Pharmacokinetic-Pharmacodynamic Analysis of Lopinavir-Ritonavir in Combination with Efavirenz and Two Nucleoside Reverse Transcriptase Inhibitors in Extensively Pretreated Human Immunodeficiency Virus-Infected Patients


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The steady-state pharmacokinetics and pharmacodynamics of two oral doses of lopinavir-ritonavir (lopinavir/r; 400/100 and 533/133 mg) twice daily (BID) when dosed in combination with efavirenz, plus two nucleoside reverse transcriptase inhibitors, were assessed in a phase II, open-label, randomized, parallel arm study in 57 multiple protease inhibitor-experienced but non-nucleoside reverse transcriptase inhibitor-naive human immunodeficiency virus (HIV)-infected subjects. All subjects began dosing of lopinavir/r at 400/100 mg BID; subjects in one arm increased the lopinavir/r dose to 533/133 mg BID on day 14. When co-dosed with efavirenz, the lopinavir/r 400/100 mg BID regimen resulted in lower lopinavir concentrations in plasma, particularly C_{\text{min}} than those observed in previous studies of lopinavir/r administered without efavirenz. Increasing the lopinavir/r dose to 533/133 mg increased the lopinavir area under the concentration-time curve over a 12-h dosing interval (AUC{\text{12h}}), C_{\text{predose}} and C_{\text{min}} by 46, 70, and 141%, respectively. The increase in lopinavir C_{\text{max}} (33%) did not reach statistical significance. Ritonavir AUC{\text{12h}}, C_{\text{max}}, C_{\text{predose}}, and C_{\text{min}} values were increased 46 to 63%. The lopinavir predose concentrations achieved with the 533/133-mg BID dose were similar to those observed with lopinavir/r 400/100 mg BID in the absence of efavirenz. Results from univariate logistic regression analyses identified lopinavir and efavirenz inhibitory quotient (IQ) parameters, as well as the baseline lopinavir phenotypic susceptibility, as predictors of antiviral response (HIV RNA < 400 copies/ml at week 24); however, no lopinavir or efavirenz concentration parameter was identified as a predictor. Multiple stepwise logistic regressions confirmed the significance of the IQ parameters, as well as other baseline characteristics, in predicting virologic response at 24 weeks in this patient population.

The introduction of human immunodeficiency virus (HIV) protease inhibitors (PIs) has led to a dramatic decline in the morbidity and mortality associated with HIV infection (36). PI-based combination regimens can lead to profound and sustained suppression of viral replication (9, 13, 20); however, these regimens eventually fail to control replication in a significant portion of patients, leading to the eventual development of resistant viruses (10, 14, 18). Although failure of PI-based therapy has a complex and multifactorial etiology, inadequate drug concentrations in plasma due to poor or variable pharmacokinetics and/or inconsistent adherence appear to be important factors (1, 7, 16, 19, 26, 38, 42).

Lopinavir is a new HIV PI that is rapidly and essentially exclusively metabolized by cytochrome P450 3A isoenzymes (CYP3A) (29–31; lopinavir/r [Kaletra] package insert). The in vitro \( V_{\text{max}} \) of lopinavir is 60-fold faster than that of ritonavir. Lopinavir, when given alone, yields very low concentrations in plasma. However, when administered with ritonavir, a potent inhibitor of lopinavir metabolism (\( K_c = 13 \text{nM} \) or 0.009 \( \mu g/ml \)) (31), at the standard lopinavir-ritonavir (lopinavir/r) dose of 400/100 mg twice a day (BID), lopinavir predose concentrations achieved in HIV-positive subjects typically exceed the serum protein binding-adjusted 50% inhibitory concentration (IC_{50}) for wild-type HIV type 1 by at least 50-fold (4, 35; lopinavir/r package insert). In antiretroviral agent-naive patients lopinavir/r, in combination with two nucleoside reverse transcriptase inhibitors (NRTIs), has demonstrated substantial and durable antiviral activity (76%, <50 copies/ml at week 144, intent-to-treat [ITT] analysis) through 3 years in a phase II clinical trial (35; L. Perrin, M. King, S. Brun, S. Yerly, T. Marsh, N. Travers, K. Real, A. Japour, and E. Sun, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1927, 2001). Comparable data have been generated in children 6 months to 12 years old (8). In a randomized, double-blind, phase III clinical trial conducted in adult antiretroviral-naive HIV patients, lopinavir/r appeared to exhibit superior antiviral activity compared to nelfinavir (63% versus 51%, <50 copies/ml at week 60, ITT analysis, \( P < 0.001 \)) (41).

The pharmacokinetic profile of lopinavir/r is characterized by high trough concentrations in plasma relative to the IC_{50} for wild-type virus, as determined in the presence of human serum. This suggests that lopinavir/r may also be effective against HIV with reduced susceptibility to lopinavir resulting from mutations associated with prior PI therapy. Therefore, prospective, randomized, controlled clinical trials were performed to inves-
tigate the use of lopinavir/r in PI-experienced patients (2, 12). Given that these patients had limited treatment options, the studies were designed to minimize the likelihood of further drug resistance by maximizing antiviral suppression. Since non-NRTI (NNRTI) use was still relatively uncommon at the initiation of these studies, PI-experienced but NNRTI-naive patients received lopinavir/r in combination with an NNRTI and additional NRTIs. In the first study, patients who had failed a single PI-containing regimen were treated with lopinavir/r in combination with nevirapine plus two NRTIs. A substantial virologic response was observed, although the response rate was lower than that observed in studies of lopinavir/r plus NRTIs in antiretroviral-naive patients. Through 3 years, 49% (ITT analysis) of the NNRTI-naive patients enrolled in that study maintained a viral load in plasma of <50 copies/ml (3).

