Pharmacokinetic and Safety Evaluation of BILR 355, a Second-Generation Nonnucleoside Reverse Transcriptase Inhibitor, in Healthy Volunteers[▽]

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BILR 355 is a second-generation nonnucleoside reverse transcriptase inhibitor (NNRTI) under clinical development for the treatment of human immunodeficiency virus infection, particularly in those who harbor virus resistant to the currently available NNRTIs. Two single-center, double-blinded, placebo-controlled, parallel dose-escalation studies were conducted to evaluate the pharmacokinetics and safety of oral BILR 355 administration alone and after coadministration with ritonavir (RTV) at 100 mg. Following a single dose of BILR 355 in oral solution, the mean half life ($t_{1/2}$) was 2 to 4 h, with peak concentrations occurring at 0.5 to 1 h postadministration. The mean apparent clearance (CL/F) ranged from 79.2 to 246 liters/h for administered doses of 12.5 mg to 100 mg. This observed nonlinearity in CL/F resulted from the increased bioavailability attributed to a saturated absorption and/or elimination process at higher doses. In contrast, after the coadministration of single doses of 5 mg to 87.5 mg of BILR 355 with RTV, the mean CL/F ranged from 5.88 to 8.47 liters/h. Over the dose range (5 to 87.5 mg) studied, systemic BILR 355 exposures were approximately proportional to the doses administered when they were coadministered with RTV. With RTV coadministration, the mean $t_{1/2}$ increased to 10 to 16 h, and the mean time of the maximum concentration in plasma lengthened to 1.5 to 5 h. Compared to the values for BILR 355 given alone, the mean area under the concentration-time curve from time zero to infinity, the maximum concentration in plasma, and the $t_{1/2}$ of BILR 355 achieved after coadministration with RTV increased 15- to 30-fold, 2- to 5-fold, and 3- to 5-fold, respectively. In both studies, BILR 355 appeared to be safe and well tolerated in healthy volunteers when the outcomes in the treated volunteers were compared with those in the placebo group.

Since the introduction of highly active antiretroviral therapy in the mid-1990s, there have been marked reductions in the rates of mortality and morbidity among individuals infected with human immunodeficiency virus (HIV) (19), and HIV infection is now considered a manageable chronic disease (14). However, the emergence of viral resistance to the approved therapies, mainly due to suboptimal drug potency and pharmacokinetics (PK), poor adherence to complex highly active antiretroviral therapy regimens, and/or compromised immune function, has challenged the early success of treatment of HIV infection by severely limiting the treatment options available (8). Viruses are particularly sensitive to the development of resistance to members of the first generation of nonnucleoside reverse transcriptase inhibitors (NNRTIs) because of their low genetic barrier to the development of drug-resistant reverse transcriptase (RT) variants. Mutant viruses resistant to one member of the current NNRTIs are also usually cross-resistant to the whole class. Thus, patients may be left without a treatment option among this class of compounds (1, 2). Recent surveys of the status of HIV infection alarmingly revealed that the prevalence of drug-resistant variants, particularly those resistant to NNRTIs in newly infected (treatment-naïve) pa-

tients, has increased significantly in the past few years (3, 11, 18), suggesting that the resistance issue is even more critical than before. Therefore, there is an urgent need to develop new antiretroviral therapies that combine potency against resistant and wild-type viruses with safety in a convenient dosing regimen.

BILR 355 {11-ethyl-5,11-dihydro-5-methyl-8-[2-(1-oxido-4-quinolinyl)oxy]ethyl)-6*H*-dipyrido[3,2-*b*:2',3'-*e*][1,4]dizepin-6-one; molecular weight, 477.52} is a second-generation NNRTI under development for the treatment of HIV infections, particularly those caused by strains resistant to current NNRTIs, in adults and children (5, 22). BILR 355 is highly specific toward HIV type 1 (HIV-1) RT and exhibits activity toward HIV-1 RT several magnitudes greater than that toward other viral and mammalian polymerases, including β -human polymerase, γ -human polymerase, and hepatitis C virus polymerase. The in vitro data show that the 50% effective concentration of BILR 355 against wild-type HIV-1 is 0.26 ng/ml, while the 50% effective concentrations against common NNRTI-resistant viruses range from 1.5 to 13 ng/ml. BILR 355 exhibits an attractive resistance profile against both laboratory strains and clinical isolates of HIV-1. In vitro data indicate that BILR 355 exhibits a low level of cross-resistance to a broad spectrum of viruses that are highly resistant to nevirapine, efavirenz, and delavirdine (5).

Single-dose PK studies with several animal species have indicated that BILR 355 has a short half-life ($t_{1/2}$; <2 h) and volume of distribution (V ; 1 to 2 liters/kg). In preclinical studies, the absolute bioavailability of BILR 355 varied widely

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among animal species, from 4.2% in the rhesus monkey to 100% in the rat. The plasma protein binding for BILR 355 is 95.8% (4).

The purposes of the two studies described here were to assess the safety, tolerability, and preliminary PKs (including a preliminary assessment of the dose linearity) of BILR 355 after the administration of single ascending oral doses alone or in combination with ritonavir (RTV) at 100 mg.

