Association of total bilirubin with indinavir and lopinavir plasma concentrations in HIV-infected patients receiving three different double-boosted dosing regimens

Robert Dicenzo¹²*, Amneris Luque², Panupong Larppanichpoonphol² and Richard Reichman²

¹Department of Pharmacy and Pharmaceutical Science, University at Buffalo, Buffalo, NY, USA; ²Infectious Disease Unit, Department of Medicine, University of Rochester, Rochester, NY, USA

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Objectives: The purpose of this study was to determine the pharmacokinetics and tolerability of three different indinavir and lopinavir/ritonavir dosing regimens.

Methods: HIV-infected adults receiving lopinavir/ritonavir 400/100 mg twice daily with food had nine plasma samples taken over a 12 h dosing interval at baseline (BL), after adding indinavir 600 mg twice daily for 10 days (R1), indinavir 800 mg twice daily for 5 days (R2) and lopinavir/ritonavir 533/133 mg plus indinavir 600 mg twice daily for 10 days (R3). Plasma samples were assayed using HPLC.

Results: A total of 12 patients completed the BL visit [10 male; mean (SD) age = 43.9 (5.8) years] and 9, 7 and 7 completed R1, R2 and R3 visits, respectively. Two subjects discontinued treatment due to hypertriglyceridaemia. Compared with BL, the R3 lopinavir AUC (P < 0.05) and Cₘₐₓ (P = 0.0025) were significantly higher and the R2 AUC trended higher (P = 0.09). The indinavir AUC (P = 0.030) and Cₘₐₓ (P = 0.035) were significantly higher for R2 compared with R1. There was a trend for increased total bilirubin (TB) after the addition of indinavir (P = 0.09). Lopinavir and indinavir AUC, Cₘₐₓ and Cₘᵢₙ were associated with TB during univariate analyses (P < 0.01) while only lopinavir AUC (P = 0.0004) and indinavir AUC (P = 0.0028) were associated with TB during multivariate analysis. Only indinavir AUC was significant when both drugs were included in the model (P = 0.0028).

Conclusions: Elevated lopinavir and indinavir concentrations are associated with elevated TB.

Keywords: drug monitoring, HIV antiviral pharmacology, pharmacokinetics

Introduction

Boosted-protease-inhibitor-containing antiretroviral regimens play an important role in both treatment-naive and experienced HIV-infected patients in whom the viral load of HIV-1 in plasma is commonly reduced to a level below the limit of detection. However, the development of resistance often precludes the success of highly active antiretroviral therapy (HAART), and complex salvage regimens are needed to successfully treat patients with limited therapeutic options. Some patients in need of salvage therapy may benefit from a double-boosted-protease-inhibitor-containing regimen in which the pharmacokinetics of two active protease inhibitors are boosted by ritonavir. One such option is the combination of lopinavir/ritonavir and indinavir. Potential pharmacokinetic advantages of combining these two protease inhibitors include increased plasma concentrations since both are substrates for and inhibitors of cytochrome P450 3A4 and differences in distribution to potential viral sanctuary sites since indinavir is less bound to protein and both are substrates for transporter proteins.

Although double-boosted-protease-inhibitor-containing regimens are being used in HIV-infected patients, little is known about the achievable plasma concentrations or tolerability. Investigations have been primarily limited to small pharmacokinetic trials in which the results are compared with historical controls. These studies are further limited by inherent wide inter- and intrapatient variability in plasma concentrations making it difficult to determine the best candidate regimen. Accurate estimates of achievable plasma concentrations are of particular importance when weighing the risk and benefits of using higher doses.
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For example, there is evidence that boosting indinavir may lead to decreased tolerability and results from the ATHENA trial demonstrated that the benefit of adjusting indinavir regimens based on plasma concentrations may have been due to decreased toxicity. Moreover, the knowledge of achievable trough concentrations will be crucial if ongoing clinical trials support the use of therapeutic drug monitoring to maximize the ratio of achievable trough concentration and a measure of viral sensitivity.

Potential dosing regimens for lopinavir/ritonavir combined with indinavir include lopinavir/ritonavir/indinavir 400/100/600, 400/100/800 and 533/133/600 mg twice daily, which are currently being used in a prospective clinical trial designed to determine the usefulness of therapeutic drug monitoring in salvage patients (Adult AIDS Clinical Trials Group Study 5146). To date, there have been no prospective trials comparing the pharmacokinetics of these regimens in which steady state plasma concentrations of each candidate regimen were determined in all subjects. The purpose of this study was to determine the pharmacokinetics and tolerability of three different regimens of lopinavir/ritonavir and indinavir co-administration in HIV-infected adults.