A second study was undertaken to explore the safety, efficacy, pharmacokinetics, and pharmacodynamics of a regimen of lopinavir/r, efavirenz, and NRTIs in a more advanced patient population (i.e., NNRTI-naive patients who had failed multiple PIs). The safety and efficacy of this regimen have been described elsewhere (12). The pharmacokinetic characterization of this regimen was essential because efavirenz, like lopinavir/r, is both an inhibitor and an inducer of CYP-mediated metabolism (efavirenz [Sustiva] package insert). Thus, a potential drug-drug interaction with efavirenz may result in increased or decreased concentrations of PIs (efavirenz package insert). Furthermore, Burger et al. have shown that coadministration of nevirapine, another CYP inducer, with indinavir/ritonavir (800/100 mg BID) significantly lowers concentrations of indinavir in plasma (6). The pharmacokinetic interaction between lopinavir/r and efavirenz was previously studied in healthy volunteers but was incompletely characterized due to the small sample size of that study. Nonetheless, the available results suggest that efavirenz decreased the lopinavir area under the concentration-time curve (AUC) and C_{max} values (by ~19 and 39%, respectively) (lopinavir/r package insert). Thus, the present study was designed to further characterize the interaction between lopinavir/r and efavirenz in HIV-infected subjects and to identify a lopinavir/r dose that was likely to yield lopinavir predose levels similar to those achieved at the standard dose of lopinavir/r without efavirenz.

The correlation between PI predose levels and virologic response has been demonstrated for several PIs, both retrospectively and prospectively (1, 7, 16, 26, 33, 40, 42, 44). In general, these observations have been made in PI-naive patients in whom a relatively uniform viral susceptibility to the PI being studied may be expected. Since drug resistance testing has become widely available, a number of studies have also demonstrated that baseline viral susceptibility to individual drugs, whether expressed as a function of genotype or phenotype, also correlates with virologic response in patients previously treated with antiretroviral medications (11, 16, 37, 40, 44). The correlation of baseline phenotype and genotype with the virologic response to lopinavir/r has been characterized elsewhere (27, 28a). We hypothesized that the pharmacodynamic parameter most relevant to PI-based treatment response is the relationship of PI concentrations to viral susceptibility, expressed as the inhibitory quotient (IQ) (17). The present study provided an opportunity to test this hypothesis because it represented patients with both a population distribution of drug concentrations and a wide range of drug susceptibilities at baseline.

**MATERIALS AND METHODS**

**Study design, subject selection, and dosing.** This was a phase II, open-label, randomized, parallel arm study conducted at multiple centers in Europe and the United States (12). Fifty-seven multiple PI- and NRTI-experienced, but NNRTI-naive, HIV-infected subjects with plasma viral loads of >1,000 copies/ml were enrolled in the study. Subjects were randomized equally to receive one of two lopinavir/r dosage regimens: regimen A (400/100 mg BID) and regimen B (333/133 mg BID). Subjects in both arms were to initiate these regimens with lopinavir/r dosing of 400/100 mg (as three coformulated capsules) BID, 600 mg of efavirenz given once daily at bedtime, and investigator-selected NRTIs. On day 14, subjects assigned to Arm B increased the lopinavir/r dose to 533/133 mg (as four coformulated capsules) BID, while maintaining the efavirenz and NRTI regimens. Subjects in Arm A continued with the original treatment regimens. Subjects were instructed to take lopinavir/r with food. Both regimens were continued through at least 24 weeks.

Subjects were required to have previously received at least two PIs for at least 12 weeks (sequentially or concomitantly), including treatment with a stable PI regimen for at least 8 weeks prior to study entry. They were to have HIV RNA loads greater than 1,000 copies/ml, have had no prior NNRTI exposure in the 6 months prior to study entry, have had a Karnofsky score of ≥70, and have a Karnofsky score of >70. Exclusion criteria included a hemoglobin level <8.0 g/dl, an absolute neutrophil count of <750 cells/µl, a platelet count of <50,000 cells/µl, an alanine transaminase or aspartate transaminase level of ≥3 times the upper limit of normal, a creatinine level of ≥1.5 times the upper limit of normal, and prior treatment with lopinavir/r. Women had to agree to use barrier birth control methods and were excluded if pregnant or lactating. All subjects gave written informed consent to participate in the present study.

