Pharmacokinetic Study of Human Immunodeficiency Virus Protease Inhibitors Used in Combination with Amprenavir

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In an open-label, randomized, multicenter, multiple-dose pharmacokinetic study, we determined the steady-state pharmacokinetics of amprenavir with and without coadministration of indinavir, nelfinavir, or saquinavir soft gel formulation in 31 human immunodeficiency virus type 1-infected subjects. The results indicated that amprenavir plasma concentrations were decreased by saquinavir soft gel capsule (by 32% for area under the concentration-time curve at steady state [AUC_{ss}] and 37% for peak plasma concentration at steady state [$C_{max,ss}$]) and increased by indinavir (33% for AUC_{ss}). Nelfinavir significantly increased amprenavir minimum drug concentration at steady state (by 189%) but did not affect amprenavir AUC_{ss} or $C_{max,ss}$. Nelfinavir and saquinavir steady-state pharmacokinetics were unchanged by coadministration with amprenavir compared with the historical monotherapy data. Concentrations of indinavir, coadministered with amprenavir, in plasma decreased in both single-dose and steady-state evaluations. The changes in amprenavir steady-state pharmacokinetic parameters, relative to those for amprenavir alone, were not consistent among protease inhibitors, nor were the changes consistent with potential interactions in CYP3A4 metabolism or P-glycoprotein transport. No dose adjustment of either protease inhibitor in any of the combinations studied is needed.

In vivo inhibition of human immunodeficiency virus (HIV) replication via combination antiretroviral therapy can be achieved with drugs targeting different HIV enzymes (i.e., reverse transcriptase and protease) or with drugs targeting the same viral enzyme (i.e., reverse transcriptase or protease). Although the combined use of HIV reverse transcriptase and protease inhibitors in HIV-infected individuals has been associated with significant virologic and clinical benefits (2, 3, 8, 15; British HIV Association Guidelines for the Treatment of HIV Disease with Antiretroviral Therapy [http://www.bhiva.org] and Department of Health and Human Services and Henry J. Kaiser Family Foundation Panel on Clinical Practices for the Treatment of HIV Infection, Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents [http://www.hivatis.org]), the high anti-HIV potency of the protease inhibitors provides a rationale for examining the clinical utility of dual protease inhibitor combinations.

The success of a dual protease inhibitor combination approach, however, may depend on potential drug-drug interactions. All of the currently available HIV protease inhibitors are metabolized in the liver and gastrointestinal tract, primarily by the cytochrome P450 system (1). In addition, all protease inhibitors are substrates for transport by the P-glycoprotein drug transport protein (10, 11). The actions of each protease inhibitor in a dual combination regimen on the pharmacokinetics of the partner protease inhibitor must be assessed to determine whether drug-drug interactions that affect plasma drug concentrations will occur and whether two specific protease inhibitors can be safely coadministered.

Amprenavir (Agenerase, formerly 141W94) is a potent HIV protease inhibitor that was originally synthesized using a structure-based drug design process (9). The mean 50% inhibitory concentration of amprenavir against 334 HIV clinical isolates is 29 nM (19). Amprenavir binds to proteins in normal human plasma or serum to the extent of ~90%, with the greatest degree of binding to α_1-acid-glycoprotein (AAG) (89%) and albumin (42%) (12). The metabolism of amprenavir, like that of the other approved HIV protease inhibitors, appears to be primarily dependent upon the 3A4 isozyme of the hepatic cytochrome P450 system (CYP3A4), based on in vitro and in vivo studies (6; J. Woolley, S. Studenberg, C. Boehlert, G. Bowers, A. Sinhababu, and P. Adams, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-60, 1997). These and other drug interaction studies have shown that amprenavir inhibits CYP3A4 to a degree comparable to that exhibited by indinavir and nelfinavir.

