Safety and Pharmacokinetics of Brecanavir, a Novel Human Immunodeficiency Virus Type 1 Protease Inhibitor, following Repeat Administration with and without Ritonavir in Healthy Adult Subjects[▽]

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Brecanavir (BCV) is a novel, potent protease inhibitor in development for the treatment of human immunodeficiency virus (HIV-1) infection with low nM in vitro 50% inhibitory concentrations (IC₅₀s) against many multiprotease inhibitor resistant viruses. This study was a double-blind, randomized, placebo-controlled repeat-dose escalation to evaluate the safety, tolerability, and pharmacokinetics of BCV, with or without ritonavir (RTV), in 68 healthy subjects. Seven sequential cohorts (*n* = 10) received BCV (50 to 600 mg) in combination with 100 mg RTV (every 12 h [q12h] or q24h) or alone at 800 mg q12h for 15 days. BCV alone or in combination with RTV was well tolerated, with no serious adverse events reported. The most common drug-related adverse event was headache. BCV was readily absorbed with median time to maximum concentration of drug in serum values ranging from 2.5 to 5.0 h postdose following single- and repeat-dose administration of BCV alone and BCV with RTV 100 mg. Geometric mean BCV accumulation ratios ranged from 1.4 to 1.56 following BCV-RTV q24h regimens and from 1.84 to 4.93 following BCV q12h regimens. BCV steady state was generally achieved by day 13 in all groups. All day 15 BCV-RTV trough concentration values in q12h regimens reached or surpassed the estimated protein-binding corrected in vitro IC₅₀ target BCV concentration of 28 ng/ml for highly resistant isolates. The pharmacokinetic and safety profile of BCV-RTV supports continued investigation in HIV-1-infected subjects.

Protease inhibitors (PIs) have represented a major advancement in human immunodeficiency virus type 1 (HIV-1) therapy and are recommended for use as part of first-line therapy. However, viral drug resistance remains an impediment to long-term suppression of viral replication. Coadministration of a protease inhibitor with low-dose ritonavir (RTV), a potent CYP3A4 inhibitor, improves virological suppression by significantly enhancing the plasma exposure of the coadministered PI and by contributing to better adherence by decreasing the pill burden (2).

Brecanavir (BCV) is a novel, potent PI with low nM in vitro 50% inhibitory concentrations (IC₅₀s) against many multi-PI-resistant viruses. Using an MT-4 assay, BCV demonstrated 20- to 100-times-higher potency against both wild-type and PI-resistant HIV than other marketed protease inhibitors, including lopinavir, saquinavir, indinavir (IDV), nelfinavir, and amprenavir. In addition, BCV exhibited greater potency than these other PIs against a panel of 55 recombinant viruses with median of eight PI mutations (range, 4 to 14) indicative of a highly resistant group of viruses (presence of PRO or protease mutations at codons 10, 32, 46, 47, 50, 54, 84, and/or 90) from PI-experienced HIV-infected patients. BCV maintained low nM IC₅₀s for all 55 PI-resistant isolates, 80%

of which had an IC₅₀ at or below 0.8 nM (A. W. Spaltenstein and R. Hazen, Second IAS Conference on HIV Pathogenesis and Treatment, Paris, France, 13 to 16 July 2003).

BCV is ~98% bound to human plasma proteins as assessed by ex vivo equilibrium dialysis and is associated with a sixfold increase in IC₅₀ measured in the presence of 50% human serum. A conservative approach, therefore, is to use a protein binding correction factor of 50-fold, based on the free fraction of BCV, 2%. Eighty percent of the 55 clinical PI resistant isolates surveyed would have predicted relevant in vivo BCV IC₅₀s of ≤40 nM (50 × 0.8 nM) or 28 ng/ml (Spaltenstein and Hazen, Second IAS Conf. HIV Pathog. Treat.). Therefore, a minimum PI resistance clinical target trough of 28 ng/ml was chosen a priori to determine the viability of BCV as an anti-retroviral agent in the target population.

