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Pharmacokinetic and Safety Evaluation of High-Dose Combinations of Fosamprenavir and Ritonavir

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High-dose combinations of fosamprenavir (FPV) and ritonavir (RTV) were evaluated in healthy adult subjects in order to select doses for further study in multiple protease inhibitor (PI)-experienced patients infected with human immunodeficiency virus type 1. Two high-dose regimens, FPV 1,400 mg twice a day (BID) plus RTV 100 mg BID and FPV 1,400 mg BID plus RTV 200 mg BID, were planned to be compared to the approved regimen, FPV 700 mg BID plus RTV 100 mg BID, in a randomized three-period crossover study. Forty-two healthy adult subjects were enrolled, and 39 subjects completed period 1. Due to marked hepatic transaminase elevations, predominantly with FPV 1,400 mg BID plus RTV 200 mg BID, the study was terminated prematurely. For FPV 1,400 mg BID plus RTV 100 mg BID, the values for plasma amprenavir (APV) area under the concentration-time profile over the dosing interval (τ) at steady state [$AUC_{(0\text{-}\tau)}$], maximum concentration of drug in plasma (C_{max}), and plasma concentration at the end of τ at steady state (C_τ) were 54, 81, and 26% higher, respectively, and the values for plasma RTV $AUC_{(0\text{-}\tau)}$, C_{max}, and C_τ were 49% higher, 71% higher, and 11% lower, respectively, than those for FPV 700 mg BID plus RTV 100 mg BID. For FPV 1,400 mg BID plus RTV 200 mg BID, the values for plasma APV $AUC_{(0\text{-}\tau)}$, C_{max}, and C_τ were 26, 48, and 32% higher, respectively, and the values for plasma RTV $AUC_{(0\text{-}\tau)}$, C_{max}, and C_τ increased 4.15-fold, 4.17-fold, and 3.99-fold, respectively, compared to those for FPV 700 mg BID plus RTV 100 mg BID. FPV 1,400 mg BID plus RTV 200 mg BID is not recommended due to an increased rate of marked hepatic transaminase elevations and lack of pharmacokinetic advantage. FPV 1,400 mg BID plus RTV 100 mg BID is currently under clinical evaluation in multiple PI-experienced patients.

The use of low-dose ritonavir (RTV) in combination with human immunodeficiency virus type 1 (HIV-1) protease inhibitors (PIs) is routine in the treatment of HIV-1 infection, especially in treatment-experienced subjects (2, 3). Heavily treatment-experienced patients, especially patients with PI mutations, may benefit from higher plasma HIV-1 PI exposure than that achieved with the standard RTV-boosted dosage regimens in order to overcome resistance and maximize efficacy. For example, higher-than-approved dose combinations of lopinavir-ritonavir demonstrated antiviral activity in patients with multiple prior antiretroviral regimens (D. Podzamczer, R. Tressler, C. Flexner, C. Katlama, D. Havlir, S. Letendre, J. Eron, L. Weiss, J. Gatell, A. Simon, E. Ferrer, M. King, R. Bertz, K. Robinson, and S. Brun, Abstr. XV Inter. AIDS Conf., abstr. TuB4555, 2004).

Fosamprenavir (FPV) calcium is the phosphate ester prodrug of the HIV-1 PI amprenavir (APV). FPV has demonstrated antiviral efficacy, durability, and tolerability in antiretroviral therapy-naïve and PI-experienced subjects (4, 10; R.C. Elston, P. Yates, M. Tisdale, N. Richards, S. White, and E. DeJesus, Abstr. XV Inter. AIDS Conf., abstr. MoOrB1055, 2004). FPV 700 mg twice a day (BID) plus RTV 100 mg BID is approved for the treatment of HIV-1 PI-experienced patients.

The results from previous studies conducted with APV at lower doses indicated that increasing the dose of RTV and keeping the same dose of APV did not increase plasma APV exposure (12; S. Piscitelli, S. Bechtel, B. Sadler, J. Falloon, and the Intramural AIDS Program, Abstr. 7th Conf. Retroviruses and Opportunistic Infect., abstr. 78, 2000). However, plasma APV pharmacokinetic data following the coadministration of the APV prodrug, FPV, and RTV at doses higher than the standard regimen were not available. Therefore, this study evaluated two strategies to increase plasma APV exposure, including doubling the dose of FPV that is coadministered with RTV and doubling the doses of both FPV and RTV, in healthy subjects. Doses for further study in multiple PI-experienced, HIV-1-infected patients were to be selected by evaluating the safety and pharmacokinetics of these high-dose combinations in healthy adults.

MATERIALS AND METHODS

Study design. A phase 1, open-labeled, randomized, balanced, three-period, crossover, steady-state pharmacokinetic study was planned in healthy male and female adult subjects. The planned study design is shown in Table 1. Forty-two subjects were randomized to one of six treatment sequences, each consisting of three periods, in which the subjects were to receive FPV 700 mg BID plus RTV 100 mg BID (treatment A), FPV 1,400 mg BID plus RTV 100 mg BID (treatment B) and FPV 1,400 mg BID plus RTV 200 mg BID (treatment C). All study treatments were to be dosed BID for 14 days with a washout period of 21 to 28 days between treatments. Serial pharmacokinetic profiles were evaluated on day 14, when the morning dose was administered after a 10-h fast and fasting was maintained for 4 h after dosing. Serial blood samples were collected for APV and RTV plasma concentrations at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h postdose on day 14. Predose samples were collected for APV and RTV plasma concentrations on days 11, 12, 13, and 14. Samples were processed within 1 h of collection, and plasma was stored at −20°C or lower. Adverse events (AEs), vital signs, and clinical laboratory tests were obtained throughout each treatment period.

