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Pharmacokinetic and Pharmacodynamic Study of the Human Immunodeficiency Virus Protease Inhibitor Amprenavir after Multiple Oral Dosing

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In a dose-ranging study of amprenavir (formerly 141W94), an inhibitor of the protease enzyme of human immunodeficiency virus (HIV) type 1, single-dose and steady-state pharmacokinetic parameters were estimated from plasma samples collected on day 1 and during week 3, respectively. Amprenavir was administered on either a twice-daily (b.i.d.) or three-times-daily dosage schedule to 62 HIV-infected adults, 59 of whom had pharmacokinetic data. Log-log regression analysis (the power model) revealed that the steady-state area under the curve (AUC_{ss}) and the maximum, minimum, and average concentrations at steady state ($C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$, respectively) increased in a dose-proportional manner over the 300- to 1,200-mg dose range. Steady-state clearance was dose independent. $AUC_{ss}/AUC_{0\rightarrow\infty}$ decreased linearly with dose and correlated significantly with treatment-associated decreases in α_1-acid glycoprotein. After 3 weeks, the dose of 1,200 mg b.i.d. provided a median amprenavir $C_{min,ss}$ (0.280 μg/ml) that was higher than the median in vitro 50% inhibitory concentration for clinical HIV isolates (0.023 μg/ml), even after adjustment for protein binding. The median amprenavir $C_{min,ss}$ was also greater than the estimated in vivo trough concentration calculated to yield 90% of the maximum antiviral effect (0.228 μg/ml) over 4 weeks. A pharmacodynamic analysis of the relationship between steady-state pharmacokinetic parameters and safety revealed headache and oral numbness to be the only side effects significantly associated with C_{max}. The pharmacodynamic relationship defined in this study supports the use of 1,200 mg b.i.d. as the approved dose of amprenavir.

As a drug class, the human immunodeficiency virus type 1 (HIV-1) protease inhibitors are potent and selective inhibitors of the HIV-1 protease enzyme (19) and are capable of potent in vivo antiretroviral activity when combined with HIV-1 reverse transcriptase inhibitors (5). Nevertheless, there are differences in the pharmacokinetic profiles and differences in the type, severity, and frequency of adverse events associated with the currently available protease inhibitors. New protease inhibitors are needed to increase the therapeutic options available to HIV-infected individuals, as treatment failure is not uncommon and intolerability or side effects can restrict use of the currently available protease inhibitors.

Amprenavir (formerly 141W94), an N,N-disubstituted hydroxyethylamino sulfonamide protease inhibitor (molecular mass, 506 Da), is a potent inhibitor of recombinant HIV-1 protease (K_i = 0.6 nM) (8) and possesses potent in vitro antiviral activity as demonstrated in a number of cell culture systems using laboratory HIV strains and clinical isolates (1; G. R. Painter, M. H. St. Clair, P. DeMiranda, D. Reynolds, S. Ching, R. Dornsife, D. J. Livingston, S. Pazhanisamy, and R. Tung, Abstr. 2nd Int. Conf. Hum. Retroviruses Relat. Infect., abstr. LB5, 1995). Amprenavir is highly (~90%) bound to proteins in normal human plasma or serum, with the greatest degree of fractional binding to α_1-acid glycoprotein (AAG) (89%) and albumin (42%) (10). The mean 50% inhibitory concentration (IC_{50}) of amprenavir against 334 HIV clinical isolates is 14.6 ± 12.5 ng/ml (B. M. Sadler, P. J. Piliero, S. L. Preston, Y. Lou, M. Sale, and D. S. Stein, Abstr. 7th Conf. Retroviruses Opportunistic Infect., abstr. 77, 2000). The metabolism of amprenavir appears to be primarily dependent upon the CYP3A4 isozyme of the hepatic cytochrome P450 system, based on in vitro and in vivo studies (4; J. Woolley, S. Studenberg, C. Boehlert, G. Bowers, A. Sinhababu, and P. Adams, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-60, 1997).

Single-dose pharmacokinetic studies of amprenavir in HIV-infected adults and children have been conducted and have shown that amprenavir can be given safely at doses of up to 1,200 mg and 20 mg/kg, respectively (15; B. M. Sadler, G. E. Chittick, R. Yogev, A. Kovacs, Y. Lou, C. Pilati-Stevens, W. T. Symonds, and S. V. Hetherington, submitted for publication). We report here the multiple-dose pharmacokinetics and pharmacodynamics of five escalating, parallel, oral doses of amprenavir administered alone or in combination with the HIV reverse transcriptase inhibitor abacavir in HIV-infected adult subjects (Glaxo Wellcome Protocol PROA1002). The relationship between steady-state pharmacokinetic parameters and antiviral activity was examined, as it has been suggested that a direct correlation exists between protease inhibitor drug exposure and the magnitude of reduction of HIV RNA (3, 11, 17; G. L. Drusano, B. M. Sadler, J. Millard, W. T. Symonds, M. Tisdale, C. Rawls, A. Bye, and the 141W94 International Product Development Team, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-16, 1997). The relationship between steady-state pharmacokinetic parameters and safety was also examined. Together, these two pharmacodynamic

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analyses helped in the characterization of doses of amprenavir that warranted further exploration in subsequent phase II and phase III studies.

(Preliminary pharmacokinetic data from this study were originally reported at the 9th International Conference on Antiviral Research, Fukushima, Japan, 19 to 24 May 1996 [16]. Preliminary pharmacodynamic data from this study were reported at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada, 28 September to 1 October 1997 [Drusano et al., 37th ICAAC]. Preliminary clinical safety and efficacy data from this study were reported at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996 [R. T. Schooley and the International 141W94 Study Team, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. LB8, 1996], and the final clinical safety and efficacy data have been submitted for publication [R. T. Schooley, N. Clumeck, R. Haubrich, M. Thompson, S. A. Danner, M. E. van der Ende, D. Sereni, F. Antunes, D. Blake, R. E. Myers, M. Tisdale, J. Millard, N. Mustafa, and P. Nacci, submitted for publication].)