Methods

Study subjects

HIV-1-infected male or female subjects 18–55 years of age receiving an antiretroviral regimen containing lopinavir/ritonavir 400/100 mg twice daily (baseline regimen) for at least 3 weeks prior to enrolment were recruited at the University of Rochester Medical Center. Additional criteria for inclusion included no clinically significant deviation from normal in medical history, physical examination and clinical laboratory determinations that in the opinion of the investigator would preclude the subject from safely participating in the study. Criteria for exclusion included ingestion of indinavir within 30 days, women who are pregnant or breastfeeding, women of childbearing potential who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period, current or recent (within 3 months) history of gastrointestinal disease or a history of any gastrointestinal surgery that could impact upon the absorption of study drug, and history of recent (within 6 months) drug or alcohol abuse.

Study design

The Institutional Review Boards at the University of Rochester and the University at Buffalo approved the study and all subjects were required to provide informed consent before any study procedures were initiated. Subjects received 600 mg of indinavir twice daily on days 1–10 (regimen 1), 800 mg twice daily on days 11–15 (regimen 2) and 600 mg twice daily on days 16–25 (regimen 3). Lopinavir/ritonavir 400/100 mg was administered with indinavir twice daily on days 1–15 while subjects received 533/133 mg on days 16–25. Subjects arrived in the General Clinical Research Center (GCRC) at the University of Rochester Medical Center on days 0, 10, 15 and 25 after fasting since midnight the night before and received the study drug within 5 min of eating a standardized light breakfast of 40 g of Cheerios®, 350 g of 2% milk, 43 g of toasted white bread, 9 g of margarine and 4 g of sugar. Blood samples were drawn before and 1, 2, 3, 4, 6, 8, 10 and 12 h after the morning dose of study drug. Clinical laboratory tests including plasma chemistry, cell blood counts and lipid profiles were drawn at each visit before study drug administration. Crixivan® 200 mg capsules (Merck and Co., Inc, Whitehouse Station, NJ, USA) and Kaletra® 400/100 mg capsules (Abbott Laboratories, North Chicago, IL, USA) were dispensed by the University of Rochester investigational pharmacy.

Drug assays

Lopinavir and indinavir plasma concentrations were measured using an NYS certified HPLC method for plasma protease inhibitor quantification utilized within the Pharmacotherapy Research Center Core Analytical Laboratory at the University at Buffalo. The lower limit of quantification for lopinavir and indinavir was <0.2 and <0.1 µg/mL, respectively.

Pharmacokinetic analyses

Standard non-compartmental techniques were used to calculate pharmacokinetic parameters using WinNonlin™, version 4.1 (Pharsight, Palo Alto, CA, USA). The steady state area under the concentration-time curve during a 12 h dosing interval (AUC) was determined using the linear trapezoidal method and the maximum observed concentration (Cmax) was determined by visual inspection. If the sample drawn at the end of the dosing interval (Cmin) was not available the concentration reported was determined by extrapolation using the estimated terminal elimination rate.

Statistical analysis

Mixed effects modelling was used to identify predictors of changes in laboratory results. The candidate variables assessed in univariate analysis were drug regimen, pharmacokinetic parameters (AUC, Cmax and Cmin), age, gender and baseline weight. Multivariate analyses was performed using a step-wise backward elimination method first with the pharmacokinetic results of each drug separately and then combined. Only those pharmacokinetic parameters that were significant during individual drug multivariate analysis were included when lopinavir and indinavir were combined. Since dosing regimen, AUC, Cmin and Cmax are correlated variables, only the most predictive of these variables was included in the multivariate analyses; however, the multivariate analysis was repeated including all pharmacokinetic variables to confirm that the same variables remained in the final model. Mixed effects modelling was also used to identify predictors of pharmacokinetic parameters (AUC, Cmin and Cmax) in which candidate variables were baseline weight, age, gender and dosing regimen. Least square mean differences and their corresponding 95% confidence intervals (95% CI) were calculated for all fixed categorical parameters that remained in the final model. The Bonferroni method was used to correct for multiple comparisons made during the analysis of laboratory values and indinavir pharmacokinetic parameters whereas the Dunnett–Hsu method was used to correct for multiple comparison between lopinavir pharmacokinetic parameters on days 10, 15 and 25 and baseline (day 0). All statistical analyses were performed using SAS® System v9.1 (SAS Institute, Cary, NC, USA).

