The P450 enzyme, CYP3A4, extensively metabolizes both amprenavir and clarithromycin. To determine if an interaction exists when these two drugs are coadministered, the pharmacokinetics of amprenavir and clarithromycin were investigated in healthy adult male volunteers. This was a Phase I, open-label, randomized, balanced, multiple-dose, three-period crossover study. Fourteen subjects received the following three regimens: amprenavir, 1,200 mg twice daily over 4 days (seven doses); clarithromycin, 500 mg twice daily over 4 days (seven doses); and the combination of the above regimens over 4 days (seven doses of each drug). Twelve subjects completed all treatments and the follow-up period. The erythromycin breath test (ERMBT) was administered at baseline, 2 h after the final dose of each of the three regimens and at the first follow-up visit. Coadministration of clarithromycin and amprenavir significantly increased the mean amprenavir AUCss, Cmax,ss, and Cmin,ss by 18, 15, and 39%, respectively. Amprenavir had no significant effect on the AUCss of clarithromycin, but the median T1/2 for clarithromycin increased by 2.0 h, renal clearance increased by 34%, and the AUCss for 14-((R)-hydroxyclarithromycin decreased by 35% when it was given with amprenavir. Amprenavir and clarithromycin reduced the ERMBT result by 85 and 67%, respectively, and by 87% when the two drugs were coadministered. The baseline ERMBT value did not correlate with clearance of amprenavir or clarithromycin. A pharmacokinetic interaction occurs when amprenavir and clarithromycin are coadministered, but the effects are not likely to be clinically important, and coadministration does not require a dosage adjustment for either drug.

Amprenavir (Agenerase, 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 (1, 14, 21). All of the currently available protease inhibitors are metabolized by the hepatic microsomal P450 enzyme, CYP3A4, which is the major isozyme involved in the metabolism of many drugs (5). In vitro data indicate that amprenavir is also extensively metabolized by CYP3A4 (4, 20), and investigations in humans reveal that <2% of the administered dose appears in the urine as unchanged drug (27). Preclinical studies in rats in which amprenavir was administered in combination with ritonavir, a potent CYP3A4 inhibitor, resulted in an approximately eight-fold increase in the area under the concentration-time curve from 0 to 8 h (AUC0–8) of amprenavir (11). In addition, human studies have demonstrated that the AUC of amprenavir is increased when it is administered with ketoconazole, another potent inhibitor of CYP3A4 (16).

Mycobacterium avium complex (MAC) disease is one of the most common opportunistic infections affecting AIDS patients at the terminal stage of illness, and the U.S. Public Health Service has recommended chemoprophylaxis when a patient's CD4+ cell count decreases to below 50 cells/μl (22). Clarithromycin is indicated for the chemoprophylaxis and treatment of disseminated MAC disease, and significant numbers of HIV-infected patients receiving amprenavir may also be treated with clarithromycin. Because clarithromycin is a well-known inhibitor of CYP3A4 and has been shown to result in a pharmacokinetic interaction when it is given with other protease inhibitors (5, 15; prescribing information for indinavir [Crixivan; Merck and Company Pharmaceuticals, West Point, Pa.], ritonavir [Norvir; Abbott Laboratories, Abbott Park, Ill.], and saquinavir [Invirese; Roche Laboratories, Nutley, N.J.]), this study was undertaken to determine if a pharmacokinetic interaction occurs when amprenavir and clarithromycin are coadministered.

