Population Pharmacokinetics of Lopinavir Predict Suboptimal Therapeutic Concentrations in Treatment-Experience Human Immunodeficiency Virus-Infected Children

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In adult protease inhibitor (PI)-experienced patients, a lopinavir (LPV) phenotypic inhibitory quotient (PIQ) of >15 has been associated with a higher likelihood of viral suppression. The aims of this study were to develop a population pharmacokinetic (PK) model of LPV in children to estimate the probability of achieving a PK of >15. HIV-infected, PI-experienced children receiving LPV were intensively sampled for 12 h to measure plasma LPV. The data were fitted to candidate PK models (using MM-USCPACK software), and the final model was used to simulate 1,000 children to determine the probability of achieving an LPV PK of >15. In 50 patients (4 to 18 years old), the median LPV plasma 12-hour-postdose concentration was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter) lower than that reported in adults. After a delay, LPV was absorbed linearly into a central compartment whose size was dependent on the weight and age of the patient. Elimination was dependent on weight. The regression line of observed versus predicted LPV had an R² of 0.99 and a slope of 1.0. Visual predictive checks against all available measured concentrations showed good predictive ability of the model. The probability of achieving an LPV PK of >15 was >90% for wild-type virus but <10% for even moderately resistant virus. The currently recommended dose of LPV/ritonavir appears to be adequate for children infected with wild-type virus but is unlikely to provide adequate inhibitory concentrations for even moderately resistant human immunodeficiency virus (HIV). PI-experienced HIV-infected children will likely benefit from longitudinal, repeated LPV measurement in plasma to ensure that drug exposure is most often near the maximal end of the observed safe range.

Lopinavir/ritonavir (LPV/RTV) (Kaletra) is the first and only coformulated RTV-boosted protease inhibitor (PI) approved for use in children. The recommended doses for children who weigh more than 15 kg are 10 mg/kg of body weight or 230 mg/m² (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, 28). Introduced as a salvage agent (22), LPV/RTV has become one of the preferred PI choices for first-line regimens in children >6 months of age in the countries with access to the drug. Despite evidence for good antiviral efficacy among children in a clinical trial setting (16, 17, 23), the standard dose may not be adequate for every child. Noncompartmental analysis has shown that the average LPV plasma 12-hour-postdose concentration (C_{trough}) in children given the currently recommended pediatric dose of LPV is 67% lower than in adults (24). Recently, Jullien et al. published a population pharmacokinetics (PK) model of LPV in children aged 0 to 18 years (15). The model was based on a retrospective analysis of LPV plasma measurements in 157 children, with a median of 3 samples (range, 1 to 14) per patient, obtained for monitoring purposes after self-reported LPV intake. In that model, clearance (CL) and volume were dependent on body weight, and there was an additional increase in CL in boys after the age of 12 relative to girls. The study suggested that a distinct possibility of LPV underdosing exists in young infants and adolescent males. PK measures of LPV exposure, such as area under the time-concentration curve (AUC) or C_{trough}, are most relevant to clinical practice in the context of an estimated concentration-response relationship. Different LPV C_{trough} targets have been proposed for PI-naïve patients (1 mg/liter) (20) and for PI-experienced patients (3 to 5.7 mg/liter) (5, 6, 8, 27). A more comprehensive target, the phenotypic inhibitory quotient (PIQ), incorporates viral drug susceptibility, which is measured as the concentration of drug required to achieve 50% in vitro inhibition of replication of the virus (IC₅₀) relative to the replication of human immunodeficiency virus (HIV) in drug-free medium. The PIQ is the ratio of a patient’s C_{trough} to the IC₅₀ of the dominant viral strain. For LPV, a PIQ target of 15 has been associated with more than 90% chance of achieving <400 HIV copies/ml when combined with active background
antiretroviral therapy (ART) comprising efavirenz (13) or two nucleoside reverse transcriptase inhibitors (21).

We conducted a prospective study to develop a population PK model of LPV in a large cohort of HIV-infected children, with intense PK sampling after observed LPV intake. Additionally, we used Monte Carlo simulation to estimate the proportion of all children who would fail to achieve the LPV PIQ target of 15 when given the currently recommended dose of LPV.

MATERIALS AND METHODS

Patients. The patients were children with HIV type 1 infection who were receiving care at the large metropolitan pediatric HIV program at Children's National Medical Center, Washington, DC. The research protocol, parental consent, and assent documents (for children older than 7 years of age) were approved by the institutional review board.

All subjects in this study were on uninterrupted, twice-daily doses of LPV/RTV-based ART for at least 4 weeks prior to study entry. Blood samples for PK measurement were obtained during the 12-hour admission to the Pediatric Clinical Research Center. The subjects were administered their standard prescribed LPV/RTV dose under direct observation with a standard light snack. Plasma samples were obtained before and 0.5, 1, 2, 4, 8, and 12 h after observed intake of LPV/RTV.

