	T20 + TDF + 3TC	M41L, T69D, L74V, L210W, T215Y, K101E, V118I, Y181C, G190A	L10I, L24I, M46I, I54V, L63P, A71V, V82T, I84V	32 000	< 50
12	T20+DDI	M41L, D67N, K70R, L210W, T215Y, K219E, Y181C, G190A	L10I, M46L, F53L, I54V, A71V, V82A, I84V	88 000	< 50
13	D4T+ABC	M41L, E44D, K65R, L100I, K103N, V118I, L210W, T215S	No mutations	160 000	< 50
14	D4T+ABC	M41L, D67N, K70R, V75M, R211K, T215F, K219Q, K101E, G190C	L10I, L24I, M46I, I54V, L63P, A71V, V82A, I93L	6500	3300
15	D4T+ABC	–	–	< 50	< 50
16	TDF	–	–	< 50	< 50

[a]Antiretroviral therapy (ART) combined with ATV+LPV+RTV.
[b]HIV RNA after 1 month of therapy. At this time the patient discontinued treatment according to personal decision. ABC, Abacavir; ddI, didanosine; d4T, stavudine; TDF, tenofovir; T20, enfuvirtide.

ATV and LPV/RTV, the resistance patterns and patient follow-up.

The study drugs were withdrawn in two patients: one because of virological failure and one by personal choice. Another patient had an undetectable viral load at 3 months of treatment, but presented HIV RNA rebound at 6 months. This patient continued with the same treatment, waiting for new therapeutic options. After 6 months of follow-up, 13 of 16 patients (81%) had HIV RNA values less than 50 copies/ml (intent-to-treat). At week 24, the mean reduction in plasma HIV-1 RNA in patients who initiated treatment with a detectable viral load was 2.9 $\log_{10}$ copies/ml and the mean increase in CD4 cell count was 118 cells/μl. In the two patients who initiated treatment with a viral load of < 50 copies/ml, viral load remained undetectable after 24 weeks of treatment and CD4 lymphocyte count remained steady. No deaths occurred during the study period.

Pharmacokinetic analysis

Results of pharmacokinetic analyses are summarized in Fig. 1, Fig. 2, and Table 3. Comparison of the concentration–time profiles for LPV when LPV/RTV was administered in combination with ATV (arm A) or alone (arm B) or in combination with SQV (arm C), revealed that LPV concentrations were significantly higher in arm A (Fig. 1). Specifically, the following parameters showed higher values in arm A as compared with arm B: median LPV AUC_{0-12} (115.7 versus 85.2 μg/ml/h; $P = 0.019$), median LPV C_{max} (12.2 versus 9.5 μg/ml; $P = 0.043$), median LPV C_{trough} (10.2

Fig. 1. Median concentration–time curves of LPV at steady-state in patients treated with LPV/RTV (400/100 mg b.i.d.) in combination with ATV (300 mg q.d.), in combination with SQV (1000 mg b.i.d.), and alone. Error bars indicate IQR.

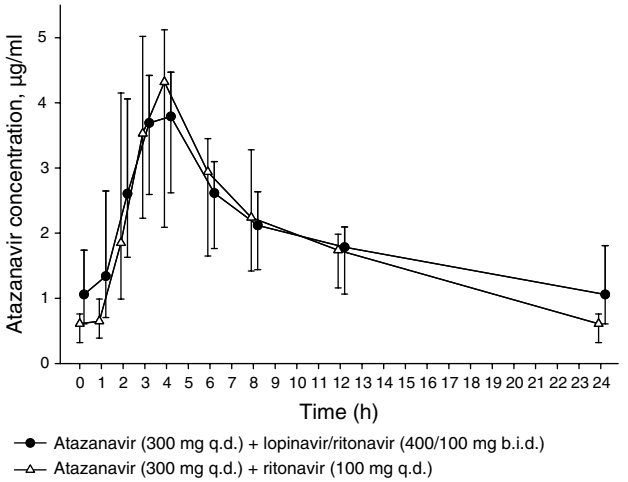

Fig. 2. Median concentration–time curves of ATV at steady state in patients treated with ATV (300 mg q.d.) in combination with LPV/RTV (400/100 mg b.i.d.) and with RTV (100 mg q.d.). Error bars indicate IQR.