The erythromycin breath test (ERMBT) is a measure of hepatic CYP3A4 activity (26; ERMBT product information, Metabolic Solutions Inc., Nashua, N.H.) and has previously been used to measure the inhibition of hepatic CYP3A4 activity by drugs used in the treatment of HIV (2). 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 is the relative potency of amprenavir as an inhibitor of hepatic CYP3A4, compared to clarithromycin? (iii) Do the results of the ERMBT help explain the pharmacokinetics of clarithromycin and amprenavir? (This work was presented at the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., September 1998.)
The ERMBT was administered up to 1 week before the first treatment (to establish a baseline), on the fourth dosing day of each treatment period (i.e., days 4, 8, and 12), and at the follow-up evaluation. The ERMBT was performed according to the product information from Metabolic Solutions Inc. Each subject received an intravenous injection over 1 min of a trace amount of [N-methyl-14C]erythromycin (3 μCi in 0.5 ml of 100% ethanol, USP, diluted in 4.5 ml of 5% Dextrose Injection, USP). On days 4, 8, and 12, the injection was given 2 h after administration of the seventh dose of each treatment, immediately after collection of the 2-h postdosing pharmacokinetic blood sample(s). Twenty minutes administration of the seventh dose of each treatment period, immediately after collecting 2 ml of heparinized plasma. Erythromycin and any missed doses while self-administering the study drug(s) at home or at work. When they were at the center study drug(s) was calculated as 1−(treatment period value/baseline value).

Pharmacokinetic samples. On each of dosing days 4, 8, and 12, serial blood samples were drawn from each subject for evaluation of the plasma concentration-time profiles of ampravir and/or clarithromycin or its 14-hydroxy metabolite [14( R)-hydroxyclarithromycin]. Blood samples were collected by peripheral venous catheter at 5 min predosing to establish a baseline and thereafter at the following intervals: 0.25, 0.5, 0.75, 1.0, 1.50, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 h postdosing. Each blood sample for ampravir analysis was collected in a pre-chilled K3 EDTA tube (containing sodium heparin). Each sample was centrifuged at 3,000 rpm for 10 min to separate the plasma.

Urine was collected predosing to establish a baseline and thereafter over the following intervals: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h postdosing on each of days 4, 8, and 12. For the predosing sample, subjects voided their bladders 15 min prior to dosing. For all postdosing collection intervals, subjects were allowed to void their bladders as needed during and at the end of the collection interval.

Individual plasma and urine samples were aliquoted into propylene storage tubes, labeled, and stored upright in a non-self-decorting freezer (−20°C or lower) until they were shipped to Glasgow, Inc., for analysis of ampravir by International Bioanalytical (Glaso Wellcome) or to BAS Analytics, Atlanta, GA, for analysis of clarithromycin or 14( R)-hydroxyclarithromycin.

Plasma analytical methods. Plasma concentrations of ampravir were determined by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Calibration standards were prepared in blank human plasma containing ampravir at concentrations ranging from 0.1 to 1,000 ng/ml; the plasma calibration standard concentrations were linear from 10 to 1,000 ng/ml; the ampravir plasma control concentrations were 30, 400, and 800 ng/ml. The clinical samples were diluted into the appropriate range of the calibration curves with blank human plasma and reassayed if they exceeded the upper limit of quantitation (1,000 ng/ml).

Upon validation of the ampravir assay technique, the interassay precision, assessed from spiked validation control samples (n = 6) at four concentrations over four analytical runs with human plasma and expressed as percent coefficient of variation (% CV), ranged from 1.8% to 7.7%; the intraday precision ranged from 1.8 to 11.3%. The percent recovery of ampravir was determined in human plasma at concentrations of 75, 400, and 800 ng/ml (n = 6 at each concentration) by injecting analytical standards (with internal standard) directly onto the column and comparing the area resulting from the injection of one to 3% across the concentration range of 75 to 800 ng/ml.

Concentrations of clarithromycin and 14( R)-hydroxyclarithromycin in plasma were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods as described. Calibrators were prepared in human plasma containing clarithromycin at concentrations ranging from 0.1 to 1,000 ng/ml of heparinized plasma by liquid-liquid extraction at an alkaline pH. Erythromycin B served as an internal standard. After the addition of carbonate solution and internal standard to the plasma, the macrolides were extracted into methyl- t-butyl ether. The ether layer was transferred to a clean tube and reconstituted with a pH 6 buffer-acetonitrile mixture. The reconstituted extract was Pass and injected into an LC-MS-MS system with atmospheric pressure chemical ionization.