Tandem mass spectrometry analysis. For each measurement of the plasma LPV concentration, 2 ml of venous blood was obtained. The blood was collected in a heparin tube and separated by centrifugation at 4,000 rpm for 10 min. The plasma was stored at -70°C pending analysis. The plasma samples were incubated for 30 min at 56°C to deactivate the HIV particles. Heating of the samples has been proven not to produce degradation of LPV (12, 26).

The LPV plasma concentrations were determined by a tandem-mass spectrometric method using the Applied Biosystems/Sciex API-2000 (12, 26). Within-run error was below 7%, and between-day error was below 10% for all analytes at the tested concentrations. The lower limit of quantification was 0.1 mg/liter. The laboratory at Children's National Medical Center that performed the LPV assays is an accredited member of the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection (University Medical Center, Nijmegen, The Netherlands) (1, 11).

PK modeling and simulation. MM-USCPACK, a parametric/nonparametric population-modeling software collection (available by license through the University of Southern California at http://www.lapik.org) was used to fit candidate PK models to the time-concentration data for LPV (14). The models were evaluated on the basis of maximization of the log likelihood, visual inspection of observed versus predicted plots, visual predictive checks, and parsimony.

To minimize model bias from uncertainty in dose times (9, 19, 25), for each patient, the initial amount of LPV in the PK dosing compartment was set equal to zero under the assumption that the LPV plasma trough concentration was nearly perfectly correlated (R = 0.995; P < 0.0001) with BSA calculated using the more familiar height- and weight-based Mosteller formula (18). The median peak was 10.3 mg/liter (range, 0.6 to 28.8 mg/liter), the median trough was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter); the median peak was 10.3 mg/liter (range, 0.6 to 28.8 mg/liter), the median trough was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter). The median plasma LPV post-dose C_{trough} was 5.9 mg/liter (range, 0.03 to 16.2 mg/liter); the median peak was 10.3 mg/liter (range, 0.6 to 28.8 mg/liter).
FIG. 1. Individual observed LPV time-concentration curves for each of the 52 patient visits (gray lines). The median observed time-concentration curve for the entire population is superimposed (black line).

TABLE 1. Estimated and calculated PK parameters in the final PK model

<table>
<thead>
<tr>
<th>Parametera</th>
<th>Median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated</td>
<td></td>
</tr>
<tr>
<td>t_{lag} (h)</td>
<td>0.37 (0.12–0.86)</td>
</tr>
<tr>
<td>k_{a} (h^{-1})</td>
<td>0.64 (0.21–1.71)</td>
</tr>
<tr>
<td>V (liters) = \theta_a \cdot wt/age^{0.25}</td>
<td>25.15 (14.00–32.88)</td>
</tr>
<tr>
<td>CL (liters/h)</td>
<td>0.69 (0.45–0.98)</td>
</tr>
<tr>
<td>\theta_1</td>
<td>15.04 (10.30–17.79)</td>
</tr>
<tr>
<td>\theta_2</td>
<td>11.44 (3.69–90.66)</td>
</tr>
<tr>
<td>\theta_3</td>
<td>0.09 (0.07–0.15)</td>
</tr>
<tr>
<td>\theta_4</td>
<td>0.22 (0.18–0.37)</td>
</tr>
<tr>
<td>AUC_{0–12} (mg·h/liter)</td>
<td>96.09 (62.73–114.30)</td>
</tr>
<tr>
<td>AUC_{inf} (mg·h/liter)</td>
<td>190.40 (123.50–321.20)</td>
</tr>
</tbody>
</table>

| Calculated |                              |
| CL (liters/h) = dose/AUC | 1.79 (1.00–2.57) |
| (liters/kg^{0.75}) | 0.11 (0.07–0.20) |
| CL (liters/h) = k_{a} \cdot V | 2.17 (1.39–3.18) |
| (liters/kg^{0.75}) | 0.14 (0.09–0.24) |
| t_{lag} (h) = \ln(2)/k_{a} | 7.69 (4.63–10.44) |

a t_{lag}, lag time; k_{a}, rate constant of absorption.
Biosciences, Inc., CA) or Vircotype (Virco Labs, Inc., NJ) cutoffs between fully sensitive and intermediate virus (ninefold and sixfold increases in IC50, respectively), only 32% or 48% of children are likely to achieve a PIQ of >15. If resistance increases to 20-fold, well within the “partially sensitive” range, which extends up to 55- and 51-fold, respectively, fewer than 10% of children are likely to achieve a PIQ of >15, given the currently recommended LPV dose.