We report here the steady-state pharmacokinetics of amprenavir administered alone and as part of three dual protease inhibitor combinations with indinavir, nelfinavir, or saquinavir soft gel capsule (sgc) formulation in protease inhibitor-naïve, HIV-infected adults (Glaxo Wellcome protocol PROA2001). The pharmacokinetics of each partner protease inhibitor used in combination with amprenavir were also assessed and compared with historical monotherapy data for each drug as indicated: nelfinavir (B. Kerr, personal communication), indinavir (Merck & Co., complete prescribing information for indinavir [Crixivan]), and saquinavir (16). Preliminary data from this study have been previously presented (B. M. Sadler, J. Eron, J. Wakeford, G. Pagano, C. Rawls, J. McCrea, K. Mazina, and P. J. Deutsch, Abstr. 37th Intersci. Conf. Antimicrob. Agents

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Chemother., abstr. A-56, 1997; B. Sadler, C. Gillotin, G. E. Chittick, and W. T. Symonds, Abstr. 12th World AIDS Conf., abstr. 12389, 1998). This 3-week, phase I trial was part of a larger 48-week phase I-II study to explore the antiviral effect and evaluate the long-term safety and tolerability of amprenavir-containing dual protease inhibitor therapy (7). The pharmacokinetic interactions of ritonavir and amprenavir were studied separately (19).

Study population. The study entry criteria were per the work of Eron et al. (7). Briefly, subjects were enrolled in the study if they were ≥18 years of age and were HIV-seropositive adults with $CD4^+$ cell counts of ≥200 cells/mm^3 and plasma HIV RNA levels of >10,000 copies/ml. Subjects were excluded from the study if they had prior treatment with a protease inhibitor or received any antiretroviral therapy within 2 weeks prior to enrollment. Subjects with malabsorption, acute opportunistic infections, or standard laboratory values outside of prespecified ranges were excluded. Women of childbearing potential were required to have a negative serum human chorionic β-gonadotropin test within 7 days of the start of dosing. Prior antiretroviral therapy (with drugs other than protease inhibitors) was permitted, but subjects were required to discontinue antiretroviral therapy 2 weeks prior to enrollment. All subjects provided written informed consent to participate in the study. All subjects were monitored for clinical adverse experiences and/or abnormal laboratory test findings throughout the study period, including the follow-up evaluation.

Study design and drug administration. A single-dose, phase IA interaction study of amprenavir and indinavir was conducted prior to the start of the 3-week, phase I multiple-dose study of the dual protease inhibitor combinations. The single-dose study was carried out to determine the pharmacokinetics of amprenavir and indinavir when administered in combination, to assess whether pharmacokinetic interactions exist between amprenavir and indinavir when administered as a single-dose combination, and to determine the dose of indinavir to be used in the later multiple-dose phases (phases I and II) of this study. Subjects were given a single oral dose of indinavir (800 mg) and amprenavir (800 mg) simultaneously, after an overnight fast of at least 8 h and 1.5 h before a meal.

This phase I study was an open-label, randomized, multicenter, multiple-dose trial of amprenavir, alone and in dual combination with indinavir, nelfinavir, and saquinavir sgc. HIV type 1 (HIV-1)-positive subjects who met the study entry criteria were enrolled and randomly assigned to one of the following four treatment groups: amprenavir plus indinavir (800 mg), amprenavir plus nelfinavir (750 mg), amprenavir plus saquinavir sgc (800 mg), or amprenavir alone. All treatments were administered three times daily, and no concomitant nucleoside therapy was allowed for the first 3 weeks of the study.

Although amprenavir is approved as a twice-daily regimen, it was administered on a three-times-a-day dosing schedule for the convenience of the participants (as the partner protease inhibitors had to be administered as a three-times-a-day medication). Initially, the amprenavir dose was 800 mg but was reduced to 750 mg during the study secondary to a change in amprenavir formulation. All but three subjects received the 800-mg dose at the time of their pharmacokinetics evaluation. At the time of the study, on recommendation of the drug's manufacturer, Roche Laboratories, saquinavir sgc was given as 800 mg, rather than the now-approved 1,200 mg, three times daily. Subjects assigned to receive nelfinavir or saquinavir sgc were instructed to take both study medications with food; those assigned to receive indinavir or amprenavir monotherapy were instructed to take both study medications either 1 h before or 2 h after a meal. Subjects assigned to receive indinavir were also instructed to drink at least 2 liters of water per day. Subjects were provided with a standard breakfast meal that consisted of 58 g of carbohydrate, 33 g of protein, and 67 g of fat.

