ORIGINAL ARTICLE

Pharmacokinetics of Lopinavir Determined with an ELISA Test in Youths with Perinatally Acquired HIV

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Abstract

Objective To investigate the plasma levels of lopinavir by enzyme-linked immunosorbent assay (ELISA) in a cohort of patients who were vertically infected with human immunodeficiency virus 1 (HIV).

Methods Plasma levels of lopinavir (C_{min}) were determined by ELISA test in patients treated with lopinavir/ritonavir-based combined antiretroviral therapy who had achieved virological response after 4 wk of therapy. Reference lopinavir concentrations were C_{min} 1–8 µg/mL. Correlation between lopinavir plasma concentration and continuous variables was evaluated by mean of Pearson correlation coefficient. Differences in lopinavir (LPV) concentration for binary categorical variables were assessed by Mann-Whitney test, while for variables with more than two categories Kruskal-Wallis test was used.

Results Thirty-four patients were enroled; median age was 133 mo (15–265). The median lopinavir dose tested was 383.5 mg/kg (IQR: 266.6–400 mg/kg), with a median plasma concentration of 8.8 µg/mL (IQR: 5–14 µg/mL). Lopinavir C_{min} was <1 µg/mL in only one sample (2.9 %), while 14 samples had C_{min} between 1 and 8 µg/mL (41.2 %) and 19 (55.9 %)>8 µg/mL. No significant correlations were found between plasma concentrations of lopinavir and the continuous variables considered in the study. A negative but, not completely significant, correlation was found between plasma drug concentration and body mass index ($r=-0.29$; $p=0.09$).

Conclusions The use of a simple and relatively cost-effective methodology might render therapeutic drug monitoring (TDM) appeal in the daily clinical practice.

Keywords Lopinavir/Ritonavir · Vertical transmission · Therapeutic drug monitoring · ELISA-TDM · HIV-infected patients · Adherence to cART

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Introduction

The therapeutic drug monitoring (TDM) is a tool generally used to monitor plasma drug levels of antiretroviral drugs, being the plasma concentration of protease inhibitors (PIs) and non-nucleosidic reverse transcriptase inhibitors (NNRTIs) associated with the levels of viral suppression and drug toxicity. Unfortunately data about pharmacokinetics of these drugs in pediatric patients are limited [1, 2].

TDM is also applicable to measure the adherence to the therapy, allowing to check if the medication is actually taken by the patient. United States and Europe HIV treatment guidelines recognize the potential benefit of TDM in selected groups of patients, including children, pregnant women, patients with a change in physiologic state, potential for drug-drug or food interaction, use of alternative dosage or drug related toxicity [3, 4].

However, the standard TDM methodology, the high performance liquid chromatography (HPLC), is expensive and technically-demanding. An alternative method to check concentrations of the drugs in plasma samples is by enzyme-linked immunosorbent assay (ELISA)-TDM, cheaper and easier to perform than HPLC. The aim of the study was to

evaluate the plasma levels of lopinavir (LPV) by TDM-ELISA in a cohort of vertically HIV-infected patients.

Material and Methods

This was an open-label and descriptive study in children, adolescents and young adults perinatally infected with HIV-1. The authors defined children as those younger than 12 y, adolescents 12 to 18 y and the young adults older than 18 y. All patients who had achieved virological response after 4 wk of combined antiretroviral therapy (cART) containing LPV/ritonavir plus two nucleoside reverse transcriptase inhibitors (NRTIs) were included in the study. Virological response was defined as viral load (VL) <50 copies/mL or decrease of >1 $\log_{10}$ in the first 4 wk of treatment.

Blood samples (5–7 mL) for pharmacokinetic evaluation were drawn in EDTA-containing tubes. At the time of the sample collection the patients, helped from parents or legal guardians, filled an adherence questionnaire regarding adherence during the last 4 d, modality of drugs consumption (empty/full stomach, with adequate water intake), concomitant medication, compliance to the therapeutic schedule.

In adolescents and young adults weighing more than 40 kg, dosage of LPV/ritonavir was 400/100 mg twice daily; in children, 230 mg/57.5 mg LPV/r/m^2 of body surface area per dose twice daily.

