Pharmacokinetic Interaction between Amprenavir and Rifabutin or Rifampin in Healthy Males

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The objective of this study was to determine if there is a pharmacokinetic interaction when amprenavir is given with rifabutin or rifampin and to determine the effects of these drugs on the erythromycin breath test (ERMBT). Twenty-four healthy male subjects were randomized to one of two cohorts. All subjects received amprenavir (1,200 mg twice a day) for 4 days, followed by a 7-day washout period, followed by either rifabutin (300 mg once a day [QD]) (cohort 1) or rifampin (600 mg QD) (cohort 2) for 14 days. Cohort 1 then received amprenavir plus rifabutin for 10 days, and cohort 2 received amprenavir plus rifampin for 4 days. Serial plasma and urine samples for measurement of amprenavir, rifabutin, and rifampin and their 25-O-desacetyl metabolites, were measured by high-performance liquid chromatography. Rifabutin did not significantly affect amprenavir’s pharmacokinetics. Amprenavir significantly increased the area under the curve at steady state (AUCss) of rifabutin by 2.93-fold and the AUCss of 25-O-desacetylrifabutin by 13.3-fold. Rifampin significantly decreased the AUCss of amprenavir by 82%, but amprenavir had no effect on rifampin pharmacokinetics. Amprenavir decreased the results of the ERMBT by 83%. The results of the ERMBT after 2 weeks of rifabutin and rifampin therapy were increased 187 and 156%, respectively. Amprenavir plus rifapten was well tolerated. Amprenavir plus rifabutin was poorly tolerated, and 5 of 11 subjects discontinued therapy. Rifampin markedly increases the metabolic clearance of amprenavir, and coadministration is contraindicated. Amprenavir significantly decreases clearance of rifabutin and 25-O-desacetylrifabutin, and the combination is poorly tolerated. Amprenavir inhibits the ERMBT, and rifampin and rifabutin are equipotent inducers of the ERMBT.

Amprenavir (USAN approved, VX-478, 141W94; Glaxo Wellcome Inc., Research Triangle Park, N.C.) is a new human immunodeficiency virus type 1 (HIV-1) protease inhibitor which has potent in vitro and in vivo activity for HIV (2). In vitro data indicate that amprenavir is extensively metabolized by the microsomal P450 enzyme, CYP3A4, to oxidized products (8, 28, 38). In humans, less than 2% of an oral dose of amprenavir appears in urine as unchanged drug (38).

The CYP3A4 isozyme is the major route of metabolism for many drugs, including all of the currently available protease inhibitors (9, 10, 19, 24). The rifamycin antibiotics rifabutin and rifampin are well-known inducers of this isozyme (16, 18, 23, 24, 31; J. A. Smith, T. C. Hardin, T. F. Patterson, M. G. Rinaldi, and J. R. Graybill, Abstract 2nd Natl. Conf. Hum. Retrovir. Relat. Infect., abstr. 126, p. 77, 1995), although rifabutin is believed to cause substantially less isozyme induction than rifampin (16, 23). In addition, the multidrug transporter, P-glycoprotein contributes to elimination of the protease inhibitors, and rifampin increases the activity of this transporter (17, 20, 35, 37). The potential for an interaction between amprenavir and these rifamycin antibiotics is clinically important because HIV-infected patients may receive rifampin or rifabutin for the prevention and treatment of opportunistic infections caused by mycobacteria (4, 6). The prevalence of infection caused by Mycobacterium tuberculosis, including multi drug-resistant tuberculosis, has increased with the emergence of AIDS (6). This has made treatment difficult, because rifampin induces metabolism of the available HIV-1 protease inhibitors and reduces the mean area under the curve (AUC) for individual agents by 35 to 92% (4, 6). Guidelines from the Centers for Disease Control and Prevention recommend that rifampin not be used with most protease inhibitors, and if rifabutin is used in place of rifampin, that it be given at a reduced dose (6). This study was undertaken to determine if a pharmacokinetic interaction exists when amprenavir and rifabutin or rifampin are coadministered.