versus 6.6 μg/ml; $P = 0.019$) and median LPV C_{min} (9.1 versus 5.6 μg/ml; $P = 0.013$). Furthermore, the following parameters were higher in arm A as compared with arm C: median LPV AUC_{0-12} (115.7 versus 85.1 μg/ml/h; $P = 0.006$), median LPV C_{max} (12.2 versus 10.0 μg/ml; $P = 0.037$), median LPV C_{trough} (10.2 versus 7.3 μg/ml; $P = 0.016$) and median LPV C_{min} (9.1 versus 5.5 μg/ml; $P = 0.001$). As has been shown in a previous study [15], there were no significant differences in any of the pharmacokinetic parameters between arm B and arm C.

Plasma RTV concentrations were similar in the three arms (A, B, and C) where the same dose was administered (100 mg b.i.d.). Patients in arm D received 100 mg q.d. and the median C_{trough} and C_{min} for RTV were significantly lower than in the other three groups. The AUC_{0-24} of RTV in group D was similar to the AUC_{0-12} of RTV in the other three groups. (Table 3). In arm A no significant correlations were found for any of the pharmacokinetic parameters between LPV and RTV. In arms B and C there was a strong positive linear correlation between the two drugs for the AUC_{0-12}, C_{max}, C_{trough}, and C_{min}, as reported previously [15].

Comparison of the concentration–time profiles for ATV when ATV was administered in combination with LPV/RTV (400/100 mg b.i.d.) (arm A) or with RTV (100 mg q.d.) (arm D), revealed that C_{trough} and C_1 ATV concentrations were significantly higher in arm A (Fig. 2). Specifically, median ATV C_{trough} (1.14 versus 0.61 μg/ml; $P = 0.020$) and median ATV C_{min} (1.07 versus 0.58 μg/ml; $P = 0.017$) showed higher values in arm A as compared with arm D. There were no significant differences in ATV AUC_{0-24} or in ATV C_{max} between arms A and D. In arm A we observed a moderate correlation between the RTV C_{min}, and the ATV AUC_{0-24} (r, 0.57; $P = 0.020$) and C_{min} (r, 0.46; $P = 0.042$), and in arm D, between the RTV C_{min} and ATV C_{min} (r, 0.62; $P = 0.015$). No significant correlations were found for any of the pharmacokinetic parameters between LPV and ATV.

Seven of 16 patients in arm A were taking tenofovir as part of their treatment. The median AUC, C_{max} and C_{trough} values for ATV in these patients as compared to those who were not taking tenofovir were 47.8 (IQR, 25.6–58.5) and 48.6 (IQR, 36.4–55.5) μg/ml/h ($P = 0.68$), 3.87 (IQR, 2.36–4.17) and 3.98 (IQR, 2.56–4.27) μg/ml ($P = 0.54$), and 0.98 (IQR, 0.77–1.28) and 1.14 (IQR, 0.52–2.04) μg/ml ($P = 0.68$), respectively. Nine of 15 patients in arm D were taking tenofovir. The median AUC, C_{max} and C_{trough} values for ATV in these patients as compared to those who were not taking tenofovir in arm D were 38.3 (IQR, 20.5–45.7) versus 49.3 (IQR, 31.2–78.5) μg/ml/h ($P = 0.020$), 3.82 (IQR, 2.36–4.17) versus 5.46 (IQR, 2.56–4.27) μg/ml ($P = 0.011$),

Table 3. Steady-state pharmacokinetic parameters of lopinavir (LPV), ritonavir (RTV) and atazanavir (ATV) for all study arms.[a]

