Pharmacokinetics and Pharmacodynamics of Nelfinavir Administered Twice or Thrice Daily to Human Immunodeficiency Virus Type 1–Infected Children


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We studied the pharmacokinetics and pharmacodynamics of nelfinavir administered 2 or 3 times per day to human immunodeficiency virus type 1 (HIV-1)–infected children receiving highly active antiretroviral therapy containing nelfinavir. The geometric mean trough concentrations of nelfinavir for the thrice- and twice-daily regimens were 1.55 mg/L and 1.11 mg/L, respectively (P = not significant). Nelfinavir concentrations did not correlate with total daily dose, dose per kilogram of weight, age, weight, previous protease inhibitor (PI) experience, or CD4+ cell percentage. In the 25 PI-naive children, the virus load reductions at 24 weeks of treatment with the twice- and thrice-daily regimens were comparable. A significantly higher percentage of children in the twice-daily group had a trough concentration of nelfinavir of less than the inhibitory concentration of 95% (P = .042). The decrease in the virus load at 24 weeks of treatment was not correlated with the trough concentration of nelfinavir. The variability of trough concentrations was extremely high, particularly among recipients of the twice-daily regimen, resulting in a higher number of patients with subinhibitory concentrations of nelfinavir in this group.

Although several aspects of HIV infection are similar for patients of all age groups, pediatric HIV infection requires special considerations with respect to epidemiological, clinical, pharmacokinetic, and therapeutic issues [1]. Nelfinavir is one of the currently available protease inhibitors (PIs) used for the treatment of HIV infection in both adults and children [2–8]. The pharmacokinetics of nelfinavir have been investigated in healthy volunteers and in HIV-infected adults [2, 9, 10]. Data on the pharmacokinetics of nelfinavir in children with HIV infection are scarce [3, 4, 6, 11–13]. In older children, administration of nelfinavir at a dosage of ∼20–30 mg/kg 3 times per day provides exposure similar to a dosage of 750 mg 3 times per day for adults.

Nelfinavir has recently been approved for a twice-daily dose regimen for adults, whereas, for pediatric patients, twice-daily administration is being studied [1]. However, the pharmacokinetics of twice-daily administration of nelfinavir to children is limited to preliminary reports [4, 11, 12]. Because a twice-daily regimen may improve adherence to treatment, additional evaluation of the pharmacokinetics of twice-daily regimens of nelfinavir in children is timely and relevant.

The present study was designed to evaluate pharmacokinetic and pharmacodynamic data for HIV-1–infected
children receiving HAART that involves twice- or thrice-daily administration of nelfinavir. The pharmacokinetic study aimed to assess the variability of plasma concentrations of nelfinavir for the administered regimen. In the pharmacodynamic study, we focused on the relationship between the trough concentration of nelfinavir and the virologic response in PI-naive children.

**PATIENTS, MATERIALS, AND METHODS**

This open-label prospective study was conducted at 2 large Italian children’s hospitals (Istituto G. Gaslini, Genoa, and Ospedale Pediatrico Bambin Gesù, Rome). The study protocol was approved by the local institutional review boards of both institutions. Written informed consent was obtained from the parents or guardians of the children before study entry. The pharmacokinetic portion of the study aimed to evaluate the variability of plasma concentrations of nelfinavir after twice- or thrice-daily administration and to correlate nelfinavir concentrations with potential predictors of patient exposure to the drug. The pharmacodynamic portion of the study was limited to PI-naive children and designed to evaluate the relationship between trough concentration of nelfinavir and virologic response after twice- or thrice-daily administration of nelfinavir.

**Study subjects.** The study population included 35 children vertically infected with HIV-1, regardless of disease classification [14]. Twenty-five children were PI naive, and the remaining 10 were PI experienced. To be eligible for enrollment in the study, children had to have been receiving nelfinavir for ≥1 month before study entry. Before study entry, a complete medical history was obtained, a physical examination was done, and a panel of laboratory tests, which consisted of a chemistry screening and a complete blood cell count with differential and platelet counts, was performed.

**Virologic and immunologic assessment.** Virus load, CD4+ lymphocyte count, and CD4+ cell percentage were measured at baseline and every 12 weeks. The plasma virus load was determined using the branched DNA assay (Chiron), which has a lower limit of quantification of 400 copies/mL. The CD4+ lymphocyte count and the percentage of CD4+ lymphocytes, with respect to the overall lymphocyte count (CD4+ cell percentage), were determined using Coulter (Coulter Electronics) or Ortho (Ortho Diagnostic System) flow cytometry kits.

**Drug administration.** All children enrolled in the study were receiving nelfinavir tablets in combination with 2 nucleoside analogue reverse-transcriptase inhibitors (NRTIs) or NRTI(s) plus a nonnucleoside reverse-transcriptase inhibitor. The dosage of nelfinavir administered to each patient was the closest approximation to the recommended dosages of 20–30 mg/kg every 8 h (for 15 children) and 50 mg/kg every 12 h (for 20 children). The drug was taken with food, as recommended by the manufacturer.