The erythromycin breath test (ERMBT) is a measure of hepatic CYP3A4 activity (14, 36; ERMBT assay product information, Metabolic Solutions Inc., Nashua, N.H.). The inclusion of the ERMBT in this study was intended to evaluate the following questions. (i) Is amprenavir an inhibitor of hepatic CYP3A4 in vivo? (ii) What are the effects of rifabutin and rifampin on CYP3A4 activity as measured by the ERMBT? (iii) Do the results of the ERMBT help explain the pharmacokinetics of amprenavir when administered alone and in combination with rifabutin and rifampin?

MATERIALS AND METHODS

Subjects. Nonsmoking men, aged 18 to 55 years, were eligible for this study, which was approved by the Committee on the Conduct of Human Research at Virginia Commonwealth University (VCU). Each subject gave written informed consent. A complete medical history, physical examination that included vital...
signs; and routine laboratory tests that included a 13-test chemistry screen, complete blood count with differential, urinalysis (dipstick), urine drug screen for illicit controlled substances, test for HIV antibodies, and electrocardiogram were completed for each subject. Subjects were ineligible if they had a clinically significant abnormality at the screening evaluation, had donated blood within the past month, were taking concomitant medication(s) which could not be withheld for the duration of the study, or had a prior adverse reaction to rifabutin, rifampin, or another rifamycin antibiotic. Subjects were instructed to use a barrier method of contraception (condoms) while enrolled in the study and for a minimum of 1 month after administration of their last dose of study drugs. Additionally, subjects abstained from taking concomitant medications and from consuming alcohol 6 h before each treatment until discharge from the study center. The same restrictions were placed on consumption of grapefruit and grapefruit juice. Tea, coffee, chocolate, and other beverages and foods containing methyl xanthines were prohibited on each pharmacokinetic sampling day.

Experimental design and procedures. This was an open-label, parallel-group, three-period study conducted at the School of Pharmacy Center for Drug Studies, VCU/Medical College of Virginia Campus. Following the screening evaluation, the study consisted of a treatment phase of up to 5 weeks' duration and a follow-up evaluation comprising up to four separate visits over a 3-month period. The screening evaluation was scheduled within 14 days before administration of the first dose of study drug. Subjects eligible after screening were randomized to two dosing cohorts (dosing cohort 1 [DC1] and DC2) and received the following treatments:

(i) DC1. Subjects in DC1 were treated as follows: dosing days 1 to 4 (treatment 1), amphenavir (1,200 mg twice daily) for 3½ days, followed by a 7-day washout period; dosing days 5 to 18 (treatment 2), rifabutin (300 mg every morning) for 14 days; dosing days 19 to 28 (treatment 3), amphenavir (1,200 mg twice daily) plus rifabutin (300 mg every morning) for 10 days.

(ii) DC2. Subjects in DC2 were treated as follows: dosing days 1 to 4 (treatment 1), amphenavir (1,200 mg twice daily) for 3½ days, followed by a 7-day washout period; dosing days 5 to 18 (treatment 4), rifampin (600 mg every morning) for 14 days; dosing days 19 to 22 (treatment 5), amphenavir (1,200 mg twice daily) plus rifampin (600 mg every morning) for 4 days.

Subjects received the first dose of amphenavir (treatment 1, DC1 and DC2) under the supervision of the study center staff. Subjects then left the center with sufficient drug for the next four doses and were instructed to complete a daily diary card to record the exact time they took their doses, the number of capsules taken, and any missed doses. The evening (6:00 p.m.) before dosing day 4, subjects returned to the study center, where a breath alcohol test was performed, in addition to a review of concomitant medications and other substances significant at last visit. Diary cards and drug containers were inspected and deviations from the dosing schedule (e.g., missed doses) were recorded. Subjects remained overnight in the study center.