MATERIALS AND METHODS

Study population and design. Sixty HIV-positive subjects (male and female, 18 years of age or older) were planned to be enrolled into six treatment cohorts. Study entry criteria were as described by Schooley et al. (submitted). Briefly, 62 HIV-positive subjects were sequentially enrolled into one of five amprenavir-only groups, with enrollment in the only combination treatment group conducted concurrently with that of the group receiving only amprenavir at 1,050 mg twice daily (b.i.d.). The six treatment cohorts were as follows: amprenavir at 300 mg b.i.d., amprenavir at 300 mg three times daily (t.i.d.), amprenavir at 900 mg b.i.d., amprenavir at 1,050 mg b.i.d., amprenavir at 1,200 mg b.i.d., and amprenavir at 900 mg b.i.d. in combination with abacavir at 300 mg b.i.d. After completion of the 4-week treatment period, zidovudine (300 mg b.i.d.) and lamivudine (150 mg b.i.d.) were added to the regimens of subjects who remained in the amprenavir-only treatment groups, or zidovudine (300 mg b.i.d.), lamivudine (150 mg b.i.d.), and abacavir (300 mg b.i.d.) were added to the regimen of subjects who remained in the amprenavir-abacavir group.

The study's major inclusion criteria were $CD4^+$-cell counts of $\geq$150 and $\leq$400/mm^3, no prior HIV protease inhibitor use, no active infections, and no evidence of malabsorption syndrome. It should be noted that amprenavir was supplied as a hard gelatin capsule in this study, a formulation that has since been shown to have the same bioavailability as the more stable soft gelatin capsule, which is the formulation now in clinical use. A relative bioavailability study comparing the pharmacokinetics of the two amprenavir formulations found no significant difference in area under the concentration-time curve (AUC) between the two formulations (15).

Concurrent medications, such as chemoprophylaxis for HIV-related conditions, were permitted during the study. All concurrent medications and blood products administered during the study were recorded. Either an independent ethics committee or the institutional review board affiliated with each study center approved the study protocol. All subjects had to provide written informed consent prior to study participation.

Blood collection. Blood samples were collected to determine amprenavir concentration, abacavir concentration, plasma HIV RNA levels, and hematology and clinical chemistry. Blood samples for single-dose and multiple-dose plasma drug concentrations were collected during the scheduled visits on day 1 (amprenavir-only groups) and during week 3 (all groups), respectively. At both times, samples were taken prior to the subjects' first dose of the day (predose) and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 8.0 h postdose. Day 1 sampling also included collection at 10.0, 12.0, and 24.0 h postdose. Plasma samples from groups receiving study medication on a b.i.d. schedule were also collected at 10 and 12 h postdose during week 3. All subjects were instructed to fast overnight ($\geq$ 8 h) before the scheduled morning dosing and collection of plasma sample. Water was permitted ad libitum. After the predose plasma sample collection, subjects took their study medication with 200 ml of water and fasted until 3 h postdose.

Safety evaluation. Safety and tolerability were evaluated and are described elsewhere (Schooley et al., submitted). Briefly, evaluations were conducted by physical examination, ophthalmologic examination, vital signs, electrocardiogram, hematology, clinical chemistry, AAG, urinalysis, and clinical adverse events.

Efficacy evaluation. Efficacy was evaluated as the time-weighted average decrease in $\log_{10}$ HIV RNA from baseline (AAUCMB) (6, 17, 18; Drusano et al., 37th ICAAC). Plasma samples for viral load determination were collected at predose and at day 4 and weeks 1, 2, 3, and 4. Viral load was measured using the quantitative HIV-1 RNA PCR assay (Roche Amplicor HIV-1 MONITOR); the limit of quantification was 400 copies/ml (12).

Pharmacokinetic evaluation. Model-independent pharmacokinetic parameters for amprenavir and abacavir after single or multiple oral dosing were calculated using WinNonlin Pro version 1.5 (SCI, Cary, N.C.). Single-dose pharmacokinetic parameters included maximum concentration (C_{max}), time of the maximum concentration (T_{max}), apparent terminal elimination rate constant (λ_z), and terminal elimination half-life ($t_{1/2}$). Individual estimates of λ_z for amprenavir were obtained by log-linear regression of the terminal portions of the plasma concentration-time curves; $t_{1/2}$ was then calculated as $\ln(2)/\lambda_z$. The $AUC_{0\rightarrow t}$, from time zero to the time of the last quantifiable sample (t_{last}), was calculated for each subject using the linear trapezoidal rule. $AUC_{0\rightarrow t}$ was extrapolated from t_{last} to infinity ($AUC_{0\rightarrow\infty}$) by adding C_{last}/λ_z. The apparent total clearance (CL/F) was calculated as dose/$AUC_{0\rightarrow\infty}$. The apparent volume of distribution during terminal elimination (V_z/F) was calculated as (CL/F)/λ_z.

Amprenavir steady-state pharmacokinetic parameters included the maximum, minimum, and average concentrations in plasma ($C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$, respectively), time of maximum concentration in plasma ($T_{max,ss}$), steady-state AUC (AUC_{ss}), and apparent total clearance (CL/F_{ss}). The CL/F_{ss} was calculated as dose/AUC_{ss}. $C_{avg,ss}$ was calculated by dividing the AUC_{ss} by the dosing interval (τ). The $C_{max,ss}$ and $T_{max,ss}$ were obtained by inspection of the individual plasma concentration-time data. $C_{min,ss}$, the trough drug concentration at steady state, was calculated as $(C_0 + C_\tau)/2$, where C_0 is the concentration in plasma before dosing and C_τ is the concentration in plasma for the last sample of the steady-state dosing interval. The $AUC_{0\rightarrow t}$ from the time of the predose sample to the last sample of the steady-state dosing interval was calculated for each subject using the linear trapezoidal rule. When the last quantifiable sample of the steady-state dosing interval was not taken at $t = \tau$, C_τ was estimated as $C_\tau = C_t \cdot e^{-\lambda_z(\tau - t)}$ and $AUC_{0\rightarrow t}$ was extrapolated to τ (AUC_{ss}), the steady-state dosing interval, by the formula $AUC_{ss} = AUC_{0\rightarrow t} + C_t/\lambda_z \cdot [1 - e^{-\lambda_z(\tau - t)}]$. Actual sampling times were used to calculate both single-dose and steady-state pharmacokinetic parameters.

