Lopinavir/ritonavir exposure in treatment-naive HIV-infected children following twice or once daily administration

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Objectives: Lopinavir/ritonavir is approved for treatment of HIV-infected children at a dosage regimen of 230/57.5 mg/m² twice daily. However, once daily administration could increase convenience and patient adherence. Our study aimed at evaluating whether inhibitory concentrations are maintained in plasma following administration of lopinavir/ritonavir once daily.

Patients and methods: Lopinavir/ritonavir was administered at the standard twice daily regimen to 21 HIV-infected children, as a component of their antiretroviral treatment. Following at least 1 month of administration, seven patients received a dose of 460/115 mg/m² once daily for three consecutive days. After the third dose of once daily administration, blood samples were drawn at the following times: 0 (pre-dose), 1, 2 and 4 h following administration. The pre-dose (Cmin) and the peak (Cmax) concentrations were compared with the values obtained following twice daily administration in all the study patients.

Results: Median (interquartile range) Cmin with the once daily regimen was 1.59 (0.77–6.85) mg/L versus 7.90 (5.45–9.77) mg/L with the twice daily regimen (P < 0.05). Cmin was considered inhibitory for wild-type virus (>1.0 mg/L) in four out of seven patients. Cmax did not differ significantly between the once daily and twice daily regimens.

Conclusions: Our small pilot study suggests that lopinavir/ritonavir once daily may be a suitable regimen for antiretroviral-naive children. However, due to the high interindividual variability and low concentrations in some patients, therapeutic drug monitoring may be necessary to ensure that concentrations are adequate to inhibit viral replication. A formal clinical study of lopinavir/ritonavir once daily in treatment-naive children is warranted.

Keywords: pharmacokinetics, protease inhibitors, therapeutic drug monitoring

Introduction

Highly active antiretroviral therapy (HAART) has dramatically improved the prognosis for children infected with human immunodeficiency virus type 1 (HIV-1) in the developed world.¹⁻⁴ Therapeutic options continue to increase with the availability of new drugs and new strategies. However, treatment of HIV-infected children has become increasingly complex: maintenance of efficacy, long-term toxicity and adherence are keystones in the management of children.

Lopinavir/ritonavir is a combination of two protease inhibitors (PIs): lopinavir and ritonavir. Lopinavir, in combination with ritonavir, is characterized by high trough concentrations (Cmin) with respect to the IC50 for wild-type virus,⁵ as a consequence of metabolism inhibition due to ritonavir.

Lopinavir/ritonavir has been studied, using the standard twice daily dosage regimen, both in treatment-naive and experienced children.⁶⁻⁸ Current guidelines for antiretroviral treatment in

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paediatric patients, as well as in adults, include lopinavir/ritonavir, administered twice daily in combination with other antiretroviral agents, as a component of initial or salvage therapy.9,10

Although not proven, it is likely that adherence may be improved using once daily regimens in some patients. However, the efficacy of once daily regimens is highly dependent on the maintenance of inhibitory concentrations throughout the entire dosing interval. In fact, administration of the total daily dose in a once daily regimen is generally associated with lower $C_{\text{min}}$, and higher $C_{\text{max}}$, with respect to administration in a twice daily regimen.11

In treatment-naive adult patients the administration of lopinavir/ritonavir total daily dose in one single administration resulted in antiretroviral and immunological activity similar to the one observed with the standard twice daily regimen, when combining lopinavir/ritonavir with twice daily nucleoside analogues (NRTIs).12 Pharmacokinetic analysis showed that $C_{\text{min}}$ values were significantly lower with once daily therapy, but still several fold higher than wild-type virus IC$_{50}$. $C_{\text{max}}$ and AUC obtained with the once daily and twice daily regimens were comparable.12 The usage of once daily lopinavir/ritonavir for antiretroviral-naive adult patients has recently been approved by the FDA.13

Thus, based on adult data, it can be hypothesized that lopinavir/ritonavir once daily could also be used in children due to its potential for improving adherence at no cost in terms of efficacy. However, this hypothesis needs to be supported by pharmacokinetic data obtained in the relevant population. Pharmacokinetic data of once daily lopinavir/ritonavir in children are very limited: only the preliminary results of an ongoing study have been made available to date.14

Our study aimed at evaluating the pharmacokinetics of lopinavir/ritonavir administered once daily in comparison with twice daily administration in HIV-infected naive children and adolescents.

