Published in final edited form as:

Ther Drug Monit. 2008 June ; 30(3): 306–313. doi:10.1097/FTD.0b013e318177209e.

Lopinavir/Ritonavir Pharmacokinetics in HIV/HCV-Coinfected Patients With or Without Cirrhosis

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Abstract

Liver disease may alter the pharmacokinetics of antiretrovirals and produce changes in plasma protein binding. The aim was to evaluate the pharmacokinetics of total and unbound lopinavir (LPV) in HIV-infected patients with and without hepatitis C virus (HCV) coinfection. Fifty-six HIV+ patients receiving lopinavir/ritonavir (LPV/r) (group I = 24 controls; II = 23 HIV/HCV-coinfected; III = 9 cirrhotic HIV/HCV-coinfected) were included.

Total (n = 56) and unbound (n = 36) LPV pharmacokinetic parameters were determined at steady-state using validated high-performance liquid chromatography with ultraviolet detection and high-performance liquid chromatography-tandem mass spectrometry methods, respectively. Pharmacokinetic parameters (plasma concentration just before drug administration, peak concentrations in plasma, times to maximum plasma concentration, areas under the plasma concentration-time curve from 0 to 12 hours, and CL/F/kg) of both total and unbound LPV were calculated by standard noncompartmental methods and differences among groups evaluated (Kruskal-Wallis test).

LPV apparent oral clearance normalized to body weight (median, interquartile range) was 55 (40–68), 59 (44–69), and 71 (53–78) mL/h/kg for groups I, II, and III, respectively (II vs. I, $P = 0.52$; III vs. I, $P = 0.16$). The areas under the plasma concentration-time curve from 0 to 12 hours were 110.4 (80.9–135.2), 103.4 (85.5–131.3), and 92.8 (87.4–116.3) $\mu\text{g}\cdot\text{h}/\text{mL}$ for groups I, II, and III, respectively (II vs. I, $P = 0.68$; III vs. I, $P = 0.71$).

Chronic liver impairment produced a slight, although not significant, decrease in plasma protein binding. The free-fraction of LPV increased (~21%) from 0.97% (0.80–1.06) in HIV+/HCV- patients to 1.18% (0.89–1.65) in HIV/HCV+ cirrhotic patients. The apparent oral clearance of unbound LPV ($\text{CL}_u/\text{F}/\text{kg}$) in cirrhotic patients did not change significantly, supporting the concept

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Data partially presented at the 3rd IAS Conference on HIV Pathogenesis and Treatment, Rio de Janeiro, Brazil, July 24–27, 2005 (poster WePe3.2C06).

that the clearance of unbound LPV in liver disease is not affected after being inhibited by low-dose ritonavir co-administration.

LPV total and unbound pharmacokinetics were not affected by hepatic impairment, suggesting that no adjustment of LPV/r dose is required for HIV/HCV-coinfected patients with and without cirrhosis and moderate impairment of liver function.

Keywords

Lopinavir/ritonavir; hepatic impairment; HIV/hepatitis C virus coinfection; unbound drug; pharmacokinetics; therapeutic drug monitoring

INTRODUCTION

Highly active antiretroviral therapy (HAART) has modified the clinical scenario of HIV-1 infection, decreasing AIDS-related morbidity and mortality.^{1,2} However, an ongoing problem is the emergence of clinical complications associated with comorbidities.

Both hepatitis C and B virus (HCV and HBV) represent an urgent issue in HIV management because of the same route of transmission that results in a higher incidence of these infections in HIV-positive subjects compared with the general population; (prevalence of HIV/HCV coinfection ranges from <15% in northern European countries to >50% in Italy and Spain).³ Liver toxicity is a common side effect of antiretroviral therapy and is expected to occur more frequently in subjects with hepatitis virus coinfections.⁴⁻⁶

Lopinavir/ritonavir (LPV/r) is a potent HIV protease inhibitor (PI) characterized by both a high genetic barrier and inhibitory quotient, resulting in low rates of PI-associated drug resistance mutations if administered as the first-line PI. The guidelines on antiretroviral therapy in HIV-1 positive adults recommend the inclusion of LPV/r in the initial therapeutic regimen.⁷

The administration of LPV/r in combination with other antiretroviral agents provides adequate and durable suppression of viral load in both antiretroviral therapy-*naïve* and -*experienced* HIV-1 infected patients.⁸⁻¹¹ The recent approval of a new formulation (meltrex), with a lower pill burden and the absence of a need for refrigeration may contribute to an improved use in the future.¹²

LPV is extensively and rapidly metabolized by the cytochrome P450 (CYP) 3A4 isoenzyme in the liver. Its co-administration with low-dose ritonavir (100 mg), a potent inhibitor of hepatic CYP3A4, significantly improves its pharmacokinetic properties and its antiviral activity, ensuring plasma concentrations well above the mean half maximal effective concentration for wild-type HIV-1 in vitro, both in the presence and absence of 50% human serum.^{13,14}