Clarithromycin and 14( R)-hydroxyclarithromycin calibration standard concentrations ranged from 15.6 to 8,000 ng/ml, and the quality control concentrations were 40, 400, and 1,000 ng/ml in human plasma. For the clarithromycin calibration standards, the interday CV was 9.6%; the intraday CV ranged from 5.3 to 9.9%. For the 14( R)-hydroxyclarithromycin calibration standards, the interday CV was 8.6%; the intraday CV ranged from 1.7 to 7.4%. Standard curve correlation coefficients for both compounds were ≥0.985.

ERMBT analytical procedures. All ERMBT samples were assayed at the VCU School of Pharmacy Biopharmaceutical Analysis Laboratory. Liquid scintillation counting was used to measure the content of [N-methyl-14C]erythromycin. Solid-phase extraction was performed on Waters MilliLab Workstation with a Waters MilliLab Workstation. The reconstituted extract was washed with hexane and injected into an LC-MS-MS system with ambient ionization.

sample was loaded, and the cartridge was washed with water and methanol (65:35, vol/vol). The compound was eluted from the cartridges with 2.5 ml of acetonitrile. The volume of the eluate was reduced by evaporation under nitrogen at 37°C. A reisolated sample in the mobile phase was then loaded on the Waters Symmetry C18 column (3.5 by 150 mm) maintained at 40°C and eluted with a mobile phase consisting of acetonitrile-water in a 45:55 (vol/vol) ratio at a flow rate of 1.0 ml/min. Ampravir was detected at 254 or 260 nm (εmax = 340 nm). The ampravir calibration standard concentration standards were linear from 10 to 1,000 ng/ml; the ampravir plasma control concentrations were 30, 400, and 800 ng/ml. The clinical samples were diluted into the appropriate range of the calibration curves with blank human plasma and reassayed if they exceeded the upper limit of quantitation (1,000 ng/ml).

Upon validation of the ampravir assay technique, the interassay precision, assessed from spiked validation control samples (n = 6) at four concentrations over four analytical runs with human plasma and expressed as percent coefficient of variation (% CV), ranged from 1.8% to 7.7%; the intraday precision ranged from 1.8 to 11.3%. The percent recovery of ampravir was determined in human plasma at concentrations of 75, 400, and 800 ng/ml (n = 6 at each concentration) by injecting analytical standards (with internal standard) directly onto the column and comparing the area resulting from the injection of one to 3% across the concentration range of 75 to 800 ng/ml.
Pharmacokinetic analyses. The observed peak plasma drug concentrations at steady state (C_{max}) and the time for each drug to reach peak concentrations (T_{max}) were obtained by inspection of the individual plasma concentration-time data. The minimum drug concentration at steady state (C_{min}) was calculated as (C_{0} + C_{e})/2, where C_{0} is the plasma concentration before the last dose and C_{e} is the plasma concentration of the last sample of the steady-state dosing interval. The AUC at steady state (AU_{C}), from the time of the predosing sample to the last sample of the steady-state dosing interval was calculated for each volunteer using the linear trapezoidal rule. The apparent total clearance at steady-state (CL/F) was calculated as dose/AU_{C}. Similar formulae were used to determine 14-(R)-hydroxyclarithromycin pharmacokinetic parameters. The ratio of the metabolite AUC to the parent drug AUC (AU\_{C14-OH-clar}/AU\_{Cclar}) was also calculated based on the AU_{C}.