Patients and methods

Study design and patient characteristics

The study was conducted in order to examine the pharmacokinetics of once daily and twice daily lopinavir/ritonavir in combination with two NRTIs.

Patients were enrolled at three large Italian Hospitals (San Martino, Genoa; Bambin Gesù, Rome; and L. Sacco, Milan). The study protocol was approved by the local Institutional Review Board of participating Centres. Written informed consent was obtained from the parents or guardians of the children 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.

Age, weight and height were recorded at entry and at each follow-up visit. General physical examination and laboratory evaluation, including haematological, biochemical and immuno-virological parameters, were conducted at study entry (baseline) and at 4, 12 and 24 weeks after study initiation.

Eligible subjects included children >1 and ≤15 years of age, with documented vertical HIV-1 infection and plasma HIV-RNA levels >50 copies/mL [b-DNA (Bayer®)]. Patients were eligible for study participation regardless of their CD4+ lymphocyte count or percentage.

All patients were naive to PIs and non-nucleoside analogues (NNRTIs). Drug resistance testing was not performed.

Drug administration and blood sample collection

All 28 patients enrolled in the study were treated with a lopinavir/ritonavir-containing HAART, based on the decision of the attending physician. The NRTIs to be administered in combination with lopinavir/ritonavir were chosen on the basis of each patient treatment history. Lopinavir/ritonavir was administered twice daily at a dosage of 230/57.5 mg/m².

A total of 21 patients underwent blood sampling for lopinavir pharmacokinetic assessment after at least 1 month of twice daily treatment; 7 patients withdrew informed consent. The liquid formulation of lopinavir/ritonavir was used in nine children. Once daily administration was tested in a subgroup of seven patients, representative of the study population who underwent pharmacokinetic sampling following twice daily dosing, in terms of age and gender (Table 1). The subgroup of patients who underwent once daily dosing evaluation was switched to receive lopinavir/ritonavir 460/115 mg/m² once daily for 3 days. The switch to once daily lopinavir/ritonavir was carried out after at least 1 month of lopinavir/ritonavir twice daily treatment. Blood sampling for pharmacokinetic assessment of once daily lopinavir/ritonavir was carried out at the end of the 3 day once daily administration. Following pharmacokinetic assessment the patients of the once daily subgroup resumed the standard lopinavir/ritonavir twice daily treatment.

Blood samples (5–7 mL) for pharmacokinetic evaluation were drawn in EDTA-containing tubes within 30 min before a morning dose of lopinavir/ritonavir (pre-dose) and at 1, 2, and 4 h after administration. Blood was centrifuged within 4 h after collection and plasma was stored at –20°C until analysis.

All children and parents/guardians were instructed to rigorously adhere to dosing schedule and standard dietary requirements for at least 3 days before blood sample collection.

Analytical methods

Lopinavir plasma concentrations were assessed using a specific HPLC assay with ultraviolet (UV) detection. The HPLC analysis used a reverse-phase C18 analytical column and a mobile phase consisting of a 60:40 (v/v) solution of acetonitrile in 0.1% phosphoric acid, adjusted to a pH of 7.2. A liquid–liquid extraction procedure was used to separate the drug from the plasma fractions of samples, standards and quality controls. The standard curves for lopinavir were linear within the range of 0.100–12 mg/L in plasma, with a limit of quantification of 0.050 mg/L. Interday and intra-day coefficients of variation were <10% for all the quality control samples.