Considering the LPV contribution to virologic effect as a continuous function of the PIQ, it can be seen in Fig. 6B that the standard dose of LPV will maintain >90% LPV activity against wild-type virus in >75% of children. Children whose LPV dose is sufficient to ensure a Ctrough of 16.1 mg/liter (the 75th percentile for the population) will maintain at least 50% LPV effect until resistance increases to >27 times that of the wild type; in contrast, children whose Ctrough is only 2.9 mg/liter (the 25th percentile for the population) will lose 94% of their LPV effect at this same level of resistance. Alternatively, children whose LPV Ctrough is at the 25th percentile will fall below a PIQ of 15 at a 2.8-fold increase in the IC50 compared to those at the 75th percentile, who will not drop below a PIQ of 15 until their viral IC50 is increased 15.4-fold relative to the wild type.

DISCUSSION

In this study, a model of LPV disposition in children and adolescents 4 through 18 years of age was developed and validated by a visual predictive check of the distribution of LPV concentrations in 1,000 model-simulated patients compared with the distribution of LPV concentrations in the study population. In the final model, LPV was rapidly absorbed after an initial formulation-dependent delay, with an apparent V that increased nonlinearly as a function of weight over age.
Overall, the drug CL varied according to weight$^{0.75}$, in accordance with the allometric scaling principles in children suggested by Anderson et al. (2), among others.

Because of high correlation between $V$ and CL, the model was parameterized in terms of fractional elimination ($k_{el}$) that declined with increasing weight. The CL-$V$ correlation indicates that in the model clearance of LPV was driven primarily by the apparent $V$, which raises the possibility that LPV bioavailability may have been variable. Due to the lack of an intravenous formulation, the absolute bioavailability of LPV has not been established. Close inspection of Fig. 1 reinforces the fact that most of the LPV PK variability is in absorption and bioavailability, since after an individual trajectory peaks, it generally declines consistently thereafter. In other words, between-individual variability is larger than within-individual variability, which enables the close fit shown in Fig. 2B versus that in Fig. 2A.

In adults given the standard dose of 400/100 mg LPV/RTV twice daily, the Kaletra package insert reports a steady-state LPV AUC of 92.6 mg $\cdot$ h/liter, a $C_{max}$ of 9.8 mg/liter occurring 4 h after a dose, and a $C_{trough}$ of 7.1 mg/liter. For children given 230/57.5 mg/m$^2$ LPV/RTV twice daily, the package insert reports a steady-state LPV AUC of 72.6 mg $\cdot$ h/liter, a $C_{max}$ of 8.2 mg/liter occurring 4 h after a dose, and a $C_{trough}$ of 3.4 mg/liter. The children in our study received a median LPV dose of 275 mg/m$^2$, which is 20% higher than the recommended dose. The median LPV AUC, $C_{max}$, and $C_{trough}$ were correspondingly higher than those reported for children in the package insert and were similar to adult values, with the exception of the $C_{trough}$, which was slightly lower than that of adults.

With large interpatient variability in LPV PK, it is very important to estimate the chance of a child on ART having suboptimal LPV exposure. Other studies have suggested that the currently recommended dose of LPV/RTV might result in suboptimal exposure in HIV-infected children (4, 10, 24). Based on our PK model and the LPV pharmacodynamic models by Hsu et al. and Podzamczer et al. (13, 21), we have shown that the majority of children are unlikely to achieve therapeutic plasma LPV concentrations against virus that is moderately resistant to LPV, at degrees far below the clinical cutoffs suggested by current phenotypic resistance testing. Furthermore, patients with fewer than two active background antiretroviral agents would have even higher IQ targets (13, 21) and would require correspondingly higher doses of LPV. It is this population of PI-experienced patients who would most likely derive direct benefit from measurement of plasma LPV concentrations and dose adjustment if necessary to avoid excessive dependence on the remaining drugs in the therapeutic regimen. Separate analysis in this cohort of HIV-infected children will compare the measured relationships of individual LPV PK and phenotypic and genotypic IQs to virologic responses.

**Conclusion.** In this study, a model of LPV disposition in pediatric patients $\geq$4 years of age was developed and validated by a visual predictive check of the distribution of LPV concentrations in 1,000 model-simulated patients compared with the distribution of LPV concentrations in the study population. Based on this PK population model, the currently recom-
mended dose of LPV/RTV appears to be adequate for children infected with wild-type virus but is unlikely to provide adequate inhibitory concentrations for even moderately resistant HIV. PI-experienced HIV-infected children would likely benefit from longitudinal, repeated LPV drug measurement in plasma in combination with resistance evaluation to ensure that LPV dosing is sufficient to maximize the contribution of LPV to virologic control.

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