In vitro and in vivo studies have shown that BCV is a CYP3A4 substrate. This compound exhibits high first-pass elimination with subsequent low oral bioavailability. Coadministration with low-dose RTV has been shown to improve oral bioavailability by decreasing the clearance of the second protease inhibitor. In a single-dose escalation study, coadministration of 300 mg BCV with 100 mg RTV increased plasma BCV area under the concentration-time curve from 0 h to infinity (AUC_{0-∞}) and maximum concentration (C_{max}) 26-fold and 11-fold, respectively (1). In addition, BCV exposure was approximately threefold higher following single-dose administration of 150 mg BCV (tablet)–100 mg RTV (capsule) with a high-fat, high-calorie meal (approximately 50% of total caloric content of the meal and approximately 800 to 1,000 cal; me-

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dian $AUC_{0-\infty}$, 1,519 ng · h/ml; range, 967 to 2,487 ng · h/ml) compared across studies to the fasted state (median $AUC_{0-\infty}$, 460 ng · h/ml; range, 419 to 614 ng · h/ml). Based on these results, a moderate-fat, moderate-calorie standard meal was given prior to dosing in this repeat-dose escalation study.

The purpose of this study was to evaluate the pharmacokinetics and safety of BCV oral tablets administered with or without RTV for 15 days.

(These data were presented in part at the 12th Conference on Retroviruses and Opportunistic Infections, Boston, MA, February 2005.)

MATERIALS AND METHODS

Study design. This was a double-blind, randomized, placebo-controlled, repeat-dose escalation study of healthy subjects. Doses were selected based on safety and pharmacokinetics of the single-dose escalation study (1). BCV was available as 50-mg and 150-mg strength tablets. Healthy, HIV-seronegative men and women of nonchildbearing potential between the ages of 18 and 55 were recruited based on the investigator's review of the subject's medical history, physical examination, clinical laboratory tests, and electrocardiogram (ECG). All subjects provided written informed consent, and the protocol was approved by the Institutional Review Board of Covance in Madison, WI.

Doses were escalated in a sequential fashion, and projected doses were modified upon review of actual pharmacokinetic and safety data. There were 7 cohorts (planned, $n = 10$ subjects/cohort; 8 active, 2 placebo) that received BCV in doses of 50 mg, 150 mg, 300 mg, and 600 mg in combination with 100 mg RTV every 12 h (q12h) or placebo–100 mg RTV q12h, in doses of 100 mg and 250 mg in combination with 100 mg RTV q24h or placebo–100 mg RTV q24h, or alone at 800 mg q12h or placebo for 15 days. Subjects received their doses with a standard moderate-fat breakfast (30% fat calories). They consumed the meal within 25 min, and BCV was administered within 5 min of meal completion. Subjects were admitted to the research unit the evening prior to dosing and remained there until the 48-h postdose study assessments were completed.

Safety assessments. Vital signs, ECG, hematology, clinical chemistry, urinalysis, and clinical adverse experiences were collected prior to dosing, during the 15-day dosing period, and through the 48-h postdose period. Physical examinations were performed predose and prior to discharge for each treatment period. Adverse events occurring during the trial were evaluated by the investigator and graded according to protocol toxicity scales and for relationship to study drug.

PK assessments. Serial blood samples were collected to determine plasma concentrations of BCV and RTV (as applicable). On day 1, only one dose, i.e., the morning dose, was administered, and pharmacokinetic (PK) samples were collected prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, and 24 h postdosing. The 24-h sample immediately preceded the next oral dose. On days 3 through 14, trough PK samples were collected each day just prior to the morning dose. After receiving the morning dose on day 15 of the study, PK samples were collected prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 18, and 24 h postdosing. Samples were processed within 1 h of collection, and plasma was stored at -70°C prior to analyses.

Bioanalysis. Following extraction from plasma by protein precipitation, BCV and RTV concentrations were determined simultaneously by a validated high-performance liquid chromatography-mass spectrometry/mass spectrometry method (PE Sciex Analyst, version 1.1; SMS2000, version 1.4) using Turbo-Ionspray and multiple-reaction monitoring at GlaxoSmithKline. [$^2\text{H}_6$]BCV and [$^{13}\text{C}_3$ - $^2\text{H}_2$ - ^{15}N]RTV were used as internal standards. The validated linear concentration range was 1 to 1,000 ng/ml for BCV and 10 to 10,000 ng/ml for RTV. Three concentrations of quality control samples were included in each run for BCV, 4, 100, and 800 ng/ml, and RTV, 40, 1,000, and 8,000 ng/ml. Based on the results of the analysis of these quality controls, the bias for BCV ranged from -1.7 to 2.7% and the bias for RTV ranged from -2.4 to 3.6% . The within-run and between-run precision for BCV were less than or equal to 10.9% and 4.7% , respectively. The within-run and between-run precision for RTV were less than or equal to 7.7% and 3.8% , respectively.