LPV C_{min}, were determined 4 wk after the initiation of the therapy, to ensure steady-state plasma concentrations and all samples were collected 30 min before the morning dose of LPV/ritonavir (pre-dose). LPV C_{min} were evaluated with TDM ELISA, an immunoenzymatic test (Lopinavir TDM-ELISA®, by Biostrands srl, Trieste, Italy), according to the procedures described below and detailed in the lopinavir TDM ELISA package insert [5]. TDM-ELISA technology is based on a competition between the drug in plasma sample and an analogue conjugated to the detecting enzyme. A species–specific solid phase captures the specific antibody, while excess sample and reagents are removed by washing. The conjugate bound to the solid phase is detected by adding a chromogen solution. The enzymatic activity produces a colored solution whose adsorbance can be read by a microplate reader at 450 nm. Absorbance values are inversely proportional to the drug concentration on the sample. A calibration curve is tested in exactly the same way, with the following concentrations: −0.625 µg/mL, −2.5 µg/mL, −6.25 µg/mL, −12.5 µg/mL, −25 µg/mL, −50 µg/mL; negative plasma samples were included, the standard 0 was total binding. The dynamic range for the LPV test is 1 to 8 µg/mL.

Statistical analysis was completed both for the real LPV plasma concentration and for the LPV plasma concentration divided in two classes (<=8 µg/mL and >8 µg/mL).

Correlation between continuous LPV plasma concentration and continuous variables such as age in months, disease duration, weight, body mass index (BMI), lymphocyte CD4 cell count, drug dosage, time between last dose and sampling was evaluated by mean of Pearson correlation coefficient while for number of tablets, Spearman's rank correlation coefficient was used.

Differences in LPV concentration for binary categorical variables were assessed by mean of non-parametric Mann-Whitney test, while for categorical variables with more than two categories (i.e., : formulation) non-parametric Kruskal-Wallis test was used.

The study protocol was approved by the local Institutional Review Board of participating centres. Written informed consent was obtained before study entry, and human experimentation guidelines of the US Department of Health and Human Services and/or those of the authors' institutions were followed.

Results

In one year of observation, a total of 34 HIV-infected young patients naïve to LPV were enroled in the study. Twenty-five patients (73.5 %) were Caucasians, 4 (11.8 %) were Africans, 4 (11,8 %) were Latin Americans, and 1 (2.9 %) was Asian. All patients showed good immuno-virological response to LPV-containing cART, with a decrease in viral load of ≥1 $\log_{10}$ in the first 4 wk of treatment. According to the Centers for Disease Control classification, 13 (38.2 %) samples were collected in patients who were in stage A (A1=5; A2=5 and A3=3), 14 (41.2 %) were in stage B (B1=5; B2=5 and B3=4), 6 (17.6 %) in stage C (C2=1 and C3=5) and 1 (3 %) in stage N (N2). The median age of patients was 133 mo (r=15–264 mo) and the weight ranges comprised between 11 and 76 kg with median weight of 34.1 kg (IQR: 24.3–52), and median BMI of 17.6 kg/cm^2 (IQR 16.2–20), as showed in Table 1.

All patients received LPV in twice-daily regimen; tablet formulation in 18 cases (53 %), capsules in 7 (20.6 %) and syrup in 9 (26.4 %). A variety of antiretroviral combinations were co-administered with LPV, including zidovudine/lamivudine in 10 patients (29.4 %), tenofovir/lamivudine in 6 (17.6 %), abacavir/lamivudine in 6 (17.6 %), tenofovir/emtricitabine in 5 (14.7 %) and other regimens in the remaining seven patients (20.6 %).

The median time between last LPV dose and sample collection was 12.5 h (IQR: 12–13 h) and median LPV dose tested was 383.5 mg/m^2 (IQR: 266.6–400 mg/m^2), with a median plasma concentration of 8.8 µg/mL (IQR: 5–14 µg/mL). LPV plasma concentrations resulted erratic, with big inter-patient variability: the average LPV C_{min} of the cohort was 9.2 µg/mL (SD=±4.96, r=0.6–18 µg/mL); in patients

Table 1 Demographic and clinical data of the study population at baseline

Variable	Median	Range
Gender – no. (%)		
Males 16 (45.7)	–	–
Female 19 (54.3)	–	–
Age (mo)	133	15–265
Weight (kg)	31	11–76
BMI	17	12–28
CD4+ (cells/mm^3)	569	258–1,945
CD4+%	28	16–45
Viral load (copies/mL)	<50	<40–33,000
LPV dosage (mg/kg)	368	200–800
LPV C$_{min}$ (μg/mL)	8.2	0.6–35.0

BMI Body mass index, *LPV* Lopinavir

reporting complete adherence average LPV C$_{min}$ was 9.8 μg/mL (SD=±4.94; r=0.6–18 μg/mL).