MATERIALS AND METHODS

Subjects. The studies were conducted at the Human Pharmacology Centre of Boehringer Ingelheim Pharma KG in Ingelheim, Germany, following the provision of approval by the local ethics committee. The study was conducted by following the guidance of the Declaration of Helsinki. After signing the written informed consent, the healthy male volunteers (age range, 21 to 50 years; body mass index range, 18.5 to 29.9 kg/m²) entered into the studies. The subjects were in generally good health, as judged by the medical history, a physical examination, and clinical laboratory data. Clinically abnormal laboratory results, evidence of existing diseases or disorders, or any observations or conditions (e.g., smoking of more than 10 cigarettes per day, excessive consumption of alcohol, and drug abuse) which might interfere with the PKs of the study drug were reasons for exclusion. The subjects abstained from all grapefruit products and other foods or beverages that might affect enzyme function. The subjects could be withdrawn from the study at any time due to the occurrence of adverse events (AEs), inclusion or exclusion criteria violations and a failure to report for the study, withdrawal of consent, smoking, and/or the onset of illness. Volunteers dropped from the trial were not replaced unless the number of volunteers in a dose group was less than six.

Study design. Two studies with similar designs were conducted to evaluate the safety and PKs of BILR 355 after BILR 355 was administered alone and after it was coadministered with RTV.

BILR 355 alone in single ascending doses. The single-center, randomized, double-blind, placebo-controlled, single dose-escalation (1, 5, 12.5, 25, 50, 75, and 100 mg) study of BILR 355 alone was designed to evaluate the PKs of BILR 355 and document the safety and tolerability of BILR 355 in healthy male volunteers. For each dose level, six volunteers received active drug dissolved in a polyethylene glycol (PEG) solution and two received a placebo PEG control. One dose level was tested within each group. The subject groups were dosed and evaluated sequentially, beginning with the lowest-dose group and proceeding stepwise to the highest-dose group. The next higher dose was administered only if no safety concerns arose during treatment of the preceding group. The placebo or BILR 355 in PEG solution was administered with 200 ml of tap water on the morning of each study day after an overnight fast; a standard breakfast was served 60 to 90 min after study drug administration. BILR 355 did not change the taste of the PEG solution.

BILR 355 coadministered with RTV in single ascending doses. The study of BILR 355 coadministered with RTV was similar to the single-ascending-dose study, except that each dose group received one dose of RTV at 100 mg 10 h prior to the administration of BILR 355 and a second dose of RTV at 100 mg with the BILR 355 dose on the morning of the study day (day 1). Additionally, the effect of food on the PKs of BILR 355 in PEG solution was assessed in the study cohort that received the 50-mg dose of BILR 355, after completion of the study with the highest-dose (87.5 mg) group. The subjects in this fed cohort were given a high-fat breakfast 30 min before drug administration. The recipe of the high-fat meal was obtained from FDA guidance for food-effect bioavailability and fed bioequivalence studies. This meal derived approximately 150, 250, and 500 to 600 cal from proteins, carbohydrate, and fat, respectively.

Sampling and analytical methods. (i) Blood sampling. To study the effects of a single dose of BILR 355 administered alone, blood samples were taken predosing (0 h) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 32, 48, 72, 96, 120, and 144 h after drug administration. For dose groups receiving 100 mg or more of BILR 355 alone or BILR 355 with RTV, blood samples were drawn before drug administration on day 1 (predosing) and at 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, 24, 32, 48, 96, and 144 h postdosing. The total amount of blood collected from any individual in each study during the trial was approximately 300 ml. At each time point, about 9 ml of blood was drawn into evacuated collection tubes containing EDTA anticoagulant and labeled with sample identification information. For the preparation of plasma, the samples were centrifuged within 60 min after blood sampling at 4°C (refrigerator) and 2,000 × g (equivalent to 3,000 rpm) for 10 min. Two aliquots of plasma (about 2 ml in polypropylene tubes) were available

for the drug assays. The plasma was stored in individually labeled polypropylene tubes at <−18°C (sample stability data support the stability of the drug for up to 1 year of storage under this condition).

(ii) Urine sampling. Urine was collected quantitatively by weight. Two 10-ml aliquots of urine were stored at −20°C until analysis (stability data support the stability of the drug when it is stored for up to 1 year under this condition). Urine was collected from 0 to 72 h after drug administration over the following collection intervals: prior to drug administration (blank sample) and at 0 to 6 h, 6 to 24 h, and 24 to 72 h following drug administration. The bladder was voided before the beginning of each collection interval.

(iii) Bioanalytical method. A validated high-pressure liquid chromatography-tandem mass spectrometry/mass spectrometry method was used for the assay of plasma and urine samples. An aliquot of human plasma or urine containing BILR 355 with an internal standard was extracted by a protein precipitation/online switch-valve procedure. The extracted samples were analyzed with a high-pressure liquid chromatograph equipped with a PE Sciex API 3000 mass spectrometer. Positive ions were monitored in the selected reaction-monitoring mode. Quantification was performed by use of the peak area ratio. The calibration range was 1.99 ng/ml to 497.00 ng/ml. The lower limit of quantification was 2 ng/ml for both urine and plasma samples. For plasma, the between-batch precision and accuracy were 5.3% to 5.5% and 95.8% to 98.0%, respectively; the within-batch precision and accuracy were 1.9% to 9.3% and 91.1% to 100.3%, respectively. For urine, the between-batch precision and accuracy were 2.7% to 5.4% and 110.5% to 113.7%, respectively; the within-batch precision and accuracy were 2.1% to 4.6% and 103.2% to 106.4%, respectively.