On dosing day 4, drug was administered at 8:00 a.m. with 240 ml of tap water after an overnight fast (from at least 12:00 midnight). Water was prohibited for 4 h predosing until 4 h postdosing. Standard, balanced meals were served 4 and 10 h postdosing. Before dosing on day 4, blood samples for hematometry and serum chemistry were obtained for dipstick and therapeutic monitoring purposes. Serial blood samples for pharmacokinetic evaluation of amphenavir were drawn 5 min before dosing and up to 12 h postdosing (see schedule below). Urine for pharmacokinetic evaluation of amphenavir was collected 15 min before dosing and then at intervals up to 12 h postdosing (see below for exact timing of samples). Subjects were discharged from the study center after completion of all 12-h-postdosing procedures.

After a washout period of 7 days, subjects returned to the study center. The first dose of either rifabutin (treatment 2, DC1) or rifampin (treatment 4, DC2) was administered to each subject under staff supervision, after which subjects were given enough drug for the next 5 days. Subjects then left the study center. During this period, subjects self-administered either rifabutin or rifampin as directed and were asked to complete a diary card as before. Subjects returned to the study center on the morning of dosing day 22 (DC2) or 28 (DC1) as described before and an ERMBT (below). Diary cards and drug containers were inspected for compliance monitoring. After subjects were determined to be well, they were given enough rifabutin or rifampin sufficient for the next 6 days and were allowed to leave the study center.

The evening before dosing day 18, subjects returned to the study center and remained there for 2 nights, until completion of all 24-h-postdosing procedures. On arrival, diary cards and drug containers were again inspected for compliance and a breath alcohol test was performed. Subjects fasted after midnight. On the morning of dosing day 18, subjects received their last dose of rifabutin or rifampin. Clinical and laboratory evaluations were conducted as before. The only difference was that urine and serial blood samples for measurement of rifabutin and rifampin and their respective 25-O-desacyl metabolites were collected up to 24 h postdosing. Once all 24-h-postdosing procedures were completed, if the subjects were well, they were allowed to leave the study center. Before leaving, the final pharmacokinetic and laboratory evaluations were completed (treatment 5, DC2) was administered to subjects under staff supervision. Subjects were then given enough drug to self-administer over the next 2 days (DC2) or 8 days (DC1). Subjects completed a daily diary card as previously described.

Subjects were instructed to return to the study center the evening before dosing day 22 (DC2) or 28 (DC1), where they were to remain for 2 nights, until completion of all 24-h-postdosing procedures. On arrival, diary cards and drug containers were again inspected for compliance and a breath alcohol test was performed. Subjects fasted after midnight. On the morning of dosing day 22 (or 28), subjects received their last doses of amphenavir and rifampin (or amphenavir and rifabutin). Clinical and laboratory evaluations were conducted as before, with serial blood samples collected for up to 12 h postdosing and urine collected up to 12 h (for amphenavir) and up to 24 h postdosing for rifabutin, rifampin, and their respective metabolites.

The first follow-up evaluation occurred within 7 to 10 days after completion of the final treatment period. This comprised a physical examination, vital signs, electrocardiogram, clinical laboratory evaluations, and an ERMBT. If a subject's liver function tests (LFTs) were not clinically significant, then subsequent visits for follow-up LFTs were scheduled 1, 2, and 3 months after completion of the treatment phase. If a subject's LFTs were significantly elevated, additional follow-up visits were scheduled at weekly intervals until resolved.

Administration and analysis of the ERMBT. The ERMBT (Metabolic Solutions, Inc.) was administered within 1 week before treatment period 1 (to establish baseline), at 2 h after the final dose of amphenavir (treatment 1), at 2 h after the morning dose of either rifabutin or rifampin at 7 and 14 days, at 2 h after the final dose of combined treatment with amphenavir and rifampin or rifabutin, and at the follow-up evaluation. For each ERMBT, subjects received an intravenous injection containing 3 μCi of [N-methyl-14C] erythromycin as a 1-min bolus infusion according to the manufacturer's directions. Twenty minutes later, subject exhaled through a straw into a vial containing 20 ml of a 50:50 hyamine-ethanol solution containing thymolphthalein until there was a color change, from blue to clear, indicating the trapping of 2 mmol of CO2.