Urine pharmacokinetic parameters were determined for clarithromycin and 14-(R)-hydroxyclarithromycin only. Renal clearance (CL\_R) was calculated as A_{e}/AU_{C}, where A_{e} is the amount of drug excreted in the urine over the dosing interval. The percentages of clarithromycin and its metabolite eliminated in the urine were calculated based on clarithromycin weight equivalents. The molecular sizes of clarithromycin and 14-(R)-hydroxyclarithromycin were 747.96 and 763.96 Da, respectively.

The pharmacokinetic profiles obtained when the two drugs were administered together were compared with the profiles obtained when the drugs were administered alone (i.e., amprenavir plus clarithromycin versus amprenavir alone; amprenavir plus clarithromycin versus clarithromycin alone).

Statistical analysis. The primary analysis of pharmacokinetic parameters (other than T_{max}) was performed after log transformation. Analyses of variance (ANOVA) considering subject within sequence as the random effect, were performed using the Mixed Linear Models procedure (SAS PROC MIXED, version 6.12; SAS Institute, Cary, N.C.). The geometric least-squares mean and 90% confidence intervals were calculated for each pharmacokinetic parameter, along with their 90% confidence intervals. There were no statistically significant differences between treatments. There were no serious adverse events reported during this study, and all three treatments were generally well tolerated. The 14 subjects reported a total of 188 adverse events. The most common adverse events for amprenavir were mild gastrointestinal events (50%) and oral numbness (43%). Clarithromycin was most commonly associated with a bad taste (31%). Combination treatment with amprenavir plus clarithromycin resulted in greater subject intolerance than treatment with either drug alone, with any gastrointestinal events (71%) and oral numbness (50%) accounting for the majority of adverse effects. There was no apparent effect of the study drugs on hematology, clinical chemistry, or urinalysis laboratory values, nor any apparent changes in vital signs, physical examination findings, or electrocardiogram data from screening to follow-up.

Pharmacokinetics. (i) Amprenavir. Concentrations of amprenavir immediately before the final dose (C_{0}) were not different from concentrations 12 h after the final dose, indicating that steady state had been achieved. Figure 1 illustrates the effect of clarithromycin on mean plasma amprenavir concentrations. There were statistically significant increases in the amprenavir AU\_{C} (18%), C_{\text{max}} (15%), and C_{\text{min}} (39%), and a decrease in CL/F (15%), when amprenavir was administered with clarithromycin (Table 1). There was a nearly significant negative correlation between the baseline amprenavir AU\_{C} and the percent change in the amprenavir AU\_{C} when amprenavir was given with clarithromycin (r^{2} = 0.30; P = 0.065). There was a significant negative correlation between the AU\_{C} for clarithromycin and the magnitude of percent change from baseline in the amprnavir AU\_{C} (r^{2} = 0.44; P = 0.02). There was no significant association between subject weight and the AU\_{C} for amprenavir (r^{2} = 0.24; P = 0.10). The medians of T_{\text{max}} were not different between treatments.

RESULTS

Study subjects. A total of 14 HIV-seronegative, healthy males (12 Caucasian and 2 African-American) were enrolled in this study. Thirteen subjects received all three treatments, but only 12 subjects completed all phases of the study. One subject was withdrawn midway through his second treatment (amprenavir plus clarithromycin) after complaining of nausea and vomiting. The other subject withdrew during the third treatment (amprenavir plus clarithromycin) for personal reasons.

Adverse events. There were no serious adverse events reported during this study, and all three treatments were generally well tolerated. The 14 subjects reported a total of 188 adverse events. The most common adverse events for amprenavir were mild gastrointestinal events (50%) and oral numbness (43%). Clarithromycin was most commonly associated with a bad taste (31%). Combination treatment with amprenavir plus clarithromycin resulted in greater subject intolerance than treatment with either drug alone, with any gastrointestinal events (71%) and oral numbness (50%) accounting for the majority of adverse effects. There was no apparent effect of the study drugs on hematology, clinical chemistry, or urinalysis laboratory values, nor any apparent changes in vital signs, physical examination findings, or electrocardiogram data from screening to follow-up.