Liver impairment caused by hepatitis infections may impact on the absorption of the drug, its first-pass metabolism by the intestine and the liver, binding to plasma proteins, and plasma protein concentrations, thereby resulting in an increased exposure to LPV/r and the risk of developing adverse events. The correlation between plasma concentrations and adverse effects is documented for some PIs, such as indinavir,¹⁵ atazanavir,¹⁶ and the non-nucleotide reverse-transcriptase inhibitors (NNRTIs) efavirenz¹⁷ and nevirapine,¹⁸ but it still remains equivocal with regard to LPV/r. The incidence of severe liver toxicity in patients administered PIs is variable, depending both on the drug and the prevalence of HCV coinfection in different study populations.

Data on the impact of HIV/HCV coinfection on LPV/r plasma concentrations are currently based on a few studies involving a small number of patients and has resulted in conflicting conclusions.^{19,20} Most studies have not considered cirrhosis, which in HIV/HCV coinfection could impact on both LPV/r absorption and clearance.^{21,22}

The objective of this study was to evaluate LPV/r pharmacokinetics in HIV-positive patients with hepatitis C coinfection, with or without cirrhosis, and in a control group of HIV-positive patients without coinfection.

MATERIAL AND METHODS

Subjects

HIV+ outpatients, attending the Second Department of Infectious Diseases of L. Sacco Hospital in Milan between September 2004 and December 2005 and who were going to start LPV/r as part of HAART therapy including nucleoside reverse-transcriptase inhibitors and excluding NNRTIs, were asked to participate in this study. Each patient was informed about the schedule of sequential blood collection for the pharmacokinetic evaluation and received additional counseling about adherence to treatment. The protocol was approved by the Ethics Committee of L. Sacco Hospital (7/05/79/04), and written informed consent was obtained from each patient before enrollment.

A total of 56 Caucasian HIV+ men and women were included in the study. Other drugs co-administered were known to have no interference with LPV/r plasma disposition. At baseline, serologic parameters for HBV and HCV infection status were measured: hepatitis B surface antigen and antibodies against hepatitis C virus (anti-HCV). HBV and HCV chronic infection was defined by the persistent positivity and detectability of hepatitis B surface antigen and HCV serum antibodies and HCV-RNA.

The patients were stratified into three groups with regard to hepatic serology: 24 HIV+ control subjects (male/female, 18/6), and 32 HIV/HCV-coinfected subjects with (n = 9) or without (n = 23) cirrhosis currently not receiving antiviral or interferon treatment for HCV infection (1 patient HIV/HCV/HBV-coinfected; male/female, 24/8).

Study Design

In this prospective, open-label study, patients were administered capsules of LPV/r at the standard dose of 400/100 mg twice daily after a standardized continental breakfast and after a moderate fat (22.5–5.0%) meal for lunch (500–700 kcal). A dose of 266/66 mg twice daily was administered if body weight was less than 50 kg.

The pharmacokinetic analysis of LPV was performed at steady-state (2 wk after treatment initiation) with blood samples obtained at predose (t₀), 1, 2, 3, 4, 6, 8, and 12 hours after the LPV/r morning intake. The precise times of the predose sample and of the previous LPV/r evening intake were recorded to ensure accurately timed blood sampling.

Blood samples collected in EDTA-containing tubes were centrifuged (600g; 10 min) to obtain plasma samples within 2 hours of collection. The plasma samples were stored at –80°C and were sent on dry ice to the pharmacology laboratories to perform the pharmacokinetic analysis. All plasma samples were analyzed for total LPV, whereas ritonavir and free (unbound) LPV were determined in some of the patients (33 and 36 patients, respectively).

On the day of blood sampling for pharmacokinetic analysis, epidemiologic data (sex, age, risk factors for HIV infection, and HIV stage according to the 1992 revised Centers for

Disease Control and Prevention HIV classification) were collected, and anthropometric measurements [body weight, height, and body mass index (BMI)], clinical, and immune-virologic evaluation [CD4+ cell count and viral load (branched-DNA Bayer, Bayer Diagnostic, Milan, Italy, lower limit 50 HIV-1 RNA copies/mL)], and biochemical monitoring [serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ -glutamyltransferase, total bilirubin, albumin, pseudo cholinesterase, prothrombin time, and partial prothrombin time] were performed.