All ERMBT samples were assayed at the VCU School of Pharmacy Biopharmaceutical Analysis Laboratory. Liquid scintillation counting was used to measure exhaled 14CO2. Ten milliliters of Insta-Gel XF scintillation cocktail (Packard Instrument Co.) was added to decolorize samples in the scintillation vials; samples were mixed well and left in the dark at room temperature for at least 16 h. Scintillation in the samples was counted on a Packard Model Tricarb 4530 for 34C using terminators of 1% standard deviation or 10 min, whichever came first. Results of the ERMBT are expressed as percent erythromycin dose metabolized during the 1st h postinjection and are calculated from disintegrations per minute as previously described (34). The change in isoenzyme activity due to the study drug(s) was described using the equation 1 - (treatment period value/baseline value). The intra-assay precision (coefficient of variation [CV]) ranged from 3.4 to 3.8%.

Sample procurement and assay of plasma and urine samples for amphenavir, rifampin, and rifabutin and their respective metabolites. On dosing days 4 and 28 (DC1) or 22 (DC2), blood was obtained for determination of amphenavir concentrations in plasma on 15 separate occasions during the respective treat

Vol. 45, 2001 AMPRENAVIR INTERACTION WITH RIFAMPIN OR RIFABUTIN 503
day 4, urine was collected just prior to dosing and over the collection intervals: 0 to 4, 4 to 8, and 8 to 12 h postdosing. On dosing days 18 and 28 (DC1) and 22 (DC2) urine was collected at the same times for dosing day 4 with an additional collection 12 to 24 h postdosing. Samples were stabilized with ascorbic acid. Urine was stored in a refrigerator during each collection interval. At the end of each collection period, the urine was weighed and two 10-ml aliquots were transferred to 13-ml tubes and stored in an upright position at −20°C until shipment for analysis.

Concentrations of ampravir were determined at Glaxo Wellcome Research and Development with a semiautomated solid-phase extraction method. A 0.5-ml portion of plasma was combined with 0.5 ml of internal standard solution (VB 11599; 5.0 mg/ml). Solid-phase extraction was performed with a Waters MilliLab Workstation and C18 Sep Pak cartridge. Extraction cartridges were primed with methanol followed by water. The sample plus internal standard was loaded on the cartridge, and the cartridge was washed with water and methanol (65:35, vol/vol). The compound was eluted from the cartridge with 2.5 ml of acetonitrile (100%) and blown to dryness under gentle nitrogen gas at 50°C in a Turbo Vap. The sample was reconstituted with acetonitrile and water (45:55, vol/vol) and vortexed to mix, and 50 μl was injected. Ampravir was detected by fluorescence (excitation λ = 245 nm; emission λ = 340 nm). The analytical column was a Waters Symmetry C18 column (3.9 by 150 mm) maintained at 40°C.

Analysis of rifampin and 25-desacetylrifampin in human urine and plasma was performed by PPD-Development, Middleton, Wis., using validated methodologies. Aliquots of plasma and urine were stabilized with ascorbic acid solution. Internal standard (rifamycin) was added, and the analytes were isolated by liquid-liquid extraction into an organic phase (chloroform–methyl–butyl ether). The sample was evaporated, and the residues were reconstituted in a methanol–acetonitrile–water (0.2:32:68, vol/vol/vol). Further extraction of impurities was performed by liquid-liquid extraction into hexane. Concentrations were determined by high-performance liquid chromatography with UV detection from the resultant aqueous phase. The data were acquired and interpolated against a calibration curve. The range of the assay was 5 to 500 ng/ml for rifabutin in plasma, 3.7 to 300 ng/ml for 25-desacetylrifabutin in plasma, 50 to 5,000 ng/ml for rifabutin in urine, and 36.8 to 2,940 ng/ml for 25-desacetylrifabutin in urine. The specificity, accuracy, precision, limits of quantification of the method, and recovery of both rifabutin and the 25-desacetyl metabolite were evaluated with analytical standards, calibration standards, and spiked plasma standards to validate the assays. Accuracy, expressed as mean percent difference from the theoretical value, was demonstrated to be <15% for both rifabutin and 25-desacetylrifabutin in plasma and urine. Precision, expressed as a maximum CV, was demonstrated to be <10% for both rifabutin and 25-desacetylrifabutin in plasma and urine.