Assay for amprenavir. Concentrations of amprenavir in plasma were quantified using validated and cross-validated reversed-phase high-performance liquid chromatographic methods. Solid-phase extraction or protein precipitation was coupled with fluorescence detection, as previously described (15), or mass spectrometry. The range of detection of amprenavir by fluorescence spectroscopy was 10 to 1,000 ng/ml, and that by mass spectrometry was 10 to 5,000 ng/ml. At least three serially diluted quality control samples were included at the beginning, middle, and end of each assay run. All samples from the runs in which the quality control data were not within control specifications were reassayed. For reporting purposes, concentrations were expressed in micrograms per milliliter. The between-assay bias was $<$15% for all assays.

Assay for abacavir. Concentrations of abacavir in plasma were determined using a validated, reversed-phase high-performance liquid chromatographic assay with UV detection as previously described (9). The between-assay bias was $<$6%, and the range of detection of abacavir was 25 to 5,000 ng/ml. For reporting purposes, concentrations were expressed in micrograms per milliliter.

Assay for AAG. Concentrations of AAG in serum were quantified using a validated fixed-time nephelometric method (Quest Diagnostics, Capistrano, Calif.). The limits of quantification were 20 to 660 ng/dl, with an interassay variability of $<$6%.

Statistical analyses. (i) Single- and multiple-dose pharmacokinetics. Descriptive statistics were performed to compare treatment cohorts. Dose proportionality and linearity for amprenavir pharmacokinetic parameters at day 1 and week 3 were evaluated using the power model (mixed linear models procedure; SAS PROC MIXED) described by the equation $\log(Y) = a + b \cdot \log(dose)$, where Y is the pharmacokinetic parameter of interest, a and b are the estimated coefficients, and dose is the dose received by each subject (total daily dose for steady state) (7). Dose dependence and independence were determined by the inclusion of 1 and 0 in the 90% confidence interval (CI), respectively, estimated for the slope of the parameter of interest.

To evaluate the effect of abacavir on amprenavir at steady state (data were analyzed after $\log_e$ transformation), a one-way analysis of variance (SAS PROC

MIXED, version 6.12) was used to compare the ratio of the geometric least-squares (GLS) means for amprenavir pharmacokinetic parameters obtained for the 900 mg b.i.d. amprenavir monotherapy and the b.i.d. amprenavir-plus-abacavir combination therapy groups. A similar analysis was used to evaluate the effect of amprenavir on abacavir steady-state pharmacokinetics, in which the amprenavir-plus-abacavir combination therapy group was compared with a historical control group (300 mg, b.i.d., abacavir monotherapy). Two one-sided tests (90% CI) were used to compare the pharmacokinetic parameters AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, $C_{avg,ss}$, and CL/F_{ss}. An effect was considered significant if the 90% CI for the ratio of the GLS means fell between 0.70 and 1.43.

(ii) Pharmacodynamics. The relationship between the steady-state pharmacokinetic parameters $C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$ and antiviral activity (AAUCMB) was examined using simple and sigmoid E_{max} models, with and without a baseline effect. The models were described by the following equations: $AAUCMB = E_{max} \cdot C_{pk,ss}/(EC_{50} + C_{pk,ss})$ (simple), $AAUCMB = E_0 + E_{max} \cdot C_{pk,ss}/(EC_{50} + C_{pk,ss})$ (simple with baseline effect), $AAUCMB = E_{max} \cdot C_{pk,ss}^{\gamma}/(EC_{50}^{\gamma} + C_{pk,ss}^{\gamma})$ (sigmoid), and $AAUCMB = E_0 + E_{max} \cdot C_{pk,ss}^{\gamma}/(EC_{50}^{\gamma} + C_{pk,ss}^{\gamma})$ (sigmoid with baseline). In the equations, $C_{pk,ss}$ is the steady-state parameter of interest, E_{max} is the predicted maximum effect on AAUCMB, EC_{50} is the $C_{pk,ss}$ producing 50% of the maximal effect, E_0 is the baseline AAUCMB when $C_{pk,ss}$ is 0, and γ is a unitless shape parameter for sigmoid models. The equations were used to evaluate each of the parameters after appropriate substitution. Nonlinear curve fitting was performed with WinNonlin Pro version 1.5 (SCI) using an unweighted analysis. Estimates of E_{max}, EC_{50}, and γ were calculated for the models and used to determine the concentration of amprenavir that produced various percent changes in AAUCMB.

The goodness of fit between observed data and the E_{max} models was measured by the coefficient of variance (%CV) of the estimated parameters, the planar 95% CI of the estimate, the coefficient of determination (r^2), and plots of residuals. Model differentiation was defined as the r^2 of a model being high with a low Akaike information criterion (AIC) (2) together with a small %CV of the estimated parameters.

The steady-state pharmacokinetic parameters, $C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$, were categorized as being above or below the median of the distribution of each parameter. The association between these pharmacokinetic parameters and the incidence of adverse events was evaluated by Mantel-Haenszel chi-square tests and by logistic regression (SAS 6.12, PROC LOGISTIC). Odds ratios were calculated with their 95% CI.