Results

All subjects were receiving the baseline regimen (lopinavir/ritonavir 400/100 mg) prior to enrolment and all regimens were given twice daily. A total of 12 subjects completed the baseline visit [10 male; mean (SD) age = 43.9 (5.8) years] and 9 [7 male; 43.7 (6.1) years], 7 [6 male; 44.7 (6.8) years] and 7 [5 male; 44.9 (5.7) years] completed pharmacokinetic
sampling visits on regimens 1 (lopinavir/ritonavir/indinavir 400/100/600 mg), 2 (lopinavir/ritonavir/indinavir 400/100/800 mg) and 3 (lopinavir/ritonavir/indinavir 533/133/600 mg). The baseline median (range) plasma HIV-1 RNA was 3.44 log_{10} copies/mL (1.92–4.35) and six subjects had undetectable viral load at baseline. History of hepatitis virus co-infection included two subjects with a history of hepatitis C, one with hepatitis A and one with a history of hepatitis viral infection of unknown type.

Two subjects discontinued treatment early (day 14 and 15) due to elevated triglycerides; one subject due to a type I allergic reaction potentially due to administration of indinavir (day 2); and two for reasons unrelated to study drugs (study inconvenience (day 14) and drowsiness due to unapproved use of a muscle relaxant (day 8)). When considering complaints of adverse reactions, the double-boosted regimens appeared to be well tolerated by most subjects. Two subjects complained of mild upset stomach, three complained of mild to moderate diarrhoea and one complained of mild acid reflux.

Antiretroviral medications consisted of tenofovir in 41.7%, abacavir in 33.3%, lamivudine in 41.7%, stavudine in 25%, didanosine in 25%, Trizivir® (abacavir/lamivudine/zidovudine) in 8.3%, zidovudine in 8.3% and Combivir® (lamivudine/zidovudine) in 8.3% of patients. One subject who received nevirapine only completed the baseline visit due to an allergic reaction to indinavir and had lopinavir pharmacokinetic results similar to median baseline values. Other concomitant drugs included lipid lowering agents in 33.3%, trimethoprim/sulfamethoxazole in 41.6%, dapsone in 25%, antidepressants in 41.7%, antipsychotics in 16.7%, proton pump inhibitors (PPI) in 25%, antihypertensives in 25% and medications for sleep in 25% of patients.

### Pharmacokinetic results

Pharmacokinetic results for lopinavir and indinavir are listed in Table 1. Dosing regimen was a significant predictor of lopinavir AUC \( (P = 0.048) \) and \( C_{\text{min}} \) \( (P = 0.0063) \) during the multivariate analyses. Compared with baseline, lopinavir plasma concentrations were significantly higher for regimen 3 and there was a trend for higher lopinavir plasma concentrations for regimen 2. The least square mean difference (95% CI) for lopinavir AUC was 38.5 \( \mu g \cdot h/mL \) (31.4 – 47.7) and 33.8 \( \mu g \cdot h/mL \) (-4.4 – 72.0) comparing regimens 3 \( (P < 0.05) \) and 2 \( (P = 0.09) \) with baseline, respectively. Lopinavir \( C_{\text{min}} \) was also significantly higher for regimen 3 [4.3 \( \mu g/mL \) (1.5 – 7.2)] compared with baseline \( (P = 0.0025) \). Although \( C_{\text{min}} \) was numerically higher for regimen 2 compared with baseline [2.0 \( \mu g/mL \) (-0.8 – 4.9)], the difference did not reach statistical significance \( (P = 0.2) \). Regimen was not a significant predictor of lopinavir \( C_{\text{max}} \) \( (P = 0.13) \). Compared with baseline the least square mean difference (95% CI) for lopinavir \( C_{\text{max}} \) was 2.6 \( \mu g/mL \) (-1.0 – 6.2) and 3.0 \( \mu g/mL \) (-0.61 – 6.6) for regimens 2 and 3, respectively.