Prospective criteria for the discontinuation of study drug for an individual subject included an alanine aminotransferase (ALT) or aspartate aminotrans-

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TABLE 1. Planned study design

Treatment sequence[b]	Study treatment (days 1 to 14) for[a]:		
	Period 1	Period 2[c]	Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	B	A	C
6	C	B	A

[a] Treatment A was FPV 700 mg BID plus RTV 100 mg BID, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID. A 21- to 28-day washout period occurred between each two periods.
[b] There were seven subjects in each sequence.
[c] The study was prematurely terminated on day 9 of period 2.

ferase (AST) value of at least three times the upper limit of normal (×ULN) (confirmed with repeat measurement), any single ALT or AST value of at least five ×ULN, or any single ALT or AST measurement of at least two ×ULN in conjunction with a total bilirubin of more than the ULN (>1.6 mg/dl). Furthermore, if five or more subjects were withdrawn according to these criteria, the study would be terminated. All subjects provided written informed consent, and the protocol was approved by the Institutional Review Board of PPD Development, Research Consultants' Review Committee in Austin, TX.

Bioanalytical methods. Samples were analyzed for APV and RTV concentrations using a validated high-performance liquid chromatography with tandem mass spectrometry detection method following solid-phase extraction. The calibration range of the method was 10 to 10,000 ng/ml for both APV and RTV. For APV and RTV concentrations, the average accuracy (percent bias) was less than or equal to −4.3 and 2.5, respectively, and the average precision (percent coefficient of variation) was less than or equal to 5.8 and 5.6, respectively.

Pharmacokinetic analyses. A noncompartmental pharmacokinetic analysis of concentration-time data was performed by standard methods with WinNonlin Professional software version 4.1 (Pharsight Corporation, Mountain View, CA). Actual sample collection times were used in the pharmacokinetic analysis. The following plasma pharmacokinetic parameters were calculated for each treatment: maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}), area under the plasma concentration-time profile over the dosing interval (τ) at steady state [$AUC_{(0\text{-}\tau)}$], and plasma concentration at the end of τ at steady state (C_τ). The AUC was calculated using the linear-up-log-down trapezoidal rule. The C_τ was calculated as the average of the predose concentrations on days 11, 12, 13, and 14.

Statistical analyses. Assuming an intrasubject standard deviation of 0.29 (maximum value for steady-state plasma APV pharmacokinetic parameters from unpublished studies), 30 evaluable subjects were calculated to provide 90% of the power to detect a 25% difference in $AUC_{(0\text{-}\tau)}$, C_{max}, or C_τ using a two-sided test at alpha equals 0.05. This 25% difference was chosen because it represents the minimum increase in plasma APV exposure that might be clinically relevant. To account for possible dropouts, 42 subjects were enrolled in one of six sequences.

Subjects who took at least one dose of study drug were included in the safety population for safety analyses. To utilize standard grading criteria, ALT and AST results were summarized according to ACTG grading criteria. These categories did not exactly correspond to the individual discontinuation criteria.

In addition, the change in fasting total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein from day 1 to day 14 was evaluated for each study treatment. Analysis of variance (ANOVA) was performed using SAS PROC MIXED, with subject as a random effect and treatment and day as fixed effects. The estimated least squares mean difference was reported.

Subjects who completed period 1 dosing with evaluable pharmacokinetic parameters were included in the pharmacokinetic population. Descriptive statistics, including the geometric means and 95% confidence intervals (CIs), were calculated for all pharmacokinetic parameters and summarized by study treatment. ANOVA, considering study treatment as a fixed effect, was performed using SAS (version 8.2) mixed linear models procedure to compare log-transformed plasma APV and RTV C_{max}, $AUC_{(0\text{-}\tau)}$, and C_τ values between the study treatments. Race, sex, and age were included in the ANOVA as covariate variables if they were significant at 0.05 level. The impact of dose escalation was estimated by the ratio of geometric least squares means and the associated 90% confidence interval. Achievement of steady-state plasma APV and RTV concentrations was