Liver ultrasonography B-mode with convex probe (3.5 MHz) was performed within 1 year before LPV/r treatment in HCV-positive patients. The image patterns of the liver parenchyma were classified into four groups: “negative,” if no structural alterations were present; “fatty liver” (“steatosis”), defined as diffuse increased liver echogenicity (bright liver echo pattern) and possibly distal sound extinction; “fibrosis,” defined as widespread inhomogeneous liver texture and slight irregularity of the liver surface; and “cirrhosis” if three or more of the following criteria were present: lobulated boundaries, gross irregularity of the liver surface, coarse echo pattern, distorted hepatic veins, caudal liver edge deformation, left and caudal lobe enlargement, and a progressive decrease in right liver lobe size. Left liver lobe hypertrophy was assessed as the ratio of right lobe thickness to left lobe thickness. Caudal liver hypertrophy was calculated from the caudal transverse diameter lobe (C) and the right transverse diameter lobe (RL) ratio. A C/RL ratio 0.65 or greater was taken to be significant for a diagnosis of cirrhosis.

According to this evaluation, the HIV/HCV coinfecting patients ($n = 32$) were classified as the following: “negative” ($n = 10$), presenting steatosis ($n = 6$), fibrosis ($n = 7$), and cirrhosis ($n = 9$). HIV/HCV-positive patients were finally divided into groups according to biochemical, clinical, and ultrasonography data: mild hepatic impairment (group II, $n = 23$) and compensated cirrhosis (group III, $n = 9$). Furthermore, the cirrhotic patients were classified by means of the Child-Pugh score as the following: A5 ($n = 5$), B7 ($n = 3$), and C11 ($n = 1$).

Separation of Total and Unbound Lopinavir

Ultrafiltration was used to separate total and unbound LPV. Plasma (800 μ L) was applied to Centrifree Micro-partition filter devices (Millipore Corporation, Bedford, MA) before centrifugation (1500g; 60 min) at 37°C. Resulting ultrafiltrate (approximately 170 μ L obtained per sample) was retained for drug analysis. To limit nonspecific binding of drug, the filters were incubated with Tween-20 (500 μ L; 5%) at room temperature (24 hr) and washed. This step was repeated, and filters were then inverted and centrifuged (1000g; 3 min, 37°C) before injection of patient plasma samples. Mean (SD) recovery was 69% (4) and was constant across a range of concentrations of LPV (1000, 5000, 10,000, and 15,000 ng/mL); thus, correction to unbound concentrations was not applied.²³⁻²⁷

Drug Analysis

Plasma (total) LPV and ritonavir concentrations were determined by means of a validated high-performance liquid chromatography (HPLC) assay with ultraviolet detection, using a modified method.²⁸ The standard curves for LPV and ritonavir were linear within the ranges of 100 ng/mL to 16,000 ng/mL in plasma, with a lower limit of detection of 20 ng/mL for both drugs. Intra-assay coefficients of variation for LPV and ritonavir quality control (QC) concentrations were 9.7% and 9.1%, respectively, for the low QC concentration (120 ng/mL) and 8.6% and 8.9%, respectively, for the high QC concentration (12,000 ng/mL). Interassay coefficients of variation were 9.1% and 9.3%, for LPV and ritonavir, respectively, for the low QC concentration and 7.8% and 8.3%, respectively, for the high QC

concentration. The corresponding accuracy ranged between 95% to 103% for LPV and 92% to 105% for ritonavir.

Unbound LPV concentrations were determined by a validated HPLC-tandem mass spectrometry method as described previously.²⁹ Internal standard (IS, 20 μ L; 500 ng/mL; Ro31-9564, Roche Discovery, Welwyn, UK) and acetonitrile (400 μ L; VWR Laboratory Supplies, Poole, UK) were added to aliquots (100 μ L) of calibrators (13–1052 ng/mL), QCs (42, 150, 702 ng/mL), and patient ultrafiltrate. After mixing, centrifugation, and addition of ammonium formate buffer (100 μ L; 20 mM; Fisher Scientific, Loughborough, UK), samples were analyzed by HPLC-tandem mass spectrometry (10 μ L). Fragmentation of parent molecules (LPV: 629.4; IS: 674.4) into daughter ions occurred by electrospray ionization; ions were separated according to their m/z ratio and quantified by the intensity of their respective daughter ions (LPV: 447.1, 611.2; IS: 388.2, 436.1, 573.2). Interassay variation was between 6.9% and 8.4% for low, medium, and high QCs. Intra-assay variability was 5.7%, 4.7%, and 2.3%, with an accuracy of 100%, 99.9%, and 98.5% for low, medium, and high QCs, respectively. To assess intra-filter variation, spiked plasma (20,000 ng/mL) was injected into six separate Centrifree filters, ultrafiltrate collected, and LPV quantified. Mean unbound LPV was 418 ng/mL, and concentrations varied by 12%.

Both assays for total and unbound drug concentrations were performed in laboratories participating in an international quality assurance program (International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV Infection, KKG, Nijmegen, The Netherlands).

