Evaluation of the Effect of Fluconazole on the Pharmacokinetics of Ritonavir

ABSTRACT:

The effects of fluconazole on the pharmacokinetics of the HIV protease inhibitor ritonavir were investigated after multiple dosing in an open-label study. In this randomized, two-period crossover study, eight healthy subjects received ritonavir alone (200 mg every 6 hr for 4 days) and ritonavir with fluconazole (400 mg on day 1, 200 mg every day on days 2–5) with a 2-week washout period. Ritonavir plasma concentrations were measured during the final four ritonavir dosing intervals (24 hr) and a 12-hr washout period. There were statistically significant increases in ritonavir C_{max} and AUC_{0–24} (p < 0.02), with concurrent administration of fluconazole compared with administration of ritonavir alone. The difference between regimens in C_{max} was marginally statistically significant (p = 0.089), and t_{max} and β were not statistically significantly different. Although some ritonavir parameters were affected by fluconazole, mean increases in C_{max} and AUC were ≤15% for the 24-hr period, and only 7–19% for individual dose intervals. Thus, the pharmacokinetics of ritonavir may be influenced only to a small extent when administered with fluconazole. These changes are probably of limited clinical significance and do not necessitate dosage adjustment of ritonavir when fluconazole is added to the regimen.

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1 Abbreviations used are: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; CYP, cytochrome P450; C_{max}, maximum concentration; C_{min}, minimum concentration; t_{max}, time to maximum concentration; AUC, area under the concentration-time curve for each interval; AUC_{0–24}, area under the concentration-time curve for the entire 24-hr interval.

Send reprint requests to: Dr. Allen Cato III, Pharmacokineticist, Pharmaceutical Products Division, D–4PK, AP13A, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064.
and visual acuity were measured periodically during the study, and subjects were monitored for evidence of drug intolerance throughout the study.

Serial 5 ml blood samples were collected at the following times relative to the 7:00 a.m. dose on day 4 in each period: 0 (within 5 min before the dose), 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 26, 29, 32, and 36 hr postdose. Samples were obtained immediately before dosing when and if dosing times coincided (additional ritonavir doses were administered at 6, 12, and 18 hr; fluconazole was administered at 24 hr). Blood samples were separated by centrifugation, and plasma was collected and frozen until assayed for ritonavir.

Plasma concentrations of ritonavir were determined at Oneida Research Services, Inc. (Whitesboro, NY) using a validated HPLC assay (Journal of Chromatography, in press). Calibration standards ranged from 0.010 to 15.00 μg/ml. Quality control samples (0.150, 7.50, and 12.00 μg/ml) had coefficients of variation <6%. The lowest quantifiable concentration was 10 ng/ml.

Ritonavir pharmacokinetics for each subject were estimated with noncompartmental methods. Cmax, Cmin, and tmax were obtained directly from the observed plasma concentration-time data for each dose interval and for the entire 24-hr interval. The terminal-phase elimination rate constant (β) was estimated as the negative of the slope of the straight line obtained by regression of the logarithms of the measurable concentrations vs. time in the log-linear terminal phase of the curve (the last five samples collected, 24–36 hr), and the terminal elimination half-life was calculated as ln(2)/β. The AUC and AUC₀₋₂₄ were calculated by the linear trapezoidal method.

Statistical Analysis. An analysis of variance with effects for sequence, subjects nested within sequence, period, and regimen was performed on Cmax, Cmin, AUC₀₋₂₄, average tmax, and β for the 24-hr period and those parameters calculated for each of the 6-hr dose intervals of this 24 hr. For each of 24-hr Cmax, Cmin, and AUC₀₋₂₄ an exact 95% confidence interval for the ratio of the mean with concurrent fluconazole administration to the mean for ritonavir administered alone was calculated. Because 3 of 5 women finished the regimen of ritonavir administered alone, an analysis of covariance with effects for gender and weight was performed on the ritonavir 24-hr parameters for the regimen of ritonavir administered alone, followed by an analysis with effects for gender only.

Results and Discussion. Thirteen subjects were enrolled (5 women, 8 men), and five of these subjects (4 women, 1 man) were unable to complete the study. The eight subjects who completed the study were (mean ± SD) 29.6 ± 9.7 years old (range: 19–43 years), weighed 81.9 ± 10.6 kg (70.3–100.7 kg), and were 179 ± 9.1 cm tall (161–189 cm). Of the subjects that did not complete the study, only one subject withdrew from the study during concurrent administration of ritonavir and fluconazole after completing the regimen of ritonavir and that subject was experiencing adverse events similar to those during treatment with ritonavir alone (nausea and vomiting).

The high proportion of women withdrawing from the study (four of five discontinued) suggests that women may tend to have higher ritonavir concentrations, possibly due to smaller body size compared with men. However, ritonavir pharmacokinetic parameters for the regimen of ritonavir administered alone were not statistically significantly different between men (N = 5) and women (N = 3) in this study. Additionally, previous studies have failed to detect gender-dependent differences in ritonavir pharmacokinetics (18). Thus, statistical analyses and mean values of pharmacokinetic parameters for this study may be calculated without regard to gender. Statistical comparisons of adverse events across gender were not possible in this study due to the limited number of subjects.