TABLE 2. Demographics summary by study treatment

Demographic characteristics[a]	Study treatment[b]			Total
	A	B	C	
Safety population (all subjects, no.)	25	26	25	42
Mean age (yr) (range)	30 (18–50)	34 (18–50)	35 (19–49)	34 (19–49)
Sex (%)				
M	56	50	56	55
F	44	50	44	45
Race (%)				
W	60	69	44	55
B	20	15	32	24
H	20	15	24	21
Height, mean (cm)	168 (150–186)	172 (153–198)	167 (150–198)	169 (150–198)
Weight, mean (kg)	72 (52–94)	76 (53–99)	76 (52–94)	73 (52–99)
Body mass index, mean (kg/m^2)	25 (19–30)	25 (20–30)	25 (19–30)	25 (19–30)
Pharmacokinetic population (no.)[c]	13	13	13	39
Mean age (yr) (range)	34 (16–46)	26 (18–50)	41 (21–49)	34 (18–50)
Sex (%)				
M	77	38	45	54
F	23	62	54	46
Race (%)				
W	69	62	38	56
B	15	23	23	21
H	15	15	38	23
Height, mean (cm)	168 (154–182)	172 (153–198)	166 (150–184)	168 (150–198)
Weight, mean (kg)	75 (54–94)	71 (53–83)	76 (52–94)	72 (52–94)
Body mass index, mean (kg/m^2)	26 (21–30)	24 (20–30)	25 (19–30)	25 (19–30)

[a] The total number of subjects for the study was 42. M, male; F, female; W, white; B, black; H, Hispanic.
[b] Treatment A was FPV 700 mg BID plus RTV 100 mg BID for 14 days, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID for 14 days, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID for 14 days.
[c] Subjects with evaluable pharmacokinetic data in period 1.

TABLE 3. Most commonly reported adverse events by study treatment and system organ class[a]

System organ class or type of adverse event	No. of events for study treatment[b]			Total
	A (n = 25)	B (n = 26)	C (n = 25)	
Any event	17 (68)	20 (77)	22 (88)	39 (93)
Gastrointestinal disorders	13 (52)	16 (62)	18 (72)	31 (74)
Loose stools	11 (44)	11 (42)	13 (52)	24 (57)
Nausea	6 (24)	8 (31)	8 (32)	18 (43)
Abdominal pain	3 (12)	6 (23)	3 (12)	10 (24)
Flatulence	5 (20)	3 (12)	3 (12)	9 (21)
Vomiting	1 (4)	4 (15)	3 (12)	8 (19)
Nervous system disorders	10 (40)	14 (54)	18 (72)	30 (71)
Paraesthesia oral	6 (24)	12 (46)	13 (52)	24 (57)
Headache	8 (32)	5 (19)	6 (24)	16 (38)
Dizziness	1 (4)	1 (4)	2 (8)	4 (10)
Skin and subcutaneous tissue disorders	5 (20)	5 (19)	7 (28)	15 (36)
Pruritus	3 (12)	4 (15)	7 (28)	12 (29)
Maculopapular rash	4 (16)	3 (12)	5 (20)	11 (26)
Respiratory, thoracic, and mediastinal disorders	4 (16)	4 (15)	4 (16)	11 (26)
Throat irritation	3 (12)	2 (8)	1 (4)	5 (12)
Dyspnea	1 (4)	1 (4)	2 (8)	4 (10)
General disorders and administration site	3 (12)	4 (15)	3 (12)	10 (24)
Fatigue	2 (8)	0	3 (12)	5 (12)
Clinical laboratory investigations	3 (12)	1 (4)	5 (20)	8 (19)
ALT increased	1 (4)	1 (4)	4 (16)	6 (14)
AST increased	2 (8)	1 (4)	3 (12)	6 (14)
Psychiatric disorders	5 (20)	3 (12)	1 (4)	8 (19)
Insomnia	3 (12)	2 (8)	1 (4)	5 (12)
Metabolism and nutrition disorders	0	4 (15)	3 (12)	7 (17)
Decreased appetite	0	4 (15)	3 (12)	7 (17)

[a] Adverse events were considered common if they affected ≥10% of subjects overall. Data are reported as number of subjects experiencing event (percentage of subjects experiencing event).
[b] Treatment A was FPV 700 mg BID plus RTV 100 mg BID for 14 days, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID for 14 days, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID for 14 days.

assessed by calculating the 90% CI of the slope of the linear regression of predose concentrations from days 11, 12, 13, and 14 versus the day for each study treatment. Pearson correlation was used to measure the strength of the relationship between the plasma APV and RTV pharmacokinetic parameters and the individual maximum ALT and AST values during period 1.

TABLE 4. Number of subjects with ALT and AST ACTG toxicity grades 1 to 3 by study treatment[a]

ACTG toxicity grade[b]	No. (%) of subjects for study treatment[c]		
	A	B	C
ALT			
0	24/25 (96)	23/26 (88)	17/25 (68)
1	1/25 (4)	2/26 (8)	4/25 (16)
2	0	1/26 (4)	3/25 (12)
3	0	0	1/25 (4)
Grades 1 to 3 combined	1/25 (4)	3/26 (12)	8/25 (32)
AST			
0	24/25 (96)	23/26 (88)	19/25 (76)
1	0	3/26 (12)	4/25 (16)
2	1/25 (4)	0	2/25 (8)
3	0	0	0
Grades 1 to 3 combined	1/25 (4)	3/26 (12)	6/25 (24)

[a] For each treatment, the highest toxicity grade for each subject was used.
[b] ALT and AST ACTG toxicity grade 1 was 1.25 to 2.5 ×ULN, grade 2 was >2.5 to 5 ×ULN, and grade 3 was >5 to 10 ×ULN.
[c] Treatment A was FPV 700 mg BID plus RTV 100 mg BID, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID.