Dosing regimen was also a significant predictor of indinavir AUC \( (P = 0.032) \) and \( C_{\text{max}} \) \( (P = 0.020) \). Indinavir AUC and \( C_{\text{max}} \) were significantly higher for regimen 2 compared with regimen 1. The least square mean difference (95% CI) was 11.7 \( h^2 \mu g/mL \) (1.0 – 22.3) and 1.4 \( \mu g/mL \) (0.085 – 2.6) when comparing regimen 2 AUC \( (P = 0.030) \) and \( C_{\text{max}} \) \( (P = 0.035) \) with regimen 1, respectively. Although the indinavir \( C_{\text{min}} \) for regimens 2 and 3 were numerically higher than regimen 1, the differences did not reach statistical significance \( (P = 0.16) \). With the exception of weight, none of the other variables tested was associated with any of the pharmacokinetic variables during multivariate analysis. Weight was borderline significant for lopinavir AUC \( (P = 0.053) \) and indinavir \( C_{\text{max}} \) \( (P = 0.054) \), and although weight was a significant predictor of lopinavir \( C_{\text{min}} \) \( (P = 0.019) \), weight did little to improve the predictive value of the model \((r^2 = 0.67 \text{ versus } 0.62)\).

#### Laboratory results

Laboratory results for each visit are listed in Table 2. Using the standard Division of AIDS Clinical Trials Group Adverse Event Grading Table published in December 2004, two subjects developed grade 2, one developed grade 3 and one developed grade 4 hypertriglyceridaemia after the addition of indinavir. Two of these subjects discontinued treatment early due to hypertriglyceridaemia (1379 and 883 mg/dL) while receiving regimen 2, which is the most likely reason for the lower triglyceride concentration reported for regimen 3. Both subjects had elevated baseline triglycerides (559 and 626 mg/dL), which returned to baseline after discontinuation of indinavir. Of the five subjects that had grade 1 elevated baseline cholesterol, two developed grade 2 while another subject with grade 2 developed grade 3. One subject with grade 2 elevated LDL developed grade 3. Of the four subjects who had elevated liver function tests at baseline,

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**Table 1. Lopinavir and indinavir pharmacokinetic results**

<table>
<thead>
<tr>
<th>Drug/parameter</th>
<th>Baseline</th>
<th>Regimen 1</th>
<th>Regimen 2</th>
<th>Regimen 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (( \mu g/mL ))</td>
<td>67.3 (20.8 – 142.4)</td>
<td>72.0 (51.6 – 160.0)</td>
<td>106.2 (63.0 – 168.9)</td>
<td>106.0 (51.7 – 181.1)</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu g/mL ))</td>
<td>8.4 (4.0 – 15.5)</td>
<td>8.3 (6.0 – 15.8)</td>
<td>10.3 (6.4 – 18.2)</td>
<td>11.6 (5.5 – 16.6)</td>
</tr>
<tr>
<td>( C_{\text{min}} ) (( \mu g/mL ))</td>
<td>3.9 (0.20 – 8.5)</td>
<td>4.6 (1.4 – 12.5)</td>
<td>5.4 (3.0 – 11.4)</td>
<td>8.4 (4.5 – 15.4)</td>
</tr>
<tr>
<td><strong>IDV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AUC (( \mu g/mL ))</td>
<td>26.4 (12.3 – 83.3)</td>
<td>30.6 (25.0 – 114.1)</td>
<td>28.4 (13.7 – 114.2)</td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}} ) (( \mu g/mL ))</td>
<td>4.8 (2.5 – 12.5)</td>
<td>5.8 (4.2 – 13.9)</td>
<td>4.6 (2.4 – 14.1)</td>
<td></td>
</tr>
<tr>
<td>( C_{\text{min}} ) (( \mu g/mL ))</td>
<td>0.48 (0.13 – 3.2)</td>
<td>0.56 (0.30 – 4.8)</td>
<td>1.0 (0.32 – 5.0)</td>
<td></td>
</tr>
</tbody>
</table>

Lopinavir (LPV) and indinavir (IDV) median (range) pharmacokinetic results.

A total of 12 subjects for the baseline visit and 9, 7 and 7 for regimens 1, 2 and 3, respectively.

AUC, area under the concentration-time curve over 12 h at steady state; \( C_{\text{max}} \), the maximum concentration during a dosing interval; \( C_{\text{min}} \), the minimum concentration before the next dose.
two developed a higher grade. One subject whose aspartate aminotransferase (AST) was grade 1 at baseline developed grade 2 while another who had a baseline grade 2 AST, grade 1 ALT and a history of hepatitis C virus (HCV) developed grade 3 AST and grade 2 ALT. The only other subject with a known history of HCV had a baseline grade 2 AST and grade 1 ALT, but neither increased during this trial. Lastly, one subject whose baseline total bilirubin was 0.3 mg/dL developed a grade 1 elevated total bilirubin (1.8 mg/dL).

