Higher plasma lopinavir concentrations are associated with a moderate rise in cholestasis markers in HIV-infected patients

Elena Seminari¹*, Gianluca Gentilini², Laura Galli¹, Hamid Hasson¹, Anna Danise¹, Elisabetta Carini¹, Fernanda Dorigatti², Armando Soldarini², Andriano Lazzarin¹ and Antonella Castagna¹

¹Infectious Disease Department, San Raffaele Scientific Institute, Via Stamira d’Ancona 20, 20122 Milan, Italy; ²Laboraf, San Raffaele Scientific Institute, Via Olgettina 60, 20122 Milan, Italy

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Objectives: The aim of this study was to evaluate the correlation between liver function markers (necrosis and cholestasis) and plasma lopinavir levels in a cohort of HIV-infected patients treated with lopinavir and ritonavir.

Patients and methods: The blood samples for determining steady-state Cₜrough lopinavir levels and analysing liver function were drawn from fasting patients. Steady-state Cₜrough lopinavir levels, liver function and immuno-virological markers were assessed on the same day. Plasma lopinavir and ritonavir levels were determined by means of high-performance liquid chromatography.

Results: One hundred and forty-nine patients were included in the analysis [57 were HCV co-infected (34%) and 10 were HBV co-infected (6.7%)]; they had been treated with lopinavir/ritonavir for a median of 232 days (range 132–282). All patients received lopinavir/ritonavir [400/100 mg twice daily or 533/133 mg twice daily if amprenavir or a non-nucleoside reverse transcriptase inhibitor (NNRTI) was part of therapy] and concomitant therapy with NRTI(s). Median (interquartile) lopinavir trough levels were 6391 ng/mL (4121–8726), 5662 (3585–8893) and 6819 ng/mL (5324–8726) in the patients with HIV alone and those with HIV/HCV (or HBV) co-infection, respectively (P = not significant). Univariate analysis showed a significant association between the cholestasis markers and Cₜrough lopinavir level. Multivariate analysis selected only gamma glutamyltransferase (GGT) (OR = 1.010, 95% CI: 1.002–1.021) as being independently associated with plasma lopinavir levels of >6425 ng/mL; alkaline phosphatase (OR = 1.004, 95% CI: 1.000–1.010; P = 0.08) and total bilirubin (OR = 3.118, 95% CI: 0.980–11.715; P = 0.07) were not associated.

Conclusions: Elevated lopinavir concentrations are associated with raised GGT.

Keywords: pharmacokinetics, drug monitoring, HIV antiviral pharmacology

Introduction

The plasma concentrations of all antiretroviral drugs vary widely from one patient to another for a large number of reasons, including genetic factors that determine the activity of CYP isoenzymes and drug-transporting proteins, drug–drug and drug–food interactions, and concomitant diseases such as kidney and liver insufficiency. The inter-patient Cₜrough variability of lopinavir ranges from 50% to 70%, but increased lopinavir concentrations have only been observed in patients with mild or moderate cirrhosis, furthermore, no difference in plasma lopinavir levels has been observed between HIV/HCV co-infected patients and those infected by HIV alone. Despite the higher incidence of hepatotoxicity in co-infected patients, it has been reported that there is no correlation between necrosis markers and plasma lopinavir levels, but there are no published data concerning cholestasis markers and lopinavir pharmacokinetics.

The aim of this retrospective study was to evaluate the correlation between liver function markers (necrosis and cholestasis) and plasma lopinavir levels in a cohort of drug-experienced HIV-infected patients treated with lopinavir and ritonavir.
Lopinavir plasma concentrations and cholestasis markers

Methods

All patients that underwent a lopinavir plasma analysis at the Infectious Disease Department, San Raffaele Scientific Institute, Milan, Italy, between 2002 and 2004, were included in this study. The blood samples for determining steady-state C_{trough} lopinavir levels and analysing liver function were drawn from fasting patients who had been instructed not to take their morning medication before the sampling. Exclusion criteria were: age below 18, not being at steady state when sample was collected, use of co-medication known to induce an increase in liver function tests or to interfere with cytochrome P-450 system activity, pregnancy.

Their fasting status, last dose intake, and all concomitant therapies (including herbal therapies) were verified by the study nurse involved in the Therapeutic Drug Monitoring (TDM) programme; their epidemiological data were acquired from an electronic database.