RESULTS

Subject accountability and demographics. Forty-two subjects were enrolled, and 12 subjects withdrew prior to termination of the study. The study was prematurely terminated on day 9 of period 2, based on the prospective study stopping criteria for ALT and AST values. Subjects' demographic information for the safety and pharmacokinetic populations are included in Table 2. For the safety population, demographic characteristics appeared to be similar between study treatments. As a consequence of restricting the pharmacokinetic population to period 1 of the study (i.e., before subjects crossed over to another treatment), the demographic characteristics were somewhat different across study treatments for the pharmacokinetic population. More female subjects received treatments B and C, more Hispanic subjects received treatment C, and median age occurred in the following descending rank order: treatment C > treatment A > treatment B.

Safety. (i) Adverse events. Seven subjects prematurely withdrew due to adverse events prior to study termination: five due to increased ALT and/or AST, one due to a rash, and one due to decreased hemoglobin. One additional subject had hepatic transaminase elevations after investigational product discon-

TABLE 5. Means and changes from baseline by study treatment for fasting lipid parameters[a]

Parameter	Baseline (n = 38)	Lipid mean (by day) or change value for study treatment (n = 13)[b]									Follow-up (n = 38)
		A			B			C			
		Day 1	Day 14	Change[c]	Day 1	Day 14	Change	Day 1	Day 14	Change	
Total cholesterol	182	180	198	18.2	179	189	NS[d]	186	197	NS	187
Triglycerides	80.0	85	151	65.9	78.8	114	34.8	87.0	168	81.2	93.5
HDL	45.3	41.2	37.7	−3.54	47.5	39.5	−8.08	47.4	36.0	−11.4	48.9
LDL	120	122	130	NS	115	126	11.2	122	127	NS	119
vLDL	16.0	17.1	30.2	13.1	15.8	22.7	6.92	17.4	33.6	16.2	18.8

[a] Data are from period 1 only and are presented as least squares mean value. Data are reported as milligrams/deciliter. vLDL, very low-density lipoprotein.
[b] Treatment A was FPV 700 mg BID plus RTV 100 mg BID, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID.
[c] Change values are presented as least squares mean difference. Value for least squares mean difference indicates statistical significance ($P < 0.05$).
[d] NS, change was not statistically significant.

tinuation and study termination. Thirty-nine subjects (93%) reported at least one AE that was considered drug related by the investigator. No serious adverse events or deaths were reported during this study. The most common adverse events reported according to study treatment are presented in Table 3. All AEs, especially gastrointestinal and nervous system events, were most frequent with treatment C, followed by treatment B, and then treatment A.

(ii) Liver chemistry tests. No abnormal bilirubin levels were seen in any subject during study treatment. Marked hepatic transaminase elevations, defined as an ALT or AST of >2.5 ×ULN (ACTG grade 2 or higher), were observed in 6 of the 42 subjects, predominantly while receiving FPV 1,400 mg BID plus RTV 200 mg BID (treatment C), (Table 4). Four subjects receiving treatment C had marked ALT elevations after 5 to 9 days of dosing (maximum values ranged from 3.0 to 5.7 ×ULN), with concomitant marked AST elevations in two subjects (maximum values ranged from 3.1 to 3.9 ×ULN). One subject receiving treatment B had marked ALT (3.5 ×ULN as maximum value) and AST elevations (2.3 ×ULN as maximum value). During period 2, one subject receiving treatment A had an AST elevation (4.9 ×ULN) with concomitant increases in ALT (1.4 ×ULN) and creatine phosphokinase (38 ×ULN) after previously receiving treatment C in period 1 with no elevations in transaminases. This profile was likely consistent with muscle enzyme elevation rather than hepatic injury and differed from the predominant ALT elevations noted for subjects receiving treatments B and C.

At the follow-up visit, 21 to 28 days after study drug discontinuation, the ALT and AST changes resolved for three subjects and decreased to below a grade 1 for one subject. Two subjects did not return for the follow-up visit. However, ALT levels had decreased to grade 1 and AST levels had returned to normal by 7 days after the discontinuation of study drugs.

(iii) Fasting lipid parameters. Mean fasting serum triglycerides increased during all treatments ($P < 0.05$), as presented in Table 5. Mean fasting total cholesterol increased during treatment A and mean LDL decreased during treatment B ($P < 0.05$). Mean HDL cholesterol decreased during all treatments ($P < 0.05$). These lipid changes tended to decline or return to baseline during the follow-up period.

Pharmacokinetics. The median plasma APV and RTV concentration-time profiles are shown in Fig. 1 and 2, respectively. Plasma APV and RTV pharmacokinetic parameters are presented in Table 6. Steady-state plasma APV and RTV concentrations were achieved by day 14 for all three study treatments (data not shown).