Dosing regimen, pharmacokinetic parameters (AUC, \(C_{max}\) and \(C_{min}\)), weight, age and gender were tested for association with laboratory values. Lopinavir AUC (\(P = 0.0004; r^2 = 0.63\)), \(C_{max}\) (\(P = 0.0008; r^2 = 0.63\)) and \(C_{min}\) (\(P = 0.0021; r^2 = 0.55\)) and indinavir AUC (\(P = 0.0028; r^2 = 0.84\)), \(C_{max}\) (\(P = 0.011; r^2 = 0.81\)) and \(C_{min}\) (\(P = 0.0065; r^2 = 0.82\)) were associated with total bilirubin concentration during preliminary univariate analyses (Table 3). However, only lopinavir AUC (\(P = 0.0004; r^2 = 0.63\)) and indinavir AUC (\(P = 0.0028; r^2 = 0.84\)) were associated with total bilirubin concentration during the multivariate analysis of each drug separately. When both drugs were included in the multivariate analyses only indinavir AUC was associated with total bilirubin (\(P = 0.0028; r^2 = 0.84\)). Although not statistically
significant \((P = 0.09)\), total bilirubin increased after the addition of indinavir (Table 1). The least square mean difference (95% CI) for R2 and R3 compared with baseline was 0.396 mg/dL \((-0.0306–0.822)\) and 0.385 mg/dL \((-0.0616–0.831)\), respectively. Although lopinavir AUC failed to stay in the final model, lopinavir AUC \((P = 0.024, r^2 = 0.41)\) and \(C_{\text{max}}\) \((P = 0.025, \ r^2 = 0.41)\) were associated with total bilirubin prior to the initiation of indinavir, and covariance between indinavir and lopinavir plasma concentrations confound the results. Regimen 1 lopinavir AUC was associated with regimen 1, 2 and 3 \((P < 0.005)\) indinavir AUC and regimen 2 lopinavir AUC was associated with regimen 1 and 2 indinavir AUC \((P < 0.02)\).

Regimen \((P = 0.022; r^2 = 0.89)\) and gender \((P = 0.025; r^2 = 0.84)\) were significantly associated with haematocrit (HCT) during univariate analysis. Regimen \((P = 0.023)\) and gender \((P = 0.025)\) were also associated with HCT in the multivariate analysis \((r^2 = 0.89)\); however, the influence of gender on HCT is limited by the small number of women (\(n = 2)\) enrolled. The least square mean HCT (95% CI) at baseline was 2.9% (0.39–5.4) higher compared with regimen 2 \((P = 0.017)\) and the HCT was 6.4% \((0.98–11.74)\) lower in women. None of the pharmacokinetic parameters tested \((\text{AUC, } C_{\text{max}} \text{ and } C_{\text{min}})\) was associated with HCT \((P > 0.2)\) and the decrease in HCT may have been influenced by blood sampling \((-50\text{ mL of blood per visit})\). Regimen \((P = 0.031; r^2 = 0.93)\) and gender \((P = 0.013; r^2 = 0.91)\) were also associated with red blood cell count (RBC) during univariate analysis whereas regimen \((P = 0.023)\), weight \((P = 0.0096)\) and gender \((P = 0.007)\) were associated with RBC during multivariate analysis \((r^2 = 0.93)\). The least square mean RBC (95% CI) at baseline was slightly higher \([0.29 \times 10^6\) cells/mm\(^3\) \((0.032–0.56)\)] compared with regimen 2 \((P = 0.022)\); the least square mean RBC for women was 1.24 \(\times 10^6\) cells/mm\(^3\) \((0.71–1.78)\) lower \((P = 0.007)\); and the covariate (95% CI) for weight \((P = 0.0096)\) was 0.0137 \(\times 10^8\) cells/mm\(^3\) \((0.00433–0.0231)\) per kg. Although there was a trend for a decrease in haemoglobin compared with baseline, the difference did not reach statistical significance \((P = 0.081)\).

Even though two subjects were discontinued due to elevated triglycerides, dosing regimen was not associated with triglyceride concentrations \((P > 0.3)\); however, the results may have been influenced by the early withdrawal of two subjects due to hypertriglyceridaemia. One subject was discontinued from the study on day 14 and the other on day 15 for which triglyceride concentration was available on day 18 and day 20, respectively. Including these additional values failed to influence the results \((P > 0.3)\). Protease inhibitor plasma concentrations increased significantly for both subjects. The indinavir AUC, \(C_{\text{max}}\) and \(C_{\text{min}}\) increased from regimen 1 to 2 in one subject (15.1 versus 30.6 h\(\times\)µg/mL; 2.5 versus 6.7 µg/mL; and 0.22 versus 0.30 µg/mL, respectively) while the other showed a noticeable increase in regimen 1 lopinavir AUC, \(C_{\text{max}}\) and \(C_{\text{min}}\) compared with baseline (20.8 versus 55.1 h\(\times\)µg/mL; 4.0 versus 6.9 µg/mL; and 0.2 versus 1.4 µg/mL).