Pharmacokinetic analysis. Plasma BCV and RTV pharmacokinetic parameters following repeat-dose administration were determined by noncompartmental analysis of individual concentration-actual time data using WinNonLin Professional, version 3.2 (Pharsight Corporation, Mountain View, CA) for extravascular administration (model 200) with linear up/log down implementation of the trapezoidal rule. Estimated PK parameters included C_{max} , time of C_{max} (T_{max}), time to first quantifiable concentration (T_{lag}), apparent terminal-

phase elimination rate constant (λ_z), estimated terminal-phase half-life ($t_{1/2}$), AUC from time zero to the time of last quantifiable concentration (AUC_{0-t}), $AUC_{0-\infty}$, and $AUC_{0-\tau}$ (AUC from time zero to the end of the dosing interval [12 or 24 h post dose depending on regimen]). Individual λ_z values were estimated by log-linear regression of the terminal phases of plasma concentration-time curves using a minimum of 3 data points; $t_{1/2}$ was calculated as $\ln 2/\lambda_z$. AUC_{0-t} was determined from time zero to the last quantifiable concentration (T_{last}). $AUC_{0-\infty}$ was calculated by adding C_{last} (last quantifiable concentration)/ λ_z to AUC_{0-t} .

Statistical analysis. Plasma BCV and RTV PK parameters were summarized for each dose level using descriptive statistics. There was no formal calculation of power for this study. An estimation approach was taken and sample size was based on expected confidence interval widths of PK parameters AUC_{0-t} and C_{max} . Dose proportionality was assessed for BCV $AUC_{0-\infty}$ (day 1), BCV $AUC_{0-\tau}$ (day 15), and C_{max} (day 1 and day 15) using the power model $y = \alpha \times \text{dose}^{\beta}$, where y denoted the PK parameter being analyzed and α depends on the subject and period. The log_e-transformed model, $\log(y) = \log(\alpha) + \beta \times \log(\text{dose})$, was used to estimate β along with its 90% confidence interval (CI). A value of $\exp(\beta)$ near 1 implied dose proportionality. Hence, if the 90% CI for $\exp(\beta)$ included 1, dose proportionality was considered confirmed. Dose proportionality was assessed for day 1 measures by combining both q24h and q12h regimens administered in combination with RTV. For day 15 assessments, only q12h regimens administered with RTV were included.

Time invariance of BCV and RTV was assessed by comparing $AUC_{0-\tau}$ for day 15 and $AUC_{0-\infty}$ for day 1 separately for each dose using analysis of variance (ANOVA) with a random effect for subject and a fixed effect for day. The dependent variable was the appropriate log-transformed AUC value. A 90% confidence interval for the true difference (on the log scale) across all subjects was estimated. The estimated difference and endpoints of the 90% CI on the log scale were exponentiated to obtain the estimated ratio and corresponding 90% CI. For BCV and RTV, the accumulation ratio (R) was assessed by comparing the $AUC_{0-\tau}$ on day 15 to the $AUC_{0-12\text{ h}}$ on day 1 for q12h dosing or $AUC_{0-24\text{ h}}$ on day 1 for q24h dosing using ANOVA with a random effect for subject and a fixed effect for day. The dependent variable was the appropriate log-transformed AUC value. A 90% confidence interval for the true accumulation difference (on the log scale) across all subjects was estimated. The estimated difference and endpoints of the 90% CI on the log scale were exponentiated to obtain the estimated ratio and corresponding 90% CI. For the assessment of accumulation and time invariance, the model failed to converge for the 600 mg BCV–100 mg RTV treatment group. The subject was treated as fixed effect for this group to resolve the convergence problems.

Steady state was assessed by examination of the 90% confidence interval for the slope of the linear regression of log-transformed trough levels versus day for days 3 to 15. Subject and day were included in the model as fixed effects. The analysis was repeated, dropping the earliest day, until it was either shown that steady state was achieved or until only days 13 to 15 were included in the regression. Steady state was considered to be achieved when the 90% confidence interval for the slope included zero.

RESULTS

Demographics. A total of 68 subjects were enrolled and received at least one dose of investigational product, and 64 subjects completed the study. The majority of subjects were male (57 subjects; 84%). Fifty-two subjects (76%) were white, 12 subjects (18%) were African-American, 3 subjects (4%) were Hispanic, and 1 subject (1%) was American Hispanic. The median (range) age, weight, height, and body mass index were 36.0 years (19 to 55 years), 76.7 kg (51.6 to 94.3 kg), 176 cm (154 to 189 cm), and 25.5 kg/m^2 (19.2 to 29.6 kg/m^2), respectively.