For plasma APV pharmacokinetics, age, sex, and race were not significant in the ANOVA. Therefore, the results of the statistical comparison of the steady-state plasma APV phar-

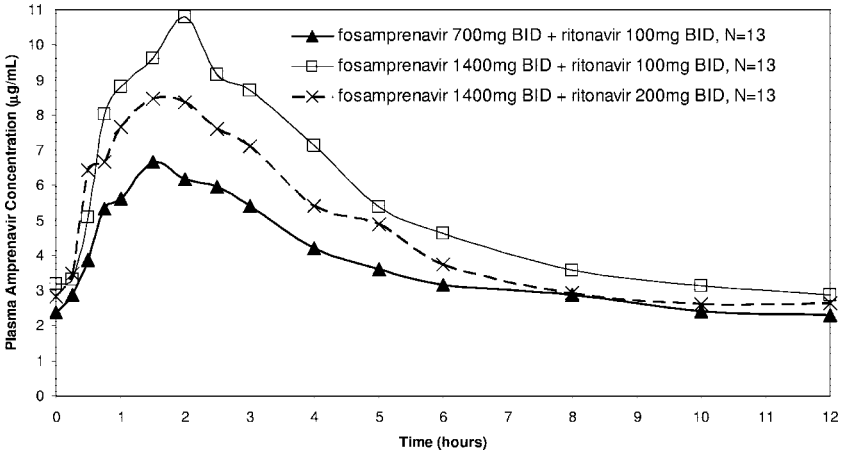

FIG. 1. Median steady-state plasma amprenavir concentration-time profiles in healthy subjects.

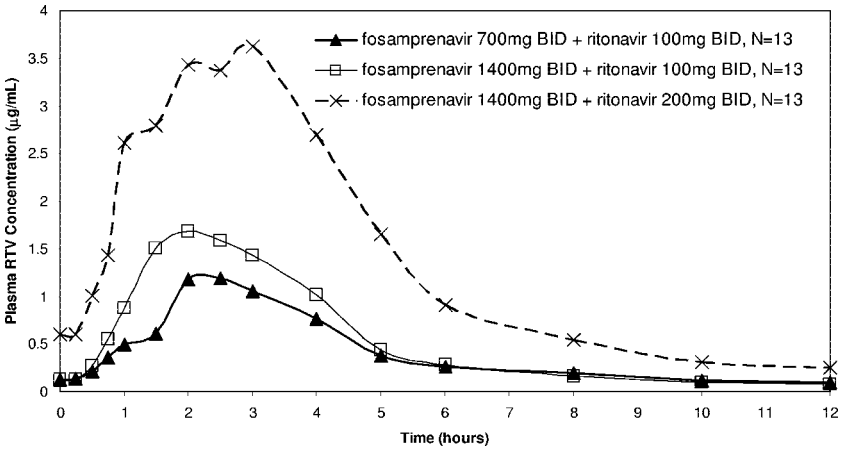

FIG. 2. Median steady-state plasma ritonavir concentration-time profiles in healthy subjects.

macokinetic parameters without these covariates are presented in Table 7. Doubling the FPV dose from 700 mg BID (treatment A) to 1,400 mg BID (treatment B), while maintaining the RTV dose at 100 mg BID, increased plasma APV $AUC_{(0\text{-}\tau)}$ by 54%, C_{max} by 81%, and C_τ by 26%. Doubling the FPV dose and the RTV dose from 100 mg BID to 200 mg BID (treatment C) increased plasma APV $AUC_{(0\text{-}\tau)}$ by 26%, C_{max} by 48%, and C_τ by 32%. Treatment C delivered slightly lower plasma APV exposures compared to those for treatment B, providing no pharmacokinetic advantage.

The results of the statistical comparison of steady-state plasma RTV pharmacokinetic parameters are presented in Table 7. Relative to treatment A, treatment B increased plasma RTV $AUC_{(0\text{-}\tau)}$ by 49% and C_{max} by 71% and reduced C_τ by 11%. For treatment C, values for plasma RTV $AUC_{(0\text{-}\tau)}$, C_{max}, and C_τ were increased 4.15-fold. 4.17-fold, and 3.99-fold, respectively, compared to those observed for treatment A.

Because the study was prematurely terminated in period 2, the analysis of correlation between plasma APV and RTV pharmacokinetic parameters and individual maximum ALT or AST values was limited to period 1 and did not include subjects who prematurely withdrew prior to pharmacokinetic sampling on day 14. No correlation between plasma APV or RTV pharmacokinetic parameters and individual maximum ALT or AST values was observed in this study; however, an increased frequency of ALT and AST elevations was observed with higher FPV and RTV doses, as described in Table 4.

DISCUSSION

The use of low-dose RTV (100 to 400 mg/day) in combination with HIV-1 PIs is routine in the treatment of HIV-1 infection, especially for treatment-experienced subjects (3, 7). Multiple PI-experienced patients may benefit from higher, sustainable HIV-1 PI concentrations to suppress viruses with multiple resistance mutations. Coadministration of PIs with low-dose RTV is generally well tolerated, but it is not without risk. Adverse events, such as gastrointestinal side effects, hepatotoxicity, and blood lipid abnormalities, may be more prevalent and increase in severity as doses increase. Therefore, finding a dose combination for multiple PI-experienced patients that optimizes pharmacokinetics with an acceptable safety profile is challenging but may be an alternative to increasing the number of drugs administered to this population, including the use of dual RTV-boosted PIs.