**Discussion**

To our knowledge, these are the first data on the pharmacokinetics and tolerability of concomitant lopinavir/ritonavir and indinavir administration in HIV-infected patients in which each subject received three different regimens. Lopinavir concentrations at baseline and for regimens 1 and 2 were similar to those previously reported for lopinavir/ritonavir 400/100 mg twice daily. Kaletra\(^a\) prescribing information shows a mean \pm SD AUC\(_{0–12}, \ C_{\text{max}}\) and \(C_{\text{min}}\) of 92.600 \pm 36.700 µg·h/mL, 9.800 \pm 3.700 µg/mL and 5.500 \pm 2.700 µg/mL, respectively.\(^7\) Lopinavir plasma concentrations for regimen 1 were also similar to those previously reported for lopinavir/ritonavir/indinavir 400/100/600 mg twice daily. Harris et al. showed a median (range) lopinavir \(C_{\text{max}}\) of 10.925 µg/mL \((5.62–16.64)\) and \(C_{\text{min}}\) of 5.195 µg/mL \((0.299–11.733)\) when combining lopinavir/ritonavir 400/100 mg with indinavir 600 mg twice daily in 10 HIV-infected subjects.\(^8\) However, their results are pooled with non-nucleoside-reverse-transcriptase-inhibitor (NNRTI)-containing regimens in which lopinavir/ritonavir was increased to 533/133 twice daily. When combined with indinavir 600 mg twice daily one additional Kaletra\(^a\) tablet appeared to achieve higher lopinavir plasma concentrations than baseline. The most likely reason is the increased lopinavir and ritonavir dose. The least square mean difference (95% CI) for lopinavir AUC and \(C_{\text{max}}\) was 38.5 h\(\times\)µg/mL \((0.31–76.7)\) and 4.3 µg/mL \((1.5–7.2)\), respectively, when comparing lopinavir/ritonavir/indinavir 533/133/600 mg twice daily with baseline.

Antoniou and colleagues raised concern of a potential negative interaction by reporting slightly lower than expected lopinavir plasma concentrations when combining lopinavir/ritonavir 400/100 mg with indinavir 800 mg twice daily in five HIV-infected patients. They reported a median (range) AUC\(_{0–12}, \ C_{\text{max}}\) and \(C_{\text{min}}\) of 53.44 µg·h/mL \((10.29–78.09)\), 6.09 µg/mL \((1.75–8.49)\) and 4.26 µg/mL \((0.22–7.39)\), respectively.\(^7\) However, other investigators have failed to show an influence or reported an increase in indinavir plasma concentrations and the results of Antoniou and colleagues are limited by small sample size and a comparison with historical controls.\(^0,11\) Similar to previous reports in which adding indinavir 400 mg twice daily resulted in a trend towards higher lopinavir plasma concentrations, our results show a trend towards higher lopinavir plasma concentrations on regimen 2 compared with the baseline regimen; therefore, the addition of indinavir 800 mg twice daily does not appear to decrease lopinavir exposure.\(^12\)

Similar to previous studies of indinavir plus lopinavir, our results demonstrated a higher \(C_{\text{min}}\), lower \(C_{\text{max}}\) and similar 24 h AUC compared with unboosted indinavir 800 mg thrice daily.\(^13\) Indinavir pharmacokinetic parameters were similar to those previous reported for indinavir and lopinavir/ritonavir co-administration. Previous reports of indinavir steady state pharmacokinetics in HIV-infected patients include a median (range) \(C_{\text{max}}\) and \(C_{\text{min}}\) of 3.418 µg/mL \((2.15–5.96)\) and 0.348 µg/mL \((<0.085–1.63)\) for lopinavir/ritonavir/indinavir 400/100/600 mg and median (range) AUC\(_{0–12}, \ C_{\text{max}}\) and \(C_{\text{min}}\) of 51.84 µg·h/mL \((29.63–64.45)\), 7.58 µg/mL \((5.35–9.25)\) and 0.61 µg/mL \((0.05–1.91)\) for lopinavir/ritonavir/indinavir 400/100/800 twice daily, respectively.\(^6,9\) However, half of the subjects receiving indinavir 600 mg twice daily had an NNRTI-containing regimen, which may explain the lower than expected \(C_{\text{max}}\).\(^8\)