**Blood collection and assays.** On approximately day 35 (pharmacokinetic day), blood samples for lopinavir and ritonavir concentration evaluations in plasma were obtained prior to the morning dose (0 h) and at 2, 4, 6, 8, 10, and 12 h postdosing. Predose lopinavir and ritonavir concentrations were also obtained on day 0. Samples for efavirenz were collected on days 14 and 35, ca. 12 h after the evening dose. The concentrations of lopinavir and ritonavir in plasma were simultaneously analyzed by using a validated liquid chromatography method with UV absorption detection at a wavelength of 205 nm (43). Abbott-86093, a proprietary compound of Abbott Laboratories, was used as an internal standard. The in-study calibration curves contained seven standards at lopinavir and ritonavir concentrations of 0.006 to 3.500 µg/ml. The correlation coefficient was equal to or greater than 0.9985 and 0.9982 for the respective lopinavir and ritonavir standard curves. The coefficient of variation for the quality control samples supplemented with concentrations of 0.04 to 7.00 µg of lopinavir and ritonavir/ml ranged from 4% to 12% for lopinavir and 5% to 7.5% for ritonavir. The lower limits of quantitation for lopinavir and ritonavir were both 0.006 µg/ml. Calculated concentrations above the upper limit of quantitation determined by the highest standard accepted in the standard curve were diluted and reassayed.

The concentration of efavirenz in plasma was analyzed by using a validated liquid chromatography method with UV detection. YZ214, a proprietary compound of Bristol-Myers Squibb, was used as an internal standard. The calibration curves contained nine standards with concentrations ranging from 0.05 to 10.00 µg of efavirenz/ml. The correlation coefficient was equal to 1.0000 for efavirenz standard curves. The coefficient of variation of the quality control samples supplemented with concentrations of 0.120 to 8.00 µg/ml of efavirenz ranged from 1.8 to 7.1%. The lower limit of quantitation for efavirenz in human plasma was 0.050 µg/ml.

**Noncompartmental pharmacokinetic analysis.** Values for the pharmacokinetic parameters of lopinavir and ritonavir were obtained by using the following noncompartmental pharmacokinetic methods. (i) The maximum observed concentration in plasma (C_{max}), the time to the maximum observed concentration (T_{max}), the minimum observed concentration in plasma (C_{min}), and the predose concentration (C_{predose}) were taken directly from the plasma concentration measurements. (ii) The area under the plasma concentration-time curve over a 12-h dosing interval (AUC_{12}) was calculated by the linear trapezoidal method. (iii) The apparent oral plasma clearance (CL/F, where F is the bioavailability) was calculated by dividing the administered dose by the AUC_{12}. (iv) The peak-to-trough elimination rate constant (β) was estimated from log-linear regression of concentrations from C_{min} to C_{12}. The corresponding t_{1/2} was calculated as ln(2)/β.

**Pharmacodynamic analysis.** The IQ was calculated as the ratio of individual drug concentration to individual baseline IC_{50} (e.g., IQ C_{plasma}, IQ C_{min}, IQ C_{max}, and IQ AUC). The individual baseline IC_{50} was defined as the product of
individual baseline phenotype and protein binding-corrected IC₅₀ for wild-type virus. The individual baseline phenotype is defined as the fold change in IC₅₀ relative to wild-type virus and was determined for each viral isolate by using the PhenoSense HIV assay (Virologic, Inc.) (39). The protein binding-corrected IC₅₀ for wild-type virus, measured in vitro in the presence of 50% human serum, was estimated to be 0.07 μg/ml and 0.014 μM (or 4.42 ng/ml) for lopinavir and efavirenz, respectively (34). It should be noted that the IC₅₀ for wild-type virus used in the calculation of individual IQ values is assumed to be constant for all subjects.

Statistical methodology. (i) Pharmacokinetic data analysis. Analysis of covariance (ANCOVA) was performed on the logarithms of Cₚ₅₀, Cₚ₅₀ × AUC, and Cₚ₅₀ max of both lopinavir and ritonavir. The initial model included the following explanatory variables: gender, body weight, baseline CD4 count, lopinavir/r dose level, week 2 trough concentrations of lopinavir (or ritonavir) and week 2 concentrations of efavirenz. Since gender and baseline CD4 count were not statistically significant, they were not included in the final ANCOVA model. The final model included body weight, lopinavir/r dose level, week 2 efavirenza concentration and week 2 lopinavir Cₚ₅₀ (for lopinavir parameters) or week 2 ritonavir Cₚ₅₀ (for ritonavir parameters) as covariates. It should be noted that Cₚ₅₀ represents the predose concentration, and the median (interquartile range) collection times of the predose samples were 13.1 h (range, 12.6 to 14.3 h) and 12.2 h (range, 11.8 to 12.7 h) for the week 2 and week 5 Cₚ₅₀ values, respectively. For each variable, the point estimate and the 95% confidence interval (95% CI) for the Cₚ₅₀, Cₚ₅₀ × AUC, and Cₚ₅₀ max, and AUC values of the 533–133 mg BID regimen relative to that of the 400/100 mg BID regimen were obtained within the framework of the ANCOVA model.

An ANCOVA was performed on the logarithms of the week 5 efavirenz concentrations to test the effect of lopinavir/r dose on efavirenz concentrations. The model included body weight and week 2 efavirenz concentrations as covariates.

(ii) Pharmacodynamic analysis. Three statistical approaches were used to investigate the relationship between virologic response (binary response variable with 400 copies of HIV RNA/ml as the cutoff) at week 24 and pharmacodynamic-phenotypic parameters. Since the pharmacokinetic parameters (and hence their correspond-

RESULTS

Subject disposition. A total of 57 subjects with HIV RNA levels in plasma of at least 1,000 copies/ml were enrolled in the present study. Six subjects were excluded from the pharmacodynamic analysis: four subjects who discontinued participation in the study on or before day 18 for reasons not related to virologic failure, one subject for whom baseline lopinavir and efavirenz phenotypic data were not available, and one subject for whom baseline efavirenz phenotypic change was not available. Fifty subjects had evaluable pharmacokinetic data. In addition, the IQ values for three subjects with no pharmacokinetic data were imputed by using the median pharmacokinetic results from their respective lopinavir/r regimen group.