Adherence to the treatment regimen and dietary requirements was evaluated at every visit by an interview conducted by the caregivers. Only patients defined as compliant with treatment (compliance of ≥95%) were included in the analysis.

**Obtainment of blood samples for nelfinavir concentration analysis.** Blood samples (5–7 mL) were drawn into heparinized vacutainer tubes before a morning dose of nelfinavir was administered (Cmin) and 3–4 h (Cmax) after administration of the morning dose. All samples were obtained 9–16 weeks after the initiation of nelfinavir treatment. At the time of study, none of the children had diarrhea or had received drugs known or suspected to interact with nelfinavir.

**Analytical methods.** Plasma concentrations of nelfinavir were determined using a validated high-performance liquid chromatography (HPLC) assay. In brief, standard curves and quality-control samples were prepared using nelfinavir powder (kindly donated by Agouron Pharmaceuticals). Samples were loaded onto a 10-μg, 96-well Oasis MCX Extraction Plate (Waters), washed with acidified water, and then washed with acetonitrile. Elution was performed with a basic mixture of acetonitrile and methanol; the samples were evaporated to dryness under a gentle stream of nitrogen. The extracts were redissolved in a mobile phase of phosphate buffer (25 mmol/L; pH, 5) and acetonitrile (55:45 v/v) for direct injection into the HPLC system. Nelfinavir was separated with a 5-μm Symmetry Shield RP_{3.9} × 150-mm Column (Waters) at a flow rate of 1.5 mL/min (L-7100 LaChrom Pump; Merck-Hitachi). The detector (L-4200 UV-VIS; Merck-Hitachi) was set at a wavelength of 210 nm. Chromatography was performed at room temperature (20°C–25°C). All reagents were HPLC gradient grade.

Peaks of interest were quantified using a TurboChrom Navigator 4.0 Chromatography Data System (Perkin Elmer). The concentration standard curve was prepared in the range of 0.1–15 μg/mL. Precision (intraday and interday variabilities; n = 9) of the assay was <10% at each of the quality-control concentrations (0.75, 3, and 10 μg/mL). The average recovery was 78.6%, and the lower limit of quantification was 0.1 μg/mL.

**Pharmacokinetic, pharmacodynamic, and statistical analysis.** The data obtained from all 35 study patients were used for pharmacokinetic analysis. The baseline characteristics of children receiving the twice- or thrice-daily nelfinavir regimen were analyzed using χ² test, Fisher’s exact test, and Student’s t test. Peak and trough concentrations of nelfinavir after twice-daily administration were compared with concentrations after thrice-daily administration using the Mann-Whitney U test. Correlations of plasma concentrations of nelfinavir with demographic and other potentially predictive variables were explored using the Pearson correlation coefficient, which was...
Table 1. Baseline demographic and clinical characteristics of children in the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pharmacokinetic study group, by nelfinavir dosing schedule</th>
<th>Pharmacodynamic study group&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (&lt;i&gt;n&lt;/i&gt; = 35)</td>
<td>Thrice daily (&lt;i&gt;n&lt;/i&gt; = 15)</td>
</tr>
<tr>
<td>Male sex, % of patients</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>8.1 ± 3.5</td>
<td>7.6 ± 3.9</td>
</tr>
<tr>
<td>Weight, mean kg ± SD</td>
<td>23.7 ± 8.8</td>
<td>23.2 ± 10.0</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; cell value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % ± SD</td>
<td>20.6 ± 11.0</td>
<td>23.4 ± 11.9</td>
</tr>
<tr>
<td>Mean cells/μL ± SD</td>
<td>620 ± 560</td>
<td>746 ± 611</td>
</tr>
<tr>
<td>Virus load, mean log&lt;sub&gt;10&lt;/sub&gt; copies/mL ± SD</td>
<td>5.1 ± 0.6</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>CDC HIV disease stage, no. (%) of patients</td>
<td>Not available</td>
<td>2 (5.7)</td>
</tr>
<tr>
<td>A</td>
<td>10 (28.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>B</td>
<td>13 (37.1)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>C</td>
<td>10 (28.5)</td>
<td>4 (26.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients were protease inhibitor naive.

NOTE. CDC, Centers for Disease Control and Prevention.

RESULTS

Study populations. Thirty-five children were enrolled in the study during the period of February 1999 through September 2000. Baseline patient characteristics are shown in table 1. Of the 35 children, 10 were PI experienced and 25 were PI naive. This subgroup of 25 patients was included in the pharmacodynamic analysis.

