High Variability of Plasma Drug Concentrations in Dual Protease Inhibitor Regimens

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Ritonavir (RTV) strongly increases the concentrations of protease inhibitors (PIs) in plasma in patients given a combination of RTV and another PI. This pharmacological interaction is complex and poorly characterized and shows marked inter- and intraindividual variations. In addition, RTV interacts differently with saquinavir (SQV), indinavir (IDV), amprenavir (APV), and lopinavir (LPV). In this retrospective study on 542 human immunodeficiency virus-infected patients, we compared inter- and intraindividual variability of plasma PI concentrations and correlations between the Cmin (minimum concentration of drug in plasma) values for RTV and the coadministered PI Cmin values. Mean RTV Cmin values are significantly lower in patients receiving combinations containing APV or LPV than in combinations with SQV or IDV. With the most common PI dose regimens (600 mg of IDV twice a day [BID], 800 mg of SQV BID, and 400 mg of LPV BID), the interindividual Cmin variability of patients treated with a PI and RTV seemed to be lower with APV and LPV than with IDV and SQV. As regards intraindividual variability, APV also differed from the other PIs, exhibiting lower Cmin variability than with the other combinations. Significant positive correlations between RTV Cmin and boosted PI Cmin were observed with IDV, SQV, and LPV, but not with APV. Individual dose adjustments must take into account the specificity of the pharmacological interaction of each RTV/PI combination and the large inter- and intraindividual variability of plasma PI levels to avoid suboptimal plasma drug concentrations which may lead to treatment failure and too high concentrations which may induce toxicity and therefore reduce patient compliance.

Ritonavir (RTV), a human immunodeficiency virus (HIV) protease inhibitor (PI), is relatively poorly tolerated at the full dose (600 mg twice a day [BID]) and has a potent inhibitory effect on the cytochrome P450 enzyme CYP3A, the major enzyme responsible for the metabolism of the PIs and many other drugs, in the intestinal wall and liver. RTV also inhibits P-glycoprotein transport, which may increase the absorption of PIs by this pathway. Even at a reduced dose, RTV strongly increases the plasma drug concentrations of other PIs administered concomitantly (10).

This property of RTV in which it strongly increases the concentrations of other PIs is exploited therapeutically: low-dose RTV is increasingly used to improve the pharmacokinetic profiles of other PIs by ensuring higher and more stable plasma drug concentrations, while at the same reducing the number of daily doses and the number of tablets or capsules in each dose (11). These pharmacological interactions are complex and poorly characterized and show marked interindividual variations (1).

In addition, RTV interacts differently with saquinavir (SQV), indinavir (IDV), amprenavir (APV), and lopinavir (LPV).

Precise individual tailoring of the dose regimens of these PI combinations is crucial to avoid subtherapeutic plasma drug concentrations (which are predictive of virologic breakthrough) and excessively high concentrations (which may induce toxicity and therefore reduce patient compliance) (1–3, 11, 13).

In this study of patients receiving various PIs in combination with RTV, we compared the pharmacological interactions of the different dual PI regimens.

MATERIALS AND METHODS

This retrospective study involved HIV-infected patients managed in the infectious diseases department of St. Antoine and Tenon hospitals (Paris, France) from September 1999 to August 2002. They were treated with RTV (100 mg BID) (“baby dose”) in combination with SQV (600 to 800 mg BID), IDV (400 to 800 mg BID), APV (600 mg BID), or LPV (400 mg BID). Patients receiving combinations of more than two PIs (salvage therapy) were excluded from the study, as were those receiving nonnucleoside reverse transcriptase inhibitors (owing to their interactions with PIs). If patients had received successive PIs/RTV dual combinations and undergone successive drug determinations during the study period, only the pharmacological data collected for the first PI/RTV dual combination were used in this study in order to preserve statistical independence between the groups in the interindividual variability analysis.

When several drug assays were done in a given patient receiving the same PI combination at the same doses, only the initial values were used for the analysis of interindividual variability. The analysis of intraindividual variability was exclusively based on values obtained in a given patient receiving the same PI combination at the same doses at each assay.