Steady-state C_{trough} lopinavir levels, liver function [total bilirubin, alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT) and alkaline phosphatase (AP)] and immuno-virological markers (CD4+ and HIV RNA) were assessed on the same day.

Plasma lopinavir and ritonavir levels were determined by means of high-performance liquid chromatography after liquid–liquid plasma extraction, separation on a C18 column and UV detection.

Plasma lopinavir standard and control solutions were prepared by dissolving the lopinavir pure substance (kindly provided by Abbott Laboratories, Abbott Park, IL, USA) in H2O/methanol, at a unique level for calibrator and control (~10 000 ng/mL and 5000 ng/mL, respectively). The limit of detection (LOD); the lowest drug concentration giving a signal-to-noise ratio greater than 3:1) was about 20 ng/mL, whereas the limit of quantification (LOQ) was 50 ng/mL. Precision was calculated after the analysis of six replicate spiked plasma samples on the same analytical run (intra-assay) and after six repeated analyses along different analytical runs (inter-assay), at the unique lopinavir quality control concentration (5000 ng/mL) and was expressed as the percentage coefficient of variation (CV%). For the intra-assay precision, the CV was 4.6%, and for the inter-assay precision, the CV was 7.8%.

The standard AIDS Clinical Trial Group toxicity table was used to grade the levels of AP, GGT, total bilirubin and ALT.

Statistical analysis

The data are expressed as median and interquartile values.

Fisher’s exact test and the Mann–Whitney rank-sum test were used for the univariate analysis. The multivariate analysis was made using a logistic regression model to assess the independent contributions of some risk factors [age, HBV or HCV co-infection, time on lopinavir-containing therapy (delta t), the concomitant use of a non-nucleoside reverse transcriptase inhibitor (NNRTI) or another protease inhibitor, and ALT, and AP, or GGT or total bilirubin levels] to outcome, which was defined as a median C_{trough} lopinavir level of more than 6425 ng/mL (median lopinavir trough level of our cohort). This value was consistent with Kaletra® prescribing information that shows a lopinavir C_{trough} of 5500 ± 4000 ng/mL.

The odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated, and the significant values are reported.

The analyses were performed using SAS software (version 8.0, SAS Institute). A two-sided P value of <0.05 was considered statistically significant.

Results

In the period between 2002 and 2004, lopinavir plasma level was determined in 149 patients (115 males and 34 females; median age 39 years, range 37–44), 57 co-infected with HCV (38%) and 10 with HBV (6.7%), as part of the TDM programme and all of them were included in the analysis. Their overall CD4+ cell count was 302 cells/mm³ (range 179–493) and they had 2.98 log_{10} HIV RNA copies/mL (range 1.89–4.38). They had been treated with lopinavir/ritonavir for a median of 232 days (range 132–282) (Table 1). Concomitant therapies included: lipid-lowering agents in 89 patients (60%); co-trimoxazole in 34 patients (23%); and night sedation in 18 patients (12%).

All of the patients received lopinavir/ritonavir 400/100 mg twice daily (or 533/133 mg twice daily if amprenavir or an NNRTI was part of the therapy) and concomitant therapy with NRTI(s).

Twenty-nine patients were concomitantly treated with an NNRTI (18 efavirenz, and 11 nevirapine) and 25 with a third protease inhibitor (5 indinavir, 11 amprenavir, 8 saquinavir, and 1 nelfinavir).

Median AP was 205 U/L (176–243), median GGT was 44 U/L (27–98), median total bilirubin was 0.69 mg/dL (0.51–0.89), and median ALT was 32 U/L (19–65). Twenty-two patients (14.8%) patients had AP levels of more than 279 U/L (324 U/L; 303–376) (grade 1 in 21 and grade 2 in one); 52 (35%) had GGT levels of more than 50 U/L (92 U/L; 67–124) (grade 1 in 29, grade 2 in 20, and grade 3 in three); 22 (14.8%) had total bilirubin levels of more than 1 mg/dL (1.33 mg/dL; 1.14–1.52) (grade 1 in 17 and grade 2 in five), and 37 (24.8%) had ALT levels of more than 55 U/L (89 U/L; 75–119) (grade 1 in 30 and grade 2 in seven).

The overall median lopinavir trough level was 6391 ng/mL (4121–8726), and 5662 (3585–8893) and 6819 ng/mL (5324–8726) in the patients with HIV alone and those with HIV/HCV (or HBV) co-infection, respectively (P = not significant).