PROC CORR (SAS 6.12) was used to examine the correlation between AUC_{ratio} ($AUC_{ss}/AUC_{0\rightarrow\infty}$) and baseline $CD4^+$-cell count, baseline viral load ($VL_{baseline}$), AAUCMB for $CD4^+$-cell count ($AAUCMB_{CD4}$), AAUCMB for viral load ($AAUCMB_{VL}$), and the absolute or percent change in AAG (ΔAAG or %AAG, respectively). A stepwise regression (SAS PROC Reg.) was applied to the full model, $\ln(AUC_{ratio}) = \Delta AAG$ (or %AAG) + $CD4_{baseline}$ + $VL_{baseline}$ + $AAUCMB_{CD4}$ + $AAUCMB_{VL}$ + race + sex + ln(dose), to evaluate the association between the single-dose and steady-state amprenavir AUC_{ratio} and the absolute or percent change from baseline in AAG concentrations. A reduced model with absolute or percent change in AAG and ln(dose) would be used to estimate the association with AUC_{ratio} if other covariates were not significant.

RESULTS

Subject demographics and accountability. This study was conducted between November 1995 and November 1997 at eight centers in Portugal, Belgium, France, Holland, and the United States. Subject demographics were similar across all treatment groups at baseline. Baseline characteristics were also comparable among all cohorts (median $CD4^+$-cell count range, 254 to 305 cells/mm^3; median plasma HIV RNA range, 4.71 to 5.08 $\log_{10}$ copies/ml).

Of the 62 enrolled subjects, 56 completed the 4 weeks of amprenavir treatment to which they were assigned. Five subjects prematurely discontinued treatment; one of these subjects was never treated, and four withdrew due to the following adverse events (Schooley et al., submitted): skin rash (amprenavir at 300 mg t.i.d., one subject), abdominal discomfort and pain with diarrhea (amprenavir at 1,200 mg b.i.d., one subject), skin rash and paresthesia (amprenavir at 1,200 mg b.i.d., one subject), and dysarthia and erythematous skin condition (amprenavir-abacavir, one subject). Fifty-nine subjects had day 1 and/or week 3 plasma amprenavir profiles, 53 subjects had single-dose (day 1) concentration-time profile data, and 55 subjects had multiple-dose (week 3) concentration-time profile data. Irregularities in plasma sampling (i.e., deviations in sampling time) and dosing at individual clinical sites prompted a duplicate analysis of both the single- and multiple-dose plasma profiles used to estimate pharmacokinetic parameters. Two populations were therefore analyzed; one consisted of all subjects and one consisted of "well-characterized" subjects.

Subjects were excluded from the well-characterized population on day 1 because of dosing deviations. Either they received the wrong dose to start or they took a second (or third) dose during the 24-h single-dose pharmacokinetic profile. Subjects were excluded from the well-characterized population on week 3 because their dosing history or pharmacokinetic profile clearly indicated that they were not at steady state. Either the recorded time of the prior dose did not fall within 2 h of the dosing interval specified by the protocol or there were gross (i.e., 5- to 10-fold) differences between C_0 and C_τ (these should be equal under true steady-state conditions). Analysis of pharmacokinetic profiles from either all subjects or well-characterized subjects yielded similar interpretations of the study results.

In this paper, 36 well-characterized day 1 plasma drug concentration profiles were included in the analysis of single-dose pharmacokinetic parameters, and 42 well-characterized week 3 plasma drug concentration profiles were included in the analysis of the multiple-dose pharmacokinetic parameters.

Single-dose pharmacokinetics. Figure 1 shows the median plasma amprenavir concentration over time after single oral doses of amprenavir (amprenavir-only cohorts). In individual profiles for each of the amprenavir-only cohorts (data not shown), the curve was typically biphasic, with a small second peak occurring between 6 and 12 h after administration. Variations in the timing of the peak between individuals damped the effect on the median plasma drug concentration curves in Fig. 1. It is unclear whether the second peak frequently observed in the amprenavir single-dose profiles is from a second site of absorption or from recirculation. C_{max} was rapidly reached at ~1.5 h after dosing; the terminal-phase $t_{1/2}$ was ~6 h.

The mean values of amprenavir single-dose pharmacokinetic parameters are presented in Table 1. Data from 36 well-characterized single-dose plasma drug concentration profiles showed that the C_{max} increased in a dose-proportional manner, while the $AUC_{0\rightarrow\infty}$ increased in a slightly greater-than-dose-proportional manner (slope = 1.239; 90% CI = 1.022 to 1.455). CL/F decreased with increasing dose (slope = −0.239; 90% CI = −0.455 to −0.022). Both $t_{1/2}$ and V_z/F were dose-independent.

Multiple-dose pharmacokinetics. The median plasma amprenavir concentrations over time, after 3 weeks of dosing for each of the five monotherapy regimens, are shown in Fig. 1. For all regimens, C_{max} was reached within 1 to 2 h after dosing and amprenavir concentrations declined in a biphasic manner.

The estimated values of the steady-state pharmacokinetic parameters for amprenavir after multiple oral dosing, both alone and in combination with abacavir, are presented in Table