Pharmacokinetic analysis. The observed peak concentrations in plasma at steady state (Cmax) of ampravir, rifabutin, and rifampin and the time to Cmax (Tmax) were obtained by inspection of the individual plasma concentration-time data. Individual estimates of the apparent terminal elimination rate constant (k) for each drug were obtained by log-linear regression of the terminal portions of the plasma concentration-time curves. Half-lives were calculated as 0.693k. The steady-state AUC (AUCss) from time zero to the last quantifiable sample at 24 h for rifabutin and rifampin or at 12 h for ampravir was calculated by the linear trapezoidal method. The AUC from 0 to infinity was calculated by adding C∞ to AUCss. The apparent total clearance from plasma at steady state (CL/F) was calculated as dose/AUC. Similar formulae were used to determine the pharmacokinetic parameters for the metabolites (with the exception of CL/F). For each metabolite, the ratio of the metabolite AUC to that of the parent drug was also calculated based upon the AUC.

Urine pharmacokinetic parameters were determined for rifabutin, rifampin, and their 25-O-desacetyl metabolites. Renal clearance (CLR) was calculated as AUC/AUCss, where AUC∞ is the amount of drug excreted in the urine over the steady-state dosing interval. The percentages of rifabutin, rifampin, and their 25-O-desacetyl metabolites eliminated in the urine were calculated based on parent compound equivalent weights.

Statistical analysis. Data are presented as mean values ± standard deviations. Pharmacokinetic parameters other than Tmax were log transformed, and analysis of variance with treatment as the fixed effect and subject as the random effect was performed on the calculated parameters. The geometric least-squares (LS) mean ratio and its 90% CI were calculated for each pharmacokinetic parameter and for the results of the ERMBT. Two one-sided t tests (90% CI) were performed on

### Table 1. Summary of results of geometric LS means for ampravir pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Treatment no. or ratio</th>
<th>Description</th>
<th>AUC0–∞ (µg/ml)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (h)</th>
<th>CL/F (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 24)</td>
<td>APV 1,200 mg BID</td>
<td>27.32 (24.01, 31.08)</td>
<td>9.20 (7.89, 10.74)</td>
<td>0.32 (0.25, 0.39)</td>
<td>1.00 (0.87, 1.38)</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>APV 1,200 mg BID + RFB 300 mg OD</td>
<td>29.12 (17.05, 28.69)</td>
<td>8.56 (7.17, 10.23)</td>
<td>0.25 (0.14, 0.42)</td>
<td>1.12 (0.73, 1.50)</td>
</tr>
<tr>
<td>5 (n = 11)</td>
<td>APV 1,200 mg BID + RFB 600 mg OD</td>
<td>4.99 (3.96, 6.28)</td>
<td>2.78 (2.07, 3.72)</td>
<td>0.02 (0.02, 0.03)</td>
<td>0.75 (0.62, 1.00)</td>
</tr>
<tr>
<td>Ratio 3:1 (90% CI)</td>
<td>0.85 (0.72–1.00)</td>
<td>0.93 (0.75–1.10)</td>
<td>0.85 (0.62–1.17)</td>
<td>0.00 (~0.64–0.38)</td>
<td>1.18 (1.00–1.38)</td>
</tr>
<tr>
<td>Ratio 5:1 (90% CI)</td>
<td>0.30 (0.24–0.38)</td>
<td>0.30 (0.24–0.38)</td>
<td>0.86 (0.05–0.11)</td>
<td>−0.25 (~0.76–0.00)</td>
<td>5.45 (4.64–6.39)</td>
</tr>
</tbody>
</table>

* Unless otherwise indicated data in parentheses indicate 95% CI.  
* Abbreviations: APV, ampravir; RFB, rifabutin; RFP, rifampin; BID, twice daily; OD, once daily.  
* Median and median difference.

* Based on 6 subjects only.