The baseline virus for two of the three subjects displayed wild-type susceptibility to lopinavir, producing imputed IQ values of 64 and 56, respectively. The baseline virus from the third subject exhibited 96-fold-reduced susceptibility to lopinavir, producing an imputed IQ value of 0.47. This subject had viral load in plasma of >400 copies/ml at the time of study discontinuation (week 22) and was considered as a nonresponder.

Pharmacokinetics. (i) Demographic and baseline characteristics. Sixty-eight percent of baseline viral isolates demonstrated a ≥4-fold increase in IC₅₀ to ≥3 licensed PIs by phenotypic analysis. The mean susceptibility to lopinavir at baseline was 16-fold above the susceptibility of the wild-type virus. The mean numbers of prior PIs and total antiretroviral agents used were 2.9 and 7.1, respectively. Of the 50 subjects with evaluable pharmacokinetic data, 86% were Caucasian (with 10% black and 4% Hispanic) and 82% were male. The mean ± the standard deviation age, body weight, and height of these subjects were 41.8 ± 9.0 (range, 25 to 63) years, 71.6 ±
10.3 (range, 48 to 92) kg, and 173.6 ± 8.4 (range, 145.4 to 191) cm, respectively.

(ii) Pharmacokinetics of lopinavir/r. The mean steady-state concentration-time profiles in plasma for lopinavir determined on day 35 for both lopinavir/r dose groups are shown in Fig. 1. The steady-state median (interquartile range) pharmacokinetic parameters determined on day 35 and the predose concentrations determined on day 14 for lopinavir and ritonavir are presented in Table 1. Point estimates and 95% CIs for the ratio of the central estimates based on pharmacokinetic data obtained on day 35 are presented in Table 2. Overall, increasing the lopinavir/r dose by 33% during coadministration with efavirenz increased lopinavir and ritonavir concentrations in plasma more than proportionally. Lopinavir AUC12, Cpredose, and Cmin central values were increased by 46, 70, and 141%, respectively (P = 0.055, 0.048, and 0.008, respectively). The lopinavir Cmax central value was also higher in the 533/133-mg dose group (33%); however, the difference did not reach statistical significance (P = 0.123). Ritonavir AUC12, Cmax, Cpredose, and Cmin values in the 533/133-mg dosing arm were 46 to 63% higher than those in the 400/100-mg dosing arm, and the differences were marginally or statistically significant (P < 0.057).

In addition to the dose effects described above, lopinavir and ritonavir AUC12, Cpredose, Cmax, and Cmin values were either marginally or significantly correlated with their respective week 2 predose concentrations (P < 0.058). Efavirenz week 2 concentrations were negatively correlated with lopinavir pharmacokinetic parameter values. Also, all pharmacokinetic parameters were positively and significantly or marginally significantly correlated with body weight (P < 0.064), with a larger body weight associated with lower pharmacokinetic parameter values.

### Table 1. Steady-state median (interquartile range) pharmacokinetic parameters of lopinavir and ritonavir during coadministration with efavirenz

<table>
<thead>
<tr>
<th>Day and parameter</th>
<th>Arm A (400/100 mg BID)* (n = 24)</th>
<th>Arm B (533/133 mg BID)* (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lopinavir</td>
<td>Ritonavir</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpredose (μg/ml)</td>
<td>1.94 (0.86–3.39)</td>
<td>0.10 (0.06–0.13)</td>
</tr>
<tr>
<td>Day 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean T&lt;sub&gt;max&lt;/sub&gt; ± SD (h)</td>
<td>4.00 ± 1.87</td>
<td>3.57 ± 1.78</td>
</tr>
<tr>
<td>Cmax (μg/ml)</td>
<td>8.40 (5.99–9.97)</td>
<td>0.52 (0.33–0.83)</td>
</tr>
<tr>
<td>Cmin (μg/ml)</td>
<td>1.97 (0.73–3.43)</td>
<td>0.09 (0.05–0.15)</td>
</tr>
<tr>
<td>Cpredose (μg/ml)</td>
<td>3.13 (1.45–4.95)</td>
<td>0.14 (0.08–0.25)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;12&lt;/sub&gt; (μg·h/ml)</td>
<td>63.50 (43.28–75.75)</td>
<td>3.46 (2.05–4.50)</td>
</tr>
<tr>
<td>CL/F (liter/h)</td>
<td>6.3 (5.3–9.3)</td>
<td>29.5 (22.2–49.2)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>3.61</td>
<td>3.49</td>
</tr>
</tbody>
</table>

* Dose in milligrams of lopinavir/r, BID, for days 14 to 35; the dose was 400/100 mg BID before day 14 for both arms. Interquartile range values are given in parentheses.

* Harmonic means.