PK analyses. Plasma BILR 355 concentration-time data were analyzed by a noncompartmental approach with the WinNonlin program (version 4.01; Cary, NC). Standard formulas for noncompartmental PK analysis provided by WinNonlin were used to determine the maximum concentration of drug in serum (C_{max}), the time to C_{max} (T_{max}), oral clearance (CL/F), mean residence time after oral administration ($MRT_{p.o.}$), and the apparent V in the terminal phase (V_z/F). The area under the plasma concentration-time curve (AUC) was calculated by using a linear up/log down trapezoidal algorithm. The last predicted concentration was used for extrapolation of the AUC and the area under the first moment of the concentration-time curve. The V at steady state (V_{ss}) was calculated as $CL/F \cdot (MRT_{p.o.} - 1/K_a)$, where K_a (the absorption rate constant) was determined by compartmental modeling of the data. The renal clearance (CL_R) was calculated as the ratio of the amount of unchanged drug excreted in urine (A_e) over a time interval to the AUC over the same time interval. The fraction excreted as unchanged drug in urine (f_e) was calculated as the percentage of A_e compared to the administered dose. The plasma concentrations below the lower limit of quantification were ignored during the PK analysis and calculation of the mean plasma concentration, unless they were in the lag phase between the predose sampling time (0 h) and the time of the first measurable concentration, which were treated as 0. For a scheduled sampling time point of any treatment group, if less than two-thirds of the subjects had concentrations above the lower limit of quantification, then the mean concentration of this time point was not presented in the mean plasma-time plots.

Statistical analysis. The power model was used to analyze dose proportionality in terms of the AUC from time zero to infinity ($AUC_{0-\infty}$) and C_{max} . The model is described by the following equation: $Y_{km} = \alpha \cdot D_k^\beta \cdot e_{km}$, where Y_{km} is the response ($AUC_{0-\infty}$, C_{max}) measured for subject m receiving dose k ; α is the intercept; β is the slope; D_k is the effect of the k th dose, where k is equal to 1, 2, . . . ; and e_{km} is the random error associated with the m th subject who received dose k . Logarithmic transformation yields the linear regression equation, as follows: $\ln Y_{km} = \ln \alpha + \beta \cdot \ln D_k + \ln e_{km}$. Dose linearity requires that β equal 1. Statistical analysis was performed with SAS program (version 8.2, Cary, NC).

RESULTS

Subjects. A total of 54 eligible subjects participated in the study of single ascending doses of BILR 355 given alone. The PK evaluation included 40 subjects treated with active drug. The median age, weight, and height of the volunteers were 31 years (range, 21 to 48 years), 79 kg (range, 60 to 106 kg), and 179 cm (range, 169 to 195 cm), respectively. There were no major differences in demographic or baseline data among the subjects in the different treatment groups (including the placebo group).

Sixty-two subjects entered the study of single ascending

TABLE 1. AEs observed after administration of single oral ascending doses of BILR 355 or placebo by treatment

AE	No. (%) of subjects with AEs or no. (%) of AEs observed after administration of:							
	BILR 1 mg	BILR 5 mg	BILR 12.5 mg	BILR 25 mg	BILR 50 mg	BILR 75 mg	BILR 100 mg	Placebo
Subjects with at least one AE	1 (16.7)	2 (33.3)	1 (20.0)	3 (50.0)	1 (16.7)	4 (66.7)	1 (20.0)	2 (14.3)
Fatigue	1 (16.7)	0	1 (20.0)	0	1 (16.7)	1 (16.7)	1 (20.0)	0
Headache	0	0	0	1 (16.7)	1 (16.7)	0	0	1 (7.1)
Abdominal pain	0	0	0	1 (16.7)	0	1 (16.7)	0	0
Nausea	0	0	0	0	0	0	0	1 (7.1)
Vomiting	0	0	0	0	0	0	0	1 (7.1)
Migraine	0	0	0	0	0	0	0	1 (7.1)
Increased alanine aminotransferase level	0	1 (16.7)	0	0	0	0	0	0
Maculopapular rash	0	0	0	1 (16.7)	0	0	0	0
Constipation	0	0	0	1 (16.7)	0	0	0	0
Neurological eyelid disorder	0	0	0	0	0	1 (16.7)	0	0
Flatulence	0	0	0	0	0	1 (16.7)	0	0
Palpitations	0	0	0	0	0	1 (16.7)	0	0
Herpes simplex	0	1 (16.7)	0	0	0	0	0	0
Frequent bowel movements	0	0	0	0	0	1 (16.7)	0	0

doses of BILR 355 coadministered with RTV; all subjects were treated and completed the planned assessments according to the protocol. In all, eight dose groups were studied. In all dose groups except the 5-mg and 62.5-mg dose groups, eight subjects were treated. Only seven subjects were treated in the 5-mg and 62.5-mg groups because of screening failures. The median age, weight, and height of the subjects were 33 years (range, 21 to 49 years), 77 kg (range, 63 to 102 kg), and 178 cm (range, 168 to 195 cm), respectively. There were no major differences in the demographic or baseline data between treatment groups in this study.

Safety and tolerability. The AEs are summarized in Tables 1 and 2 for BILR 355 alone and BILR 355 plus RTV treatment, respectively. For both studies, all AEs were, overall, of mild to moderate intensity and transient in nature, and in the majority of cases they resolved completely without treatment. There were no severe or serious AEs, and no subject withdrew from the trial because of the occurrence of an AE. The AEs were almost evenly distributed over all treatment groups, including the placebo group (or the group receiving placebo plus RTV), with a slightly higher incidence of six AEs in four subjects (66.7%) in the 75-mg group of BILR 355 alone in the dose-escalation study. There was no evidence of any clinically relevant, drug-related changes according to physical examination, skin inspection, vital signs, neurological assessment, and testing for occult blood in both studies. Additionally, no clinically relevant electrocardiographic changes were detected in

either study. No drug- or dose-related changes were observed in the global tolerability assessment. Six days after dosing of BILR 355, one subject (16.7%) in the 5-mg dose group in the study of BILR 355 alone had an increase in the alanine aminotransferase level of more than double the normal upper limit. It was considered unlikely to have been due to the BILR 355 treatment.