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There were no significant period or sequence effects in any of the ANOVA comparisons.

(ii) Clarithromycin. Concentrations of clarithromycin immediately before the final dose ($C_0$) were not different from concentrations 12 h after the final dose, indicating that steady state had been reached. Amprenavir had no significant effect on the geometric least-squares means for the clarithromycin AUC<sub>ss</sub>, $C_{\text{min,ss}}$, and CL/F (Fig. 2; Table 2). The median $T_{\text{max,ss}}$ following administration of the combined treatment was 2.0 h later than that after the administration of clarithromycin alone ($P < 0.05$). There was a 34% increase in CL<sub>R</sub> with the combined treatment over that with clarithromycin alone ($P < 0.05$). There was no significant linear correlation between the baseline apparent oral clearances for clarithromycin and amprenavir ($r^2 = 0.22; P = 0.11$). Weight was able to explain a significant amount of variability in the AUC<sub>ss</sub> for clarithromycin ($r^2 = 0.34; P = 0.04$); larger subjects had a lower AUC<sub>ss</sub>.

(iii) 14-($R$)-Hydroxyclarithromycin. Figure 3 illustrates the effect of amprenavir on mean plasma 14-($R$)-hydroxyclarithromycin concentrations. A summary of the results for 14-($R$)-hydroxyclarithromycin parameters is presented in Table 3. Amprenavir clearly reduced the formation of the main metabolite for clarithromycin, resulting in statistically significant decreases in the 14-($R$)-hydroxyclarithromycin AUC<sub>ss</sub> (35%) and $C_{\text{max,ss}}$ (32%). There was a 37% decrease in the $AUC_{14-OH-clar}/AUC_{clar}$ ratio. The median $T_{\text{max,ss}}$ following administration of the combined treatment was 2.0 h later than that following the administration of clarithromycin alone. The percentage of the dose excreted in the urine as 14-($R$)-hydroxyclarithromycin was 16% lower with the combined treatment than with clarithromycin alone.

The AUC<sub>ss</sub> for amprenavir given alone did not predict the magnitude of the percent reduction in the baseline AUC<sub>ss</sub> for 14-($R$)-hydroxyclarithromycin ($r = 0.17; P = 0.61$).

TABLE 1. Summary of results for amprenavir pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLSM (arithmetic mean CV&lt;sup&gt;a&lt;/sup&gt;) for:</th>
<th>Treatment 3 GLSM/treatment 1 GLSM ratio (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;ss&lt;/sub&gt; (h·µg/ml)</td>
<td>27.40 (29.08, 26%)</td>
<td>32.28 (32.98, 24%)</td>
</tr>
<tr>
<td>$C_{\text{max,ss}}$ (µg/ml)</td>
<td>8.42 (8.98, 26%)</td>
<td>9.65 (10.10, 26%)</td>
</tr>
<tr>
<td>$C_{\text{min,ss}}$ (µg/ml)</td>
<td>0.38 (0.41, 45%)</td>
<td>0.53 (0.53, 38%)</td>
</tr>
<tr>
<td>CL/F (ml/min)</td>
<td>730 (754, 38%)</td>
<td>619 (649, 30%)</td>
</tr>
<tr>
<td>$T_{\text{max,ss}}$ (h&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>1.14 (1.25, 53%)</td>
<td>1.38 (1.34, 38%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> GLSM, geometric least-squares mean.  
<sup>b</sup> CV, coefficient of variation.

Amprenavir, 1,200 mg twice a day.

Amprenavir, 1,200 mg twice a day, plus clarithromycin, 500 mg twice a day.

Median and median difference.

FIG. 2. Mean plasma clarithromycin concentrations (± standard deviations) versus time ($n = 12$ subjects) when clarithromycin was given alone (solid circles) or coadministered with amprenavir.