Safety and tolerability. (i) Adverse events. BCV and BCV-RTV were well tolerated at all repeat doses for 15 days. Twenty-four subjects (35%) experienced adverse events (AEs) considered to be drug-related by the investigator; all were mild or moderate in intensity. The most commonly reported drug-related AEs were headache (12%), dizziness (7%), flatulence (6%), loose stools (6%), and generalized pruritus (6%). There were no serious adverse events or deaths during the study.

Four subjects were withdrawn from the study due to AEs. Three subjects in the cohort receiving 800 mg BCV q12h alone experienced a diffuse, erythematous macular rash that appeared on days 5, 10, and 10, respectively. Two of these subjects received 800 mg of BCV, whereas 1 received placebo. These rashes were considered mild in intensity and resolved within 2 days. One subject receiving 250 mg BCV–100 mg RTV q24h was withdrawn on day 10 due to dental caries; this adverse event was not considered drug related.

(ii) Laboratory safety data. There were no clinically significant changes in clinical chemistry or hematology values. Three of eight subjects receiving 50 mg BCV–100 mg RTV q12h had asymptomatic, grade 1 elevations of thyroid stimulating hormone on routine clinical laboratory tests drawn on day 15. These abnormalities returned to baseline over 2 to 3 weeks. There were no clinically significant trends in free T3 and T4 values associated with the elevated thyroid stimulating hormone results. Thyroid function values from the remaining treatment groups were comparable to those from the placebo-treated subjects. One subject who received 50 mg BCV–100 mg RTV q12h had an abnormal urinalysis at the follow-up visit. The investigator did not consider this to be related to the study drug; the subject subsequently was lost to follow-up. Overall, there were no clinically significant changes or trends observed in blood pressure, heart rate, or ECG intervals in subjects who received repeat doses of BCV over 15 days, with or without RTV.

BCV pharmacokinetics. The day 1 and day 15 BCV PK parameters are shown in Table 1, and the median day 15 BCV plasma concentration-time profiles are shown in Fig. 1. Distributions of BCV trough concentration (C_T) values following BCV-RTV q12h regimens are compared to the distribution of protein-binding-adjusted IC_{50} s for the panel of 55 clinical isolates used to estimate the clinical target in Fig. 2.

BCV alone. Following single-dose administration of 800 mg BCV, the geometric mean BCV C_{max} was 4 ng/ml, and the median (range) T_{max} occurred 2.5 h (2.0 to 4.0 h) postdose. The day 1 BCV $AUC_{0-\infty}$ and $t_{1/2}$ were not estimated due to low BCV plasma exposure following single-dose administration. Following repeat-dose administration of 800 mg BCV q12h, the median (range) day 15 BCV T_{max} occurred at 3.75 h (1.5 to 5.0 h) postdose. The accumulation ratio for this regimen was not determined due to low BCV exposure on day 1; however, comparison of the BCV C_{max} from day 15 to day 1 suggested significant BCV accumulation (approximately 12.5-fold).

BCV with RTV. Following single-dose administration of BCV with 100 mg RTV on day 1, BCV T_{max} values ranged from 3.75 to 5.0 h across BCV-RTV treatments and appeared to be prolonged with increasing BCV dose. Geometric mean BCV terminal-phase $t_{1/2}$ values ranged from 4.5 to 10 h for BCV-RTV regimens. Using the power model, mean slope (90% CI) estimates for BCV $AUC_{0-\infty}$ and C_{max} were 0.80 (0.61, 0.98) and 0.74 (0.58, 0.91), respectively, indicating that both BCV $AUC_{0-\infty}$ and C_{max} increased less than proportional to BCV dose following single-dose administration of BCV-RTV.

Following repeat-dose administration of 100 mg BCV–100 mg RTV q24h, the day 15 geometric mean $AUC_{0-\tau}$ for BCV was 1,750 ng · h/ml. In comparison, the geometric mean day 15 BCV $AUC_{0-\tau}$ for 250 mg BCV–100 mg RTV q24h was ~49% higher at 2,599 ng · h/ml. BCV accumulation and time in-

TABLE 1. Day 1 and day 15 BCV pharmacokinetic parameters following single- and repeat-dose administration of BCV alone and with RTV^a