Pharmacokinetics. The mean plasma concentration-time profiles for BILR 355 after oral administration of single doses of BILR 355 alone and coadministration of 100 mg RTV are depicted in Fig. 1 and Fig. 2, respectively; the mean (median and percent coefficient of variation [CV]) PK parameters of BILR 355 alone and of BILR 355 coadministered with RTV are summarized in Table 3 and Table 4, respectively. Dose-normalized plots of $AUC_{0-\infty}$ and C_{max} versus dose are presented in Fig. 3 and Fig. 4 for BILR 355 alone and BILR 355 coadministered with RTV, respectively. BILR 355 was rapidly absorbed and eliminated following oral administration, with median T_{max} values of 0.5 to 2.0 h and mean $t_{1/2}$ values of 1.86 to 5.02 h. The mean C_{max} showed a trend toward more than a dose-proportional increase in relation to the dose. A nonlinear relationship was also observed between the mean exposure ($AUC_{0-\infty}$) and dose (36.3 ng · h/ml at the 5-mg dose to 1,308.4 ng · h/ml at the 100-mg dose). Increasing oral doses led to decreases in CL/F from a mean of 246 liters/h with the 12.5-mg dose to a means of 79.2 liters/h with the 100-mg dose. The very high mean CL/F at the 75-mg dose level was the result of a very

TABLE 2. AEs observed after single oral ascending doses of BILR 355 or placebo coadministered with 100 mg RTV by treatment

AE	No. (%) of subjects with AEs or no. (%) of AEs observed after administration of:									
	BILR 5 mg	BILR 12.5 mg	BILR 25 mg	BILR 37.5 mg	BILR 50 mg	BILR 62.5 mg	BILR 75 mg	BILR 87.5 mg	BILR 50 mg (fed)	RTV only
Subjects with at least one AE	1 (14.3)	0	1 (16.7)	1 (16.7)	0	0	0	2 (33.3)	1 (16.7)	1 (8.3)
Aphthous stomatitis	1 (14.3)	0	1 (16.7)	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	1 (16.7)	0	1 (8.3)
Fatigue	0	0	0	0	0	0	0	0	1 (16.7)	0
Maculopapular rash	0	0	1 (16.7)	1 (16.7)	0	0	0	1 (16.7)	0	0

FIG. 1. Mean plasma BILR 355 concentration-time profiles after the administration of single ascending oral doses of BILR 355.

large CL/F in a single subject, which might be considered an outlier. Less than 1% of the BILR 355 doses administered were excreted in the urine as unchanged drug. The majority of the urinary excretion of BILR 355 was completed within 6 h after drug administration, and almost all renally excreted BILR 355 was recovered within 24 h postdosing (data not shown). It appears that the f_e of BILR 355 increased with an increase in the dose. The median T_{max} decreased from 2.0 h to 0.50 h as the dose increased from 5 mg to 100 mg, indicating that T_{max} is dose dependent. The mean $t_{1/2}$ values for the BILR 355 doses of 5, 12.5, 25, 50, 75, and 100 mg administered were 5.0, 4.1, 3.4, 2.5, 2.2, and 1.9 h, respectively; there was a gradual decrease in the $t_{1/2}$ with an increase in the dose. The mean apparent V_z/F values were 955, 1,425, 1,037, 459, 831, and 213 liters for the BILR 355 dose groups of 5, 12.5, 25, 50, 75, and 100 mg, respectively, indicating a trend of decreasing volume with an increase in the dose. A similar pattern was observed for the apparent V_{ss}/F .

Statistical analyses of the doses versus exposures revealed that over the BILR 355 dose range of 5 to 100 mg, the point estimate and 95% confidence interval (CI) of the regression slopes based on a power model (analysis of covariance) were 1.98 (95% CI, 1.58 to 2.38), 1.58 (95% CI, 1.22 to 1.94), and 1.41 (95% CI, 1.08 to 1.76) for C_{max} , AUC from 0 to the last quantifiable plasma concentration (AUC_{0-last}), and $AUC_{0-\infty}$, respectively. As the 95% CI did not contain a value of unity (1.00) for either exposure parameters, a conclusion for dose proportionality could not be determined statistically for the dose range studied.