TABLE 2. Summary of results for clarithromycin pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLSM (arithmetic mean, CV&lt;sup&gt;a&lt;/sup&gt;) for:</th>
<th>Treatment 3 GLSM/treatment 2 GLSM ratio (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;ss&lt;/sub&gt; (h·µg/ml)</td>
<td>21.40 (22.31, 29%)</td>
<td>20.51 (20.92, 21%)</td>
</tr>
<tr>
<td>$C_{\text{max,ss}}$ (µg/ml)</td>
<td>2.57 (2.70, 29%)</td>
<td>2.31 (2.56, 19%)</td>
</tr>
<tr>
<td>$C_{\text{min,ss}}$ (µg/ml)</td>
<td>1.03 (1.06, 33%)</td>
<td>1.04 (1.09, 28%)</td>
</tr>
<tr>
<td>CL/F (ml/min)</td>
<td>390 (405, 30%)</td>
<td>406 (417, 25%)</td>
</tr>
<tr>
<td>$T_{\text{max,ss}}$ (h&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>2.25 (2.46, 73%)</td>
<td>4.99 (4.79, 29%)</td>
</tr>
<tr>
<td>CL&lt;sub&gt;R&lt;/sub&gt; (ml/min)</td>
<td>114 (121, 32%)</td>
<td>154 (159, 21%)</td>
</tr>
<tr>
<td>% Dose&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31.25 (31.62, 35%)</td>
<td>38.82 (38.63, 19%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> GLSM, geometric least-squares mean; CV, coefficient of variation.

<sup>b</sup> Consists of 500 mg of clarithromycin twice a day.

<sup>c</sup> Consists of 1,200 mg of amprenavir twice a day plus 500 mg of clarithromycin twice a day.

<sup>d</sup> Median and median difference.

<sup>e</sup> Least-squares mean and least-squares mean ratio.
**ERMTB.** The mean reduction in the ERMTB result was 85% (95% CI, 78 to 92%) after the administration of amprenavir, 67% (95% CI, 59 to 74%) for clarithromycin, and 87% (95% CI, 79 to 94%) for both drugs administered concurrently (Fig. 4). These data are consistent with evidence that drug interactions between clarithromycin and CYP3A4 substrates are of a lower magnitude compared with the effects of HIV-1 protease inhibitors (5). There was no significant correlation between the baseline ERMTB result and the CL/F for protease inhibitors (5). There was no significant correlation between the percent reduction in the ERMTB result following clarithromycin treatment and the percent reduction in the clearance of amprenavir (r = 0.34; P = 0.06). There was a nearly significant negative correlation between the percent reduction in the ERMTB result following clarithromycin treatment and the percent reduction in the clearance of amprenavir (r² = 0.34; P = 0.06). The mean ERMTB result at follow-up (2.08% ± 0.63% metabolized/h) was not significantly different from baseline (2.31% ± 0.68% metabolized/h; P = 0.107).

**DISCUSSION**

The pharmacokinetics of amprenavir and clarithromycin when given alone are in agreement with the findings of previous investigations (3, 19). Clarithromycin given in combination with amprenavir resulted in statistically significant changes in selected pharmacokinetic parameters for both drugs. Clarithromycin increased the amprenavir AUCₜss, Cₘ₈₅₉₉, and Cₘ₈₅₉₉ by 18, 15, and 39%, respectively, with an associated 15% decrease in CL/F. While this interaction is statistically significant, it is unlikely to be clinically important. An 18% increase in the CL/F is within the intersubject variability normally seen when amprenavir, 1,200 mg every 12 h, is used clinically (19). In addition, the 39% mean increase in Cₘ₈₅₉₉ is not likely to be a safety concern, since the absolute effect is small (mean increase from 0.38 to 0.53 μg/ml) and there is no known adverse event related to increased amprenavir trough concentrations.