* CL/F, apparent oral plasma clearance, where F is the bioavailability.
AUC12, navir/r at 533/133 mg BID in the presence of efavirenz yields able. However, the cross-study comparison suggests that lopinavir AUC, quanti

Although the study design allowed adequate characterization of the steady-state lopinavir and ritonavir pharmacokinetics of two lopinavir/r doses (400/100 versus 533/133 mg BID) during coadministration with efavirenz, quantification of the extent of interaction between efavirenz and lopinavir or ritonavir required cross-study comparisons. Table 3 compares steady-state lopinavir pharmacokinetic parameters obtained from the present study with those from a phase II study (35) in which lopinavir/r was dosed with stavudine and lamivudine without an enzyme-inducing NNRTI.

The comparison of lopinavir pharmacokinetic parameters determined for the 400/100-mg BID regimen across studies indicates that, although lopinavir $C_{\text{max}}$ was relatively similar, the lopinavir AUC, $C_{\text{predose}}$, and $C_{\text{min}}$ values were lower in the present study when lopinavir/r was coadministered with efavirenz, suggesting that hepatic induction by efavirenz lowers lopinavir pharmacokinetics. The reductions, based on the mean pharmacokinetic parameters, were ca. 25, 33, and 44% in AUC, $C_{\text{predose}}$, and $C_{\text{min}}$ values, respectively. The extent of interaction between lopinavir/r at 533/133 mg BID and efavirenz cannot be estimated because pharmacokinetic data for lopinavir/r at 533/133 mg BID without efavirenz are not available. However, the cross-study comparison suggests that lopinavir/r at 533/133 mg BID in the presence of efavirenz yields lopinavir AUC, $C_{\text{predose}}$, and $C_{\text{min}}$ values similar to those of lopinavir/r at 400/100 mg BID without efavirenz.

The cross-study comparison (35) also indicates that ritonavir AUC12, $C_{\text{max}}$, $C_{\text{predose}}$, and $C_{\text{min}}$ values were not substantially impacted by coadministration with efavirenz (see Table 3). These results generally agree with the previous observation that efavirenz increased ritonavir concentrations in plasma by ca. 20% (efavirenz package insert). For lopinavir/r at 533/133 mg BID, the ritonavir pharmacokinetic parameters are within expected values.

### Table 3. Comparison of steady-state lopinavir pharmacokinetic parameters obtained in the present Study to those obtained from a phase II study

<table>
<thead>
<tr>
<th>Lopinavir or ritonavir pharmacokinetic parameter</th>
<th>Mean $\pm$ SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopinavir</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ ($\mu$g/ml)</td>
<td>9.58 $\pm$ 4.41</td>
</tr>
<tr>
<td>$C_{\text{min}}$ ($\mu$g/ml)</td>
<td>3.83 $\pm$ 3.44</td>
</tr>
<tr>
<td>$C_{\text{predose}}$ ($\mu$g/ml)</td>
<td>5.49 $\pm$ 4.02</td>
</tr>
<tr>
<td>AUC12 ($\mu$g·h/ml)</td>
<td>82.8 $\pm$ 44.5</td>
</tr>
<tr>
<td>Lopinavir/r plus efavirenz in present study at:</td>
<td></td>
</tr>
<tr>
<td>400/100 mg BID</td>
<td>8.15 $\pm$ 3.04</td>
</tr>
<tr>
<td>533/133 mg BID</td>
<td>4.07 $\pm$ 4.04</td>
</tr>
<tr>
<td>533/133 mg BID</td>
<td>5.88 $\pm$ 5.53</td>
</tr>
<tr>
<td>533/133 mg BID</td>
<td>89.8 $\pm$ 65.4</td>
</tr>
<tr>
<td>Ritonavir</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ ($\mu$g/ml)</td>
<td>0.60 $\pm$ 0.35</td>
</tr>
<tr>
<td>$C_{\text{min}}$ ($\mu$g/ml)</td>
<td>0.10 $\pm$ 0.07</td>
</tr>
<tr>
<td>$C_{\text{predose}}$ ($\mu$g/ml)</td>
<td>0.21 $\pm$ 0.18</td>
</tr>
<tr>
<td>AUC12 ($\mu$g·h/ml)</td>
<td>3.8 $\pm$ 1.8</td>
</tr>
</tbody>
</table>

### Pharmacodynamics

A total of 51 subjects were included in the pharmacodynamic analyses. The median baseline viral load for the 51 subjects included in the pharmacodynamic analyses was 4.56 log10 copies/ml, and the median baseline CD4 count was 220 cells/μl. At week 24, the mean CD4 increase from baseline was 49 cells/μl, and 82% (42 of 51) of the subjects had a viral load of <400 copies/ml.

Virologic response in these subjects was statistically significantly associated with the logarithmic transformed lopinavir IQ $C_{\text{predose}}$ but not with $C_{\text{predose}}$ (Fig. 2). In particular, week 24 virologic response rates were 70, 80, and 100% for subjects with lopinavir $C_{\text{predose}}$ values of <4, 4 to 15, and >15, respectively (P = 0.047, Fisher exact test). In contrast, virologic response rates of subjects with lopinavir $C_{\text{predose}}$ values of <2.5, 2.5 to 4.5, and >4.5 μg/ml were 86, 78, and 84%, respectively (P = 0.811). Consistent with the categorical analyses described above, none of the drug concentration measures were associated with week 24 antiviral activity as determined by univariate logistic regression analysis (P = 0.574 to 0.834). In contrast, all of the lopinavir IQ parameters (IQ $C_{\text{max}}$, IQ $C_{\text{min}}$, IQ $C_{\text{predose}}$, and IQ AUC) were marginally or statistically significantly associated with week 24 virologic response, with P values ranging from 0.022 to 0.078. As detailed elsewhere, the lopinavir baseline phenotype was also statistically significantly correlated (P = 0.006) with the week 24 virologic response (28a). The baseline efavirenz IQ was also statistically significantly correlated with the week 24 virologic response (P = 0.027). Similarly, the transformed efavirenz concentration was not significantly correlated with virologic response (P = 0.207) (28a).