Subjects were instructed to fast overnight (at least 8 h) from the evening prior to the scheduled morning dosing and sampling; subjects' morning dose was to be postponed until arrival at the clinic for sampling. Although the use of medically necessary concomitant medications was permitted, it was advised that medications metabolized by CYP3A4 not be used because of the potential for serious and/or life-threatening adverse experiences. Such contraindicated medications included terfenadine, astemizole, cisapride, triazolam, midazolam, and ergotamine- and/or dihydroergotamine-containing regimens. Chemoprophylactic agents for HIV-related conditions were permitted. The study protocol was approved by the Institutional Review Board affiliated with each study center.

Plasma sampling. Blood samples were collected on the first day of dosing to determine the single-dose indinavir and amprenavir pharmacokinetic parameters (phase IA) and at the week 2 study visit to determine steady-state pharmacokinetic parameters for amprenavir, indinavir, nelfinavir, and saquinavir. Blood samples were collected at predose and then at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 8 h postdose. For amprenavir, blood was collected in EDTA-containing tubes, and for indinavir, nelfinavir, and saquinavir sgc, blood was collected in sodium heparin tubes. All plasma samples were stored frozen at −20°C until analysis. Plasma sampling for AAG was conducted at weeks 1, 2, and 3.

Safety evaluations. Safety and tolerability were evaluated at predose; day 1; and weeks 1, 2, 3, and 4 and every 4 weeks thereafter. Evaluations were conducted by physical examination, vital signs, electrocardiogram, hematology, clinical chemistry, urinalysis, and monitoring for clinical adverse experiences.

Assays for protease inhibitors. Concentrations of amprenavir in plasma were determined using cross-validated isocratic reversed-phase chromatography with fluorescence detection as previously described (18) and an automated high-performance liquid chromatography method with detection by tandem mass spectrometry. Concentrations of indinavir, saquinavir, and nelfinavir in plasma were analyzed by specific, validated high-performance liquid chromatography assays (22, 23; document no. RD1998/01807/00, RD1998/00615/00, and RD1998/01806/00, Glaxo Wellcome Inc.) with UV detection conducted by the following laboratories: Oneida Research Services, Whitesboro, N.Y. (saquinavir); BAS Analytics, West Lafayette, Ind. (indinavir); and PPD-Pharmaco, Richmond, Va. (nelfinavir and M8-nelfinavir). Calibration curves for indinavir in plasma were linear for the concentration range of 5 to 500 ng/ml. The intraday precision ranged from 1.8 to 6.3%, the interday precision was 1.8 to 2.8%, and the interday accuracy ranged from 91.2 to 94.5%. For saquinavir, calibration curves were linear for the concentration range of 10 to 350 ng/ml. Interday pre-

cision was 8.62%, and the interday accuracy ranged from 99.88 to 106.38%. Intraday precision was 8.02%, and the intraday accuracy ranged from 96.66 to 116.18%. The calibration curves for nelfinavir were linear for the concentration range of 0.05 to 10 μg/ml. The intraday precision was 1.53 to 7.21%, and the intraday accuracy was 16%, while the interday precision was 2.32 to 5.43%, and the interassay accuracy was 9.75%.