	AUC (μg/ml/h)[b]	C_{max} (μg/ml)	T_{max} (h)	C_{trough} (μg/ml)	C_{min}, (μg/ml)
LPV					
Arm A (LPV/RTV/ATV)	115.7 (99.8–136.5)[c]	12.2 (10.7–14.5)[c]	4 (3–5.5)	10.2 (7.7–11.8)[c]	9.1 (7.1–10.4)[c]
Arm B (LPV/RTV)	85.2 (68.3–109.2)	9.5 (6.8–13.9)	4 (3–6)	6.6 (5.0–8.2)	5.6 (4.7–8.2)
Arm C (LPV/RTV/SQV)	85.1 (60.6–110.1)	10.0 (6.9–13.6)	4 (3.4–6)	7.3 (4.5–9.3)	5.5 (4.2–7.5)
RTV					
Arm A (LPV/RTV/ATV)	10.4 (8.26–11.8)	1.19 (0.95–1.36)	4 (3–5.5)	0.75 (0.54–1.06)	0.59 (0.50–0.79)
Arm B (LPV/RTV)	9.48 (4.95–15.36)	1.09 (0.77–1.93)	4 (3–4.5)	0.70 (0.35–1.12)	0.55 (0.31–1.02)
Arm C (LPV/RTV/SQV)	8.39 (4.58–13.03)	1.02 (0.59–1.68)	4 (3.4–6)	0.68 (0.48–1.13)	0.53 (0.30–1.02)
Arm D (ATV/RTV)	11.9 (10.4–16.3)	1.33 (0.98–2.06)	5 (3.5–6)	0.17 (0.12–0.23)[d]	0.15 (0.11–0.22)[d]
ATV					
Arm A (LPV/RTV/ATV)	48.2 (28.6–55.0)	3.95 (2.33–4.32)	4 (3–4)	1.14 (0.66–1.88)	1.07 (0.61–1.79)
Arm D (ATV/RTV)	45.2 (31.9–50.8)	4.44 (2.97–5.43)	3 (3–4)	0.61 (0.32–0.84)[e]	0.58 (0.32–0.83)[e]

[a]Values are expressed as median (interquartile ranges).
[b]AUC_{0-12h} for LPV and for RTV arms A, B and C; AUC_{0-24h} for ATV and for RTV arm D.
[c]$P < 0.05$ (Mann–Whitney U test) for comparisons with arm B and arm C.
[d]$P < 0.05$ (Mann–Whitney U test) for comparisons with arm A, arm B and arm C.
[e]$P < 0.05$ (Mann–Whitney U test) for comparison with arm A. SQV, saquinavir.

and 0.45 (IQR, 0.28–0.72) versus 0.68 (IQR, 0.43–1.03) μg/ml ($P = 0.14$), respectively.

Adverse events

The combination of ATV (300 mg q.d.) and LPV/RTV (400/100 mg b.i.d.) was generally well tolerated. None of the patients discontinued treatment due to adverse effects. Six patients presented grade 1 diarrhoea, which was self-limited or improved without withdrawal of treatment. The most common adverse event was mild hyperbilirubinaemia. All patients experienced an increase in total bilirubin greater than the upper normal limit (UNL), with levels of 1.1–1.5 × UNL (grade 1) in one case, 1.6–2.5 × UNL (grade 2) in seven cases and 2.6–5 × UNL (grade 3) in eight cases. At different points along the follow-up period, four patients (25%) developed scleral icterus or jaundice. In contrast, none of the patients showed clinical symptoms suggesting acute hepatitis, and none had grade 3–4 hepatic transaminase elevations (> 5.1 × UNL). Median levels of bilirubin (IQR) at baseline and at months 1, 3, and 6 were 0.47 (0.38–0.67), 1.92 (1.48–2.39), 2.74 (1.80–3.53), and 2.50 (1.83–3.21) mg/dl, respectively. The lipid profile changes were mild, with a slight elevation of total cholesterol and no triglyceride changes.