Patients who forgot to take at least one drug dose during the two days prior to the pharmacological assay were not included in the analysis. The nurse who performed the blood test asked patients about their compliance, and information was based on patients’ answers. Only plasma PI concentrations measured 12 ± 2 h after the last administration (minimum concentration of drug in plasma
TABLE 1. Interindividual variability of RTV and PI C_{min}s

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>PI (ng/ml)</th>
<th>RTV (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean C_{min}</td>
<td>SD (ng/ml)</td>
</tr>
<tr>
<td>APV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,200/0</td>
<td>10</td>
<td>300</td>
<td>185</td>
</tr>
<tr>
<td>600/100</td>
<td>58</td>
<td>1,756</td>
<td>791</td>
</tr>
<tr>
<td>IDV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800/0 TID</td>
<td>29</td>
<td>361</td>
<td>659</td>
</tr>
<tr>
<td>600/100</td>
<td>104</td>
<td>1,267</td>
<td>999</td>
</tr>
<tr>
<td>400/100</td>
<td>97</td>
<td>855</td>
<td>787</td>
</tr>
<tr>
<td>NFV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,250/0</td>
<td>90</td>
<td>1,841</td>
<td>1,275</td>
</tr>
<tr>
<td>1,250/100</td>
<td>11</td>
<td>3,385</td>
<td>2,525</td>
</tr>
<tr>
<td>SQV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800/100</td>
<td>40</td>
<td>792</td>
<td>842</td>
</tr>
<tr>
<td>600/100</td>
<td>18</td>
<td>620</td>
<td>519</td>
</tr>
<tr>
<td>LPV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400/100</td>
<td>45</td>
<td>4,650</td>
<td>2,398</td>
</tr>
</tbody>
</table>

a The PI/RTV treatment is shown with the PI dose (APV, IDV, NFV, SQV, or LPV) before the slash and the RTV dose after the slash. Both doses are given in milligrams, and the drugs were administered BID unless stated otherwise.
b CV, coefficient of variation.

[C_{min}] were taken into account in patients receiving a stable antiretroviral drug regimen for at least 2 weeks before blood sampling.

These RTV and PI concentrations in plasma were determined by high-pressure liquid chromatography with quantification cutoffs of 5 ng/ml for APV and IDV and 10 ng/ml for LPV, SQV, and RTV (15).

Data were analyzed using nonparametric tests (Yates’ and Fisher’s chi-squared tests and the Mann-Whitney test); only series of assays performed on samples from at least 10 subjects were used for the analysis of interindividual variability. Correlations between the RTV C_{min}s and the PI C_{min}s were determined by linear regression curves.

RESULTS

A total of 955 plasma PI determinations were done during the 36-month study period in 542 patients. A total of 413 patients received low-dose RTV (100 mg BID) combined with another PI. The other 129 patients received only one PI (APV, IDV, or nelfinavir [NFV]) and were studied for comparison.

Interindividual variability of plasma RTV concentrations.

RTV C_{min}s in the patients who were also receiving another PI showed large interindividual variability, regardless of the combination. The calculated coefficients of variation of RTV C_{min}s observed with each RTV/PI combination were generally high (Table 1).

Using a nonparametric Kruskal-Wallis test, distributions of RTV C_{min}s are globally different between the groups (P < 0.0001). Distributions are not statistically different, however, between the three IDV/RTV groups (P = 0.45 by the Kruskal-Wallis test) and between the two SQV/RTV groups (P = 0.55 by the Mann-Whitney test) (Table 2).

TABLE 2. Comparison of mean RTV C_{min}s of RTV/PI combinations by the Mann-Whitney test

<table>
<thead>
<tr>
<th>RTV/PI treatment</th>
<th>RTV/SQV</th>
<th>RTV/IDV</th>
<th>RTV/APV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTV/SQV</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTV/IDV</td>
<td>0.697</td>
<td>0.425</td>
<td></td>
</tr>
<tr>
<td>RTV/APV</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RTV/LPV</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.009</td>
</tr>
</tbody>
</table>

* P value in comparison of mean RTV C_{min}s of RTV/PI combinations.

a Between SQV dose regimens (800 mg BID and 600 mg BID).
b Between IDV dose regimens (800 mg BID, 600 mg BID, and 400 mg BID) by the Kruskal-Wallis test.
two to six) were done in 132 patients during identical dose regimens (Table 3).