Pharmacokinetic analyses. Model-independent pharmacokinetic parameters for single and multiple oral dosing were calculated using WinNonlin Pro, version 1.5 (Scientific Consulting, Inc., Cary, N.C.). The peak plasma concentration (C_{max}), the time to reach peak plasma concentration (T_{max}), the peak plasma concentration at steady state ($C_{max,ss}$), and the time to reach maximum plasma concentration ($T_{max,ss}$) were observed from the individual plasma concentration-time data. The minimum drug concentration at steady state ($C_{min,ss}$) was calculated as $(C_0 + C_t)/2$, where C_0 is the plasma concentration before the last dose and C_t is the plasma concentration of the last steady-state dosing interval. The area under the plasma concentration-time curve ($AUC_{0 \to t}$) from the time of the predose sample to the time of the last sample was calculated using the linear trapezoidal rule. When necessary, $AUC_{0 \to t}$ was extrapolated to steady-state (AUC_{ss}) by adding $C_t/\lambda_z[1 - e^{-\lambda_z(\tau - t)}]$ to $AUC_{0 \to t}$, where t is the time of the last plasma concentration sample during the steady-state dosing interval, λ_z is the elimination rate constant, and τ is the length of the steady-state dosing interval. The apparent total clearance (CL/F) was calculated as dose/AUC_{ss}.

Statistical analyses. Only descriptive statistical analysis was performed for phase IA. The mean amprenavir AUC and mean indinavir $AUC_{0 \to 8 h}$ and C_{max} were compared with their respective historical control values. The 95% confidence intervals (CI) of the means were calculated and not considered different from their historical values if the mean used as the reference fell within the limit of the 95% CI. The single-dose historical pharmacokinetic data for indinavir monotherapy was from the work of Yeh et al. (24); that for amprenavir monotherapy, in which a single dose of 900 mg was administered, was from two studies (17, 18). Values obtained from the two amprenavir studies were recalculated to reflect the $AUC_{0 \to 8 h}$ only and normalized to an 800-mg dose for comparison purposes in this study.

AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, and CL/F for all protease inhibitors were analyzed after logarithmic transformation. Pharmacokinetic parameters for subjects who received 750 mg of amprenavir were normalized to an 800-mg dose prior to descriptive summarization and statistical analyses. The pharmacokinetic parameters of each protease inhibitor in a dual therapy regimen were compared to those of each drug administered as a single agent. For amprenavir, the monotherapy group of this study was used as the single-agent comparator; for indinavir, nelfinavir, and saquinavir, historical values were used as the single-agent comparator. The calculated arithmetic mean or geometric least squares (GLS) mean values for protease inhibitors in combination obtained in this study were considered not different from single-agent values if the latter values fell within the limit of the 95% CI.

Analyses of variance, considering treatment as fixed effects, were performed using the mixed linear model procedure (SAS PROC MIXED, version 6.12; SAS Institute, Cary, N.C.). De-

TABLE 1. Amprenavir pharmacokinetic parameters and treatment comparisons[a]

Parameter	Value for treatment:					Treatment comparison ratio		
	SQV + APV (n = 7)	IDV + APV (n = 9)	NFV + APV (n = 6)	APV (n = 9)	APV + IDV[b] (single dose)	SQV + APV/APV	IDV + APV/APV	NFV + APV/APV
AUC_{ss} (μg · h/ml)	10.52 (8.14–13.59)	20.46 (16.33–25.65)	16.76 (12.71–22.11)	15.39 (12.28–19.29)		0.68[c] (0.51–0.91)	1.33[c] (1.02–1.73)	1.09 (0.81–1.47)
$AUC_{0 \to \infty}$ (μg · h/ml)					25.32[d] (21.36–29.29)			
$C_{max,ss}$ (μg/ml)	4.23 (3.18–5.64)	7.93 (6.16–10.22)	5.78 (4.24–7.88)	6.74 (5.23–8.68)		0.63[c] (0.46–0.86)	1.18 (0.87–1.58)	0.86 (0.62–1.20)
C_{max}[b] (μg/ml)					7.16[d] (4.49–9.83)			
$C_{min,ss}$ (μg/ml)	0.27 (0.16–0.45)	0.39 (0.25–0.62)	0.90 (0.48–1.67)	0.31 (0.20–0.49)		0.86 (0.48–1.54)	1.25 (0.73–2.16)	2.89[c] (1.52–5.48)
$T_{max,ss}$ (h)	1.13 (0.50–1.75)	1.00 (0.75–1.25)	0.75 (0.50–1.25)	1.14 (0.75–2.00)		0.00 (−0.50–0.50)	−0.03 (−0.50–0.17)	−0.33[c] (−1.00–0.00)
T_{max} (h)	1,267 (981–1,637)	652 (520–817)	796 (603–1,049)	866 (691–1,086)		1.46[c] (1.10–1.94)	0.75[c] (0.58–0.98)	0.92 (0.68–1.24)
CL/F (ml/min)								