Three options exist when constructing RTV-boosted regimens to deliver higher HIV-1 PI concentrations for multiple PI-experienced patients: (i) increase the dose of the HIV-1 PI, (ii) increase the dose of RTV, or (iii) increase the doses of both the HIV-1 PI and RTV. The pharmacokinetic outcome of each option is dependent upon the individual HIV-1 PI. For LPV-RTV, either increasing the doses of both LPV and RTV or increasing just the RTV dose results in an increase in plasma LPV exposure (8; C. Flexner, Y.-L. Chiu, C. Foit, P. Perez, E. Tillman, D. Podzamczer, C. Renz, S. Brun, and R. Bertz,

TABLE 6. Plasma amprenavir and ritonavir pharmacokinetic parameter estimates[a]

Parameter	Pharmacokinetic estimate for[b]:					
	Amprenavir			Ritonavir		
	Treatment A	Treatment B	Treatment C	Treatment A	Treatment B	Treatment C
$AUC_{(0\text{-}\tau)}$ (μg · h/ml)	43.02 (36.07–51.32)	66.27 (59.37–73.97)	54.19 (46.44–63.23)	4.33 (2.85–6.60)	6.44 (4.73–8.78)	17.99 (12.05–26.87)
C_{max} (μg/ml)	6.37 (5.20–7.80)	11.53 (9.98–13.32)	9.42 (7.99–11.10)	1.03 (0.59–1.79)	1.76 (1.29–2.40)	4.29 (2.89–6.36)
C_τ (μg/ml)	2.30 (1.91–2.77)	2.91 (2.42–3.49)	3.04 (2.35–3.92)	0.137 (0.104–0.181)	0.122 (0.072–0.208)	0.547 (0.343–0.874)
T_{max} (h)	1.50 (0.75–3.00)	1.50 (1.00–3.00)	1.57 (0.75–2.50)	2.00 (0.50–5.00)	2.00 (1.00–3.00)	2.50 (1.57–4.00)

[a] Results are shown as geometric mean (95% confidence interval) except for T_{max}, which is shown as median (range).
[b] Treatment A was FPV 700 mg BID plus RTV 100 mg BID, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID. There were 13 subjects for each study treatment.

TABLE 7. Steady-state plasma amprenavir and ritonavir pharmacokinetic treatment comparisons[a]

Parameter	APV treatment comparisons[b]			RTV treatment comparisons		
	B/A	C/A	C/B	B/A	C/A	C/B
$AUC_{(0\text{-}\tau)}$ (μg · h/ml)	1.54 (1.31–1.81)	1.26 (1.07–1.48)	0.818 (0.694–0.963)	1.49 (0.980–2.25)	4.15 (2.74–6.30)	2.79 (1.84–4.24)
C_{max} (μg/ml)	1.81 (1.50–2.19)	1.48 (1.22–1.78)	0.817 (0.677–0.986)	1.71 (1.06–2.75)	4.17 (2.60–6.70)	2.44 (1.52–3.92)
C_{τ} (μg/ml)	1.26 (1.00–1.59)	1.32 (1.05–1.66)	1.04 (0.828–1.31)	0.889 (0.549–1.44)	3.99 (2.47–6.47)	4.49 (2.77–7.27)

[a] Results are shown as geometric least squares mean ratio (90% CI).
[b] Treatment A was FPV 700 mg BID plus RTV 100 mg BID, treatment B was FPV 1,400 mg BID plus RTV 100 mg BID, and treatment C was FPV 1,400 mg BID plus RTV 200 mg BID.

Abstr. 2nd ISA Conf. on HIV Pathogenesis and Treatment, abstr. 843, 2003), but the AUC of indinavir 800 mg BID was not significantly increased by increasing the RTV dose from 200 mg to 400 mg (11). For saquinavir-RTV combinations, increasing the dose of saquinavir, but not RTV, increased plasma exposure of saquinavir (1, 6).

Based on pharmacokinetic studies of APV (formulated as Agenerase [AGN]) in combination with RTV, increasing the AGN dose above 600 mg BID, to 900 mg BID in combination with RTV 100 mg BID did not appear to increase plasma APV exposure (R. Schooley, R. Haubrich, M. Sension, A. Taege, S. Becker, D. Richman, M. B. Wire, L. Yu, K. Pappa, and A. Pierce, 41st Intersci. Conf. Antimicrob. Agents and Chemother., abstr. 1924, 2001), whereas the data suggested the achievement of higher plasma APV exposure for AGN 900 mg BID plus RTV 100 mg BID relative to AGN 450 mg BID plus RTV 100 mg BID (12). Similarly, existing data suggested that increasing just the RTV dose administered in combination with AGN provided no pharmacokinetic benefit. For example, there was no advantage to increasing RTV from 200 mg BID to 500 mg BID while maintaining AGN at 1,200 mg BID (plus efavirenz 600 mg once a day [QD]) (S. Piscitelli, S. Bechtel, B. Sadler, J. Falloon, and the Intramural AIDS Program, Abstr. 7th Conf. on Retroviruses and Opportunistic Infect., abstr. 78, 2000) and plasma APV exposure was similar between APV 450 mg BID regimens combined with either RTV 100 mg BID or RTV 300 mg BID (12).

However, plasma APV pharmacokinetic data following the coadministration of the APV prodrug, FPV, and RTV at doses higher than the standard regimen were not available; therefore, this study of healthy subjects evaluated two strategies to increase plasma APV exposure, including doubling the dose of FPV that is coadministered with RTV and doubling the doses of both FPV and RTV. The option to increase just the RTV dose (i.e., FPV 700 mg BID plus RTV 200 mg BID) was not explored, given the low probability of success and the decision to evaluate double doses of both drugs.