In order to evaluate these associations in the context of other baseline parameters, three stepwise logistic regression analyses were performed (see Materials and Methods and Table 4). In the first model, none of the various transformed lopinavir and efavirenz concentration parameters were found to be associated with virologic response in any of the models. In model 2, which included both the transformed lopinavir $C_{\text{predose}}$ and IQ $C_{\text{predose}}$ and the transformed efavirenz concentration and IQ, the efavirenz log$_{10}$IQ and lopinavir log$_{10}$IQ $C_{\text{predose}}$ values were confirmed to be statistically significant predictors of response (P = 0.027 and 0.011, respectively). In the third model, the IQ and concentration variables were investigated in the context of baseline lopinavir and efavirenz resistance parameters (phenotype and genotype), which have previously been shown to be predictive of response (27, 28a).
In this model (model 3), the baseline lopinavir phenotype superceded the IQ parameters in predicting viral suppression, whereas the efavirenz IQ parameter remained as a significant predictor of viral response in this highly PI-experienced NNRTI-naive population. Essentially identical results were obtained for models 2 and 3 when other pharmacokinetic and IQ parameter pairs (i.e., $C_{\text{max}}$ and IQ $C_{\text{max}}$, etc.) were considered instead of $C_{\text{predose}}$ and IQ $C_{\text{predose}}$.

Since the IQ values for three subjects with no pharmacokinetic data were imputed by using the median pharmacokinetic results from their respective lopinavir/r regimen group, to demonstrate that the inclusion of the three subjects did not alter the conclusions of the statistical analyses, sensitivity analyses were conducted in which the 10th and 90th percentiles were each used to impute the missing pharmacokinetic parameters for the three subjects. The results from the sensitivity analyses were essentially identical to those obtained when median pharmacokinetic results from their respective lopinavir/r regimen group were used to impute the missing pharmacokinetic parameters, thus supporting the inclusion of the three subjects. When the three subjects with missing pharmacokinetic data were excluded from the pharmacodynamic analyses, the significance levels were reduced in all analyses. However, lopinavir and efavirenz log$_{10}$ IQ were still statistically significant predictors for virologic response in model 2, whereas lopinavir log$_{10}$-fold change in susceptibility and efavirenz log$_{10}$ IQ were predictors in model 3 as determined by stepwise logistic regression.

Since IQ $C_{\text{predose}}$ was found to predict the week 24 antiviral activity, point estimates (with corresponding 95% CIs) for the probability of week 24 virologic response as a function of log$_{10}$ IQ $C_{\text{predose}}$ were computed using univariate logistic regression (Fig. 3). The week 24 virologic response rate was estimated to be ca. 67% (95% CI = 45 to 84%) for a lopinavir IQ $C_{\text{predose}}$ value of 1. Thus, with a conservative measure (i.e., the lower 95% CI), an IQ $C_{\text{predose}}$ of approximately 1 predicted a 45% response rate for this regimen in this particular patient population. With a lopinavir IQ $C_{\text{predose}}$ of approximately 4, the estimated response rate was 74%.

**DISCUSSION**

This was a prospective, dose-randomized trial in multiple PI- and NRTI-experienced, NNRTI-naive subjects, with a regimen of lopinavir/r, efavirenz, and NRTIs. The effect of efavirenz on lopinavir pharmacokinetics was characterized, at both the standard lopinavir/r dose and at a higher dose selected to compensate for the expected reduction of lopinavir $C_{\text{predose}}$ by efavirenz. Additionally, the relationships between pharmacokinetic variables, baseline viral characteristics, and virologic response were evaluated. In particular, we tested the hypoth-

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**TABLE 4. Predictors of week 24 virologic response of lopinavir/r-based regimens from stepwise logistic regression models**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model 1 (PK) P</th>
<th>Parameter</th>
<th>Model 2 (PK + IQ) P</th>
<th>Parameter</th>
<th>Model 3 (PK + IQ + phenotype + genotype) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline viral load</td>
<td>0.034</td>
<td>Log$_{10}$ efavirenz IQ</td>
<td>0.101</td>
<td>Log$<em>{10}$ fold lopinavir IC$</em>{50}$ change</td>
<td>0.019</td>
</tr>
<tr>
<td>No. of active NRTIs</td>
<td>0.036</td>
<td>Log$<em>{10}$ lopinavir IQ $C</em>{\text{predose}}$</td>
<td>0.036</td>
<td>Log$_{10}$ efavirenz IQ</td>
<td>0.061</td>
</tr>
<tr>
<td>Body wt</td>
<td>0.034</td>
<td>Baseline viral load</td>
<td>0.083</td>
<td>No. of active NRTIs</td>
<td>0.024</td>
</tr>
<tr>
<td>Time since HIV diagnosis</td>
<td>0.074</td>
<td>No. of active NRTIs</td>
<td>0.028</td>
<td>Time since HIV diagnosis</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time since HIV diagnosis</td>
<td>0.113</td>
<td>Body wt</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Body wt</td>
<td>0.094</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PK, pharmacokinetics.
the increase in lopinavir was nonlinear. With a 33% increase of lopinavir/r dose, the predose concentrations of the PIs, similar to lopinavir, the effect of efavirenz on differential effects on $AUC$ and $C_{\text{max}}$ or $C_{\text{min}}$ may be more closely associated with virologic response than $C_{\text{max}}$. It can be estimated that, first-pass extraction for lopinavir would yield lopinavir $AUC$, $C_{\text{predose}}$, and $C_{\text{min}}$ values similar to those of a 400/100-mg BID lopinavir/r regimen without efavirenz.