Following the oral administration of BILR 355 with RTV, BILR 355 was rapidly absorbed and resulted in a median T_{max} of 3 to 6 h for subjects given lower dose levels (5 mg and 12.5 mg) and 1 to 2 h for subjects given dose levels of 25 mg and higher (Fig. 2 and Table 4). After the C_{max} was reached, the plasma BILR 355 concentration declined at a moderate rate, and the terminal $t_{1/2}$ s were approximately 14 to 16 h and 10 to 12 h for the low dose levels (5 mg and 12.5 mg) and higher dose levels (25 mg and above), respectively. The mean CL/F was relatively constant and ranged from 5.88 to 8.47 liters/h. The

FIG. 2. Mean plasma BILR 355 concentration-time profiles after the administration of single ascending oral doses of BILR 355 with 100 mg RTV.

plots of the dose-normalized $AUC_{0-\infty}$ and C_{max} versus dose (Fig. 4) suggest that increases in the dose of BILR 355 result in approximately linear increases in $AUC_{0-\infty}$ over the dose range of 5 mg to 87.5 mg studied. However, the proportional increases in C_{max} with increasing doses were observed only over the dose range of 25 mg to 87.5 mg. Statistical analyses of C_{max} and $AUC_{0-\infty}$ versus the doses revealed that over the dose range of 5 mg to 87.5 mg, the 95% CI of the regression slope contained the value of unity only for $AUC_{0-\infty}$ (point estimate, 0.97; 95% CI, 0.90 to 1.04) but not for C_{max} (point estimate, 1.27; 95% CI, 1.20 to 1.33), suggesting that dose proportionality is demonstrated only with the extent of exposure for this dose range. In the case of the dose range of 25 mg to 87.5 mg, the 95% CI of the slope attained the value of unity for both $AUC_{0-\infty}$ and C_{max} . About 5% of the BILR 355 dose administered was recovered in urine as unchanged drug during the time interval from 0 to 72 h for all dose levels. Table 5 compares the mean PK parameters for BILR 355 after the administration of single doses of BILR 355 with or without RTV boosting. After RTV boosting, the increases in $AUC_{0-\infty}$ ranged from 15-fold (50 mg and 75 mg) to 31-fold (12.5 mg and 25 mg); the mean C_{max} increased about 2- to 3-fold and 5- to 6-fold for high (50 and 75 mg) and low (12.5 and 25 mg) dose levels, respectively. The $t_{1/2}$ s of BILR 355 increased from 2 to 4 h (without RTV boosting) to 11 to 16 h (with RTV coadministration).

The PK parameters for BILR 355 after the coadministration of BILR 355 at 50 mg with RTV in the fed state are presented in Table 4. The mean $t_{1/2}$ s of BILR 355 were approximately 11 h in subjects receiving treatments in either the fasting or the fed state. The mean C_{max} , $AUC_{0-\infty}$, and T_{max} values of BILR 355 after administration for subjects in the fed state were 91.9%, 122.0%, and 626.3%, respectively, of the values determined for subjects in the fasted state.

DISCUSSION

The objectives of the two studies described here were (i) to characterize the preliminary PK of BILR 355, a second-gen-

TABLE 3. PK parameters for BILR 355 after administration of single oral ascending doses of BILR 355

Dose (mg)	Statistic	AUC _{0-∞} (ng · h/ml)	AUC _{0-last} (ng · h/ml)	C _{max} (ng/ml)	T _{max} (h)	t _{1/2} (h)	CL/F (liters/h)	MRT _{p.o.} (h)	V _Z /F (liters)	V _{ss} /F (liters)	CL _R (liters/h)	f _e (%)	% AUC _{extrap} ^a
5	Mean	36.3	13.6	4.36	1.50	5.02	149	7.74	956	1020	0.271	0.172	42.3
	Median	28.9	16.7	4.20	2.00	4.45	173	6.83	1,110	1,150	0.286	0.130	42.3
	CV (%)	37.0	62.8	34.2	47.1	58.3	30.5	51.3	30.7	24.0	47.5	80.8	45.2
12.5	Mean	71.8	56.2	14.8	1.00	4.10	246	6.12	1,430	1,460	0.411	0.212	30.0
	Median	49.4	33.5	8.92	1.00	3.23	253	5.18	1,500	1,620	0.363	0.180	29.1
	CV (%)	83.9	107	97.3	0.00	46.7	48.6	31.0	54.7	48.7	30.6	58.1	49.3
25	Mean	142	128	41.6	0.833	3.44	204	4.52	1,040	914	0.688	0.405	11.7
	Median	123	109	32.7	1.00	3.07	210	4.31	828	846	0.620	0.255	10.0
	CV (%)	43.6	49.0	69.9	31.0	40.2	40.0	22.3	62.9	47.7	48.1	78.7	57.9
50	Mean	419	406	164	0.667	2.50	129	3.20	460	390	0.549	0.485	3.09
	Median	448	435	174	0.500	2.64	113	3.22	425	383	0.489	0.380	3.20
	CV (%)	27.5	27.7	24.4	38.7	10.8	32.2	10.9	30.0	25.3	38.6	61.2	27.4
75	Mean	779	763	348	0.667	2.21	272	2.95	832	608	0.615	0.602	2.98
	Median	835	820	349	0.500	2.16	105	2.85	344	282	0.651	0.585	1.83
	CV (%)	67.5	67.6	64.1	38.7	11.6	151	12.2	146	133	31.5	71.7	89.3
100	Mean	1,310	1,300	937	0.550	1.86	79.2	2.23	213	163	0.591	0.816	0.941
	Median	1,340	1,320	939	0.500	1.89	74.7	2.25	238	176	0.468	0.630	0.934
	CV (%)	20.8	20.8	15.1	38.0	12.6	21.3	13.0	24.3	21.6	54.0	68.6	50.8

^a AUC_{extrap}, extrapolated area for estimation of AUC_{0-∞}.

eration NNRTI, in healthy male volunteers after the administration of a single oral dose of BILR 355 alone and in combination with low-dose RTV and (ii) to document the safety and tolerability of BILR 355 in these subjects.