Pharmacokinetic and statistical analysis

Lopinavir $C_{\text{min}}$ was defined as the plasma concentration observed in the pre-dose sample, and $C_{\text{max}}$ was defined as the highest concentration observed in plasma. Statistical analysis was performed using the Epi INFO program (database and statistics software for public health professional, version 3.3.2, 09/02/2005). The correlation between systemic exposure parameters ($C_{\text{min}}$, $C_{\text{max}}$) and demographic parameters [age, weight and body mass index (BMI)] was evaluated. The Mann–Whitney/Wilcoxon two-sample test was used to compare the parameters of exposure obtained after once daily versus twice daily regimens. Results were considered significant for $P$ values <0.05.

Results

Baseline characteristics of the 28 children enrolled in the study are shown in Table 1. Out of them, 19 children were Caucasian, 5 were black and 4 were Hispanic.

1169
Baseline demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male/female</td>
<td>11/17</td>
</tr>
<tr>
<td>Male/female (twice daily)</td>
<td>10/11</td>
</tr>
<tr>
<td>Male/female (once daily)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.25 (3.50–14.98)</td>
</tr>
<tr>
<td>Age, years (twice daily)</td>
<td>7.43 (3.50–13.46)</td>
</tr>
<tr>
<td>Age, years (once daily)</td>
<td>8.67 (3.83–14.98)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>24.9 (8.3–39.0)</td>
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<tr>
<td>BMI</td>
<td>16.51 (7.68–21.64)</td>
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<tr>
<td>CD4+ lymphocyte count</td>
<td>450 (4–1452)</td>
</tr>
<tr>
<td>CD4+ lymphocyte percentage</td>
<td>20 (1–35)</td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td>121 500 (3900–1900 000)</td>
</tr>
<tr>
<td>CDC HIV disease stage: N, A, B, C</td>
<td></td>
</tr>
</tbody>
</table>

*All the continuous parameters are reported as median (range).*

After 1 month of treatment with lopinavir/ritonavir in combination with two NRTIs, 11 out of 28 patients achieved an HIV-RNA level of <50 copies/mL and 15 out of 28 patients had HIV-RNA levels of <400 copies/mL. All 28 patients had HIV-RNA levels of <400 copies/mL, and 18 patients had HIV-RNA levels of <50 copies/mL after 24 weeks of treatment. All seven children who underwent once daily dosing had HIV-RNA levels of <50 copies/mL at 24 weeks of treatment. None of them experienced virological rebound during the study. At week 24, CD4+ lymphocyte count increased from a baseline value of 448 cells/mm³ to 785 cells/mm³; CD4+ lymphocyte percentage increased from 20% to 29%. During the entire clinical follow-up period, none of the children progressed to AIDS or death.

A total of 21 children underwent blood sampling for pharmacokinetic evaluation while receiving the lopinavir/ritonavir twice daily regimen, and 7 of them underwent pharmacokinetic evaluation after switching to the once daily regimen. The remaining 7 patients (out of the 28) withdrew the informed consent.

Lopinavir Cmin values for the twice daily and once daily regimens are shown in Figure 1. Median [interquartile range (IQR)] Cmin with the once daily regimen was 1.59 (0.77–6.85) mg/L, whereas it was 7.90 (5.45–9.77) mg/L with the twice daily regimen (P < 0.05). Cmax was 11.80 (11.15–16.35) mg/L with the once daily regimen versus 14.60 (10.83–15.98) mg/L with the twice daily regimen (P = not significant). Cmin and Cmax evaluated after administration of the once daily and twice daily regimens were not correlated with age, weight or BMI.