When boosted with ritonavir, 800 mg of indinavir is the recommended starting dose; however, when combined with lopinavir/ritonavir, indinavir 600 mg may be preferred due to improved tolerability and the potential for decreased risk of toxicity since elevated indinavir plasma concentrations have been associated with urologic complications.\(^6,14–16\) In order to
justifying the risk of decreased tolerability, evidence should support higher achievable indinavir plasma concentration compared with indinavir 600 mg twice daily. This may be of particular importance if current clinical trials investigating the use of therapeutic drug monitoring, such as the Adult AIDS Clinical Trial Group Study (AACTG) 5146, demonstrate that improving the ratio of trough concentration to a measure of patient-specific viral sensitivity is of benefit for patients receiving salvage therapy. To date, there have been no prospective pharmacokinetic comparisons of indinavir 800 and 600 mg with concomitant administration of lopinavir/ritonavir. Even after correcting for multiple comparisons, our results indicate that indinavir 800 mg twice daily achieves a significantly higher C\text{max} [5.8 μg/mL (4.2–13.9) versus 4.8 μg/mL (2.5–12.5); P = 0.035] and AUC\text{0–12} [30.6 μg·h/mL (25.0–114.1) versus 26.4 μg·h/mL (12.3–83.3); P = 0.03] compared with 600 mg twice daily. Inter- and intra-patient variability and small sample size are the most likely reasons why the numerical difference in trough concentrations failed to reach statistical significance. One additional Kaletra® tablet resulted in a similar median AUC\text{0–12} [26.4 μg·h/mL (12.3–83.3)] compared with 28.4 μg·h/mL (13.7–114.2]) and C\text{max} [4.8 μg/mL (2.5–12.5) versus 4.6 μg/mL (2.4–14.1)] compared with lopinavir/ritonavir/indinavir 400/100/600 mg. Considering the inter- and intra-patient variability in plasma concentrations, studies with significantly more subjects will be necessary to determine whether these regimens are bioequivalent. Prescribers must consider both the risks and benefits when determining the optimal dosing regimen. Although the pharmacokinetic results of the present study may help guide therapeutic drug monitoring dosing decisions, only results from large clinical trials such as AACTG 5146 will be able to determine which salvage regimen is best based on safety and efficacy.

We found a surprisingly high incidence of laboratory abnormalities and an association between protease inhibitor plasma concentrations and total bilirubin. Indinavir has been reported to increase bilirubin, which predominately manifests as an asymptomatic increase of indirect bilirubin. Inhibition of bilirubin UDP-glucuronosyl transferase (UGT) activity appears to be the most likely mechanism and there is evidence of genetic predisposition. However, there are case reports of symptomatic hyperbilirubinemia in patients with higher than expected indinavir plasma concentrations. Although hyperbilirubinemia occurs less frequently with lopinavir/ritonavir, our results support an association between total bilirubin and the plasma concentrations of both indinavir and lopinavir. In our analysis, all pharmacokinetic parameters tested were associated with total bilirubin during preliminary univariate analyses while both lopinavir and indinavir AUC stayed in the model during multivariate analysis of each drug separately. Although only indinavir AUC was significantly associated with total bilirubin when both drugs were included in the analysis, the covariance between indinavir and lopinavir plasma concentrations confound the results and lopinavir plasma concentrations were associated with total bilirubin before the addition of indinavir.

Although there is no clear association between protease inhibitor concentration and aminotransferases, there is evidence that the pharmacokinetics of protease inhibitors may be influenced by changes in hepatic function and that lopinavir plasma concentrations are associated with markers of cholestasis. By measuring lopinavir trough concentrations and liver function tests at one time point in a cohort of 149 HIV-infected patients, Seminari et al. showed a significant association between lopinavir trough and cholestasis markers [γ-glutamyl transpeptidase (GGT) and total bilirubin] during univariate analysis; however, only elevated GGT was significantly associated with lopinavir plasma concentrations (>6425 ng/mL) during multivariate analysis (OR = 1.010, 95% CI: 1.002–1.021). Although the multivariate analysis by Seminari et al. only showed a trend towards association between total bilirubin and lopinavir trough (OR = 3.118, 95% CI: 0.980–11.715; P = 0.07), the difference between their results and ours is most likely methodological. We repeated measures in the same subjects using different dosing regimens, our sampling strategy allowed determination of an AUC which is more reflective of plasma concentrations over an entire dosing interval, and although 25 subjects received another protease inhibitor they did not test whether use of a double-boosted protease inhibitor was associated with markers of cholestasis. Similar to our results, they did not find an association between markers of liver necrosis and protease inhibitor plasma concentrations.