Regimen	n	$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (ng · h/ml) ^b	C_{max} (ng/ml)	T_{max} (h) ^c	$t_{1/2}$ (h) or C_T (ng/ml) ^d	CL/F (liters/h) ^e
Day 1						
800 mg BCV	8	NA ^f	3.96 (2.08, 7.39) [67]	2.50 (2.00–4.00) [21.7]	NA	NA
50 mg BCV–100 mg RTV	8	394 (245, 715) [58.5]	49.3 (33.1, 80.7) [50.0]	3.75 (3.00–5.00) [18.5]	9.99 (7.89, 12.7) [28.1]	127 (61.4, 259.7) [73.9]
100 mg BCV–100 mg RTV	8	1,331 (969, 1,875) [38.1]	188 (142, 257) [34.4]	4.50 (3.00–6.00) [22.9]	5.31 (4.64, 6.11) [16.3]	75.1 (53.9, 106.9) [39.4]
150 mg BCV–100 mg RTV	8	1,519 (967, 2,487) [52.7]	179 (109, 300) [55.8]	4.50 (3.50–6.00) [20.5]	7.49 (4.40, 12.7) [58]	98.8 (57.3, 169.5) [59.2]
250 mg BCV–100 mg RTV	7	1,741 (1,111, 2,753) [46.0]	240 (157, 360) [42.4]	5.00 (2.50–5.03) [23.3]	4.49 (3.94, 5.13) [14.2]	143.6 (64.1, 264.7) [65.9]
300 mg BCV–100 mg RTV	8	1,976 (1,253, 3,547) [57.2]	264 (157, 472) [60.1]	5.00 (3.50–6.00) [15.6]	8.38 (5.80, 12.4) [43.5]	151.8 (27.2, 382.3) [103.7]
600 mg BCV–100 mg RTV	8	3,673 (2,479, 5,563) [45.9]	391 (300, 521) [32.2]	5.00 (3.50–6.00) [20.7]	6.97 (6.23, 7.82) [13.6]	163.4 (108.6, 245.9) [47.1]
Day 15						
800 mg BCV q12h	6	199 (97.5, 368) [55.4]	49.2 (21.3, 98.7) [61.5]	3.75 (1.50–5.00) [39.9]	2.60 (1.13, 5.21) [61.3]	4,017.8 (1,313.4, 8,319.5) [69.3]
100 mg BCV–100 mg RTV q24h	8	1,750 (1,421, 2,188) [25.4]	237 (202, 280) [19.4]	4.00 (2.50–5.00) [21.5]	16.5 (8.77, 33.4) [69.9]	57.1 (44.9, 73.2) [28.6]
250 mg BCV–100 mg RTV q24h	6	2,599 (1,708, 3,737) [35.5]	398 (290, 531) [28.0]	4.00 (2.50–4.00) [16.5]	18.5 (10.9, 29.4) [43.6]	96.2 (69.1, 131.4) [29.6]
50 mg BCV–100 mg RTV q12h	8	1,171 (839, 1,677) [39.9]	161 (121, 217) [34.2]	4.50 (3.00–6.00) [24.3]	59.6 (39.0, 95.3) [50.2]	42.7 (30.2, 61.7) [41.1]
150 mg BCV–100 mg RTV q12h	8	2,040 (1,544, 2,858) [35.7]	305 (238, 408) [31.6]	4.50 (3.50–5.00) [14]	102 (66.5, 167) [51.4]	73.5 (122.5, 41.3) [59.3]
300 mg BCV–100 mg RTV q12h	8	3,203 (2,062, 4,826) [48.0]	496 (352, 690) [38.7]	4.00 (2.50–6.00) [26.3]	144 (80.4, 244) [60.4]	93.7 (123, 74.4) [29.5]
600 mg BCV–100 mg RTV q12h	8	4,122 (3,221, 5,341) [29.6]	574 (448, 746) [29.8]	4.50 (3.00–5.00) [18.5]	186 (144, 245) [30.9]	145.6 (114.5, 187.7) [29.0]

^a Results are geometric means with 95% CI in parentheses and percent coefficients of variation ($100 \times$ standard deviation/arithmetic mean) in brackets.

^b Results for day 1 are $AUC_{0-\infty}$; results for day 15 are $AUC_{0-\tau}$.

^c Results for T_{max} are medians with ranges indicated in parentheses.

^d Results for day 1 are $t_{1/2}$; results for day 15 are C_T .

^e CL, clearance.

^f NA, not applicable (parameters were not reportable for this dose level).