[a] Values are GLS means (95% CI) for pharmacokinetic parameters and GLS mean ratios (90% CI) for treatment comparisons, except for T_{max}, for which values are medians (nonparametric; 95% CI) for pharmacokinetic parameters and median differences (nonparametric; 90% CI) for treatment comparisons. Saquinavir (SQV) and nelfinavir (NFV) were given with food, while indinavir (IDV) and amprenavir (APV) were given under fasting conditions.
[b] Single-dose data were assessed only for indinavir plus amprenavir.
[c] Significantly different ($P \leq 0.05$).
[d] The amprenavir reference normalized mean for a single-dose comparison from reference 17 is 21.49 μg · h/ml.

TABLE 2. Pharmacokinetic parameters and historical references for indinavir, nelfinavir, and saquinavir[a]

Inhibitor(s) and type of valve	AUC_{ss} ($\mu g \cdot h/ml$)	$AUC_{0\rightarrow\infty}$ ($\mu g \cdot h/ml$)	$C_{max,ss}$ ($\mu g/ml$)	C_{max} ($\mu g/ml$)	$C_{min,ss}$ ($\mu g/ml$)	$T_{max,ss}$ (h)	CL/F (ml/min)
SQV + APV	2.58 (0.15–5.01)		1.15 (0.12–2.18)		0.071 (0.01–0.14)	1.0 (0.56–2.18)	12,854 (871–22,837)
Reference value (16)	3.21		0.95		0.14	2	4,237
Ratio	0.81		1.21		0.52	0.68	3.03
IDV + APV	13.63 (10.79–16.47)		7.01 (6.22–7.81)		0.13 (0.09–0.17)	0.75 (0.70–0.98)	1,052 (805–1,298)
Reference value[b]	21.85		8.98		0.18	0.80	610
Ratio	0.62		0.78		0.73	1.05	1.72
NFV + APV	20.03 (10.83–29.23)		3.58 (2.10–5.05)		1.70 (0.47–2.93)	3.63	722 (409–1,034)
Reference value[c]	17.4		3.18		1.49	—[d]	718
Ratio	1.15		1.12		1.14		1.00
IDV + APV (single dose)		11.18 (7.63–14.73)		5.50 (3.77–7.24)			
Reference value (24)		17.11		7.17			
Ratio		0.65		0.77			

[a] Values for pharmacokinetic parameters are means (95% CI), except for T_{max}, for which values are medians (nonparametric; 95% CI). SQV, saquinavir; IDV, indinavir; NFV, nelfinavir; APV, amprenavir.
[b] Merck & Co., complete prescribing information for indinavir.
[c] B. Kerr, personal communication.
[d] —, reference value unavailable.

scriptive summary statistics, GLS means, and associated 95% CI were calculated for each treatment. The ratio of the GLS means and associated 90% CI for AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, and CL/F was obtained using two one-sided tests. The analysis of $T_{max,ss}$ was performed using standard nonparametric methods, and the 95% CI for median $T_{max,ss}$ was computed. Treatment comparison of $T_{max,ss}$ was performed using a Wilcoxon rank sum test. Estimates of the median difference between treatments (combination versus alone for each protease inhibitor) and associated 90% CI were calculated. An analysis of covariance considering the fixed effects of treatment, race, weight, age, AAG, albumin, bilirubin, and HIV risk factors (i.e., injection drug use versus all other risk factors) was performed on amprenavir pharmacokinetic parameters after log transformation.