The intraindividual variability of the $C_{\text{min}}$s of RTV and PI was expressed in terms of coefficient of variation, calculated from the weighted means of drug determinations performed for each patient. Table 3 shows the mean coefficients of variation and standard deviations for RTV and PI $C_{\text{min}}$s for patients given RTV/PI combinations. This intraindividual variability of $C_{\text{min}}$s was relatively large, as the coefficients of variation of mean values observed ranged from 28.4 to 61.4% for the PI and 21.9 to 75.8% for RTV. As shown by the comparison of coefficients of variation of mean values, the intraindividual variabilities of RTV and PI $C_{\text{min}}$s are similar.

Globally, the observed values were similar to those obtained in the 26 patients treated with NFV without RTV. For the IDV/RTV combination, we observed a difference in the coefficients of variation of means according to the IDV dose prescribed (50% for 800-mg IDV BID and 600-mg IDV BID regimens and 30% for 400-mg IDV BID regimen).

Correlations between $C_{\text{min}}$s of RTV and PI in patients given different RTV/PI combinations. (i) IDV and RTV. In patients given the IDV/RTV combination, the $C_{\text{min}}$s of RTV correlated with those of IDV: the higher the RTV $C_{\text{min}}$, the higher the IDV $C_{\text{min}}$ (correlation coefficient $r = 0.72$ and $P < 0.0001$ for the combination of RTV [100 mg] and IDV [600 mg], both drugs given BID) (Fig. 1). The same correlation was found with the other IDV dose regimens (800 and 400 mg BID).

(ii) SQV and RTV. In patients given the SQV/RTV combination, the $C_{\text{min}}$s of SQV and RTV correlated in the same way as the IDV/RTV combination. The $C_{\text{min}}$s of RTV and SQV correlated with each other, despite a wider dispersion of the results ($r = 0.30$ and $P < 0.001$ for the combination of RTV [100 mg] and SQV [600 mg], both given BID) (Fig. 2).

(iii) LPV and RTV. $C_{\text{min}}$s of RTV and LPV (400 mg BID) also correlated with each other ($r = 0.51$ and $P < 0.0001$) (Fig. 3), again despite a wide dispersion of the data in this small subset of patients.

(iv) APV and RTV. In contrast, $C_{\text{min}}$s of RTV and APV appeared to be independent of each other, regardless of the APV dose regimen. At a dose of 600 mg of APV BID, the correlation coefficient $r$ was 0.025 ($P > 0.1$) (Fig. 4).

**DISCUSSION**

There has been a large increase in the use of low-dose RTV in combination with a variety of PIs over the last 2 years. SQV, IDV, and APV are now almost always prescribed in combination with RTV. RTV improves the pharmacokinetic profile of the different coadministered PIs by raising their concentrations in plasma, lengthening their half-lives of elimination, and reducing the influence of food on their gastrointestinal absorption. Therefore, an increase in drug exposure may improve, in some patients, virologic success. By reducing the number of pills and/or decreasing the frequency of doses and removing food restrictions, these RTV combinations may also improve patients’ comfort and compliance (1, 3, 11).

However, despite the widespread use of such RTV/PI combinations, few data are available on the plasma PI concentrations achieved in clinical practice. Available data on the clinical pharmacology of PIs were obtained in small groups of patients and under experimental conditions that differ somewhat from clinical practice.

**TABLE 3. Intraindividual variability of PI and RTV $C_{\text{min}}$s**

<table>
<thead>
<tr>
<th>Treatment $^a$</th>
<th>$n$</th>
<th>PI Mean CV$^b$ (%)</th>
<th>SD (%)</th>
<th>RTV Mean CV (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV, 600/100</td>
<td>11</td>
<td>37.5</td>
<td>20.4</td>
<td>58.0</td>
<td>33.4</td>
</tr>
<tr>
<td>IDV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800/100</td>
<td>14</td>
<td>55.1</td>
<td>34.7</td>
<td>45.2</td>
<td>39.5</td>
</tr>
<tr>
<td>600/100</td>
<td>31</td>
<td>52.5</td>
<td>32.5</td>
<td>45.5</td>
<td>30.4</td>
</tr>
<tr>
<td>400/100</td>
<td>18</td>
<td>28.4</td>
<td>23.4</td>
<td>21.9</td>
<td>17.8</td>
</tr>
<tr>
<td>LPV, 400/100</td>
<td>20</td>
<td>42.5</td>
<td>27.9</td>
<td>39.9</td>
<td>22.3</td>
</tr>
<tr>
<td>SQV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800/100</td>
<td>7</td>
<td>61.4</td>
<td>29.0</td>
<td>75.8</td>
<td>52.8</td>
</tr>
<tr>
<td>600/100</td>
<td>5</td>
<td>53.2</td>
<td>22.3</td>
<td>37.3</td>
<td>37.3</td>
</tr>
<tr>
<td>NFV, 1,250/0</td>
<td>26</td>
<td>50.1</td>
<td>31.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The PI/RTV treatment is shown with the PI dose (APV, IDV, LPV, SQV, or NFV) before the slash and the RTV dose after the slash. Both doses are given in milligrams, and the drugs were administered BID.