**Predictors of antiviral activity.** As expected, in this NNRTI-naïve but extensively PI-pretreated patient population, baseline lopinavir phenotype (fold resistance to lopinavir), genotype, and IQ parameters ($IQ_{C_{\text{predose}}}$, $AUC$, $C_{\text{max}}$, and $C_{\text{min}}$) were predictors of viral suppression at week 24. The viral susceptibility to efavirenz in this population was not uniform, ranging from 0.1- to 20-fold (median, 0.4-fold), although all but two baseline isolates displayed <2.5-fold reduced susceptibility to efavirenz. Consequently, efavirenz $IQ$ was also a significant predictor of viral suppression. The number of active reverse transcriptase inhibitors (<2.5-fold change in baseline phenotype) and baseline viral load were also correlated with virologic response. Body weight was a significant predictor of viral suppression, as well as a marginally or statistically significant covariate ($P < 0.064$) for lopinavir, ritonavir, and efavirenz pharmacokinetic parameters, probably because subjects with larger weight tended to have lower drug levels (5). The finding that patients with longer times since initial HIV diagnosis also experienced superior virologic response may represent a survival bias effect. This observation is discussed in more detail elsewhere (28a).

The fact that $IQ_{C_{\text{predose}}}$ was a predictor of virologic response in various statistical analyses is significant in two regards. First, this observation is consistent with previous evidence suggesting that $IQ_{C_{\text{predose}}}$ is correlated with the antiviral activity for PIs. Although the stepwise regression model 2 showed that all IQ parameters ($IQ_{AUC}$, $C_{\text{max}}$, $C_{\text{min}}$, and $C_{\text{predose}}$) were predictors of virologic response in the present study, it is reasonable to expect, based on first principles, that the $C_{\text{predose}}$ or $C_{\text{min}}$ values in plasma predominantly determine the in vivo antiviral effect of PIs: HIV turnover is rapid (22), PIs do not undergo intracellular activation (as do NRTIs), and PIs have high partition coefficients, allowing rapid intracellular equilibration (32). However, rigorous demonstration of this is not straightforward, since all concentration parameters, i.e., $AUC$, $C_{\text{max}}$, $C_{\text{min}}$, and $C_{\text{predose}}$, are generally highly correlated; thus, the relationships between these parameters and virologic response are also similar. For example, lopinavir $C_{\text{predose}}$ were highly correlated with $AUC$ ($R^2 = 0.84$), $C_{\text{max}}$ ($R^2 = 0.77$), and $C_{\text{min}}$ ($R^2 = 0.78$) in the present study. In this regard, the correlations between virologic response and the four IQ parameters were similar, based on the results of univariate logistic regression analysis. Also, essentially identical results were obtained when models 2 and 3 stepwise logistic regression analyses were performed for lopinavir $IQ_{AUC}$, $C_{\text{max}}$, and $C_{\text{min}}$. Nonetheless, the accumulated observations from multiple studies involving several PI regimens suggest that $C_{\text{min}}$ or $C_{\text{predose}}$ is more closely associated with response than $AUC$ or $C_{\text{max}}$ (21, 25, 27). In one study, despite less-frequent dosing

![Diagram](http://aac.asm.org/)
and higher AUC and $C_{\text{max}}$ values, an indinavir BID regimen (1,200 mg BID) was less efficacious than an indinavir three times daily (TID) regimen (800 mg TID), a result probably attributable to a significantly lower indinavir $C_{\text{predose}}$ value in the BID regimen (21, 25). In another study, switching patients with detectable viremia from a stable regimen of indinavir at 800 mg TID to indinavir-ritonavir at 400/400 mg BID without initially altering concomitant antiviral agents resulted in additional antiviral activity (viral load of <50 copies/ml or a >0.5-log$_{10}$ reduction in viral load below baseline) in a substantial proportion of patients (44). In that study, the indinavir pharmacokinetics were altered such that $C_{\text{predose}}$ and $C_{\text{min}}$ were increased >3-fold, but $C_{\text{max}}$ and AUC were actually decreased.