Increasing the dose of the HIV-1 PI while maintaining low-dose RTV may achieve a balance of increased plasma PI exposure with minimal increase in toxicities. In this study, doubling the FPV dose from 700 mg to 1,400 mg BID, while maintaining the RTV dose at 100 mg BID, led to less-than-dose-proportional increases in APV exposure [$AUC_{(0\text{-}\tau)}$, 54%, C_{max}, 81%, and C_{τ}, 26%] without a significant increase in toxicities. However, doubling both the FPV dose and RTV dose led to a smaller increase in APV $AUC_{(0\text{-}\tau)}$ (26%) and C_{max} (48%), a significantly increased RTV exposure, and an increased frequency of ALT and AST elevations. The reduced plasma APV exposure observed with FPV 1,400 mg BID plus RTV 200 mg BID compared to that for FPV 1,400 mg plus RTV 100 mg BID, suggests that the increased RTV dose provides additional CYP3A4 induction rather than additional inhibition.

Both higher dosage regimens increased plasma RTV exposure compared to that of the standard regimen of FPV plus RTV. The increased plasma RTV exposure observed with FPV 1,400 mg BID plus RTV 100 mg BID compared to that of FPV 700 mg BID plus RTV 100 mg BID suggests that the increased FPV dose provides some additional CYP3A4 inhibition. Maintaining the FPV dose at 1,400 mg BID, while doubling the RTV dose from 100 mg BID to 200 mg BID, resulted in greater-than-dose-proportional increases in plasma RTV exposure, consistent with the data reported for RTV alone (5). The highest RTV exposure occurred after doubling both the FPV dose from 700 mg BID to 1,400 mg BID and the RTV dose from 100 mg BID to 200 mg BID, which may represent a combined effect of higher RTV doses and additional CYP3A4 inhibition by the higher FPV dose. These pharmacokinetic findings demonstrate the complexity of combining agents with both inhibitory and induction properties for CYP3A4.

ALT and AST elevations led to the premature termination of the study. During the treatment period (excluding follow-up), six subjects had marked (ACTG grade 2 or higher) increases in ALT and AST within 5 to 9 days of dosing. Of these six subjects, four received twice the standard dosage regimen of FPV plus RTV (treatment C). The frequency of grade 2 and 3 AST and ALT elevations observed with the higher dosage regimens in this study was not observed in prior studies (13; unpublished data), where standard FPV plus RTV regimens were administered to healthy adult subjects for 14 days. Although one subject in this study experienced marked transaminase elevations while receiving the standard regimen of FPV 700 mg BID plus RTV 100 mg BID, the pattern of transaminase elevations coupled with elevated creatine phosphokinase suggests a muscle origin for these laboratory abnormalities.

From the clinical history for APV and RTV, it is likely that both compounds contribute to the observed hepatic transaminase elevations. In this study, RTV appears to be an important contributor to the hepatic transaminase elevations. AST/ALT elevations were more common for treatment C, where the RTV dose and plasma exposure were the highest of the three regimens and where the plasma APV exposure was lower than that for treatment B. Although grade 1/2 increases in AST/ALT were reported with treatment B (three subjects), a significant difference in transaminase elevations compared to those with treatment A (one subject) was not apparent with

this short course of dosing. The increase in plasma APV exposure with treatment B may provide a favorable risk/benefit ratio for the treatment of multiple PI-experienced patients, but careful monitoring of hepatic transaminases should be considered.

All AEs in this study were mild or moderate in intensity and generally similar in nature to those reported in other studies evaluating combinations of FPV and RTV in healthy adults. The frequency of AEs reported with the standard FPV 700 mg BID plus RTV 100 mg BID regimen in this study was similar to those previously reported for healthy subjects receiving either the same regimen or the regimen of FPV 1,400 mg QD plus RTV 200 mg QD (14; unpublished data). The higher doses used in this study appeared to be associated with a higher frequency of AEs overall. All AEs, especially gastrointestinal and nervous system events, occurred in the following rank order (from greatest to least): treatment C > treatment B > treatment A. For the regimen of FPV 700 mg BID plus RTV 100 mg BID (treatment A), which is approved for the treatment of PI-naïve and -experienced patients, the frequency of AEs and the increases in fasting serum total cholesterol and triglycerides were similar to those of other studies of healthy subjects using that regimen (14; unpublished data).

The reductions in HDL in this study, when considered as a percentage of change, are consistent with other studies in healthy volunteers using ritonavir at doses of 100 mg BID (5.4% decrease) or 500 mg BID (approximately 9% decrease) over 14 days (9, 13). After 48 weeks of treatment in HIV patients, HDL was increased from baseline by 21% for FPV 1,400 mg BID and FPV 1,400 mg/RTV 200 mg QD (J. Nadler, A. Rodriguez-French, P. Wannamaker, S. Tompkins, and T. Stark, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. H-156, 2004; J. Flamm, M. Lorber, D. Thomas, N. Givens, and T. Stark, Abstr. Antivir. Ther., abstr. 99, 2004). These results suggest that HIV infection may result in a different lipid response to FPV-RTV–containing regimens compared to that for healthy volunteers.