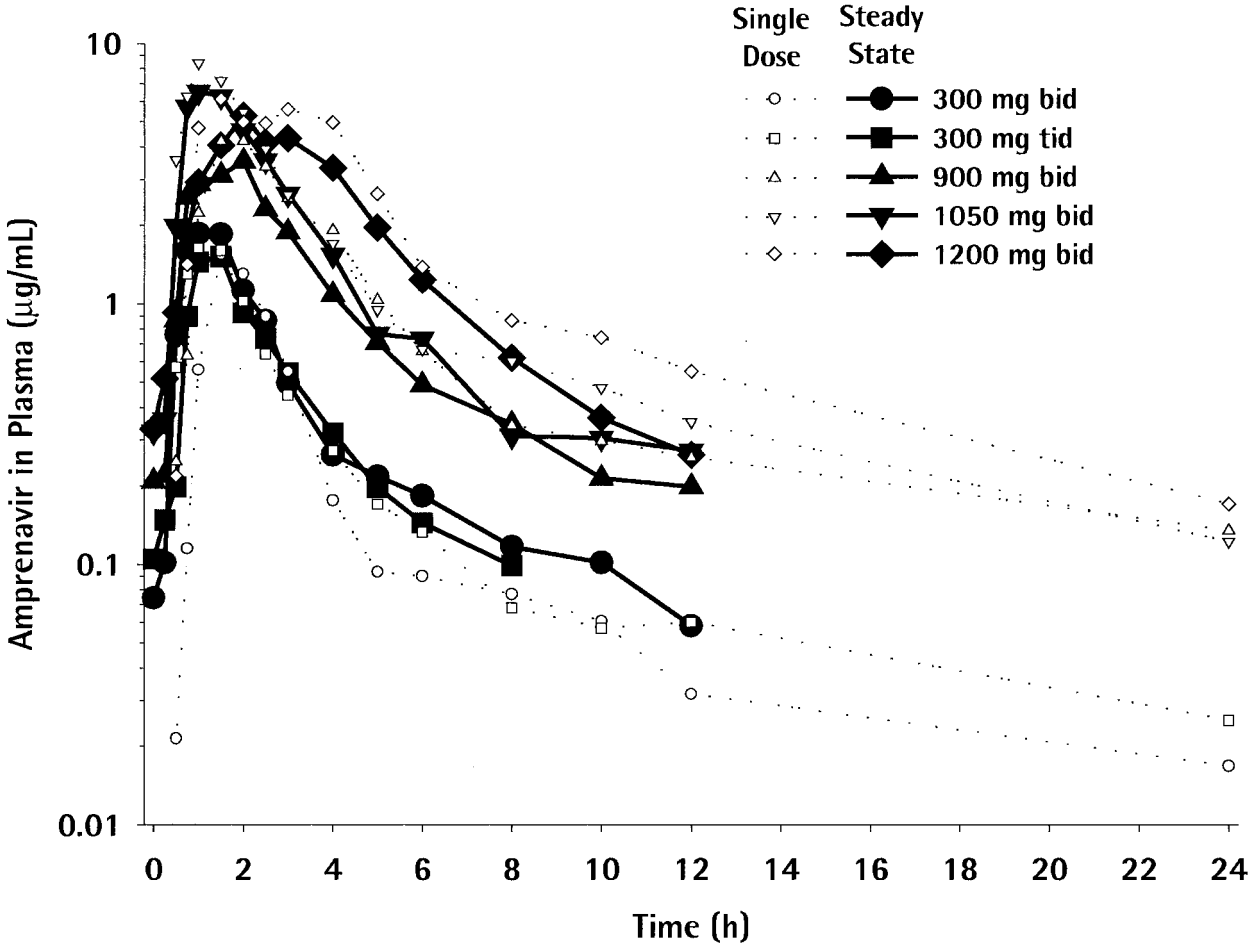

FIG. 1. Median plasma drug concentration-versus-time graphs following single-dose and multiple-dose amprenavir administration.

1. Data from 38 well-characterized multiple-dose plasma drug concentration profiles (amprenavir-only cohorts) showed that the amprenavir AUC_{ss} (slope = 1.03; 90% CI = 0.77 to 1.28), $C_{max,ss}$ (slope = 0.81; 90% CI = 0.56 to 1.07), $C_{min,ss}$ (slope = 1.02; 90% CI = 0.67 to 1.38), and $C_{avg,ss}$ (slope = 0.91; 90% CI = 0.65 to 1.17) increased in a dose-proportional manner. The CL/F_{ss} (slope = −0.15; 90% CI = −0.42 to 0.12) was dose independent. The comparatively larger CL/F_{ss} noted in the group receiving 1,200 mg of amprenavir was due mainly to the exceptionally small AUC_{ss} of one subject; exclusion of this subject from the analysis reduced the CL/F_{ss} to a value of 1,011 ml/min, which is similar to the value obtained with the 1,050-mg dose (1,031 mL/min). The mean T_{max} was reached within 2.25 h after dosing (range, 0.50 to 3.98 h). The ratio of $AUC_{ss}/AUC_{0\rightarrow\infty}$ (slope = −0.30; 90% CI = −0.49 to −0.11) decreased linearly with dose but was not dose proportional.

Amprenavir-abacavir coadministration. The multiple-dose pharmacokinetic parameters for amprenavir at 900 mg b.i.d. in the presence of abacavir at 300 mg b.i.d. were not different from those observed in the cohort receiving only amprenavir at 900 mg b.i.d. (Table 1). The $C_{max,ss}$ was reached at ~2 h after dosing, and plasma amprenavir concentrations declined in a biphasic manner. Abacavir did not alter the characteristic shape of the plasma amprenavir concentration-time curve. Moreover, neither C_{max} nor $t_{1/2}$ for amprenavir appeared to be altered by coadministration with abacavir.

Analysis of five subject profiles showed that in the presence of amprenavir, abacavir multiple-dose pharmacokinetic parameters were not different from those previously reported for monotherapy with abacavir at 300 mg b.i.d. (J. A. McDowell, W. T. Symonds, and S. W. LaFon, Abstr. XI Int. Conf. AIDS, abstr. MoB1140, 1996), with one exception: there were minor differences in $C_{max,ss}$, where the GLS mean ratio between combination and monotherapy regimens was 0.79 (90% CI = 0.63 to 0.99). The median concentrations of abacavir with and without amprenavir are very similar (data not shown).