Pharmacokinetic Analysis

Concentration-time data were analyzed using KINET-ICA software (version 4, InnaPhase Corporation, Philadelphia, PA). Pharmacokinetic parameters such as the plasma concentration just before drug administration (C_{trough}), the peak concentrations in plasma (C_{max}), and the corresponding times to maximum plasma concentration (t_{max}) of both total and unbound LPV and total ritonavir were determined by standard noncompartmental methods. The areas under the plasma concentration-time curve from 0 to 12 hours (AUC_{0-12}) were calculated by using the linear trapezoidal rule. When the 12 hour sample was not available, it was extrapolated from the decay of the terminal slope of the plasma concentration time curve. LPV apparent oral clearance (CL/F , where F is the fraction of the absorbed drug), corrected for body weight, was calculated as $dose/AUC_{(0-12)}$ divided by body weight.

The fraction of LPV unbound in plasma (f_u) was determined by $f_u (\%) = AUC_{u,(0-12)} * 100 / AUC_{(0-12)}$, where $AUC_{u,(0-12)}$ was the area under the unbound plasma concentration-time curve during the dosing interval. The apparent oral clearance of unbound LPV (CL_u/F), corrected for body weight, was calculated as $CL/F/kg * f_u$.

Statistical Analysis

The software packages Graph Pad Prism (version 3.02) and Graph Pad InStat (version 3.05) for Windows (Graph Pad Software, San Diego, CA; www.graphpad.com) were used. Factors investigated included demographic (age, sex, HIV risk group) and anthropometric (body weight and BMI) variables, clinical stage of disease, baseline CD4+ cell count, biochemical parameters (AST, ALT, total bilirubin, albumin, pseudocholinesterase, alkaline phosphatase, γ -glutamyltransferase, prothrombin time, and partial prothrombin time), and pharmacokinetic parameters (C_{trough} , C_{max} , T_{max} , $AUC_{(0-12)}$, and $CL/F/kg$) of both total and unbound LPV and total ritonavir.

All variables were compared for the three groups (HIV+/HCV-, HIV+/HCV+ patients, and HIV+/HCV+ cirrhotic patients) using one-way nonparametric analysis of variance (Kruskal-Wallis test), considering $\alpha = 5\%$. Post hoc analyses were performed by means of Mann-Whitney test; only results with $P < 0.05$ were considered to be significant, and all P values were two-sided. The correlation between total LPV and ritonavir apparent oral clearance normalized to the body weight was assessed by estimating the rho Spearman's coefficient. Results are reported as median and interquartile range (IQR) values.

RESULTS

The demographic, anthropometric, clinical, and biochemical characteristics of the patients at baseline are summarized in Tables 1 and 2. All patients were at steady-state for LPV/r treatment, at the standard dose of 400/100 mg ($n = 51$) and at the reduced dose of 266/66 mg (group I/II/III: $n = 1/1/3$) twice daily, as part of a HAART regimen including nucleoside reverse-transcriptase inhibitors and excluding NNRTIs. The most common antiretroviral therapies associated with LPV/r on the basis of the genotypic drug resistance test and previous treatments were lamivudine + didanosine ($n = 11$), lamivudine + tenofovir ($n = 10$), lamivudine + stavudine ($n = 10$), zidovudine + lamivudine ($n = 8$), didanosine + stavudine ($n = 8$), and other nucleoside/nucleotide combinations ($n = 9$). One patient was taking a dual boosted PI (hard gel saquinavir).

There were no statistically significant differences among the three patient groups in terms of baseline demographics and HIV-related clinical characteristics, except for a lower body weight and BMI in the cirrhotic group ($P = 0.03$ and $P = 0.01$, respectively), and biochemical parameters reflecting severity of hepatic status, such as AST, ALT, albumin, pseudocholinesterase, prothrombin time, and partial prothrombin time (Tables 1 and 2).

Total LPV concentrations were determined in all 56 subjects, whereas total ritonavir and unbound LPV concentrations were determined in a subset of 33 (group I, II, and III: 14, 15, and 4, respectively) and 36 (group I, II, and III: 12, 18, and 6, respectively) patients, respectively. The median (IQR) total LPV and ritonavir plasma concentrations during the 12 hour dosing interval showed no statistically significant differences among the three groups.

Pharmacokinetic parameters of both total and unbound LPV and total ritonavir for the three groups of HIV+ patients are reported in Tables 3 and 4, respectively. The values are expressed as median and IQR and corrected for the LPV/r standard doses of 400/100 mg.

