HIV Protease is a constitutive enzyme of HIV that processes viral proteins essential for the maturation of infectious virions. HIV protease is necessary for the completion of the viral life cycle, and represents a key target for intervention in the development of novel therapeutic agents for treatment of HIV infection (1).

Ritonavir is a potent HIV protease inhibitor (K_i = 15 pM) that has been tested extensively for its ability to inhibit the HIV protease enzyme and HIV viral replication in cell culture (2). Ritonavir has a broad spectrum of activity against HIV types 1 and 2 (including zidovudine-resistant HIV) in a variety of transformed and primary human cell lines, yet seems to be selective with limited inhibition of other aspartic acid proteases (2). Administration of ritonavir is associated with exponential decreases in plasma viral RNA within a few days (3–5), and ritonavir was approved by the Food and Drug Administration for mono- and combination therapy for individuals with HIV infection.

Fluconazole is used to treat fungal infections that occur frequently in patients with AIDS, including cryptococcal meningitis and candidal infections (6). Therefore, it is likely that ritonavir and fluconazole may be administered concurrently. Fluconazole inhibits fungal CYP (7), but seems to have less of an effect on mammalian CYP metabolism, as indicated by the results of in vitro studies using human hepatocytes and the lack of effect on antipyrine pharmacokinetics in vivo (8, 9). Nonetheless, potentially clinically significant effects of fluconazole have been documented on the metabolism of cyclosporin A, tolbutamide, warfarin, phenytoin, and terfenadine (9–14). Fluconazole is a moderately potent inhibitor of CYP3A4 in vitro (K_i = 15–18 μM, ~5 μg/ml), and fluconazole plasma concentrations during steady-state dosing typically range from 15 to 60 μM (15).

Because ritonavir is metabolized principally by the CYP3A and, to a lesser extent, the CYP2D6 subfamilies (16), the possibility that fluconazole could affect the metabolism of ritonavir was investigated in this study. Moreover, because 80% of fluconazole is excreted unchanged in the urine (17), whereas ritonavir is excreted primarily via the hepatobiliary route (18), it is unlikely that ritonavir would have a significant effect on fluconazole pharmacokinetics. Therefore, the purpose of this study was to evaluate the effect of fluconazole on ritonavir pharmacokinetics.

Subjects and Methods. Healthy subjects of either gender between the ages of 18 and 45 years old and weighing within 10% of the ideal weight for the subject’s height were eligible to participate in the study. Subjects were to have no recent history of drug or alcohol abuse, were not to be users of tobacco products, and were to be negative for the hepatitis B virus. Appropriate contraceptive measures were required for women. All subjects gave written, informed consent in compliance with the Food and Drug Administration’s regulations, and Institutional Review Board approval was obtained. Subjects were excluded from study participation if they had received any investigational drug, terfenadine, astemizole, loratadine, erythromycin, or clarithromycin within 6 weeks before the initial study drug administration, or had used any other drug (with the exception of oral contraceptives), including over-the-counter medications within 2 weeks before the initial study dosing.

Study Design. This was a single-center, multiple-dose, open-label, two-period, randomized, crossover study with a 2-week washout interval between periods. Subjects received ritonavir (200 mg every 6 hr for 4 days) during regimen A and a combination of ritonavir at the same dosage plus fluconazole (400 mg every day on day 1 and 200 mg every day on days 2–5) during regimen B. All fluconazole doses were administered at the same time as the morning dose of ritonavir (~7:00 a.m.). Both drugs were administered orally: fluconazole as 200 mg tablets and ritonavir as a liquid (80 mg/ml) by oral syringe. All doses were administered with ~200 ml of water and within 10 min after a meal or a snack.

Subjects remained under supervision at the study site during each period, and abstained from all food and beverages except for scheduled standardized meals and water to quench thirst; grapefruit and grapefruit juice were prohibited. Physical examinations, funduscopic, 12-lead ECG, and laboratory analyses (hematology, blood chemistry, and urinalysis) were performed; vital signs
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 sampling and 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 <6.1%. The lowest quantifiable concentration was 10 ng/ml.

Ritonavir pharmacokinetics for each subject were estimated with noncompartamental methods. $C_{\text{max}}$, $C_{\text{min}}$, and $t_{\text{max}}$ 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 ($\beta$) 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)/$\beta$. The $AUC_{0–24}$ and $AUC_{0–t}$ 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 $C_{\text{max}}$, $C_{\text{min}}$, $AUC_{0–24}$, average $t_{\text{max}}$, and $\beta$ 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 $C_{\text{max}}$, $C_{\text{min}}$, and $AUC_{0–24}$, 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 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 alone, and that subject was experiencing adverse events similar to those during treatment with ritonavir alone (nausea and vomiting). Thus, compared with the subjects completing the study, there was no indication that the subjects who were unable to complete the study were more sensitive to an interaction between ritonavir and fluconazole.

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 $t_{\text{max}}$ or $\beta$ were not statistically significant. In contrast, differences in ritonavir 0–24 hr $C_{\text{max}}$ and $AUC_{0–24}$ with concurrent administration of fluconazole were statistically significant ($p < 0.02$), and the difference in ritonavir 0–24 hr $C_{\text{min}}$ 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 $C_{\text{max}}$ and $AUC_{0–24}$ values for an individual subject were 30% and 23%, respectively.

**Mean values of $t_{\text{max}}$, $C_{\text{max}}$, $C_{\text{min}}$, and AUC** were highest for the morning dose interval, with $C_{\text{max}}$ 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 $C_{\text{max}}$ 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 $C_{\text{min}}$ and AUC. Changes in $C_{\text{max}}$ and $C_{\text{min}}$ were statistically significant ($p < 0.04$) with coadministration for the first and third dose intervals, and the change in AUC was statistically significant.
centrally altered by fluconazole, these small changes are probably of limited clinical significance, and dosage adjustment of ritonavir is unnecessary if fluconazole is added to the regimen.

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**References**