Twenty children (14 of whom were PI naive) received nelfinavir at a mean dosage of 47.2 mg/kg twice per day (range, 32.9–62.5 mg/kg b.i.d.). Fifteen children (11 of whom were PI naive) received a mean dosage of 25.8 mg/kg 3 times per day (range, 17.4–35.7 mg/kg t.i.d.). No statistically significant differences were found between patients receiving twice-daily regimens and those receiving thrice-daily regimens with regard to age, sex, previous PI experience, weight, baseline CD4<sup>+</sup> cell percentage, total number of CD4<sup>+</sup> cells, HIV-1 infection classification group (by Centers for Disease Control and Prevention criteria), and baseline virus load (table 1).

A variety of antiretroviral combinations were administered concomitantly with nelfinavir, including didanosine and stavudine (10 children); stavudine and lamivudine (12 children); zidovudine and lamivudine (5 children); stavudine, lamivudine, and nevirapine (1 child); stavudine and nevirapine (2 children); stavudine and zalcitabine (2 children); zidovudine and abacavir (2 children); and lamivudine and abacavir (1 child). At the time of initiation of nelfinavir treatment, 7 children were antiretroviral therapy naive, 21 children modified all underlying NRTIs, 6 children added nelfinavir to their treatment regimens, and 1 child added nevirapine.
Peak and trough plasma concentrations of nelfinavir.

There was a large interpatient variability in plasma concentrations of nelfinavir. Plasma samples with peak nelfinavir concentrations were obtained 3.7 ± 2.2 h after nelfinavir administration. The C_{max} was 1.53–13.7 mg/L in the twice-daily regimen group (geometric mean, 5.65 mg/L), compared with 2.24–11.72 mg/L in the thrice-daily regimen group (geometric mean, 4.00 mg/L). This difference was not statistically significant.

The relationship between trough plasma concentrations and administered dose is depicted in figure 1. The geometric mean trough concentration for the thrice-daily regimen group (1.55 mg/L; range, 0.13–5.22 mg/L) was not significantly different than that for the twice-daily regimen group (1.11 mg/L; range, nondetectable to 6.08 mg/L). In the whole population examined, peak and trough concentrations of nelfinavir did not correlate with total daily dose, dose per kilogram of weight, age, weight, previous PI experience, or CD4⁺ cell percentage.

Pharmacodynamic study. Demographic parameters (mean ± SD) for the 25 PI-naive children in the twice-daily (n = 14) and thrice-daily (n = 11) groups, respectively, were as follows: age, 8.1 ± 3.0 versus 6.3 ± 2.5 years; weight, 20.2 ± 6.9 versus 21.6 ± 7.1 kg; baseline CD4⁺ cell percentage, 20.6% ± 10.7% versus 26.3% ± 12.0%; and baseline virus load, 5.1 ± 0.4 versus 5.2 ± 0.8 log_{10} copies/mL. Mean dosages of nelfinavir were 48.7 mg/kg twice per day (range, 33.3–62.5 mg/kg b.i.d.) and 27.6 mg/kg 3 times per day (range, 21.4–35.7 mg/kg t.i.d.), respectively. Geometric mean trough concentrations of nelfinavir in the twice- and thrice-daily regimen groups were 0.94 mg/L (range, not detectable to 5.74 mg/L) and 1.63 mg/L (range, 0.13–5.22 mg/L), respectively. Seven (50%) of 14 children in the twice-daily regimen group had a trough concentration of nelfinavir of <1 mg/L (estimated IC₅₀ cutoff value), compared with 1 (9%) of 11 children in the thrice-daily regimen group (P = .042, by 2-tail Fisher’s exact test; figure 2, top and bottom).

At 24 weeks of follow-up, we observed a mean virus load reduction (± SD) of 2.8 ± 0.7 log_{10} copies/mL in the twice-daily regimen group, compared with a reduction of 2.1 ± 1.2 log_{10} copies/mL in the thrice-daily regimen group. This difference was not statistically significant.

On univariate analysis, the decrease in the virus load at 24 weeks of treatment was not correlated with trough concentration of nelfinavir. There was, however, a trend toward a decreased virus load in patients with higher trough concentrations of nelfinavir (figure 3).

DISCUSSION

We studied the variability of plasma concentrations of nelfinavir after twice-daily and thrice-daily dosing, the correlation of plasma concentrations with potential predictors of patient exposure to the drug, and the relationship between trough concentrations of nelfinavir and virologic response, according to the administered regimen.