In these studies, only male subjects were enrolled because the reproductive toxicological study had not been completed when these studies were initiated, and on the basis of regulatory requirements, no healthy female subjects (with ages comparable to those of the male subjects in the study) were allowed to enroll in the study. It is very important to evaluate the PK in female subjects; this is particularly true when a drug's metabolism is mediated by the cytochrome P450 (CYP) CYP3A4 isozyme (7). We did enroll female subjects in later phase I studies (i.e., drug interaction studies and formulation studies) of BILR 355, and data from those studies will be published at a later date. In the present studies, no heavy smokers (those who smoke <10 cigarettes/day) were allowed to participate in the study; however, during the study, the subjects were not allowed to smoke. Since CYP3A4 is the major enzyme identified to be responsible for the metabolism of BILR 355 and the literature so far contains no data suggesting that smoking has a significant impact on the activity of CYP3A4, it is believed that the enrollment of smokers in this study had a minimal impact on the evaluation of the PK of BILR 355.

For both studies, six subjects received active drugs and two received a placebo. Due to a lack of prior clinical data, it is usually not possible to accurately perform a sample size calculation for a human study conducted for the first time; however, it is generally believed that the sample size selected (six subjects receiving active drugs and two subjects receiving a placebo) is sufficient for the evaluation of the safety and preliminary PK of a new chemical entity (6).

The safety parameters investigated in these two studies included the standard parameters usually used in the first studies

to be conducted with humans, as well as other specific tests performed because of the toxicological profiles and experience with the NNRTI class of drugs. With and without coadministration with RTV, BILR 355 displayed an excellent safety profile. Analyses of AEs, laboratory parameters, and other safety data did not raise any concerns over the safety and tolerability of single oral doses of BILR 355. No dose-AE relationship was observed, and BILR 355 did not appear to affect electrocardiographic parameters, including the Q-T interval. No deaths, no severe AEs, and no AEs leading to treatment discontinuation occurred. No drug-related changes in vital signs or laboratory abnormalities were identified.

In the present study, some extreme values for the PK parameters were reported, particularly for those PK parameters obtained after the administration of BILR 355 alone. To better represent the data, both mean and median values are reported in the PK tables (Tables 3 and 4). The percentage of the extrapolated area for estimation of AUC_{0-∞} was also reported. As showed in Table 3 and 4, the percentages of the extrapolated AUC for 5 mg and 12.5 mg of BILR 355 after BILR 355 was given alone were more than 20%, suggesting a bias in the estimation of AUC_{0-∞} for these two groups. The analysis of dose proportionality was therefore performed by using both AUC_{0-∞} and AUC_{0-last} values. The assumption for the power model was checked and confirmed that the required linear relationship between the expected logarithm of the exposures (AUC and C_{max}) and the logarithm of the doses was indeed established. The residuals of the fitted analysis of covariance model (power model) on the logarithmic scale were examined and confirmed the normal distribution of the residuals; in addition, no sequence effect was found (data did not show).

By using recombinant CYP enzymes in a metabolism study, BILR 355 was found not to be the substrate of CYP2B6, CYP2D6, CYP1A2, CYP2C9, or CYP2C19; the CYP3A4

TABLE 4. PK parameters of BILR 355 after coadministration of single oral ascending doses of BILR 355 with 100 mg RTV

Dose (mg)	Statistic	AUC _{0-∞} (ng · h/ml)	AUC _{0-last} (ng · h/ml)	C _{max} (ng/ml)	T _{max} (h)	t _{1/2} (h)	CL/F (liters/h)	MRT _{p.o.} (h)	V _z /F (liters)	V _{ss} /F (liters)	CL _R (liters/h)	f _e (%)	% AUC _{extrap} ^a
5	Mean	707	615	25.3	5.71	14.7	7.33	24.8	153	166	0.362	4.78	12.7
	Median	623	567	22.6	6.00	14.6	8.02	24.5	164	173	0.363	4.57	12.0
	CV (%)	22.4	20.7	24.2	42.5	20.8	18.7	14.8	21.6	18.8	20.9	19.2	36.2
12.5	Mean	1,960	1,680	74.1	3.59	15.9	6.70	24.8	149	153	0.438	6.37	13.4
	Median	2,030	1,790	80.4	3.00	16.8	6.21	25.3	143	143	0.460	7.00	14.2
	CV (%)	22.6	19.8	19.6	67.5	20.9	24.4	14.8	20.7	18.3	37.2	38.1	34.9
25	Mean	4,340	4,140	226	1.75	11.5	5.88	18.1	97.6	102	0.390	6.62	4.41
	Median	4,230	4,070	228	1.50	11.8	5.94	18.4	92.5	99.2	0.387	5.89	4.85
	CV (%)	16.1	15.0	15.0	71.1	8.48	16.0	5.84	19.4	15.5	29.2	35.7	54.0
37.5	Mean	5,960	5,800	344	1.50	11.0	6.52	16.9	104	105	0.295	4.58	2.89
	Median	6,330	6,180	339	1.50	10.6	5.95	17.0	105	104	0.240	4.35	3.19
	CV (%)	19.8	21.1	18.3	36.5	17.2	21.5	8.71	27.6	20.6	43.6	41.9	58.3
50	Mean	6,800	6,540	468	0.958	11.5	7.84	16.7	129	125	0.292	3.58	4.08
	Median	7,140	6,730	431	0.875	11.3	7.00	16.6	120	113	0.287	3.70	5.15
	CV (%)	26.2	27.7	37.8	58.1	6.70	29.2	5.47	27.2	27.6	48.8	35.7	52.0
62.5	Mean	7,630	7,480	653	0.700	9.73	8.47	14.0	118	113	0.430	5.05	1.98
	Median	7,790	7,700	579	0.750	9.61	8.03	14.3	110	108	0.401	5.44	1.89
	CV (%)	19.9	19.9	24.2	29.9	10.4	21.4	10.2	21.0	16.4	30.8	20.2	57.3
75	Mean	12,300	12,100	812	1.63	11.9	6.35	17.4	108	105	0.351	5.44	1.90
	Median	12,100	11,800	817	1.38	11.3	6.18	16.9	103	101	0.347	5.76	1.22
	CV (%)	21.2	21.6	15.0	83.6	16.8	22.4	15.2	22.2	16.2	30.1	16.9	91.8
87.5	Mean	11,800	11,700	838	1.50	9.61	8.28	14.6	114	112	0.492	5.96	1.10
	Median	11,100	11,000	934	2.00	9.02	8.22	14.8	103	107	0.416	5.91	0.785
	CV (%)	35.4	36.1	25.4	51.6	21.5	34.8	14.7	40.8	29.4	46.9	30.5	87.0
50 (fed)	Mean	8,300	7,970	430	6.00	11.0	6.26	18.7	100	81.4	0.299	4.62	4.32
	Median	8,410	8,120	430	6.00	10.5	6.05	17.3	96.3	77.5	0.289	4.19	4.62
	CV (%)	21.1	23.1	16.3	0.00	15.6	21.5	13.3	29.0	18.5	48.1	37.4	65.5