Discussion

In the present study, administration of LPV/RTV (400/100 mg b.i.d.) with ATV (300 mg q.d.) achieved high LPV and ATV plasma levels. Co-administration of ATV and LPV/RTV substantially increased LPV exposure, producing statistically significant increases in the LPV AUC_{0-12}, C_{max}, C_{min}, and C_{trough}. RTV is a potent inhibitor and ATV a modest inhibitor of CYP3A4 metabolism [18,20]. However, ATV seems to be able to further enhance LPV exposure in the presence of RTV. Boffito *et al.* [17] reported that the addition of ATV (300 mg q.d.) to SQV/RTV (1600/100 q.d.) further increased SQV and RTV AUC_{0-24}, C_{max}, and C_{trough}, and they speculated that inhibition of P-glycoprotein mediated drug transport may be responsible for the increase in SQV and RTV exposure. Among currently available PI, ATV is the only one that exerts a clinically significant inhibiting effect while producing plasma increases in RTV-boosted PI. In this setting, SQV [15,16] or indinavir [25] do not seem to modify plasma concentrations of LPV, whereas with APV, [10,11,13,14] FPV, [12] or nelfinavir, [26] decreases in plasma LPV concentrations to varying extents have been documented.

The mechanism by which ATV further boosts LPV is unknown. LPV is metabolized by CYP3A4 and is a substrate for P-glycoprotein and other drug efflux pumps. An increase in the RTV dose produces a further increase in plasma LPV concentration [27,28]. In the present study, no increases were found in RTV concentrations in the presence of ATV, but it is possible that ATV might have produced some additional CYP3A4 inhibition, resulting in a slight increase in plasma LPV. It was demonstrated recently that ATV is an inhibitor of P-glycoprotein and multidrug resistance-associated protein, and that the inhibitory effect is greater when ATV is combined with RTV than when these drugs are used alone [29,30]. This may be the main mechanism responsible for the increase in LPV exposure when ATV is co-administered with LPV/RTV.

When double-RTV-boosted PI combinations are utilized, the subsequent effects of LPV on exposure to other PI vary. The combination of LPV/RTV plus APV or FPV has resulted in important reductions in plasma concentrations of APV [10–14]. The combination of LPV/RTV and SQV has shown favourable pharmacokinetic profiles, without any apparent modification of SQV exposure [15,16]. In our study, the AUC_{0-24} and C_{max} values of ATV were similar in patients with LPV (48.2 μg/h/ml and 3.95 μg/ml, respectively) and in patients without LPV (45.2 μg/h/ml and 4.44 μg/ml, respectively). Plasma C_{trough} and C_{min} ATV values were almost twofold higher in patients receiving ATV plus LPV/RTV than in patients receiving ATV plus RTV (1.14 versus 0.61 μg/ml, $P = 0.008$; and 1.07 versus 0.58 μg/ml, $P = 0.007$, respectively). However, patients with LPV/RTV received 200 mg of RTV (100 mg b.i.d.), whereas those with RTV alone received only 100 mg. It has been observed that the association of 300 mg of ATV with 100 mg of RTV did not significantly increase the C_{max} as compared to 400 mg of unboosted ATV, but it did produce a five- to sevenfold increase in the C_{trough} [31]. It is likely that the C_{min} increase in arm A as compared to arm D in our study was due to the fact that patients in arm A received 100 mg more RTV and had a higher RTV C_{min} than those in arm D. There was a significant correlation between RTV C_{min} and ATV C_{min}. In this setting, the combination of LPV with ATV did not appear to negatively influence exposure to ATV. Winston *et al.* [32] also observed significantly higher plasma trough ATV levels in nine patients on LPV/RTV/ATV regimens than in 72 patients on RTV-boosted ATV regimens without LPV (median values: 1.457 versus 0.604 μg/ml, $P = 0.032$).