Administration of amprenavir with clarithromycin had no statistically significant effect on the pharmacokinetic parameters AUCₜss, Cₘ₈₅₉₉, and Cₘ₈₅₉₉ for clarithromycin. However, the AUCₜss and Cₘ₈₅₉₉ of 14-(R)-hydroxyclarithromycin were decreased 35 and 32%, respectively by amprenavir; there was a 28% increase in CLₘ₉ for this metabolite; and the CLₘ₉ of clarithromycin increased by 34%. This reduced formation of 14-(R)-hydroxyclarithromycin appeared to be balanced by increased CLₘ₉ of the parent drug, resulting in no net change in the AUCₜss for clarithromycin. The metabolism of clarithromycin to 14-(R)-hydroxyclarithromycin is mediated by CYP3A4 (18), and the decreases in the 14-(R)-hydroxyclarithromycin AUC and Cₘ₈₅₉₉ are consistent with inhibition of CYP3A4 by amprenavir. Ritonavir has a similar effect on the metabolism of clarithromycin, but of greater magnitude (15). The mechanism for increased CLₘ₉ of clarithromycin is unclear but is unlikely to represent protein-binding displacement, since clarithromycin is approximately 70% bound to albumin, and binding would have to decrease to nearly zero to account for the increase in CLₘ₉. Furthermore, amprenavir has no known effects on renal function and should not alter renal secretion of clarithromycin. It is possible that the reduced formation of the metabolite may decrease competition with the parent compound for secretion, resulting in an increase in the CLₘ₉ of clarithromycin, but this remains conjectural.

It is unlikely that the changes in clarithromycin and 14-(R)-hydroxyclarithromycin pharmacokinetics are clinically relevant 14-(R)-Hydroxyclarithromycin has in vitro activity against...
some bacterial pathogens and may contribute to the clinical efficacy of clarithromycin, especially for infections caused by *Haemophilus influenzae* (8), but is less important for MAC (13). While it is possible that the therapeutic efficacy of clarithromycin may be compromised as a result of this interaction, the effect of amprenavir is less than that of other protease inhibitors (Table 4). There are no published reports of therapeutic failure when clarithromycin has been used to treat bacterial infections in HIV-infected patients receiving protease inhibitors, and dosage adjustments are not recommended for patients receiving other protease inhibitors and clarithromycin.

We have attempted to determine a mechanism for these effects. Since erythromycin (in the ERMBT), clarithromycin, and amprenavir are at least partially metabolized by hepatic CYP3A4, we hypothesized that there would be significant correlations of metabolic parameters between these three drugs. However, the mechanism(s) of the interactions described above appears to be more complex than simple alterations in hepatic CYP3A4 metabolism, as suggested by a number of observations. First, a good correlation between the ERMBT result and clearance of a CYP3A substrate has been suggested as evidence that the substrate is largely metabolized by hepatic CYP3A (7). In contrast, we found that the ERMBT results at baseline did not predict clearance of either amprenavir or clarithromycin, which suggests that nonhepatic mechanisms are more relevant (below). Second, although both amprenavir and clarithromycin significantly reduce hepatic CYP3A4 activity as measured by the ERMBT, amprenavir caused significantly greater suppression than clarithromycin (Fig. 4). However, clarithromycin had a more pronounced effect on serum amprenavir concentrations than amprenavir had on serum clarithromycin concentrations, an effect opposite to which would be expected if hepatic metabolism were of central importance. Third, a number of correlation analyses are not consistent with a hepatic mechanism to explain the interaction. For example, there was a significant negative correlation between the AUC$_{m}$ of clarithromycin and the magnitude of percent increase in the amprenavir AUC$_{m}$. Likewise, there was a near-significant (*P* = 0.06) negative correlation between the AUC$_{m}$ for amprenavir and the magnitude of reduction in the 14-(R)-hydroxycarhilthromycin metabolite, an effect also opposite to that predicted if impairment of hepatic metabolism was the main mechanism of interaction. Finally, the correlation between the clearance of amprenavir and the clearance of clarithromycin, two putative substrates of hepatic CYP3A4, was not significant.