Univariate analysis showed a significant association between the cholestasis markers (stratified into increased and low-normal values) and C_{trough} lopinavir level (stratified on the basis of its median value). The patients with a C_{trough} lopinavir level above the threshold were more likely to have increased levels of GGT (34, 57.6% versus 23, 37.7%; P = 0.04) and total bilirubin (16, 25.4% versus 6, 9.1%; P = 0.02); there was no between-group difference in ALT and AP levels. In comparison with those with lower levels, the patients with C_{trough} lopinavir levels of more than 6425 ng/mL showed increased levels of total bilirubin (0.62 versus 0.56 mg/dL; P = 0.007), AP (219 versus 206 U/L; P = 0.009) and GGT (62 versus 52 U/L, P = 0.006).

Multivariate analysis selected only GGT (OR = 1.010, 95% CI: 1.002–1.021) as being independently associated with plasma lopinavir levels of >6425 ng/mL; AP (OR = 1.004, 95% CI: 1.000–1.010; P = 0.08) and total bilirubin (OR = 3.118,
95% CI: 0.980–11.715; \( P = 0.07 \) were not associated (AP, GGT and total bilirubin were continuously considered in the model and their ORs were estimated by 1 unit increase).

**Discussion**

There is strong evidence that the pharmacokinetics of protease inhibitors is influenced by alterations in hepatic metabolism, although there is no clear association between high serum protease inhibitor concentrations and hepatotoxicity expressed as increased aminotransferase levels.\(^6\),\(^7\)

It has always been complex and difficult to prove relationships between drug levels and toxicity, and this is the first report showing an association between cholestasis markers and high plasma lopinavir levels. A plasma lopinavir level of >6425 ng/mL was independently associated with a GGT above the upper limit of normal; this observation seems also to be valid for AP and total bilirubin, even though these two markers were near to significance. On the contrary, no association was found between liver necrosis markers and plasma lopinavir levels.

The design of this study does not allow us to assume a causal relationship, at least because no baseline liver function data were available for the analysis. Other limitations may be that we determined total plasma lopinavir levels without distinguishing the total and unbound fractions, the latter being the active drug associated with antiviral activity and possibly toxicity;\(^4\) and the absence of intra-patient variability data for this cohort. No association between the use of lopinavir and increased levels of cholestasis markers has been reported previously;\(^8\) our hypothesis is that even a mild cholestatic state may have an impact on the liver metabolism of lopinavir, but whether this may be associated with greater liver toxicity, in particular, if higher lopinavir plasma levels are associated with cholestasis, remains to be fully evaluated with prospective studies.

Liver toxicity due to protease inhibitors is often primarily evaluated on the basis of increased aminotransferase levels,\(^9\) with grade 3 or 4 aminotransferase increases being developed by 7–13% of co-infected patients as opposed to 1–2% of those with HIV alone. In particular, combined lopinavir/ritonavir treatment has been associated with increased aminotransferase levels in 4–10% of patients,\(^10\) whereas a more specific sign of hepatic injury (increased serum bilirubin levels) has been found in up to 5% of highly active antiretroviral therapy (HAART)-treated patients\(^11\) and increased AP levels are probably under-reported.\(^12\) It may therefore be that a drug reaction considered ‘hepatocellular’ is actually ‘cholestatic’ or ‘mixed’.\(^12\)

The finding that cholestasis markers are associated with increased plasma lopinavir levels is not surprising as a correlation between cholestasis and the plasma levels of amprenavir was observed in a study showing that total bilirubin levels were predictive of the amprenavir AUC in subjects with liver impairment.\(^13\) There is some evidence indicating that increased baseline levels of cholestasis markers such as AP\(^14\) and GGT\(^15\) represent an independent risk factor for the development of acute increases in liver enzyme levels during HAART. Increased AP levels have also been found to be a risk factor associated with hepatic decompensation in patients with liver cirrhosis on interferon-based therapy.\(^16\)

We observed that high cholestasis marker levels are associated with increased plasma lopinavir levels. It could be suggested that patients with increased cholestasis markers have a lopinavir plasma level determination, and, possibly, also, that patients with higher values of lopinavir \(C_{\text{trough}}\) (i.e. 5500 ng/mL) should have their liver function parameters monitored.

**References**