A second implication of the relationship between lopinavir IQ and response is that combined information on drug exposure and drug susceptibility is more relevant than drug concentration alone. In univariate analyses, none of the lopinavir pharmacokinetic parameters demonstrated an association with virologic response ($P > 0.574$), whereas statistically or marginally significant associations were found for the corresponding IQ parameters ($P < 0.078$). As can be seen from Fig. 4, although there appears to be a general relationship between drug concentrations and inhibitory quotients, the inhibitory quotients may vary by up to 2 orders of magnitude at a given $C_{\text{min}}$ or $C_{\text{predose}}$ value. Thus, subjects with high drug levels do not necessarily have higher inhibitory quotients than those with lower drug levels, since differential viral susceptibility is factored into the IQ. For the same reason, it is also expected that IQ should be a better predictor of virologic response than phenotype alone. However, almost all subjects who displayed a high degree of baseline resistance to lopinavir also had low IQ values in the present study. Consequently, the lopinavir phenotype appeared to be a better predictor of response than IQ when the stepwise logistic regression analysis included lopinavir concentration and IQ parameters plus baseline lopinavir phenotype and genotype (model 3). To test whether IQ is a better response predictor than phenotype alone, it may be necessary to conduct a study within a specified range of phenotypic susceptibilities that allows modulation of IQ by dose adjustment. In reality, such studies may be limited by concerns over tolerability issues.

It is important to discuss the limitations of the present study and its conclusions. A relatively small number of subjects were included and, although appropriate statistical methodologies were applied, these findings should be confirmed in larger studies. The applicability of these results to other patient populations and other antiretroviral treatment regimens may vary. In general, the IQ $C_{\text{predose}}$ (or IQ $C_{\text{min}}$) is likely to be correlated with response in situations in which both $C_{\text{predose}}$ and viral susceptibility are variable and are in a range that will affect virologic response. For example, in treatment-naive patients, one would not anticipate a better correlation between response and IQ $C_{\text{predose}}$ compared to $C_{\text{predose}}$ alone, given the relatively narrow range of baseline viral susceptibilities expected in this patient population. The use of efavirenz in the present study should also be considered when interpreting these results. Although all subjects in the present study were required to be NNRTI naive, the baseline susceptibility to efavirenz was not entirely uniform, and efavirenz IQ was found to be a predictor of viral suppression (models 2 and 3). However, given that lopinavir IQ and lopinavir phenotype were also significant predictors of virologic response in the present study, this result cannot be solely attributed to efavirenz.

**Clinical utility of the inhibitory quotient.** The IC$_{50}$ values used in this publication were determined in a culture system containing 50% human serum in addition to 10% fetal calf serum (34). Since most PIs and NNRTIs, with the exception of indinavir and nevirapine, are highly bound to plasma proteins, and since the in vitro system cannot tolerate more than 50% human serum, the addition of 50% human serum is an attempt to account for potential protein binding effects on the IC$_{50}$ values. IC$_{50}$ values can reasonably approximate IC$_{50}$ values in 100% human serum, then the IQ values calculated by using 50% human serum-adjusted IC$_{50}$ values would be more clinically meaningful than those calculated by using IC$_{50}$ values determined in the presence of only 10% fetal calf serum. The conventional methods of accounting for 100% protein-binding effects on in vitro IC$_{50}$ values often involve adding physiologically relevant concentrations of plasma proteins such as albumin and/or $\alpha_1$-acid glycoprotein to the in vitro cell cultures (34). However, the effects of purified proteins do not neces-

![Image](http://aac.asm.org/Downloaded from http://aac.asm.org/ on February 20, 2021 by guest)
sarily recapitulate the attenuation of activity observed with human serum (34). We have recently demonstrated that free fraction of lopinavir in 50% human serum plus 10% fetal calf serum approximates the free fraction in 100% human serum (23). These results support the use of 50% human serum-adjusted IC_{50} values for the calculation of IQ as described in this report.

The inhibitory quotient calculated in this report is a simple and straightforward concept initially proposed by Ellner and Neu to quantify the inhibitory effect of anti-infective agents (17). The inhibitory quotient accounts for the contribution of individual drug concentrations and viral susceptibilities and is expected to provide a better estimation of potential drug activity for individual patients than drug concentration or viral susceptibility alone. The results of the present study demonstrate the importance of a high inhibitory quotient with respect to antiviral efficacy. Additionally, the results of the present study show that IQ_{predose} is an important parameter for predicting the antiviral activity of PIs. It should also be noted that the mutation rate during viral replication is high, and mutant HIV strains are expected to preexist as the quasispecies even prior to therapy. Based on simple pharmacological principles, mutants or quasispecies with elevated IC_{50} values are expected to have a replication advantage over wild-type virus or the baseline strain in the presence of antiretroviral therapy. Thus, to ensure sustained antiviral activity, the inhibitory quotients for potential preexisting mutants should also be considered in dosage selection (24).

Although IQ is expected to provide a better estimate of antiviral activity than drug concentration or viral susceptibility alone, it is important to appreciate the dynamic relationship between IQ and virologic response; hence, the limitations of IQ utility in clinical settings. For example, for patients with highly resistant viruses, desirable IQ values may not be achievable even with higher doses. Under such conditions, virologic response is likely to be predicted equally well by the individual baseline viral susceptibility, the individual baseline viral susceptibility, and the number of active NRTIs, the efavirenz IQ, and body weight were statistically significant predictors of antiviral response to lopinavir/r-based therapy. Validation of these pharmacokinetic-pharmacodynamic relationships in larger clinical trials could help to support the use of such metrics in treatment decision in antiretroviral agent-experienced patients.

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