^a AUC_{extrap}, extrapolated area for estimation of AUC_{0-∞}.

isozyme was identified as an isoform of human CYP responsible for the metabolism of BILR 355, with an estimated K_m of 377.2 ng/ml obtained when recombinant CYP was used. In a study with human microsomes and specific enzyme inhibitors, CYP3A (CYP3A4/5) was found to be the major isoform of the human CYP3A4 responsible for the metabolism of BILR 355 (Boehringer Ingelheim Pharmaceuticals, Inc., data on file). A preliminary transport study with Caco-2 monolayer cell cultures suggested that BILR 355 is a P-glycoprotein substrate. Given the low K_m value for CYP3A4, it is expected that saturated metabolism and/or absorption might occur during a clinical study. Indeed, a pronounced, more than dose-proportional increase in $C_{max}/AUC_{0-∞}$ was observed with increasing single doses of BILR 355 alone, particularly over the dose range of 75 mg to 100 mg (Fig. 1 and Table 3), suggesting saturated absorption and elimination. In addition, a slight increase in the f_e of unchanged BILR 355 over the studied dose was observed, indicating a saturation of nonrenal clearance. The saturation of absorption and, possibly, that of elimination after the oral administration of BILR 355 suggest that at least one of the following processes may occur at higher doses: (i) BILR 355 saturated intestinal CYP 3A4/5 and/or other CYP enzymes; (ii) BILR 355 saturated the intestinal transporters, particularly P-glycoprotein; (iii) BILR 355 had a dose-dependent formu-

lation effect; or (iv) BILR 355 saturated the metabolism enzymes (i.e., CYP3A4/5) responsible for BILR 355 elimination. Since after the coadministration of BILR 355 and RTV the nonlinear PKs observed after the administration of BILR 355 alone largely disappeared (Fig. 3 and Fig. 4), even though the same BILR 355 formulation (a powder in a solution for oral consumption) was used, it is hypothesized that the saturated PKs were probably not caused by a formulation effect but were caused by a combination of saturated absorption and elimination processes.

Interestingly, the terminal $t_{1/2}$ slightly decreased with increasing doses; however, this may be an artifact because of the fewer sampling time points obtained for the low-dose group and the larger number of sampling time points obtained for the high-dose group. A preliminary population PK analysis of these data suggested that the observed change in $t_{1/2}$ was probably an artifact.

After BILR 355 was given alone, both V_{ss} and V_z/F decreased with increasing doses. This was likely attributed to the fact that the absorption and/or the elimination process was saturated at higher dose, which led to an increase in relative bioavailability (a decrease in the apparent CL/F) with an increase in the dose, since both V_{ss} and V_z/F were related to CL/F and, therefore, the reported values of V_{ss} and V_z/F were prob-

FIG. 3. Plot of dose-normalized (norm) $AUC_{0-\infty}$ (AUC_{0-inf}) and C_{max} after the administration of single doses of BILR 355 alone versus the doses.

ably biased, given the fact that apparent CL/F was changed with the dose administered.

RTV, a mechanism-based CYP3A4/5 inhibitor, inactivates CYP3A by forming a reactive metabolite which irreversibly

FIG. 4. Plot of dose-normalized (norm) $AUC_{0-\infty}$ (AUC_{0-inf}) and C_{max} after the administration of single doses of BILR 355 with 100 mg RTV versus the doses.