Pharmacodynamics analysis of safety. Five adverse events occurring over the 4-week period, i.e., headache (n = 11), nausea or vomiting (n = 12), diarrhea (n = 12), oral numbness (n = 5), and rash (n = 4), were selected for analysis of the relationship between steady-state pharmacokinetic parameters and safety based on their incidence and potential for pharmacokinetic dependence. Categorical analysis (Table 2) indicated significant associations between increasing $C_{max,ss}$ and headache (P = 0.01) and oral numbness (P = 0.02). $C_{avg,ss}$ was also associated with oral numbness (P = 0.02). A trend toward a higher occurrence of nausea and/or vomiting with higher $C_{avg,ss}$ was noted (P = 0.05). Logistic regression analysis found

TABLE 1. Pharmacokinetic parameters of amprenavir

Amprenavir treatment	Mean (% CV)								
	C_{max}[a] (μg/ml), day 1	$C_{max,ss}$[b] (μg/ml), wk 3	T_{max}[a] (h), day 1	$T_{max,ss}$[b] (h), wk 3	$AUC_{0\rightarrow\infty}$[c] (h · μg/ml), day 1	AUC_{ss}[b] (h · μg/ml), wk 3	$AUC_{ss}/AUC_{0\rightarrow\infty}$	$C_{min,ss}$[b] (μg/ml), wk 3	CL/F[c] (ml/min), day 1	CL/F_{ss}[b] (ml/min), wk 3
300 mg, b.i.d.	2.58[d] (67)	2.27 (51)	1.25[d] (45)	1.50 (30)	4.87[d] (43)	4.66 (59)	1.00 (39)	0.07 (58)	1,027[d] (47)	1,073 (62)
300 mg, t.i.d.		1.87 (38)		1.20 (20)		3.97 (45)	0.82 (40)	0.09 (88)		1,260 (43)
900 mg, b.i.d.	5.33 (37)	4.26 (39)	1.48 (35)	2.25 (52)	18.30 (21)	12.18 (21)	0.97 (33)	0.17 (78)	819 (26)	1,232 (28)
1,050 mg, b.i.d.	8.35 (25)	7.48 (27)	1.34 (24)	1.06 (57)	26.81 (34)	20.27 (35)	0.74 (23)	0.29 (56)	655 (42)	863 (39)
1,200 mg, b.i.d.	8.57 (53)	4.63 (62)	2.00 (32)	1.94 (51)	29.14 (64)	16.15 (63)	0.55 (23)	0.28 (52)	686 (86)	1,238 (119)
900 mg, b.i.d., plus abacavir (300 mg, b.i.d.)		5.72 (58)		1.88 (26)		13.56 (58)		0.15 (86)		1,119 (46)

[a] For this day 1 parameter, n = 22, 8, 10, and 9 for the 300-, 900-, 1,050-, and 1,200-mg doses, respectively.
[b] For all week 3 parameters, n = 9, 6, 9, and 5 for the 300-mg b.i.d., 300-mg t.i.d., 900-mg, 1,050-mg, and 1,200-mg amprenavir doses, respectively, and n = 4 for the 900-mg b.i.d. amprenavir plus 300-mg abacavir doses.
[c] For this day 1 parameter, n = 14, 2, 5, 8, and 7 for the 300-, 900-, 1,050-, and 1,200-mg doses, respectively.
[d] The 300-mg dosing groups (day 1) were combined for analysis of pharmacokinetic parameters.

TABLE 2. Summary of pharmacodynamic categorical analysis of associations between concentrations and adverse events (n = 42)

Adverse event	n	Association[a] with:		
		$C_{max,ss}$	$C_{avg,ss}$	$C_{min,ss}$
Nausea and/or vomiting	12	0.21	0.05	0.15
Headache	11	0.01	0.07	0.34
Diarrhea	12	0.44	0.92	0.92
Rash	4	0.96	0.28	0.96
Oral numbness	5	0.02	0.02	0.18

[a] Values are P values from the Mantel-Haenszel chi-square test with modified Ridits scores. Parameter values were categorized as greater or less than the median.

no significant associations between the incidence of subjects with adverse events and each pharmacokinetic parameter as a continuous variable, except for borderline associations with $C_{max,ss}$ (odds ratio = 1.31; 95% CI = 0.99 to 1.82) and $C_{avg,ss}$ (odds ratio = 1.06; 95% CI = 0.99 to 1.13) for oral numbness.

Pharmacodynamics analysis of efficacy. Four E_{max} models were used to examine the relationship between $C_{max,ss}$, $C_{min,ss}$, $C_{avg,ss}$, and antiviral activity over 4 weeks in subjects with well-characterized steady-state pharmacokinetic data. All four models provided a statistically significant fit to the data ($P < 0.0001$); however, the sigmoid E_{max} model was favored over the other models because it had the lowest Akaike information criterion with a better model fit. Both $C_{min,ss}$ and $C_{avg,ss}$ were somewhat better predictors of the decrease in AAUCMB than was $C_{max,ss}$, based upon the resulting r^2. The relationship between $C_{min,ss}$ and the AAUCMB, with the modeled curve, is shown in Fig. 2.

Using the sigmoid E_{max} model, estimates of the amprenavir concentrations that provided various percentages of the maximum decrease in AAUCMB (defined as EC_{50}, EC_{75}, EC_{90}, EC_{95}, and EC_{99}) were derived. The predicted ECs calculated for $C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$ are shown in Table 3. None of the models had a significant fit for CD4 changes to amprenavir pharmacokinetic parameters.

Pharmacodynamics analysis of AAG. Over the 3-week period between obtaining day 1 and week 3 pharmacokinetic profiles, the AAG concentration declined a median of 19.8%. The absolute and percent changes in AAG level were strongly correlated with $AUC_{ss}/AUC_{0\rightarrow\infty}$ (r^2 = 0.639 and 0.664, respectively; $P < 0.0001$ for both). Of all variables tested, only the percent change in AAG was significantly associated with the $AUC_{ss}/AUC_{0\rightarrow\infty}$ (P = 0.014, full model). In the reduced model, with the percent change in AAG and the ln(dose) as independent predictors, only the percent change in AAG remained significantly associated with the $AUC_{ss}/AUC_{0\rightarrow\infty}$ (P = 0.002). Models using the absolute change in AAG gave findings similar to those using the percent change.

DISCUSSION

In this 4-week multiple-dose, dose-escalating study, amprenavir administration, either alone or in combination with abacavir, resulted in a significant exposure-related change in viral load. Safety and tolerability findings were consistent with those of previous single-dose studies (15; Sadler et al., submitted) and with subsequent phase II and III clinical studies (L.