Subject demographics and accountability. This study was conducted between January 1997 and October 1998 at three centers in the United States. A total of 34 HIV-infected subjects were enrolled in phase I and randomized to receive study medication. Of these 34 subjects, 12 (all from one center) participated in phase IA and all 12 were included in the single-dose pharmacokinetic analysis. Of the 33 subjects who initiated study treatment in phase I (one subject randomized to the amprenavir-alone group withdrew from the study before receiving the first dose), 31 were included in the multiple-dose pharmacokinetic analysis. One of the subjects not included in the analysis withdrew consent at week 3, and the other subject was lost to follow-up at week 2.

No significant differences in baseline characteristics were apparent among the treatment groups, and overall, 62% were white and 76% were male, with the median age being 38 years, the median CD4 cell count being 393 cells/mm^{3}, and the median $\log_{10}$ HIV RNA value being 4.74—although the amprenavir-saquinavir sgc group had higher percentages of subjects who were older, black (50%), female (50%) and who reported heterosexual contact as an HIV risk factor (7). Most subjects (79%) were asymptomatic (Centers for Disease Control and Prevention classification A), and only one subject had a diagnosis of AIDS (Centers for Disease Control and Prevention classification C, in the amprenavir-alone group) (4). The ma-

jority of subjects (62%) had received prior antiretroviral therapy.

Single-dose amprenavir-indinavir pharmacokinetic interactions (phase IA). Coadministration of amprenavir and indinavir produced an increase in the mean amprenavir $AUC_{0\rightarrow8\ h}$ relative to the historical amprenavir monotherapy data: 25.32 versus 21.49 $\mu g \cdot h/ml$ (17, 18) (see Table 1). This change in mean amprenavir $AUC_{0\rightarrow8\ h}$ represents an increase of 18% over that observed for amprenavir alone (18). Coadministration of amprenavir and indinavir produced a decrease in both indinavir $AUC_{0\rightarrow8\ h}$ (35%) and C_{max} (23%) compared with historical indinavir monotherapy data (24). Only the decrease in indinavir C_{max} was statistically different from indinavir control data (see Table 2).

Phase I safety and tolerability. No serious adverse events occurred during the study. A full description of the safety findings is given in the work of Eron et al. (7).

Effect of multiple-dose indinavir, nelfinavir, or saquinavir sgc on amprenavir pharmacokinetics. The steady-state pharmacokinetic values obtained for amprenavir in dual combination with indinavir, nelfinavir, or saquinavir sgc and amprenavir alone after multiple oral doses are presented in Table 1. The GLS mean ratios for AUC_{ss} (0.68 [90% CI, 0.51 to 0.91]) and $C_{max,ss}$ (0.63 [90% CI, 0.46 to 0.86]) between the saquinavir sgc-amprenavir combination and amprenavir alone are significantly different, as are the GLS means for AUC_{ss} between the indinavir-amprenavir combination and amprenavir alone, which increased by 33% (90% CI, 1.02 to 1.73; ratio, 1.33). The GLS mean ratio for $C_{min,ss}$ of the nelfinavir-amprenavir combination was also significantly increased by 2.89-fold (90% CI, 1.52 to 5.48) compared to the amprenavir-alone treatment group.

Effect of multiple-dose amprenavir on indinavir, nelfinavir, or saquinavir sgc pharmacokinetics. The steady-state pharmacokinetics of indinavir, nelfinavir, and saquinavir, estimated from subjects treated with each of these protease inhibitors in dual combination regimens with amprenavir, are presented in Table 2. Also given in the table are historical values of steady-state pharmacokinetic parameters for each of the protease inhibitors administered as monotherapy. Compared with indi-

navir-alone historical data, amprenavir was found to decrease the AUC_{ss}, $C_{max,ss}$, and $C_{min,ss}$ of indinavir by 38, 22, and 27%, respectively, and to increase the CL/F by 72% (Table 2). The decreases in indinavir AUC_{ss}, $C_{max,ss}$, and $C_{min,ss}$ and the increase in CL/F produced by coadministration with amprenavir were statistically different from historical values (indinavir alone), as indicated by historical reference values falling outside the 95% CI range of the means of these pharmacokinetic parameters for the indinavir and amprenavir combination. None of the pharmacokinetic parameter values for either saquinavir sgc or nelfinavir obtained from the historical studies fell outside the 95% CI range observed in this study.