When ATV was co-administered with LPV/RTV in arm A, plasma ATV concentrations in patients taking tenofovir were similar to those in patients who were not taking tenofovir. However, when ATV was co-administered with RTV at standard boosting dose (100 mg q.d.), without LPV, plasma ATV AUC_{0-24} and C_{max} were significantly lower in patients taking tenofovir than in patients not taking this drug. The data in the literature about the interaction between tenofovir and ATV are controversial. In healthy individuals, a significant 25% reduction in the ATV C_{trough} was observed when tenofovir was added to a regimen with ATV/RTV (300/100 mg) [33]. After

adding tenofovir to a regimen containing ATV/RTV (300/100 mg) in HIV-infected patients, Taburet *et al.* [34] found a trend toward lower ATV concentrations, but the decrease in the ATV AUC was the only difference that reached statistical significance. In other studies in HIV-infected patients, tenofovir had no effect on RTV-boosted ATV trough concentrations [17,32,35–37]. In any case the magnitude of the interaction between tenofovir and boosted ATV seems to be small and it is not necessary to increase ATV dose when it is administered with RTV boosting.

The combination of ATV and LPV/RTV was well tolerated, despite the high plasma concentrations of both ATV and LPV. None of the 16 patients had to discontinue study medication because of adverse events [18]. Digestive tolerance was good in our patients. Nevertheless, a possible selection bias in the participating patients could have led to better tolerance of LPV/RTV: a large number of patients included had already received or were receiving LPV/RTV with good tolerance, whereas patients who had previously shown poor tolerance to these drugs were not considered for this treatment. Digestive tolerance to ATV is generally good [38–41]. The most common adverse event seen after ATV/LPV/RTV administration was an elevation in total bilirubin level, predominantly unconjugated. ATV plasma concentration plays a role in causing hyperbilirubinaemia. The frequency of grade 3 or 4 elevation of total bilirubin was 20–40% in non-boosted ATV regimens [38–40,42] and 30–50% in RTV-boosted ATV regimens [39,41]. The effect of jaundice is judged by patients on an individual basis, and less than 2% of patients in clinical trials discontinued therapy because they found it cosmetically unacceptable.

The combination of ATV plus LPV/RTV had substantial antiviral efficacy in these heavily pre-treated patients, with a reduction of 2.9 $\log_{10}$ in HIV RNA load and an increase of 118 CD4 cells/μl after 24 weeks of treatment. The study was not designed to assess therapeutic efficacy and does not have sufficient statistical power for this purpose; nevertheless, it is worthy of mention that a very high proportion of patients (13/16 in the intent-to-treat analysis) showed a plasma HIV-1 RNA load < 50 copies/ml after 24 weeks of treatment. In intensively pre-treated patients it is difficult to achieve lasting treatment efficacy. In the majority of studies, the percentage of patients with undetectable viral load after 24–48 weeks of rescue treatment following numerous treatment failures ranges from 20% to 55% [22,43–48]. The substantial efficacy in the present study can be attributed to the elevated concentrations of LPV and ATV achieved, the good tolerability of the treatment and the effect of the other associated antiretroviral drugs.

In summary, the combination of ATV and LPV/RTV provided high plasma concentrations of both PI. ATV seems to be able to further enhance LPV exposure in the presence of RTV. The C_{min} of ATV was higher with the ATV/LPV/RTV combination than with standard boosting, probably because a higher dose of RTV was used when ATV was combined with LPV/RTV than in the standard boosting regimen. These high plasma concentrations of LPV and ATV seemed to be appropriate for combining these agents in a dual PI-based antiretroviral regimen for patients in whom multiple antiretrovirals had failed, yielding good tolerability and substantial antiviral efficacy. Further studies are required to confirm these encouraging preliminary results.

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