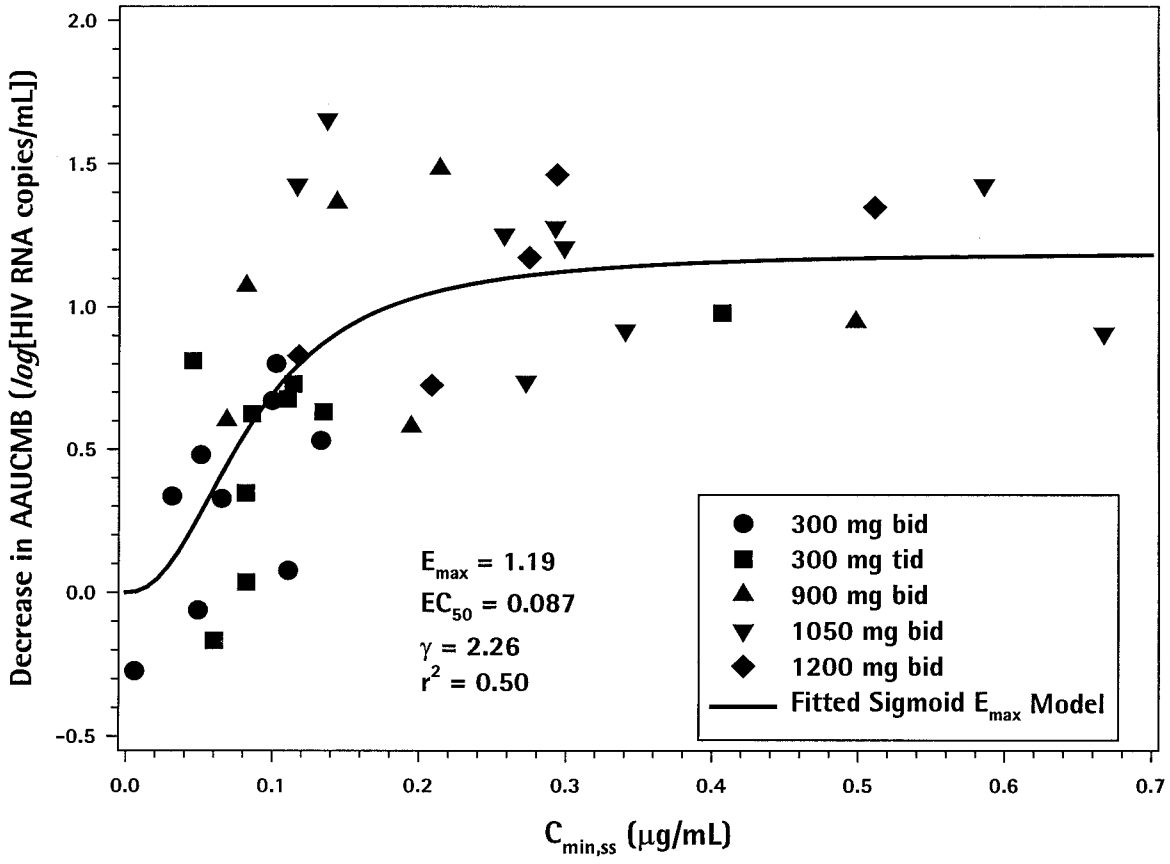

FIG. 2. Fitted curve of amprenavir $C_{min,ss}$ versus decrease in AAUCMB for plasma HIV RNA using the sigmoid E_{max} model. For the model, estimated E_{max} = 1.19 (95% CI, 0.88 to 1.5) $\log_{10}$ copies/ml, EC_{50} = 0.087 (95% CI, 0.053 to 0.12) μg/ml, γ = 2.26 (95% CI, 0.14 to 4.4), r^2 = 0.50, and $P < 0.0001$. The %CVs for the estimated E_{max}, EC_{50}, and γ were 12.9%, 18.9%, and 46.4%, respectively.

Pednealt, A. Fetter, C. Hanson, J. Wilson, P. Nacci, and J. Millard, Abstr. 6th Conf. Retroviruses Opportunistic Infect., abstr. 386, 1999). The most frequently reported drug-related adverse events in this study were of mild intensity. Headache and oral numbness were significantly associated with C_{max}. Oral numbness was also significantly associated with $C_{avg,ss}$, and a trend toward significance was observed for nausea and/or vomiting with $C_{avg,ss}$.

All single-dose and multiple-dose pharmacokinetic analyses were performed using data both from all subjects and from well-characterized subjects. No meaningful difference in the interpretation of the study resulted from the use of pharmacokinetic profiles from all subjects versus well-characterized subjects.

Analyses of the estimates of single-dose amprenavir pharmacokinetic parameters for amprenavir doses of 300, 900, 1,050, and 1,200 mg showed C_{max} to increase dose proportionally, while $AUC_{0\rightarrow\infty}$ increased with increasing amprenavir doses in a slightly greater-than-dose-proportional manner. The greater-than-dose-proportional increase in AUC was accompanied by a corresponding decrease in CL/*F* with increasing dose. These findings are consistent with those previously reported for HIV-infected adults and children (15; Sadler et al., submitted). In the present study, V_z/F was dose independent

TABLE 3. Predicted ECs of amprenavir for various decreases in plasma HIV RNA AAUCMB calculated for $C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$ using the sigmoid E_{max} model

EC level	$C_{min,ss}$ (μg/ml)	AAUCMB ($-\log_{10}$ copies/ml)	$C_{avg,ss}$ (μg/ml)	AAUCMB ($-\log_{10}$ copies/ml)	$C_{max,ss}$ (μg/ml)	AAUCMB ($-\log_{10}$ copies/ml)
EC_{50}	0.087	0.60	0.506	0.60	2.233	0.61
EC_{75}	0.141	0.89	0.793	0.90	3.702	0.91
EC_{90}	0.228	1.07	1.243	1.09	6.139	1.09
EC_{95}	0.318	1.13	1.687	1.15	8.661	1.15
EC_{99}	0.658	1.18	3.310	1.19	18.510	1.20
E_{max}		1.19		1.21		1.21

and slightly greater than previously reported; previous studies have found this parameter to be inversely related to amprenavir dose (15; Sadler et al., submitted). In the present study, $t_{1/2}$ was also dose independent.