AAG, treatment, and amprenavir pharmacokinetic parameters. Analysis of covariance revealed that, of the factors evaluated (AAG, age, albumin, bilirubin, HIV risk factor, race, treatment, and weight), only serum AAG concentrations and protease inhibitor coadministration treatment had a statistically significant effect on amprenavir steady-state pharmacokinetic parameters. AAG concentrations significantly influenced amprenavir AUC_{ss} ($P = 0.004$) and $C_{max,ss}$ ($P = 0.026$). Treatment also had a statistically significant effect on amprenavir AUC_{ss} ($P = 0.004$) and $C_{max,ss}$ ($P = 0.015$).

This pharmacokinetic evaluation of amprenavir-containing dual protease inhibitor regimens compared amprenavir steady-state pharmacokinetics calculated from subjects receiving amprenavir monotherapy with those from subjects receiving amprenavir in combination with another protease inhibitor. Historical monotherapy data for indinavir, nelfinavir, and saquinavir sgc were used as reference values for the steady-state pharmacokinetics of the partner protease inhibitor in each combination. The concurrent study design employed was used to avoid unnecessarily exposing subjects to protease inhibitor monotherapy (i.e., by a crossover design) which could potentially facilitate the development of drug resistance.

Amprenavir is approved as a twice-daily regimen, but given the three-times-a-day dosing schedule of the partner protease inhibitors, amprenavir was instructed to be taken three times daily in this study to simplify logistics and for the convenience of the participants. To characterize any drug-drug interactions after multiple dosing, steady-state pharmacokinetic data for the individual protease inhibitors in each of the dual protease inhibitor combinations were compared with the steady-state data for each protease inhibitor given alone. The decrease in amprenavir AUC_{ss} that occurred when amprenavir was coadministered with saquinavir sgc may prove to be clinically relevant. Several studies of HIV protease inhibitors have shown that AUC_{ss} and $C_{min,ss}$ are related to antiviral activity or drug resistance (4, 5, 13, 14, 21; 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; D. S. Stein, Y. Lou, M. Johnson, and S. Randall for the PROB2004 Study Team, Abstr. 2nd Int. Workshop Clin. Pharmacol. HIV Ther., no. 5.6, 2001); therefore, the lower amprenavir AUC_{ss} observed with the amprenavir-saquinavir sgc regimen could theoretically lead to reduced antiretroviral efficacy and/or the development of resistance. It is not possible to definitely attribute the decrease to an amprenavir-saquinavir sgc interaction, since food was a potential confounding factor. All amprenavir-saquinavir sgc doses were given with

food in this study, and the pharmacokinetics of the currently marketed amprenavir formulation (150-mg capsule) have been noted to be modestly affected by a high-fat meal, resulting in decreased amprenavir concentrations of approximately 25% (Glaxo Wellcome, complete prescribing information for amprenavir [Agenerase]).

The significant increase in amprenavir $C_{min,ss}$ (189% or 2.9-fold) produced by nelfinavir was not accompanied by such a large increase in $C_{max,ss}$ or AUC_{ss}. Amprenavir $C_{max,ss}$ actually decreased, although not significantly, with nelfinavir coadministration; however, as discussed above, this could have been confounded by a possible food effect. The large increase in amprenavir $C_{min,ss}$ could result in heightened antiviral activity, but longer-term evaluations of efficacy and safety are needed to determine whether this is indeed true. These changes in amprenavir concentrations produced by nelfinavir could result from a complex interaction of binding to plasma proteins—both amprenavir and nelfinavir are highly bound to the same plasma proteins, AAG and albumin—or to changes in distribution and/or metabolism. Another small study has also indicated a effect similar to what we observed from nelfinavir coadministration on amprenavir pharmacokinetics (S. Piscitelli, C. Bechtel, B. Sadler, and J. Falloon, 7th Conf. Retroviruses Opportunistic Infect., abstr. 78, 2000).