Discussion

In our study in treatment-naive children the combination of twice daily lopinavir/ritonavir (230/57.5 mg/m²) with two NRTIs was confirmed to be a potent and well-tolerated regimen. We believe, however, that once daily administration of lopinavir/ritonavir could be an attractive therapeutic option in children because of its potential for increasing convenience and patient adherence. Therefore, we conducted this small pilot study in order to evaluate whether lopinavir concentrations are sufficient to inhibit viral replication, following once daily administration. A concentration at 24 h following administration (Cmin) ≥1.0 mg/L was considered as inhibitory. This cut-off value has been previously used because it corresponds to 15 times the IC50 of lopinavir for wild-type virus, as determined in the presence of 50% human calf serum and 10% fetal calf serum, i.e. an inhibitory quotient (IQ) of 15. An IQ > 15 has been reported to be associated with improved antiretroviral response.

In our study, Cmin and Cmax values obtained with the twice daily regimen were higher than the values reported by the manufacturer in pediatric patients. However, this difference may in part be explained by the younger age of the children enrolled in the previous study (age range was 6 months to 12 years).

We found, as expected, a significantly lower Cmin with the once daily regimen with respect to the value found with the twice daily regimen. The target cut-off value (1.0 mg/L) was achieved by four out of seven children in the once daily group. One of the three children with Cmin lower than the cut-off value had a Cmin only slightly below target (0.9 mg/L), another patient had a Cmin of 0.64 mg/L and one was below the limit of assay detection.

For our results for the once daily administration are comparable to those found in an ongoing study. In fact, we found a median value for Cmin of 1.59 (IQR 0.77–6.85) mg/L, while in the other study it was 2.74 (IQR: 0.65–9.0) mg/L. It needs to be pointed out that median Tmax was 7.25 h in the other study while we performed blood sampling only until 4 h following administration. However, visual inspection of the pharmacokinetic curve obtained in the other study reveals that median plasma concentrations vary little between 4 and 12 h following administration. Thus, the plasma concentration observed at 4 h in our study can be considered representative of the true Cmax. However, Cmax in our study was comparable to the value found in the other study. In the other study, 5 out of 14 patients (36%) had Cmin <1.0 mg/L, while in our study 3 out of 7 patients (43%) showed similar values.
Lopinavir/ritonavir in children

Although a particular effort was made in order to ensure patient adherence to study protocol, the undetectable $C_{\text{min}}$ in one of our patients may be due to lack of adherence to study protocol. Considering that lopinavir concentrations at 2 and 4 h following administration were also very low (0.27 and 1.64 mg/L), this patient may be defined either non-compliant or can be considered a true population outlier, from a pharmacokinetic standpoint.

It needs to be pointed out that all patients received lopinavir/ritonavir once daily in the morning. This may not be the best approach. In fact, lopinavir absorption is dependent on food intake. Considering that breakfast in Italy is generally low in calories, evening administration could be a better option, possibly resulting in improved absorption and plasma concentrations. This has also been suggested in the previous once daily study in which some of the children with $C_{\text{min}}$ below the cut-off value were switched from morning to evening administration, with the result of an increase in $C_{\text{min}}$. In other patients with low $C_{\text{min}}$ values, the authors of the other once daily study increased lopinavir dose in order to achieve the desired concentrations.14

It needs to be pointed out that the interindividual variability of $C_{\text{min}}$ and $C_{\text{max}}$ with once daily and twice daily regimens found in our study was very high. This is not surprising: paediatric patients are considered as one of the patient categories that can particularly benefit from therapeutic drug monitoring (TDM) and dosage adjustment.15 The high variability in paediatric patients found in our study may reflect pharmacokinetic variability as well as lack of full adherence to the treatment schedule (including adherence to dietary requirements).

No significant adverse events were recorded in our study during the 3 days of switch to once daily lopinavir/ritonavir.

In conclusion, our small pilot study suggests that lopinavir/ritonavir once daily may be a suitable regimen in the treatment of antiretroviral-naive children. However, due to the high interindividual variability, TDM may be necessary to ensure that concentrations are indeed adequate to inhibit viral replication in all patients. A formal clinical study of lopinavir/ritonavir once daily in treatment-naive children is warranted.

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Transparency declarations

None of the authors has a conflict of interest.

References