Amprenavir pharmacokinetics at steady state showed AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, and $C_{avg,ss}$ to be dose proportional (increasing with increasing doses of amprenavir from 300 to 1,200 mg b.i.d.) and CL/F_{ss} to be dose independent. These findings are in contrast to those from a single-dose study in which within-subject dose proportionality was assessed (15). Comparison of the single-dose and the steady-state pharmacokinetic parameters suggests that amprenavir pharmacokinetics are time dependent, especially with respect to C_{max} and AUC at the higher doses. The $AUC_{ss}/AUC_{0\rightarrow\infty}$ ratios decreased linearly with increasing dose (Table 1), and average reductions of 29 and 48% from single dose to steady-state dose were observed for doses of 1,050 mg b.i.d. and 1,200 mg b.i.d., respectively. The median decline in the $AUC_{ss}/AUC_{0\rightarrow\infty}$ ratio between single- and multiple-dose sampling across dose groups was 19.3%, which coincided with a median decline in AAG concentration of 19.8%.

The relationship between AAG and amprenavir concentration was evaluated, since it is known that AAG levels are increased in HIV infection (13) and control of HIV replication with antiretroviral therapy could decrease these levels. The relationship was also investigated to possibly explain the decrease in amprenavir AUC observed after 3 weeks of therapy. A decrease in the AAG concentration would not be expected to produce a decrease in the free amprenavir concentration, and therefore, antiviral activity would not be affected (14). The percent change in AAG, which was almost identical to the percent change in AUC, remained significant in multivariate regression models that included the dose of amprenavir as a variable. Therefore, it seems likely that the decrease in total amprenavir concentration reflects a decrease in the concentration of the principle protein binding molecule.

The lack of dose proportionality in $AUC_{0\rightarrow\infty}$ found with the single-dose pharmacokinetics (observed in the present study and in previous single-dose studies) may be due to saturation of first-pass metabolism and/or P-glycoprotein-mediated transport. In vitro and in vivo studies have shown that the metabolism of amprenavir is mediated primarily by the 3A4 isozyme of the hepatic and intestinal cytochrome P450 system, and its expression can vary considerably between individuals. Amprenavir inhibits CYP3A4 to a degree comparable to that exhibited by indinavir and nelfinavir (Woolley et al., 37th ICAAC), and since 3A4 expression can vary considerably between individuals, it is not surprising to find slight, but not significant, differences in results from study to study. In fact, both the means and medians of pharmacokinetic parameters from a previous study (15) were all within the 95% CI of their respective parameters from the present study. In contrast to the single-dose pharmacokinetics of amprenavir, the steady-state pharmacokinetics of AUC (AUC_{ss}) are dose proportional, suggesting that either first-pass metabolism or P-glycoprotein-mediated transport may be functioning at a higher capacity after multiple dosing.

Although few subject profiles were available to examine the effect of abacavir on amprenavir multiple-dose pharmacokinetics, no effect of the presence of abacavir on amprenavir was observed, and conversely, abacavir pharmacokinetics did not appear to be significantly affected by amprenavir. The lack of an appreciable pharmacokinetic interaction between these drugs was expected, since each is metabolized by different enzymes: abacavir is metabolized primarily by alcohol dehydrogenase and UDP-glucuronyl transferase (J. R. Ravitch, B. J. Bryant M. J. Reese, C. C. Boehlert, J. S. Walsh, J. P. McDowell, and B. M. Sadler, Abstr. 6th Conf. Retroviruses Opportunistic Infect., abstr. 634, 1998). While this does not preclude that induction of UDP-glucuronyl transferase or CYP3A4 has occurred, we did not find evidence of this possible effect. Consequently, these two agents can be administered in combination without adjusting the dosage for either drug.

Analysis of the relationship between steady-state concentrations of amprenavir and decreases in plasma HIV RNA revealed that both $C_{min,ss}$ and $C_{avg,ss}$ were better predictors of the decrease in AAUCMB than was $C_{max,ss}$. C_{min} or AUC has been shown to be significantly associated with the antiviral efficacy or development of resistance with ritonavir, saquinavir, and indinavir (3, 11, 17). Modeling of viral load change versus AUC_{ss} and C_{min} of a subset of the present study's data indicated that C_{min} was a better predictor of antiviral efficacy than was AUC_{ss} (Drusano et al., 37th ICAAC). The $C_{min,ss}$ associated with the amprenavir dose of 1,200 mg b.i.d. would be greater than the IC_{50} of amprenavir as determined from 334 clinical isolates from subjects without prior protease exposure (IC_{50} = 14.6 ± 12.5 ng/ml) (Sadler et al., 7th Conf. Retroviruses Opportunistic Infect.) after adjustment for the 90% protein binding of amprenavir and would be similar to the in vivo trough concentration, estimated in the pharmacodynamic model, providing 90% of the maximum antiviral effect over the 4-week study period (EC_{90} = 0.228 μg/ml). On the basis of findings from the pharmacodynamic model described in this paper, the doses of 900, 1,050, and 1,200 mg b.i.d. were carried forward to phase II studies. The results of the pharmacodynamic model and phase II studies support 1,200 mg b.i.d. as the approved dose of amprenavir.

In summary, the steady-state pharmacokinetics of amprenavir dosed at 1,200 mg b.i.d. indicate that amprenavir can achieve adequate concentrations in plasma in a relatively short time (~2 h) and can maintain adequate trough concentrations. Pharmacodynamic modeling of steady-state parameters supports the selection of the dose of 1,200 mg b.i.d. as being effective in reducing plasma HIV RNA levels.

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