Total LPV $AUC_{(0-12)}$ for group I, II, and III were 110.4 (80.9–135.2) $\mu\text{g}\cdot\text{h}/\text{mL}$, 103.4 (85.5–131.3) $\mu\text{g}\cdot\text{h}/\text{mL}$, and 92.8 (87.4–116.3) $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, and did not differ significantly between groups (II vs. I, $P = 0.68$; III vs. I, $P = 0.71$). Furthermore, no significant differences in C_{trough} (II vs. I, $P = 0.97$; III vs. I, $P = 0.66$) or apparent oral clearance normalized to body weight ($CL/F/\text{kg}$; II vs. I, $P = 0.52$; III vs. I, $P = 0.16$) of total LPV were observed among the three groups.

Unbound LPV $AUC_{u(0-12)}$ were 0.87 (0.77–1.21) $\mu\text{g}\cdot\text{h}/\text{mL}$, 1.21 (0.73–1.43) $\mu\text{g}\cdot\text{h}/\text{mL}$, and 1.41 (1.14–1.85) $\mu\text{g}\cdot\text{h}/\text{mL}$ for group I, II, and III, respectively. Although values for group II and III were slightly higher compared with group I, this difference did not reach statistical significance (II vs. I, $P = 0.42$; III vs. I, $P = 0.16$). The concentration of unbound LPV just before administration of the following dose ($C_{u,\text{trough}}$) was 0.05 (0.03–0.08) $\mu\text{g}/\text{mL}$, 0.07 (0.05–0.10) $\mu\text{g}/\text{mL}$, and 0.08 (0.03–0.10) $\mu\text{g}/\text{mL}$ for group I, II, and III, respectively (II vs. I, $P = 0.42$; III vs. I, $P = 0.96$), and oral clearance of unbound LPV ($CL_{u/F}/\text{kg}$) was 0.56 (0.34–0.68) $\text{mL}/\text{h}/\text{kg}$, 0.55 (0.41–0.80) $\text{mL}/\text{h}/\text{kg}$, and 0.84 (0.52–1.26) $\text{mL}/\text{h}/\text{kg}$ for group I, II, and III, respectively (II vs. I, $P = 0.57$; III vs. I, $P = 0.10$). The fraction of unbound LPV did not differ significantly between patient groups: group II versus I: 0.99% (0.88–1.20).

versus 0.97% (0.80–1.06), $P = 0.33$; group III versus I: 1.18% (0.89–1.65) versus 0.97% (0.80–1.06), $P = 0.19$. Moreover, no statistically significant differences in $C_{\max}$, $t_{\max}$, and $t_{1/2}$ values for total or unbound LPV were observed between study groups (Table 3).

We observed a high interindividual variability both in total and unbound LPV and total ritonavir pharmacokinetic parameters but found no statistically significant difference among the three groups (Figs. 1, and 2, A and B, and Table 4, respectively). No differences were observed in apparent oral clearance of ritonavir among the three groups studied. There was a strong positive correlation of apparent oral clearance of LPV with ritonavir apparent oral clearance ($r = 0.71$; $P < 0.0001$).

DISCUSSION AND CONCLUSIONS

This study was designed to assess the impact of HCV coinfection with or without cirrhosis on LPV/r pharmacokinetics in HIV-infected subjects. A preliminary pilot study including 20 HIV+ patients (9 HIV+ control subjects vs. 11 HIV+/HCV+ subjects presenting with hepatic impairment including 3 with cirrhosis) highlighted that there was no difference in terms of LPV oral clearance when normalized to body weight (CL/F/kg) between HIV+/HCV+ and HIV+/HCV– patients.³⁰ We have now confirmed these results in a broader panel of patients, taking into account the wide interpatient variability that characterizes LPV/r pharmacokinetics.

The effects of liver disease on pharmacokinetics are highly variable and difficult to predict because the mechanisms are not well understood. Liver disease in humans encompasses a wide range of pathophysiologic disturbances that can lead to reduction in blood flow, extrahepatic or interhepatic blood shunting, hepatocyte dysfunction, quantitative and qualitative changes in serum proteins, and changes in bile flow. One expression of these perturbations is an altered disposition of some, but not all, drugs in patients with liver disease.

A recent study that evaluated the pharmacokinetics of nelfinavir (NFV) and its active metabolite M8 observed that NFV $AUC_{(0-12)}$ was 1.3-fold higher in HIV+/HCV+ patients and 2.5-fold higher in HIV+/HCV+ patients with cirrhosis, relative to a control group of noncoinfected HIV+ patients. The results indicated that the activity of hepatic metabolizing enzymes (particularly the CYP2C19 isoenzyme) is altered in patients with liver disease.³¹ NFV was administered without ritonavir boosting or any known cytochrome P-450 enzyme inhibitors.

In contrast, no pharmacokinetic differences caused by liver dysfunction were found in a small study involving tipranavir/ritonavir (500/200 mg twice daily). This study involved nine HIV-negative volunteers with mild liver impairment (Child-Pugh A 6) and three with moderate hepatic impairment (Child-Pugh B 7–9). Twelve HIV-non-infected control subjects without liver disease were also studied. No significant differences in tipranavir $AUC_{(0-12)}$, $C_{\max}$, or C_{trough} were found between groups.³² However, a recent study by Morello et al³³ observed a significant increase in tipranavir concentrations in patients with significant liver fibrosis, but this did not result in an increase in short-term liver toxicity.