TABLE 5. Ratios of mean BILR 355 PK parameters after administration of a single dose of BILR 355 plus RTV compared with that after administration of a single dose of BILR 355 alone

BILR 355 dose (mg)	Fold change in PK parameters after administration of BILR 355 and RTV		
	AUC	C_{max}	$t_{1/2}$
12.5	31.0	5.0	3.9
25	31.1	5.4	3.3
50	15.5	2.9	4.6
75	15.0	2.3	5.4

inactivates cytochrome activity (17). RTV is the most potent inhibitor of CYP3A4 activity of the currently available antiretroviral agents. In addition, RTV is an inhibitor of P-glycoprotein (10, 13). Because of its better safety profile and sufficient inhibitory effects, low-dose RTV (100 or 200 mg once or twice daily) has been widely used as a boosting agent to alter the PK profiles of coadministered antiretroviral agents (20). For example, saquinavir (SQV) and lopinavir (LPV) coadministration with low-dose RTV results in increased plasma concentrations of SQV and LPV, including increased C_{max} s and minimum concentrations of drug in serum (C_{min}) and prolonged elimination $t_{1/2}$ s for SQV and LPV (9, 15, 20). The increase in C_{max} is possibly the result of the inhibition of first-pass metabolism and/or P-glycoprotein transport by RTV (9, 15, 20). On the other hand, low-dose RTV increased the elimination $t_{1/2}$ s, C_{min} s, and extent of indinavir and amprenavir bioavailability but had a negligible effect on C_{max} , indicating that RTV has little effect on the absorption of these two antiretroviral agents (16, 20).

In the current study, the coadministration of BILR 355 with low-dose RTV resulted in 15- to 30-fold increases in $AUC_{0-\infty}$ s, 2- to 5-fold increases in C_{max} s, and 3- to 5-fold increases in elimination $t_{1/2}$ s. The dramatic boosting effects of RTV on the values of $AUC_{0-\infty}$, $t_{1/2}$, and C_{max} for BILR 355 indicate that RTV alters the absorption as well as the metabolism and elimination of BILR 355. The results suggest the impairment of the first-pass metabolism of CYP3A4 (intestinal) and/or the P-glycoprotein transporter, as well as systemic (hepatic) CYP3A4. The prolongation of the BILR 355 $t_{1/2}$ from 2 to 4 h to 10 to 12 h when BILR 355 is administered with a low dose of RTV may permit a reduction in the dosing frequency required while maintaining the minimum concentration of BILR 355 required for the suppression of HIV replication. An extended dosing interval should improve patient adherence. Prolongation of the $t_{1/2}$ may also reduce the fluctuations in the steady-state C_{max} to C_{min} of BILR 355 and thereby potentially improve patient tolerability of the drug.

The plasma concentration of BILR 355 at 12 h postdosing (C_{p12h}) was also markedly increased after BILR 355 was coadministered with RTV. For example, at the 75-mg dose level, the mean C_{p12h} was 4.7 ng/ml when BILR 355 was given alone, whereas the mean C_{p12h} was 343.5 ng/ml after BILR 355 was coadministered with RTV. The increase in C_{p12h} would likely result in an increase in the C_{min} at steady state ($C_{min,ss}$) with a twice-daily dosing regimen. A significant increase in the plasma $C_{min,ss}$ for BILR 355 would be expected to increase the rate of

virologic success with this agent because high C_{min} s of NNRTIs have been shown to positively correlate with rapid and sustainable viral suppression (12, 21). However, it should be pointed out that the chronic effects of the coadministration of BILR 355 and RTV need to be further examined in multiple-dosing studies either with healthy volunteers or with patients to confirm the boosting effect of RTV on the PKs of BILR 355 and to obtain more safety data.

Consistent with the belief that metabolism plays a major role in the elimination of BILR 355, the urinary excretion of BILR 355 after the administration of single doses of BILR 355 alone was found to be less than 1% of the administered dose for all dose groups. The urinary excretion of BILR 355 after oral administration of the drug alone gradually increased from 0.172% to 0.816%, a more than fourfold increase, suggesting a saturation of nonrenal clearance; however, since the urinary excretion of BILR 355 was low and the variability (60 to 80%) was quite large, more studies (data) may be needed to confirm this observation. After coadministration with RTV, the urinary recovery of BILR 355 was consistently found to be 5%, suggesting an increase in the systemic availability of BILR 355.

The effect of food on the PKs of BILR 355, after coadministration with RTV was examined with a small number of subjects ($n = 6$) at the 50-mg dose level. Because of the exploratory nature of the study, a formal inferential statistical analysis was not performed. Comparison of the relative bioavailability of BILR 355 in fed subjects and that in fasting subjects was based on the group arithmetic mean exposure ratios for the fed state versus the fasting state. The mean C_{max} , $AUC_{0-\infty}$, and T_{max} of BILR 355 in the fed state were 91.9%, 122.0%, and 626.3%, respectively, of those in the fasted state. The high-fat and high-calorie breakfast apparently somewhat decreased the rate of absorption (C_{max} , T_{max}) but increased the extent of absorption ($AUC_{0-\infty}$) for BILR 355; all mean changes in exposures ($AUC_{0-\infty}$ and C_{max}) were less than 25%.

In conclusion, after the administration of single doses of BILR 355 alone, BILR 355 was rapidly absorbed, with a T_{max} of 0.5 to 1.5 h and with a short elimination $t_{1/2}$ of 2 to 4 h. Increased doses led to a more than a dose-proportional increase in BILR 355 exposure. After coadministration with low-dose RTV, the PK profiles of BILR 355 were markedly altered. The values of $AUC_{0-\infty}$, C_{max} , and $t_{1/2}$ were increased 15- to 30-fold, 2- to 3-fold, and 5- to 6-fold, respectively. The urinary recovery of BILR 355 was increased from less than 1% to approximately 5% in the presence of RTV. Also, the administered doses of BILR 355 with RTV were approximately proportional to the systemic exposures observed. BILR 355 is safe and well tolerated in healthy male subjects after the administration of single ascending doses of BILR 355 alone or with RTV.

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