Among the 34 samples analyzed, LPV C$_{min}$ was <1 μg/mL in only one samples (2.9 %), while 14 samples had C$_{min}$ between 1 and 8 μg/mL (41.2 %) and 19 (55.9 %)>8 μg/mL.

Six out of 34 patients referred at least one omission to therapy since the introduction of LPV in cART regimen, but none of them had LPV concentration <1 μg/mL. The adherence was complete in the remaining 28 cases (82.4 %), according to the results of the questionnaires filled by patients.

The only patient with LPV C$_{min}$<1 μg/mL did not refer omissions and took the therapy on an empty stomach, the time

between the last dose and the moment in which the sample was collected was 14 h in this case and the viremia was undetectable.

Nine patients had detectable viremia after 4 wk of LPV treatment; no significant correlations were found between plasmatic concentration of LPV and the other continuous variables considered in the study, such as age, length of infection, weight, BMI, lymphocyte CD4 cell count, dosage of LPV, time passed between last drug consumption and plasma sampling. A negative but, not completely significant, correlation was found between plasma drug concentration and BMI (r=−0.29; p=0.09).

The non-parametric Kruskal Wallis test did not show statistically significant differences in LPV concentration among ethnicities (p=0.340).

Higher LPV concentrations (>8 μg/mL) were found in the majority of patients (19/34). Among them 14 received lamivudine in the back-bone regimen, 9 received zidovudine, 5 tenofovir, 2 emtricitabine, 3 abacavir, 3 didanosine, 1 stavudine and 1 efavirenz,. A not completely significant difference (p=0.09) was found between values of LPV plasma concentrations and assumption of didanosine (median : 14; IQR : 14–14.6) compared to patients who did not take this drug (median: 8.1; IQR: 4.7–11). However only three of the patients enroled in the study (8.8 %) took didanosine. No statistically significant correlation was found between hours between taking of LPV and blood sampling and LPV concentration. Considering only subjects with a strict criteria of 12 h of taking LPV/r, there are 15 patients with a mean LPV plasma concentration of 8.71±4.65, as shown in Fig. 1. Also in this

Fig. 1 LPV concentrations in samples and hour of collection (only considering a subgroup of 15 patients)
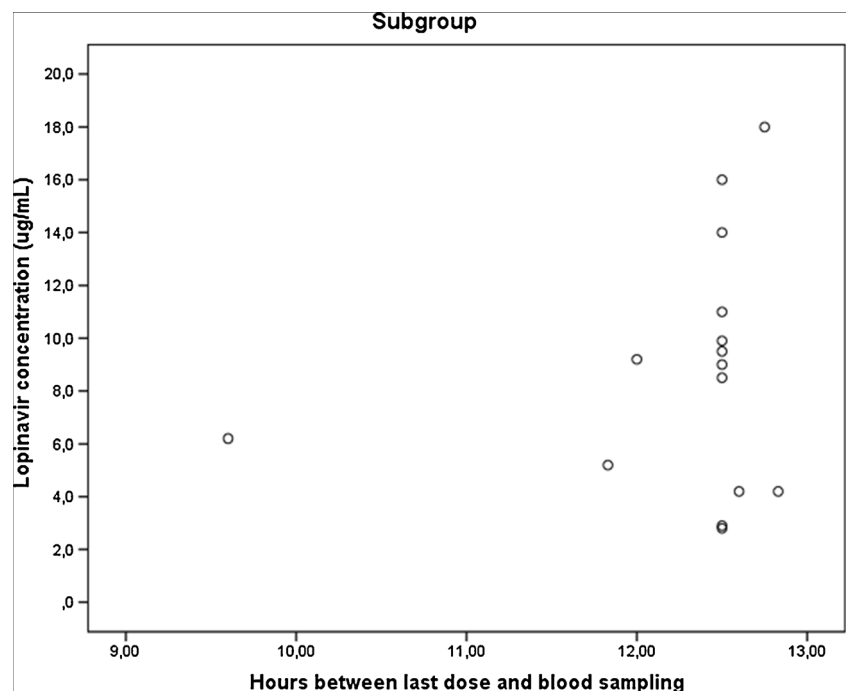
small subgroup, correlation between continuous LPV plasma concentration and time between last dose and sampling as assessed by mean of Pearson correlation coefficient did not reveal any statistical significance ($r=0.192$; $p=0.49$).