Moltò et al³⁴ studied the pharmacokinetics of LPV/r in 18 HIV-infected versus 22 HIV/HCV-coinfected subjects (Child-Pugh score < 6). The study did not include cirrhotic patients. No differences were noted in $AUC_{(0-12)}$, $C_{\max}$, or C_{trough} of LPV between the two groups. In considering that LPV/r provided similar drug exposures in both groups, LPV/r dose adjustments do not appear warranted in HIV/HCV–coinfected subjects who have no clinical/laboratory signs of liver impairment.

A recent study by Barreiro et al³⁵ investigating the influence of the stage of liver fibrosis on plasma concentrations of some antiretroviral drugs reported a slight but not significant decrease of LPV concentrations in patients with cirrhosis (Metavir score F4) compared with HCV-coinfected individuals without cirrhosis (Metavir score F0–3; 5.4 vs. 6.1 $\mu\text{g/mL}$, respectively). In contrast, cirrhosis affected drug concentrations of efavirenz, resulting in significantly higher plasma concentrations (3.4 vs. 1.9 $\mu\text{g/mL}$, $P=0.001$). Different metabolic pathways involving hepatic isoenzyme CYP2B6 for efavirenz, compared with CYP3A4 for LPV, were suggested as a potential explanation of these differences, with the activity of the latter being more preserved in liver impairment.

Peng et al³⁶ reported the results after multiple dosing of LPV/r 400/100 mg twice daily in HIV/HCV-coinfected subjects with mild to moderate hepatic impairment and found a 30% increase in LPV $\text{AUC}_{(0-12)}$, a 20% increase in C_{max} , and a 79% increase in C_{min} compared with HIV-1 infected subjects with normal hepatic function. The percentage of unbound LPV was similar in the mild (0.89%) and moderate (0.94%) hepatic groups but was statistically significantly higher than that of the control group (0.69%). The authors concluded that this difference would lead to changes in the concentration-time profiles for total or unbound drug during chronic drug intake, and, consequently, cautious interpretation of the total drug concentration measurement is required.

Most of the data available refer to the total plasma drug concentration with little emphasis on the protein binding issue. The pharmacologic activity and potential drug-related toxicity depend on the unbound concentration of a compound. An altered plasma protein binding does not necessarily result in modification of the exposure to the unbound drug at steady-state but can change the relationship between total and unbound drug concentrations (ie, the free-fraction).

In our study, there were no statistically significant differences when comparing the hepatic function groups for both total and unbound LPV concentrations or $\text{AUC}_{(0-12)}$. LPV binding to plasma proteins was extensive in all hepatic function groups, and the unbound fraction was slightly higher in subjects with cirrhosis compared with either HIV positive patients with or without HCV coinfection.

Large increases in the unbound fraction have been observed for a number of drugs in patients with liver disease. The mechanisms for decreased drug binding in patients with liver disease include decreased albumin and α_1 -acid glycoprotein concentrations because of either a decreased rate of synthesis or loss of protein from plasma to interstitial compartments, the accumulation of endogenous inhibitors such as bilirubin, and possible qualitative changes in the protein molecule.

In our study, chronic liver impairment produced a slight, although not significant, decrease in plasma protein binding; the free-fraction of LPV increased from a median (IQR) of 0.97% (0.80–1.06) in HIV+/HCV– patients to 1.18% (0.89–1.65) in HIV/HCV-coinfected patients with cirrhosis. The effect on binding results in a compensatory effect on drug clearance (~29% increase), with a fall in the total drug concentrations ($\text{AUC}_{(0-12)}$). This appears to suggest that the rate of metabolism is proportional to the free-drug concentration, without a substantial effect on intrinsic clearance.

The observation that patients with chronic liver impairment do not exhibit a decrease but rather a partial increase in CL/F/kg needs some comment. Chronic liver disease is characterized by irreversible, chronic hepatocyte damage, resulting in fibrosis, disruption of hepatic vascular architecture, and formation of nodules of regenerated hepatocytes. In patients with chronic liver disease, the pathologic changes result in an absolute decrease in hepatocyte function (50% decrease in cytochrome P450 content). In general, chronic liver

disease affects drug disposition more than any other form of liver disease. In patients with hepatic dysfunction, changes in hepatocellular mass or function may lead to a reduction in intrinsic clearance. With the coformulated LPV/r (Kaletra), because ritonavir inhibits hepatic metabolism and thus decreases LPV intrinsic clearance, the reduction in intrinsic clearance in patients with hepatic dysfunction may be absent or less pronounced than the increase in free fraction. Indeed, CL_u/F/kg in our cirrhotic patients does not change significantly [median (IQR) 0.84 (0.52–1.26) and 0.55 (0.41–0.80) mL/h/kg in HIV/HCV coinfecting patients with and without cirrhosis, respectively, vs. 0.56 (0.34–0.68) mL/h/kg in HIV mono-infected patients]. The apparent trend to a higher value of AUC_u observed in cirrhotic patients was caused mainly by the differences in body weight among the three groups (significantly lower values in the cirrhotic patients) in spite of liver disease.