Based on the half-life, administration of ritonavir should have achieved apparent steady-state concentrations by day 4; however, mean ritonavir concentrations 24-hr postdose were somewhat lower than the 0 hr concentrations, which is consistent with autoinduction after multiple dosing of ritonavir as seen in previous multiple-dose studies. Because ritonavir concentrations were measured after 4 days of dosing in each period, the results of this study should not be affected substantially by further induction in ritonavir metabolism.

In general, ritonavir pharmacokinetics (table 1) and plasma concentrations (fig. 1) were influenced only to a small extent by fluconazole. Differences between regimens in ritonavir tmax or β were not statistically significant. In contrast, differences in ritonavir 0–24 hr Cmax and AUC₀₋₂₄ with concurrent administration of fluconazole were statistically significant (p < 0.02), and the difference in ritonavir 0–24 hr Cmin was marginally statistically significant (p = 0.089). However, the actual differences in these parameters between regimens were minor (<15%), and the maximum increases in ritonavir 0–24 hr Cmax and AUC₀₋₂₄ values for an individual subject were 30% and 23%, respectively.

Mean values of tmax, Cmax, Cmin, and AUC were highest for the morning dose interval, with Cmax and AUC about one-third higher than all other intervals regardless of regimen, suggesting protracted absorption of ritonavir after evening doses (i.e. diurnal variation of absorption). It is unlikely that there was an effect due to food, because a previous study showed no difference in ritonavir bioavailability when administered after an overnight fast compared with that after a high-fat breakfast (18). In contrast, the addition of fluconazole to the ritonavir regimen did not seem to affect ritonavir plasma concentrations during the first 6 hr after the fluconazole dose any differently than for the entire 24-hr period. Compared with administration of ritonavir alone, mean ritonavir Cmax increased ~12%, 7%, 16%, and 8% during dosing intervals 1–4, respectively, with concurrent administration of fluconazole. A similar trend was observed in the changes in Cmin and AUC. Changes in Cmax and Cmin were statistically significant (p < 0.04) with coadministration for the first and third dose intervals, and the change in AUC was statistically significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ritonavir Alone</th>
<th>Ritonavir with Fluconazole</th>
<th>Estimate of Ratio of Means</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average tmax (hr)</td>
<td>3.9 ± 1.1</td>
<td>4.1 ± 1.0</td>
<td>1.038</td>
<td>NC*</td>
</tr>
<tr>
<td>0–24 hr Cmax (μg/ml)</td>
<td>8.95 ± 2.41</td>
<td>10.25 ± 2.69</td>
<td>1.145</td>
<td>1.074–1.223</td>
</tr>
<tr>
<td>0–24 hr Cmin (μg/ml)</td>
<td>4.53 ± 0.98</td>
<td>5.15 ± 1.49</td>
<td>1.137</td>
<td>0.999–1.258</td>
</tr>
<tr>
<td>AUC₀₋₂₄ (μg · hr/ml)</td>
<td>151 ± 36</td>
<td>169 ± 41</td>
<td>1.121</td>
<td>1.050–1.195</td>
</tr>
<tr>
<td>β (hr⁻¹)</td>
<td>0.232 ± 0.042</td>
<td>0.229 ± 0.041</td>
<td>0.985</td>
<td>NC</td>
</tr>
</tbody>
</table>

*NC, not calculated.

Statistically significant difference between regimens (ANOVA, p < 0.02).
(p < 0.03) for the first three dose intervals. The absence of a trend of decreasing degree of interaction with time (i.e., lower increases in mean parameter values with each dose interval after administration of fluconazole) may be due to the long half-life of fluconazole of ~30 hr (17), resulting in relatively small fluctuations in fluconazole concentrations despite once-daily dosing.

Ritonavir $t_{\text{max}}$ for individual dose intervals ($p > 0.28$), mean $t_{\text{max}}$, and $\beta$ ($p > 0.49$) were unaffected by fluconazole. Ritonavir $\beta$ was calculated from samples obtained during the time when fluconazole concentrations should have been at their highest (0, 2, 5, 8, and 12 hr after the fluconazole dose on day 5) (19). Using this study design, the presence of an effect on the ritonavir elimination rate constant due to coadministration of fluconazole should probably have been detected if ritonavir $\beta$ had been affected by fluconazole.

Although the minor effect of fluconazole on ritonavir $c_{\text{max}}$, AUC, and $c_{\text{max}}$ could have been related to decreased clearance, the lack of an effect on $\beta$ suggests that ritonavir absorption may have been affected. In addition, it is unlikely that fluconazole-induced alterations in plasma protein binding of ritonavir occurred, because the plasma protein binding of ritonavir was only limited effects on ritonavir clearance. These in vitro data confirm the in vitro results that showed ritonavir to be a high affinity substrate of CYP3A and unlikely to be affected substantially by competitive inhibition from other substrates with moderate CYP3A affinity. Thus, although some ritonavir pharmacokinetic parameters were statistically signifi-

![Graph](https://example.com/graph.png)

**Fig. 1.** Mean ($N = 8$) ritonavir plasma concentrations after administration of ritonavir concurrently with fluconazole ($\odot$) or alone ($\circ$).

References

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2. D. J. Kempf, K. C. Marsh, J. F. Denissen, E. McDonald, S. Vasanovanda,