FIG. 1. Mean ampravir concentrations in plasma following 1,200 mg administered alone (open diamond), following 2 weeks of rifampin (600 mg once daily) (open triangle), and following 2 weeks of rifabutin (300 mg once daily) (closed circle).
RESULTS

Subject characteristics. Twenty-four healthy, HIV-seronegative, male subjects were enrolled in the study (19 Caucasian, 4 African-American, 1 Asian), with 12 subjects in each dosing cohort. The subjects’ ages ranged from 18 to 49 years (mean, 27.3 years), and their weights ranged from 58.8 to 100 kg (mean, 75.9 kg). Demographic characteristics were similar between the two dosing cohorts.

Tolerability of study medications. All 24 subjects completed treatment period 1 (amprenavir alone). The most common adverse events that occurred during treatment with amprenavir alone in both cohorts were nausea, oral numbness, dizziness, diarrhea, and headache.

In DC1, one subject was removed after failing to return to the study center on the evening of dosing day 17. The combination treatment with amprenavir and rifabutin was poorly tolerated. Five of the remaining eleven subjects were withdrawn from the study between day 1 and day 9 of combination therapy due to adverse events. The adverse events consisted of chiefly flu-like symptoms (e.g., headache, nausea, fever, myalgia, tiredness, vomiting, diarrhea, chills, weakness) and laboratory abnormalities (predominantly leukopenia). There was a clear decline in white blood cell (WBC) and neutrophil counts associated with therapy. At screening, DC1 had a mean WBC count of $5.98 \times 10^3$/mm$^3$ (60% granulocytes). Following completion of amprenavir-alone, rifabutin-alone, and combination therapy, the mean WBC counts were $6.22 \times 10^3$/mm$^3$ (53% granulocytes), $3.88 \times 10^3$/mm$^3$ (46% granulocytes), and $3.24 \times 10^3$/mm$^3$ (47% granulocytes). Seven of 11 subjects starting rifabutin and amprenavir had WBC counts of less than 3,000 cells/ml compared with none of the subjects in the rifampin arm.

In DC2, 11 of 12 subjects completed the study. One subject was removed following treatment period 1 (amprenavir alone) due to development of a maculopapular rash. The combination of amprenavir and rifampin was otherwise well tolerated. There were no hematological adverse effects associated with combination therapy. The most common adverse effect seen during rifampin therapy, alone or in combination with amprenavir, was a discoloration of the urine consistent with the known effects of rifampin.

Pharmacokinetics. Twenty-four subjects were included in the pharmacokinetic analysis of amprenavir alone, 11 subjects for rifabutin alone and 11 for rifampin alone. There were 11 subjects included in the pharmacokinetic analysis of amprenavir and rifampin when given in combination, but there were only 6 subjects included in the pharmacokinetic analysis when amprenavir was given in combination with rifabutin.

Amprenavir. Table 1 provides the summary pharmacokinetic parameters, geometric LS mean ratio, and the 90% CI estimates for amprenavir pharmacokinetic parameters, alone and in combination with rifabutin and rifampin. There were no statistically significant differences in the pharmacokinetics of amprenavir when coadministered with rifabutin, but only 6 subjects were available for a full pharmacokinetic profile. In
contrast, rifampin resulted in statistically significant decreases in AUC$_{ss}$, C$_{max,ss}$ and minimum concentration in plasma (C$_{min,ss}$) (82, 70, and 92%, respectively) of amprenavir and a 5.45-fold increase in amprenavir CL/F. Figure 1 illustrates the effects of rifampin and rifabutin on the mean concentration-time profile of amprenavir.

**Rifabutin.** Table 2 provides the summary pharmacokinetic data for rifabutin alone and when administered with amprenavir. There were statistically significant 2.93-, 2.19-, and 3.71-fold increases in rifabutin AUC$_{ss}$, C$_{max,ss}$ and C$_{min,ss}$ when coadministered with amprenavir. There was a 66% decrease in rifabutin CL/F. The median $T_{max,ss}$ following administration of the combined treatment was 1.0 h longer than after administration of rifabutin alone. There was a 2.51-fold increase in the amount of rifabutin excreted in the urine as unchanged parent drug when it was administered with amprenavir. Figure 2 illustrates the effect of amprenavir on the mean concentration-time profile of rifabutin.