Discussion

International, European, and US pediatric antiretroviral therapy guidelines recommend co-formulated protease inhibitor lopinavir/ritonavir as a first-line PI agent for the treatment of HIV infection in children and infants with a post-natal age of at least 14 d [4, 6–8]. The recommended doses for children who weigh more than 15 kg are 10 mg/kg of body weight or 230 mg/m^2 (body surface area) twice daily, with a maximum of 400 mg per dose unless it is combined with drugs affecting cytochrome (CYP) P450 metabolism, which require LPV dose adjustment [4].

Models of pharmacokinetics of LPV in children have been developed on the basis of weight and age of patients [9–12]. However, the absolute bioavailability of LPV co-formulated with ritonavir in humans has not yet been established [13].

A variety of factors can interact with LPV plasma concentration. The administration of the drug with a moderate fatty food for example may increase its adsorption [13].

Conversely, a factor potentially causing a decrease in plasma LPV concentrations is its hepatic inactivation made by the cytochrome CYP 3A4, partially inhibited by ritonavir co-formulation.

Another factor influencing the kinetics of the drug is its high plasma protein binding: at steady state, LPV is 98–99 % plasma protein bound, involving possible interactions with a multitude of drugs binding plasma proteins too.

In the index study, no patient took other drugs or had co-morbidities affecting the ability to metabolize the drug. However, from the analysis, inter-individual variability in drug concentration resulted high and no factor associated significantly with drug concentration was found. These data led the authors to think that therapeutic drug monitoring should have a relevant role in routine monitoring of patients taking LPV.

The use of TDM in the management of patients infected with HIV has been widely studied, and well accepted by the patients [14–18]. TDM is a valid clinical method to avoid over or under-dosage of drugs and also to monitor compliance, in order to optimize the treatment for each patient and avoid side effects.

Besides, it could also be a useful tool to monitor which patients may be eligible for once-daily drug regimens [19]. Nevertheless, few data are available on TDM use in children and adolescents [12, 18, 20, 21]. High performance liquid chromatographic method remains the standard of care in TDM, but it is a technically-demanding and time consuming procedure, available in few laboratories and run by specifically trained expert personnel. The TDM ELISA method overcomes problems associated with peripheral HIV centers' lacking of access to pharmacokinetic test.

High inter-individual variability in trough levels of LPV was found, as already observed in all patients treated with PIs [22].

In patients with a difficult approach to cART, such as children, adolescents and young adults, TDM may also have a role in monitoring adherence during routine clinical practice. In fact, the adherence to HIV medication regimens is often suboptimal in young patients and decreases progressively when children reach adolescence, [23–26] due to a multitude of factors, such as lack of family support, denial and fear of HIV infection, lack of belief in the effectiveness of medications, regimen fatigue, adverse effects and fear of stigmatization [25, 27–32].

The main limitations of the present study were the small size of study population, the exclusion of patients which did not meet the criteria for viral response and those pre-treated with other PIs. Furthermore, the results were not compared with the gold standard TDM methodology represented by HPLC.

The use of a simple and relatively cost-effective methodology might render TDM feasible in the daily clinical practice of HIV-infected youths.

Conclusions

TDM ELISA testing could be a reliable and useful method for routine monitoring of drug plasma levels, since it is easy to perform in any laboratory and could be a valid clinical tool to avoid over or under-dosage of drugs and also to monitor compliance, in order to optimize the treatment for each patient and avoid side effects.

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