These data appear to support the concept that the intrinsic clearance of LPV in liver disease is not affected after being inhibited by low-dose ritonavir co-administration. Furthermore, no significant differences in pharmacokinetic parameters of ritonavir among any of the groups were found.

Our results suggest no significant changes in pharmacokinetics of LPV in mild liver dysfunction with morphologic evidence of cirrhosis, possibly because of the inhibitory effect of ritonavir on LPV metabolism. Variable effects on protein binding of the drug, normalization of plasma concentrations to the same dose and body weight, and the intrinsic interpatient variability of LPV all help to explain the contrasting results reported by different studies. On the basis of our pharmacokinetic data, no adjustment of LPV/r dose is required for HIV/HCV coinfecting patients with and without cirrhosis, even though additional information would be needed for more severe grades of cirrhosis.

Acknowledgments

The authors thank Dr. Rosangela Beretta, Dr. Laura Valsecchi, and Dr. Gaia Jacchetti and all the patients for their participation in the study.

LD was funded by a grant from the NIHR Biomedical Research Centre for Microbial Diseases.

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FIGURE 1. Median (interquartile range) total lopinavir (LPV) plasma concentrations over 12 hour dosing interval for three groups of HIV+ patients (n = 56).

FIGURE 2. Median (interquartile range) plasma concentrations over 12 hour dosing interval of total lopinavir (LPV) (A) and unbound (UB) lopinavir (B) for three groups of HIV+ patients (n = 36).

TABLE 1

Demographic Characteristics at Baseline for Three Groups of HIV+ Patients

	Group I (n = 24) HIV+/HCV–	Group II (n = 23) HIV+/HCV+	Group III (n = 9) HIV+/HCV+ with Cirrhosis	<i>P</i> *	<i>P</i> [†] II vs. I	<i>P</i> [†] III vs. I
Age (yr)	44 (38–55)	42 (40–44)	42 (36–45)	0.56	0.39	0.44
Sex, n (%)						
Male	18 (75)	20 (87)	4 (44)			
Female	6(25)	3 (13)	5 (56)			
CDC class						
A	8	1	2			
B	6	6	2			
C	10	16	5			
Risk factor						
Heterosexual	17	5	4			
Homosexual	5	3	—			
Intravenous drug user	2	15	5			
Child-pugh score	—	—	A5–C11			
CD4+ (cells/mm ³)	392 (224–466)	260 (185–309)	362 (110–530)	0.22	0.06	0.78
Body weight (kg)	71 (66–84)	68 (55–77)	60 (55–70)	0.08	0.34	0.03
Body mass index (kg/m ²)	23.6 (21.7–26.8)	22.6 (19.7–25.3)	20.3 (19.6–21.0)	0.06	0.18	0.01
Dose/body weight	5.7 (4.8–6.4)	5.9 (5.2–7.1)	5.7 (5.4–6.5)	0.86	0.72	0.61
Time of exposure to LPV/r (wk)	35.1 (5.7–78.1)	33.9 (6.9–66.1)	20.7 (3.2–64.2)	0.71	0.81	0.46

Values are reported as median and interquartile range (unless specified otherwise); data analyzed by
and

* Kruskal-Wallis test

[†] Mann-Whitney test (significance, $P < 0.05$).