Amprenavir plasma concentrations have also been associated with markers of cholestasis. Veronese et al. showed that total bilirubin levels were associated with amprenavir AUC in patients with impaired liver function. There is also evidence that patients receiving HAART who have elevated baseline alkaline phosphatase and GGT are at increased risk for elevated liver enzymes. However, neither our study nor any of the studies listed above was designed to determine cause and effect; therefore, larger prospective studies of longer duration are needed to determine whether elevated markers of cholestasis lead to increased protease inhibitor concentrations or vice versa. Only one subject reported a grade 1 increase in total bilirubin; therefore, the elevations reported in our study are of minor clinical significance. Considering the variability in protease inhibitor plasma concentrations along with our observation that plasma concentrations are associated with hyperbilirubinemia, more research is necessary to determine whether therapeutic drug monitoring improves outcomes for patients receiving a double-boosted protease inhibitor salvage regimen.

Protease inhibitors have been shown to elevate lipids. We found that 25% of subjects had at least a one-grade increase in hyperlipidemia using the standard Division of AIDS Clinical Trials Group Adverse Event Grading Table and two subjects were discontinued due to hypertriglyceridemia. Unlike total bilirubin, lipids were not associated with any of the pharmacokinetic parameters tested; however, this may be due to inadequate sample size. Regardless of the lack of association with pharmacokinetic parameters, the fact that each drug may cause hyperlipidemia when used separately combined with the high frequency of hyperlipidemia found in this trial supports monitoring lipids more closely when initiating an antiretroviral regimen that contains lopinavir/ritonavir and indinavir. Further study is needed to define the risk of hyperlipidemia and determine whether protease inhibitor plasma concentrations are associated with hyperlipidemia in patients receiving a double-boosted protease inhibitor containing regimen.

One obvious shortfall of our design is the small sample size. Inadequate sample size is the most likely reason that a number of variables showed a trend towards association yet failed to reach statistical significance. The small sample size was compounded by a higher than expected attrition rate which particularly affected data collection from the later visits.
and significant intra- and inter-patient variability in protease inhibitor plasma concentrations. Although we failed to find an association between HCV co-infection and total bilirubin or drug exposure, this is most likely due to small sample size and enrolment of only two subjects with documented HCV co-infection. When determining predictors of laboratory abnormalities, covariance between many of the pharmacokinetic parameters tested limited the multivariate analyses. Large intra- and inter-patient variability in pharmacokinetic parameters also limited our ability to show differences in achievable plasma concentrations when comparing dosing regimens and further support the argument that therapeutic drug monitoring may be useful for double-boosted-protease-inhibitor-containing salvage regimens. Although we found an association between protease inhibitor dosing regimens and HCT and RBC, neither appears to be clinically significant and neither was associated with protease inhibitor plasma concentrations. Also, drug regimen sequence was not randomized; therefore, the decrease in HCT and RBC for the later visits may be due to visit sequence and an aggressive plasma sampling strategy. Although PPI use may have resulted in decreased absorption of protease inhibitors, subjects stayed on them throughout the study; therefore, PPI use should not have influenced the ability to identify predictors of laboratory results or PK parameters. Lastly, drug metabolism and risk of toxicity may be linked to polymorphisms related to a single gene or the combined effect of multiple genes: therefore, our conclusions are limited by the absence of pharmacogenetic and pharmacogenomic information that may be related to the pharmacokinetics and toxicokinetics of protease inhibitors.17

In summary, impaired liver function and hyperlipidaemia are a concern when using most currently marketed protease inhibitors. Our results further support these concerns along with evidence supporting an association between elevated protease inhibitor plasma concentrations and elevated total bilirubin during lopinavir/ritonavir and indinavir co-administration. Until these supporting an association between elevated protease inhibitor plasma concentrations and elevated total bilirubin during lopinavir/ritonavir and indinavir co-administration. Until these relationships are further defined, prescribers should monitor liver function tests and lipids more closely when using a double-boosted-protease-inhibitor-containing regimen.

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