Indinavir coadministration (in the fasting condition, in contrast to the other two partner protease inhibitors) produced only a 33% increase in amprenavir AUC_{ss}. This finding, when considered together with an 18% increase in $C_{max,ss}$ and a 25% increase in $C_{min,ss}$, suggests that these changes in plasma amprenavir concentrations are unlikely to be clinically relevant. The nelfinavir and saquinavir sgc steady-state pharmacokinetic parameters obtained in this study were not different from those previously reported for nelfinavir alone and saquinavir sgc alone, as indicated by the finding that the historical reference values were within the range of the 95% CI of the means of the parameters obtained in this study (Table 2). However, although amprenavir coadministration did not have an effect on nelfinavir or saquinavir sgc steady-state pharmacokinetic parameters, amprenavir coadministration did appear to affect indinavir $C_{max,ss}$, $C_{min,ss}$, AUC_{ss}, and CL/F compared with historical data. Amprenavir coadministration resulted in a decrease in indinavir $C_{max,ss}$, $C_{min,ss}$, and AUC_{ss} and an increase in indinavir CL/F. These changes are not due to induction of hepatic metabolism or P-glycoprotein transport, since a single dose of amprenavir in the same patients had a similar effect on indinavir pharmacokinetics. A possible explanation for the observed decreases is the lipid-like formulation of amprenavir, which could affect indinavir pharmacokinetics in a manner similar to that of a food effect. It has been previously reported that a high-calorie, high-fat meal significantly decreases indinavir C_{max} and AUC by 86 and 78%, respectively (24).

The statistical analysis of the relationship between various fixed effects (such as AAG, age, albumin, and race) and amprenavir pharmacokinetic parameters revealed that the coadministered protease inhibitor treatment and AAG levels were the only variables that significantly influenced amprenavir pharmacokinetics. After controlling for AAG concentrations, no statistically significant difference in amprenavir AUC_{ss}, $C_{max,ss}$, $C_{min,ss}$, and CL/F was noted between blacks ($n = 11$) and nonblacks ($n = 23$). Gender was not evaluated because

two treatment groups had only one female each. The finding of a significant treatment effect by the partner protease inhibitor indicates that each of the protease inhibitors had different effects on amprenavir steady-state pharmacokinetics. AAG concentrations were significantly correlated with amprenavir steady-state pharmacokinetics. Decreasing AAG concentrations, as would occur with suppressive HIV therapy, were associated with decreasing total concentrations of amprenavir (i.e., protein-bound and unbound drug). Like most HIV protease inhibitors, amprenavir exhibits a high degree of high-affinity binding to AAG (~90%) (12). Changes in AAG, while affecting the measured total amprenavir concentration, are not believed to affect the unbound amprenavir concentration, since clearance of unbound drug (i.e., intrinsic clearance) is unchanged (20).

The present study was designed to evaluate the pharmacokinetics and short-term safety of multiple-dose, dual protease inhibitor therapy in protease inhibitor-naïve, HIV-infected subjects. Steady-state pharmacokinetic data for all four protease inhibitors in the three dual protease inhibitor combinations obtained in this study indicate that no drug interactions preclude the use of any of the combinations and suggest that further investigation of the dual protease inhibitor regimens as an antiretroviral therapy strategy is warranted. The results of this study have supported continued treatment of these subjects in the phase I-II follow-on of this study to evaluate longer-term safety and efficacy of the amprenavir-containing dual HIV protease inhibitor regimens.

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