Based on the pharmacokinetic and short-term safety results of this study of healthy subjects, FPV 1,400 mg BID plus RTV 200 mg BID is not recommended due to an increased rate of hepatic transaminase elevations and a lack of pharmacokinetic advantage compared to FPV 1,400 mg BID plus RTV 100 mg BID. FPV 1,400 mg BID plus RTV 100 mg BID, which increased plasma APV exposure without significant safety concerns, is now under clinical evaluation in multiple PI-experienced, HIV-1-infected patients, along with a dual-boosted PI regimen of FPV combined with lopinavir-ritonavir (TRIAD study), to allow a more robust assessment of safety and efficacy in the intended population.

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REFERENCES

1. **Autar, R. S., J. Ananworanich, W. Apateerapong, J. Sankote, A. Hill, B. Hirchel, D. Cooper, J. Lange, P. Phanuphak, K. Ruxrungtham, and D. Burger.** 2004. Pharmacokinetic study of saquinavir hard gel caps/ritonavir in HIV-1 infected patients: 1,600/100 mg once-daily compared with 2,000/100 mg once-daily and 1,000/100 mg twice-daily. J. Antimicrob. Chemother. **54:**785–790.
2. **Boffito, M., D. Maitland, Y. Samarasinghe, and A. Pozniak.** 2005. The pharmacokinetics of HIV protease inhibitor combinations. Curr. Opin. Infect. Dis. **18:**1–7.
3. **Cooper, C. L., R. P. van Heeswijk, K. Gallicano, and D. W. Cameron.** 2003. A review of low-dose ritonavir in protease inhibitor combination therapy. Clin. Infect. Dis. **36:**1585–1592.
4. **Gathe, J. C., P. Ive, R. Wood, D. Schurmann, N. C. Bellos, E. DeJesus, A. Gladysz, C. Garris, and J. Yeo.** 2004. SOLO: 48-week efficacy and safety comparison of once-daily fosamprenavir/ritonavir versus twice-daily nelfinavir in naïve HIV-1 infected patients. AIDS **18:**1529–1537.
5. **Hsu, A., G. R Granneman, G. Witt, C. Locke, J. Dennisen, A. Molla, et al.** 1997. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. Antimicrob. Agents Chemother. **41:**898–905.
6. **Kilby, J. M., G. Sfakianos, N. Gizzi, P. Siemon-Hryczyk, E. Ehrensing, C. Oo., N. Buss, and M. S. Saag.** 2000. Safety and pharmacokinetics of once-daily regimens of soft-gel capsule saquinavir plus minidose ritonavir in human immunodeficiency virus-negative adults. Antimicrob. Agents Chemother. **44:**2672–2678.
7. **Moyle, G. J., and D. Back.** 2001. Principles and practice of HIV-protease inhibitor pharmacoenhancement. HIV Med. **2:**105–113.
8. **Murphy, R. L., S. Brun, C. Hicks, J. J. Eron, R. Gulick, M. King, A. C. White Jr., C. Benson, M. Thompson, H. A. Kessler, S. Hammer, R. Bertz, A. Hsu, A. Japour, and E. Sun.** 2001. ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral naïve adults with HIV-1 infection: 48-week results. AIDS **15:**F1–F9.
9. **Purnell, J. Q., A. Zambon, R. H. Knopp, D. J. Pizzuti, R. Achari, J. M. Leonard, C. Locke, and J. D. Brunzell.** 2000. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. AIDS **14:**51–57.
10. **Rodriguez-French, A., J. Boghossian, G. E. Gray, J. P. Nadler, A. R. Quinones, G. E. Sepulveda, J. M. Millard, and P. G. Wannamaker.** 2004. The NEAT study: a 48-week open-label study to compare the antiviral efficacy and safety of GW433908 versus nelfinavir in antiretroviral therapy-naïve human immunodeficiency virus type 1 (HIV-1)-infected patients. J. Acquir. Immune Defic. Syndr. **35:**22–32.
11. **Saah, A. J., A. G. Winchell, M. L. Nessly, M. A. Seniuk, R. R. Rhodes, and P. J. Deutsch.** 2001. Pharmacokinetic profile and tolerability of indinavir-ritonavir combinations in healthy volunteers. Antimicrob. Agents Chemother. **45:**2710–2715.
12. **Sadler, B. M., P. J. Piliero, S. L. Preston, P. P. Lloyd, Y. Lou, and D. Stein.** 2001. Pharmacokinetics and safety of amprenavir and ritonavir following multiple-dose, co-administration to healthy volunteers. AIDS **15:**1009–1018.
13. **Shafran, S. D., L. D. Mashinter, and S. E. Roberts.** 2005. The effect of low-dose ritonavir monotherapy on fasting serum lipid concentrations. HIV Med. **6:**421–425.
14. **Wire, M. B., C. Ballow, S. L. Preston, C. W. Hendrix, P. J. Piliero, Y. Lou, and D. S. Stein.** 2004. Pharmacokinetics and safety of GW433908 and ritonavir, with and without efavirenz, in healthy volunteers. AIDS **18:**897–907.