FIG. 1. Day 15 median BCV concentration-time profiles.

variance ratios for BCV-RTV q24h regimens estimated by ANOVA ranged from 1.40 to 1.56 and 1.31 to 1.50, respectively (Table 2). Statistical analysis of steady-state results showed that BCV reached steady state by day 11.

Following repeat-dose administration of BCV-RTV q12h regimens, day 15 BCV median T_{max} values ranged from 4.00 to 4.50 h postdose. Using the power model, mean slope (90% CI) estimates for BCV $AUC_{0-\tau}$ and C_{max} were 0.52 (0.40, 0.64) and

FIG. 2. Distributions of BCV C_{τ} values for q12h regimens by BCV dose and compared to protein binding (PB)-adjusted (50-fold shift) *in vitro* IC_{50} s for 55 clinical isolates with high-level PI resistance mutations.

TABLE 2. BCV accumulation ratio and time invariance parameters following 100 mg RTV with BCV or placebo^a

Regimen	Accumulation ratio	Time invariance
100 mg BCV–100 mg RTV q24h	1.40 (1.17, 1.69)	1.31 (1.09, 1.59)
250 mg BCV–100 mg RTV q24h	1.56 (1.13, 2.16)	1.50 (1.07, 2.09)
50 mg BCV–100 mg RTV q12h	4.93 (3.76, 6.46)	2.97 (2.24, 3.96)
150 mg BCV–100 mg RTV q12h	2.28 (1.80, 2.89)	1.34 (1.08, 1.67)
300 mg BCV–100 mg RTV q12h	2.47 (1.70, 3.58)	1.62 (1.11, 2.38)
600 mg BCV–100 mg RTV q12h	1.84 (1.31, 2.59)	1.12 (0.76, 1.65)

^a Results are geometric least square means with 90% CI indicated in parentheses.

0.54 (0.43, 0.64), respectively, indicating that both BCV $AUC_{0-\tau}$ and C_{max} increased less than proportional to BCV dose following repeat-dose administration of BCV-RTV q12h. Statistical analysis of steady-state results showed that BCV reached steady state by day 13. BCV accumulation and time invariance ratios following BCV-RTV q12h regimens ranged from 1.84 to 4.93 and 1.12 to 2.97, respectively, and appeared to decrease with increasing BCV dose (Table 2).

RTV pharmacokinetics. The day 1 and day 15 RTV PK parameters are summarized in Table 3, and the median day 15 RTV plasma concentrations time profiles are shown in Fig. 3. Day 1 median T_{max} values ranged from 4.0 to 5.0 h, and geometric mean RTV terminal-phase half-life ranged from 5.10 to 7.34 h on day 1 across all treatments. Statistical analysis of steady-state results showed that RTV steady state was achieved by day 12 for all treatments. Following repeat administration, RTV $AUC_{0-\tau}$ and C_{max} values appeared lower following placebo–100 mg RTV than when coadministered with BCV. Significant accumulation of RTV was observed for all cohorts; geometric least square mean time invariance ratio values were >1 for all treatments (Table 4).

DISCUSSION

This was the first study that assessed the safety, tolerability, and plasma pharmacokinetics of BCV following repeat administration of escalating BCV doses administered with and without RTV to healthy subjects. Repeat administration of BCV and BCV-RTV was well tolerated across all doses. There were no deaths or serious adverse events reported during this study. The most commonly reported drug-related AEs were headache, dizziness, flatulence, loose stools, and generalized pruritus. Two subjects receiving 800 mg BCV q12h were withdrawn from the study due to drug-related rash, which resolved within 2 days of drug discontinuation. Of note, no rashes were observed in any cohorts in which RTV was administered with BCV.

PK findings suggest that RTV significantly increased BCV exposure following coadministration with BCV tablets. Dose-adjusted cross-cohort comparisons of C_{max} following single-dose administration of BCV-RTV and following 800 mg BCV alone suggest that RTV boosted BCV by 131- to 380-fold, which would require evaluation in a crossover study for confirmation of the true boost effect on BCV tablets. The impact of RTV on BCV in this study appeared higher than previously reported, probably due to the lower relative bioavailability of

TABLE 3. Day 1 and day 15 RTV pharmacokinetic parameters following repeat administration of RTV 100mg with BCV or placebo