$^b$ CV, coefficient of variation.
probably be limited by using the most recent recollection of the patient, and that is why we usually focused questions about treatment compliance on the last 2 days. All patients were informed about the pharmacological assays. One can therefore assume that the degree of inaccuracy in the results due to compliance problems was equally distributed among the groups.

**Interindividual variability of plasma RTV concentrations.** Large interindividual variability of mean RTV $C_{\text{min}}$ was observed among the different groups of patients (coefficient of variation, 57.4 to 99.9%). Variability of mean RTV $C_{\text{min}}$ was found to be lower in patients receiving combinations containing APV or LPV (59.9 and 57.4%) than in those receiving combinations containing SQV or IDV (71.7, 76.1, and 95.4% for IDV and 70.8 and 77.9% for SQV) irrespective of the dose of the coadministered PI, as shown by coefficients of variation. Mean RTV $C_{\text{min}}$ were found to be significantly lower in patients receiving combinations containing APV or LPV than in those receiving combinations containing SQV or IDV. These results could be explained by an induction effect of LPV and APV on the metabolism of RTV.

This observation should be taken into account when another PI must be added to a combination of LPV/RTV or APV/RTV in salvage therapy. Indeed, if plasma RTV concentrations are reduced by APV or LPV, the expected booster effect of RTV on the third PI contained in the combination may be correspondingly reduced (9).

In some patients receiving the SQV/RTV or IDV/RTV combination, we observed high RTV $C_{\text{min}}$, so this PI may participate directly to the overall antiretroviral activity. However, in patients receiving the APV/RTV or LPV/RTV combination, RTV $C_{\text{min}}$ always remained under its minimal effective trough level in plasma.

**Interindividual variability of plasma drug concentrations of the PI combined with RTV.** Large interindividual $C_{\text{min}}$ variability of PIs was observed during treatment without RTV among our patients (61.7, 69.3, and 182.5% for APV, NFV, and IDV, respectively), as in previous studies (4, 5, 12, 16). Interestingly, despite the pharmacokinetic enhancement of these PIs by the addition of low-dose RTV, the interindividual variability of these concentrations remains high in our study.

$$y = 13.817x + 1695.5$$
$$R = 0.511$$
$$p < 0.0001$$

**FIG. 3.** Correlation between $C_{\text{min}}$ for LPV (400 mg BID regimen) and RTV (100 mg BID).

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$$y = 1.2132x + 1442$$
$$R = 0.025$$
$$p = \text{NS}$$

**FIG. 4.** No correlation between $C_{\text{min}}$ for APV (600 mg BID regimen) and RTV (100 mg BID). NS, not significant.
relation was found between the RTV and APV C
min$ was partially due to those of RTV C
min, except for APV.

Many retrospective studies have reported concentration relationships between the exposure to PIs and antiretroviral efficacy and/or a related toxicity (2, 6–8, 14). The use of low-dose RTV in combination with various PIs improves the pharmacokinetic profile of the PI given in combination with RTV, thereby in theory reducing the risk of suboptimal or toxic plasma PI concentrations. However, the inter- and intraindividual variability of plasma drug concentrations of RTV and the coadministered PI remains high in patients thus treated. Consequently, therapeutic drug monitoring of PIs may represent an additional tool for the management of HIV-infected patients either to improve antiretroviral response or to decrease toxicity. Individual dosage adjustments must take into account the specificity of each pharmacological interaction, but the clinical relevance and benefit of therapeutic drug monitoring remain to be demonstrated.

REFERENCES

min (P < 0.0001). In contrast, no such correlation was found between the RTV and APV C
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min of IDV, SQV, and LPV can be raised by increasing the dose regimen of either RTV or PI. However, in the case of APV, only an increase in the APV dosage will increase its C
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min could be partially due to those of RTV C
min, except for APV.