In our study of 35 children, we found an extremely high interindividual variability in trough and peak concentrations of nelfinavir, as was already observed in adults and in small cohort studies of children [2, 4, 6, 11–13, 17, 18]. We did not identify factors that could explain the observed variability in plasma concentrations of nelfinavir. Despite the high dose range used, peak and trough concentrations did not correlate with total daily dose and dose per kilogram of weight, nor with age, weight, previous PI experience, and CD4⁺ cell percentage. Possible sources of variation in plasma concentrations are attributable to peculiar pharmacokinetic properties of nelfinavir. Because the drug has relatively slow gut absorption, peak and trough concentrations may occur after the C_{min} and C_{max} samples have been obtained [19]. Moreover, as already observed.
with other PIs, the variability of nelfinavir concentrations may reflect the interindividual variability in the activity of the P450 enzyme system metabolizing the drug and the extent of its saturability. It should be noted that, in our study, nevirapine was coadministered to 3 children (2 in the thrice-daily regimen group and 1 in the twice-daily regimen group); however, this drug did not appear to influence nelfinavir metabolism in vivo [20].

In our study, trough and peak concentrations did not differ between regimen groups. Although this was an observational study without randomized design, it is unlikely that the design introduced an important bias. Our results are in agreement with the results of other studies that have compared concentrations of nelfinavir after administration of twice-daily and thrice-daily regimens in children [4, 11, 12] and adults [18].

Of interest, we found that the interindividual variability in the plasma concentration of nelfinavir was even more evident in the twice-daily regimen group. Eight (40%) of 20 patients in the twice-daily regimen group, compared with 2 (13%) of 15 patients in the thrice-daily regimen group, had a trough concentration that was less than the wild-type, protein binding–corrected IC₉₅, of 1 mg/L [15, 16]. Although the limit of this approach should be acknowledged, particularly in the presence of drug-resistant virus, the finding of a trough plasma level that was less than the minimum effective concentration should be recognized as a clear indication of treatment inadequacy [19].

Because there is evidence showing a relationship between virologic outcome and plasma concentration of PIs [19], we investigated the effect of thrice-daily versus twice-daily administration of nelfinavir on virologic response. Because previous exposure history may affect the emergence of drug-resistant HIV variants, in this part of the study, we included only PI-naive children.

Figure 2. The trough concentrations of nelfinavir in protease inhibitor–naive children receiving nelfinavir thrice (top) or twice (bottom) per day. Horizontal line, protein binding–corrected concentration producing 95% inhibition of viral replication (IC₉₅) of nelfinavir (1 mg/L) for wild-type virus.
Overall, patients responded well to treatment. The mean reduction in the virus load did not differ significantly between recipients of the twice-daily regimen and recipients of the thrice-daily regimen, despite there being a significantly higher number of children in the twice-daily regimen group with trough concentrations that were less than the IC95. Also, the decrease in the virus load at 24 weeks of treatment did not correlate with trough concentrations of nelfinavir, even though we observed a trend toward a better response in patients with higher trough concentrations.

In the present study, there are several factors that can explain the lack of difference in virologic response between recipients of twice-daily regimens and recipients of thrice-daily regimens, as well as the lack of correlation between exposure and response. First of all, the number of PI-naive children was too low to detect significant differences in virologic response between treatment groups. Second, $C_{min}$ is not the ideal parameter of exposure. In fact, in some patients, the plasma concentration of PIs continues to decay after drug administration because of delayed absorption. Moreover, the utility of estimating single plasma drug concentrations (trough or random sample) is unclear at this time [19]. Third, in our study, we did not monitor the M8 nelfinavir metabolite, although this procedure does not seem to be essential for the purpose of therapeutic drug monitoring (TDM) [17]. Fourth, we did not provide data on intracellular concentration of antiretroviral drugs and on the possibility of antiretroviral drug interactions (synergy, antagonism, or indifference). Fifth, the twice-daily regimen of nelfinavir may have facilitated adherence to the treatment regimen. Questions about treatment adherence were asked at every visit; however, a more comprehensive assessment may have detected differences between regimens. Clearly, patient heterogeneity, in terms of nelfinavir daily dose and concomitant antiretroviral drugs received, may also be regarded as a source of variability. On the other hand, the study was aimed at harvesting data reflecting routine clinical practice.

Although the lack of correlation between virologic outcome and exposure would suggest that TDM may not be of benefit for this particular clinical setting, the above description of confounding factors should mitigate this conclusion. In fact, the high interindividual variability in plasma concentrations is in favor of TDM, as a recent study also suggests [21].

In conclusion, our study confirms the unpredictability of plasma concentrations of nelfinavir. The variability of trough concentrations was extremely high, particularly in the twice-daily regimen group, resulting in a higher number of patients who had a trough concentration less than the IC95 in the twice-daily regimen group, compared with the thrice-daily regimen group. Despite this difference in exposure, the virologic response at 24 weeks of treatment was similar for the 2 dosage regimens in PI-naive children. A number of confounding factors may explain the lack of correlation between nelfinavir exposure and virologic response. Therefore, our study does not exclude the utility of TDM, which is instead suggested by the high interindividual variability in plasma concentrations.

References

3. Starr SE, Fletcher CV, Spector SA, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in