Regimen	N	$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (ng · h/ml) ^b	C_{max} (ng/ml)	T_{max} (h) ^c	$t_{1/2}$ (h) or C_T (ng/ml) ^d	CL/F (liters/h) ^e
Day 1						
100 mg BCV–100 mg RTV q24h	8	4,880 (2,832, 8,524) [60.0]	546 (293, 1019) [66.1]	4.50 (2.00–6.00) [28.4]	5.19 (4.45, 6.09) [18.6]	20.5 (12.1, 35.5) [59]
250 mg BCV–100 mg RTV q24h	7	3,763 (1,562, 7,349) [61.9]	401 (190, 763) [65.1]	5.00 (1.52–6.00) [34.1]	5.18 (3.27, 7.62) [38]	26.6 (8.2, 55.7) [70.7]
Placebo–100 mg RTV q24h	3	3,402 (1,342, 8,595) [84.0]	323 (156–568) ^f [55.1]	4.00 (3.50–6.00) [29.4]	5.55 (4.60–8.04) ^g [34.3]	29.4 (–42.1, 119) [84.3]
50 mg BCV–100 mg RTV q12h	8	3,004 (1,871, 5,151) [55.9]	315 (200, 542) [55.1]	4.50 (3.50–6.00) [18]	6.22 (4.58, 8.62) [36.6]	33.3 (18.2, 60.9) [64.6]
150 mg BCV–100 mg RTV q12h	8	6,407 (4,380, 9,735) [45.4]	585 (429, 833) [38.3]	5.00 (3.50–6.00) [20.6]	7.34 (5.69, 9.81) [31.8]	15.6 (10.3, 24.3) [48.5]
300 mg BCV–100 mg RTV q12h	8	4,843 (3,270, 7,341) [45.9]	612 (367, 1002) [55.5]	4.50 (3.00–6.00) [24.5]	5.10 (4.74, 5.50) [9.0]	20.7 (14.2, 30.9) [44.2]
600 mg BCV–100 mg RTV q12h	8	6,001 (4,197, 8,948) [43.2]	634 (421, 969) [47.2]	5.00 (2.00–6.00) [32.0]	7.16 (6.24, 8.26) [16.7]	16.7 (10.6, 26.3) [51.1]
Placebo–100 mg RTV q12h	8	3,128 (1,770, 6,439) [68.0]	316 (181, 611) [64.9]	5.00 (2.00–6.00) [31.4]	5.65 (4.60, 7.00) [24.8]	32.0 (–9.1, 112.8) [140.6]
Day 15						
100 mg BCV–100 mg RTV q24h	8	9,673 (6,870, 13,729) [39.8]	1201 (857, 1764) [41.4]	3.52 (2.50–6.00) [27.5]	92.0 (52.0, 156) [60.0]	10.3 (7.5, 14.4) [37.7]
250 mg BCV–100 mg RTV q24h	6	11,808 (6,366, 19,445) [48.3]	1515 (814, 2501) [48.5]	3.75 (3.50–5.00) [14.9]	98.8 (31.2, 211) [70.8]	8.5 (5.4, 12.9) [39.2]
Placebo–100 mg RTV q24h	3	5,434 (3,415, 9,152) ^h [49.9]	746 (396, 1067) ^h [44.9]	4.00 (3.50–5.00) [18.3]	51.6 (30.4, 145) ^h [95.9]	18.4 (–2.9, 42.7) [46.2]
50 mg BCV–100 mg RTV q12h	8	11,633 (7,471–17,977) [49.4]	1785 (1240, 2679) [43.9]	3.00 (1.50–5.00) [37.0]	441 (254, 734) [58.2]	8.6 (6.1, 12.5) [41.3]
150 mg BCV–100 mg RTV q12h	8	16,810 (13,069, 22,102) [30.7]	2737 (2021, 3794) [36.5]	4.00 (3.00–5.00) [18.0]	716 (543, 979) [34.3]	5.9 (4.4, 8.1) [34.9]
300 mg BCV–100 mg RTV q12h	8	14,389 (9,963, 20,974) [42.6]	2453 (1740, 3504) [40.2]	4.00 (2.50–6.00) [30.5]	536 (347, 858) [50.7]	6.9 (5.1, 9.7) [36.7]
600 mg BCV–100 mg RTV q12h	8	15,404 (12,692, 18,928) [23.6]	2510 (2103, 3028) [21.6]	4.00 (2.00–5.00) [29.9]	614 (491, 783) [27.4]	6.5 (5.2, 8.2) [26.8]
Placebo–100 mg RTV q12h	8	9,281 (7,428, 11,767) [27.0]	1506 (1226, 1875) [25.0]	4.51 (3.00–5.00) [20.8]	366 (279, 500) [34.0]	10.8 (8.5, 13.8) [28.4]

^a Results are geometric means with 95% CI in parentheses and percent coefficients of variation in brackets.