Additional mechanisms that may explain the effects observed include alterations in CYP3A4-mediated gastrointestinal metabolism and alterations in P-glycoprotein (P-gp)-mediated gastrointestinal absorption (7). Clarithromycin has been shown to inhibit gastrointestinal CYP3A4 and thereby increase the absorption of midazolam, a substrate of CYP3A4 but not of P-gp (6). Clarithromycin has also been shown to increase the absorption of digoxin, a substrate of P-gp but not of CYP3A4 (24). Since all of the HIV-1 protease inhibitors are substrates of CYP3A4 (5) and are transported by P-gp (12, 17, 25), the increase in the AUC for amprenavir following clarithromycin pretreatment could be due to one or both of these mechanisms. There was a near-significant (*P* = 0.065) negative relationship between the baseline amprenavir AUC and the magnitude of the increase in the AUC following clarithromycin pretreatment. This suggests that those subjects with a low baseline amprenavir AUC, possibly resulting from greater first-pass clearance mediated by CYP3A4 and/or P-gp, have a larger

![FIG. 4. Percent erythromycin metabolism per hour, as measured by the ERMBT at baseline, at the end of each dosing regimen, and at follow-up. The line connects the means. APV, 1,200 mg of amprenavir given orally twice a day; CLAR, 500 mg of clarithromycin given orally twice a day; CLAR+APV, concomitant amprenavir and clarithromycin.](http://aac.asm.org/)

### TABLE 4. Comparison of protease inhibitor effects on clarithromycin pharmacokinetics

<table>
<thead>
<tr>
<th>Agent</th>
<th>% Clarithromycin increase (90% CI)</th>
<th>14-OH-CLAR$^{b}$ decrease (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>$C_{\text{max}}$</td>
</tr>
<tr>
<td>Saquinavir (1,200 mg</td>
<td>45 (17–81)</td>
<td>39 (10–76)</td>
</tr>
<tr>
<td>every 8 h$^{c}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ritonavir (200 mg</td>
<td>77 (56–103)</td>
<td>31 (15–51)</td>
</tr>
<tr>
<td>every 8 h$^{a}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indinavir (800 mg</td>
<td>53 ± 36$^{d}$</td>
<td>NS$^{f}$</td>
</tr>
<tr>
<td>every 8 h$^{a}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amprenavir (1,200 mg</td>
<td>No effect</td>
<td>−10</td>
</tr>
<tr>
<td>BID$^{d}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Results are expressed as percent change from values with clarithromycin alone.

$^{b}$ 14-OH-CLAR, 14-(R)-hydroxycarhilthromycin.

$^{c}$ Data are from saquinavir prescribing information (Roche Laboratories).

$^{d}$ Data are from ritonavir prescribing information (Abbott Laboratories).

$^{e}$ Data are from indinavir prescribing information (Merck and Company Pharmaceuticals).

$^{f}$ Mean ± standard deviation.

$^{g}$ NS, not stated.

$^{h}$ Data are from this study. BID, twice a day.
interaction with clarithromycin, since it interferes with those processes that act to reduce absorption. Similar mechanisms explain the effects when two protease inhibitors are given together, as when ritonavir is given with either saquinavir (9) or indinavir (10). Modeling of these interactions suggests that the main effect of ritonavir on indinavir is a reduction in systemic clearance via inhibition of hepatic CYP3A4 metabolism (10), whereas the effect of ritonavir on saquinavir is mediated mainly through a reduction in first-pass gastrointestinal CYP3A4 metabolism (9). It is not yet feasible to quantify the relative contribution of P-gp versus CYP3A4 to these interactions in vivo. Irrespective of the mechanisms for these interactions, these data indicate that clarithromycin and amprenavir can be given together with no need for dosage adjustment.

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REFERENCES