**25-O-Desacetylrifabutin.** Table 2 provides the summary pharmacokinetic data for 25-O-desacetylrifabutin, when rifabutin was administered alone and in combination with amprenavir. When coadministered, the AUC$_{ss}$, C$_{max,ss}$ and C$_{min,ss}$ of 25-O-desacetylrifabutin were increased by 13.35-, 7.39-, and 32.9-fold, respectively, compared to rifabutin alone. There was a 4.27-fold increase in the AUC ratio (AUC of 25-O-desacetylrifabutin to that of rifabutin). There was a 23% decrease in CLR when rifabutin was administered with amprenavir. The percent dose of rifabutin excreted in the urine as 25-O-desacetylrifabutin was 9.5-fold greater following the administration of rifabutin with amprenavir. Figure 2 illustrates the effect of amprenavir on the mean concentration-time profile of 25-O-desacetylrifabutin.

**Rifampin.** There were no significant differences in AUC$_{ss}$, C$_{max,ss}$ and CL/F when rifampin was given alone and in combination with amprenavir (Table 3). There was a 22% decrease in CLR and an 18% decrease in the amount of rifampin excreted in the urine as parent drug following coadministration with amprenavir.

**25-O-Desacetylrifampin.** There were no statistically significant differences in 25-O-desacetylrifampin pharmacokinetics when rifampin was administered alone or in combination with amprenavir (data not shown).

**Erythromycin breath test.** Amprenavir treatment reduced the LS mean ratio for the ERMBT to 17% of baseline for both dosing cohorts, and both rifampin and rifabutin significantly increased the ERMBT at 1 and 2 weeks of treatment. At follow-up, the ERMBT mean ratios had returned to baseline for both dosing cohorts. Figure 3 illustrates the ERMBT results for the rifampin group. Results for the rifabutin group were similar (data not shown), except for the combination treatment regimen of rifabutin plus amprenavir, in which the ERMBT result was significantly lower.

There was no significant linear correlation between baseline ERMBT and the AUC$_{ss}$ of amprenavir ($r^2 = 0.00; P > 0.05$) or the AUC$_{ss}$ of rifabutin ($r^2 = 0.05; P > 0.05$), nor was there a significant correlation between the magnitude of percent increase in the ERMBT result following 14 days of rifampin and

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**TABLE 3. Summary of results of geometric LS means (95% CI) for rifampin pharmacokinetic parameters**

<table>
<thead>
<tr>
<th>Treatment no. or ratio</th>
<th>Description$^a$</th>
<th>AUC$_{ss}$ (h ( \mu g/ml ))</th>
<th>C$_{max,ss}$ (( \mu g/ml ))</th>
<th>CL/F (ml/min)</th>
<th>$T_{max,ss}$ (h)$^a$</th>
<th>CLR (ml/min)</th>
<th>% Dose</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>600 mg RFP QD</td>
<td>28.08 (22.37, 35.26) 8.50 (6.55, 11.03)</td>
<td>356 (284, 447)</td>
<td>1.48 (1.00, 2.00)</td>
<td>18.33 (15.39, 21.83)</td>
<td>5.49 (4.29, 6.69)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>600 mg RFP QD + 1,200 mg APV BID</td>
<td>28.30 (22.54, 35.53) 8.41 (6.48, 10.92)</td>
<td>353 (281, 444)</td>
<td>1.50 (1.24, 2.00)</td>
<td>14.38 (12.07, 17.13)</td>
<td>4.28 (3.07, 5.48)</td>
<td></td>
</tr>
</tbody>
</table>

| Ratio 5:4 (90% CI) | 1.01 (0.90, 1.13) | 0.99 (0.87, 1.12) | 0.99 (0.88, 1.11) | 1.01 (0.49, 0.51) | 0.78 (0.68, 0.90) | 0.78 (0.69, 0.87) |

$^a$ Median and median difference.