^b Results for day 1 are $AUC_{0-\infty}$; results for day 15 are $AUC_{0-\tau}$.

^c Results for T_{max} are medians with ranges indicated in parentheses.

^d Results for day 1 are $t_{1/2}$; results for day 15 are C_T .

^e CL, clearance.

^f $n = 6$.

^g Results are geometric means with ranges indicated in parentheses.

FIG. 3. Day 15 median RTV concentration-time profiles.

unboosted BCV tablets compared to BCV solution. Significant accumulation of BCV (range, 1.4- to 5-fold) following BCV-RTV was observed after repeat administration, and BCV exhibited time-variant pharmacokinetics (>1), suggesting that BCV clearance decreased following repeat coadministration with RTV due to predominant influence of inhibition by the combination. Although an accumulation ratio for BCV was not determined for 800 mg BCV, it was estimated to be 12-fold based on $C_{\max}$.

Maintenance of $C_{\min}$ concentrations above the IC_{50} is essential for inhibition of viral replication and reduction of the possibility of selection of resistance-associated mutations. The current minimal target concentration was set using IC_{50} s obtained from a viral population consisting of variants with several PI resistance-associated mutations (a worst case population) and correcting for 98% protein binding (50-fold shift). Because the actual effect of protein binding on IC_{50} is closer to a sixfold shift and the activity of BCV against PI-sensitive viruses is on the order of 0.1 to 0.2 nM, the initial target concentration of 28 ng/ml may be considered conservative.

Despite significant accumulation, BCV given alone at 800 mg q12h did not achieve the 28-ng/ml target concentration for

resistant isolates. BCV C_T values following BCV-RTV q24h were also below the estimated target for resistant isolates but were above the estimated wild-type target of 4 ng/ml. BCV C_T values following repeat-dose coadministration with RTV in all q12h regimens reached or surpassed the estimated minimal protein-binding corrected in vitro IC_{50} target BCV concentration of 28 ng/ml for highly resistant isolates. BCV (600 mg)-100 mg RTV q12h resulted in the highest geometric mean BCV C_T value of 186 ng/ml, approximately seven times the target protein binding corrected in vitro IC_{50} value (28 ng/ml).

Plasma RTV exposures at day 15 were approximately 25 to 100% higher following coadministration with BCV than with placebo, suggesting that BCV is an inhibitor of RTV metabolism. Compared to other RTV-boosted regimens, plasma RTV exposures following BCV-RTV q12h administration were approximately 2- to 2.5-fold greater than those reported for 400 mg lopinavir-100 mg RTV q12h, 700 mg fosamprenavir-100 mg RTV q12h, and 1,000 mg saquinavir (soft gel capsules)-100 mg RTV q12h (5, 6; R. Bertz, 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 26 to 29 September 1999) and lower than RTV exposures reported for 800 mg IDV q12h or 1,200 mg IDV q12h-100 mg RTV q12h (3, 4). RTV steady state was achieved by day 12 in all relevant groups. RTV exhibited significant accumulation and time-variant PK, suggesting that BCV inhibits RTV metabolism.

Overall, the pharmacokinetic and safety data of BCV coadministered with RTV supports further investigation of several doses in HIV-infected patients. The in vitro resistance profile of BCV shows the potential for this compound to become an effective therapeutic option for the rapidly growing population of PI treatment-experienced patients.

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TABLE 4. RTV accumulation ratio and time invariance parameters following administration of BCV with RTV^a

Regimen	Accumulation ratio	Time invariance
100 mg BCV-100 mg RTV q24h	2.12 (1.64, 2.73)	1.98 (1.53, 2.57)
250 mg BCV-100 mg RTV q24h	3.08 (2.13, 4.46)	2.98 (1.89, 4.69)
50 mg BCV-100 mg RTV q12h	5.83 (4.24, 8.02)	3.87 (2.79, 5.37)
150 mg BCV-100 mg RTV q12h	4.81 (3.95, 5.85)	2.62 (2.11, 3.27)
300 mg BCV-100 mg RTV q12h	4.02 (3.43, 4.72)	2.97 (2.54, 3.48)
600 mg BCV-100 mg RTV q12h	4.13 (3.15, 5.42)	2.57 (1.96, 3.37)
Placebo-100 mg RTV q12h	4.47 (2.79, 7.17)	2.97 (1.76, 5.01)

^a Results are geometric least square means with 90% CI indicated in parentheses.

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