TABLE 2

Biochemical Parameters at Baseline for Three Groups of HIV+ Patients

	Group I (n = 24) HIV+/HCV–	Group II (n = 23) HIV+/HCV+	Group III (n = 9) HIV+/HCV+ with cirrhosis	<i>P</i> *	<i>P</i> [†] II vs. I	<i>P</i> [†] III vs. I
Aspartate aminotransferase (AST) (UI/L)	26 (21–32)	41 (30–61)	70 (46–128)	0.0002	0.002	0.0007
Alanine aminotransferase (ALT) (UI/L)	27 (18–37)	74 (30–96)	69 (31–137)	0.003	0.002	0.03
Total bilirubin (mg/dL)	0.67 (0.55–0.83)	0.80 (0.49–1.06)	1.10 (0.60–1.37)	0.31	0.79	0.14
Albumin (g/dL)	4.4 (4.1–4.5)	4.2 (3.5–4.3)	3.9 (3.1–4.1)	0.008	0.02	0.03
Pseudocholinesterase (pCHE) (mg/dL)	7791 (6,655–10,249)	6912 (4,983–7,906)	3857 (2,116–6,295)	0.006	0.06	0.003
Alkaline phosphatase (AP) (UI/L)	173 (89–2,51)	163 (108–268)	133 (106–243)	0.96	0.99	0.78
γ-glutamyltransferase (GGT) (UI/L)	58 (25–79)	69 (30–122)	78 (66–86)	0.23	0.26	0.11
Prothrombin time (PT) (INR)	1.00 (0.90–1.03)	1.10 (1.00–1.20)	1.30 (1.21–1.35)	0.003	0.06	0.003
Partial prothrombin time (PPT) (INR)	0.98 (0.90–1.03)	1.05 (0.96–1.13)	1.25 (1.15–1.33)	0.002	0.06	0.003

Data analyzed by

and

INR, international normalized ratio.

* Kruskal-Wallis test

† Mann-Whitney test (significance, $P < 0.05$).

TABLE 3

Median (Interquartile Range) Total and Unbound Lopinavir Pharmacokinetic Parameters for Three Groups of HIV+ Patients

	Group I, HIV+/HCV–	Group II, HIV+/HCV+	Group III, HIV+/HCV + with cirrhosis	<i>P</i> [*]	<i>P</i> [†] II vs. I	<i>P</i> [†] III vs. I
Total lopinavir						
No. of patients	24	23	9			
AUC _(0–12) (μg*h/mL)	110.4 (80.9–135.2)	103.4 (85.5–131.3)	92.8 (87.4–116.3)	0.88	0.68	0.71
C _{trough} (μg/mL)	7.4 (4.1–10.0)	6.4 (4.5–10.1)	7.0 (5.7–7.6)	0.94	0.97	0.66
C _{max} (μg/mL)	12.8 (10.9–15.7)	11.0 (9.8–16.3)	10.2 (8.8–13.9)	0.65	0.54	0.37
t _{max} (h)	4.0 (3.0–6.0)	4.0 (4.0–6.0)	4.0 (4.0–6.0)	0.24	0.13	0.34
t _{1/2} (h)	7.2 (6.5–8.7)	6.5 (6.0–7.6)	7.1 (6.6–10.2)	0.21	0.12	0.85
CL/F/kg (mL/h/kg)	55 (40–68)	59 (44–69)	71 (53–78)	0.28	0.52	0.16
Unbound lopinavir						
No. of patients	12	18	6			
f _u (%)	0.97 (0.80–1.06)	0.99 (0.88–1.20)	1.18 (0.89–1.65)	0.34	0.33	0.19
AUC _{u(0–12)} (μg*h/mL)	0.87 (0.77–1.21)	1.21 (0.73–1.43)	1.41 (1.14–1.85)	0.36	0.42	0.16
C _{u, trough} (μg/mL)	0.05 (0.03–0.08)	0.07 (0.05–0.10)	0.08 (0.03–0.10)	0.74	0.42	0.96
C _{u, max} (μg/mL)	0.12 (0.10–0.14)	0.13 (0.10–0.18)	0.16 (0.14–0.18)	0.31	0.33	0.15
CL _u /F/kg (mL/h/kg)	0.56 (0.34–0.68)	0.55 (0.41–0.80)	0.84 (0.52–1.26)	0.32	0.57	0.10

Data analyzed by

and

CL_u/F/kg is expressed as CL/F/kg*fu.

* Kruskal-Wallis test

[†] Mann-Whitney test (significance, *P* < 0.05)

TABLE 4

Median (Interquartile Range) Total Ritonavir Pharmacokinetic Parameters for Three Groups of HIV+ Patients

Pharmacokinetic Parameters	Group I, HIV+/HCV–	Group II, HIV+/HCV+	Group III, HIV+/HCV+ with Cirrhosis	<i>P</i> *
No. of patients	14	15	4	
AUC _(0–12) (μg*h/mL)	8.7 (6.8–9.5)	8.2 (4.0–10.4)	11.8 (10.5–14.9)	ns
C _{trough} (μg/mL)	0.57 (0.51–0.77)	0.50 (0.23–0.73)	0.70 (0.65–0.72)	ns
C _{max} (μg/mL)	1.11 (0.95–1.33)	1.10 (0.77–1.37)	1.68 (1.55–1.97)	ns
T _{max} (h)	4.0 (4.0–5.5)	4.0 (3.5–6.0)	5.0 (3.8–6.0)	ns
CL/F/kg (L/h/kg)	0.19 (0.13–0.23)	0.17 (0.13–0.36)	0.18 (0.15–0.19)	ns

ns, not significant.

* Data analyzed by Kruskal-Wallis test (significance, $P < 0.05$).