$^b$ See footnote $b$ of Table 1 for abbreviations.
DISCUSSION

The pharmacokinetics of amprenavir, rifabutin, and rifampin when administered alone are in agreement with those reported in previous studies (1, 2, 5, 29). The effects of rifabutin and rifampin on amprenavir pharmacokinetics are consistent with observations for other protease inhibitors, indicating that amprenavir is a substrate for CYP3A4 (8, 28), rifabutin is a less potent inducer of metabolic clearance for protease inhibitors than is rifampin (4, 6, 10, 16, 23, 24), and rifabutin and its 25-O-desacetyl metabolite are metabolized by CYP3A4 (15, 32).

**Rifabutin and amprenavir.** Amprenavir significantly decreased clearance of rifabutin and 25-O-desacetyl rifabutin. These results are similar to the effects of ritonavir (5) and other protease inhibitors (4, 6, 10, 24). on the pharmacokinetics of rifabutin. An assessment of the effect of rifabutin on the pharmacokinetics of amprenavir is confounded by the poor tolerability of the combination and the relatively few subjects who completed combination therapy. Although there is a 15% mean reduction in amprenavir AUC\textsubscript{ss} only six subjects were able to provide full pharmacokinetic data. However, even a true difference of this magnitude is unlikely to be clinically important.

The effects of amprenavir on the pharmacokinetics of rifabutin are clinically significant, and 5 of 11 subjects were unable to complete treatment due to adverse drug events. These flu-like symptoms and neutropenia have been previously reported when high doses of rifabutin are given alone (22, 27) or coadministered with CYP3A4 inhibitors such as HIV-1 protease inhibitors (E. Sun, M. Heath-Chiozzi, D. W. Cameron, A. Hsu, R. G. Granneman, and C. J. Maurath, Proc. Abstr. XI Int. Conf. AIDS, 1996), fluconazole (33), and clarithromycin (12, 13). These adverse effects are likely caused by increased concentrations of rifabutin and/or 25-O-desacetyl rifabutin, such as those observed in this study. It is therefore recommended that the dose of rifabutin be decreased by at least 50% if medically indicated for concomitant use with amprenavir. In addition, patients should be observed for uveitis and flu-like symptoms and monitored for leukopenia.

**Rifampin and amprenavir.** The coadministration of amprenavir and rifampin resulted in significant changes in the pharmacokinetics of amprenavir, including an 82% decrease in the AUC\textsubscript{ss} and an increase of greater than fivefold in amprenavir CL/F. This most likely reflects induction of hepatic and intestinal CYP3A4 by rifampin, and possibly enhancement of P-glycoprotein transport, resulting in an increase in clearance of amprenavir. Decreases in amprenavir concentrations in plasma of the magnitude observed in this study are of probable clinical relevance as trough concentrations are below the 95% inhibitory concentration for HIV isolates (2). Of the steady-state pharmacokinetic parameters for the HIV-1 protease inhibitors, C\textsubscript{min,ss} has been shown to be the best predictor of antiviral response, as well as being associated with the development of resistance (7, 21, 30). Because rifampin markedly increases amprenavir’s metabolism, coadministration of these drugs is contraindicated (4, 6).

The administration of amprenavir with rifampin had no effect on the pharmacokinetics of rifampin or its metabolite 25-O-desacetyl rifampin. This is consistent with statements that rifampin is not a substrate for CYP3A4 (4). There was a 22% decrease in rifampin and a 12% decrease in 25-O-desacetyl rifampin CL\textsubscript{R}. Amprenavir may slightly inhibit the CL\textsubscript{R} of rifampin, but the effect is not clinically significant.

**ERMBT results.** Amprenavir reduced hepatic CYP3A4 activity, as measured by the ERMBT, to 17% of baseline. Similar results were observed in our other studies with amprenavir (3, 25). Compared with the baseline, the mean ERMBT increased at 7 and 14 days of rifabutin and rifampin treatment by 181 and 187% and 164 and 156%, respectively. It appears that induction is not greater following 2 weeks of rifamycin therapy than following 1 week. These results are similar to the findings of a prior investigation with rifampin that revealed a mean 186% increase in the ERMBT (11). The effects of rifabutin on the ERMBT have not been previously reported.
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