ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1997, p. 898–905 Vol. 41, No. 5
0066-4804/97/$04.00+0

Multiple-Dose Pharmacokinetics of Ritonavir in Human Immunodeficiency Virus-Infected Subjects

ANN HSU,[1]* G. RICHARD GRANNEMAN,[1] GALEN WITT,[1] CHARLES LOCKE,[1] JON DENISSEN,[1] AKHTERUZZAMAN MOLLA,[1] JOAQUIN VALDES,[1] JUDY SMITH,[1] KEITH ERDMAN,[1] NICK LYONS,[1] PING NIU,[1] JEAN-PHILLIPE DECOURT,[2] JEAN-BERNARD FOURTILLAN,[2] JACQUES GIRAULT,[2] AND JOHN M. LEONARD[1]

Abbott Laboratories, Abbott Park, Illinois,[1] and CEMAF s.a., 86000 Poitiers, France[2]

Received 21 June 1996/Returned for modification 24 November 1996/Accepted 31 January 1997

The multiple-dose pharmacokinetics of ritonavir were investigated in four groups of human immunodeficiency virus-positive male subjects (with 16 subjects per group) under nonfasting conditions; a 3:1 ritonavir: placebo ratio was used. Ritonavir was given at 200 (group I), 300 (group II), 400 (group III), or 500 (group IV) mg every 12 h for 2 weeks. The multiple-dose pharmacokinetics of ritonavir were moderately dose dependent, with the clearance for group IV (6.8 ± 2.7 liters/h) being an average of 32% lower than that for group I (10.0 ± 3.2 liters/h). First-pass metabolism should be minimal for ritonavir. The functional half-life, estimated from peak and trough concentrations, were similar among the dosage groups, averaging 3.1 and 5.7 h after the morning and evening doses, respectively. The area under the concentration-time curve at 24 h (AUC_{24}) and apparent terminal-phase elimination rate constant remained relatively time invariant, but predose concentrations decreased 30 to 70% over time. Concentration-dependent autoinduction is the most likely mechanism for the time-dependent pharmacokinetics. The K_m and initial maximum rate of metabolism (V_{max}) values estimated from population pharmacokinetic modeling (nonlinear mixed-effects models) were 3.43 μg/ml and 46.9 mg/h, respectively. The group IV V_{max} increased to 68 mg/h after 2 weeks. The maximum concentration of ritonavir in serum (C_{max}) and AUC after the evening doses were an average of 30 to 40% lower than the values after the morning doses, while the concentration at 12 h was an average of 32% lower than the predose concentration, probably due to protracted absorption. Less than 2% of the dose was eliminated unchanged in the urine. Triglyceride levels increased from the levels at the baseline, and the levels were correlated with baseline triglyceride levels and AUC, C_{max}, or predose concentrations.

The human immunodeficiency virus (HIV) protease, a virally encoded enzyme, is responsible for the posttranslational cleavage of the *gag* and *gag-pol* polyproteins necessary for the maturation of infectious virions (15, 16, 30). Ritonavir is a synthetic protease inhibitor that has been shown to have activities against HIV type 1 (HIV-1) and HIV-2, including zidovudine-resistant strains, in in vitro experiments with primary and transformed human cell lines. The 50% inhibitory concentrations (IC_{50}s) against laboratory and clinical strains of HIV-1 and HIV-2 were determined to be between 0.016 and 0.115 μg/ml, respectively (14). Ritonavir is highly bound to plasma proteins, with unbound fractions ranging from 1 to 2% (14). The IC_{90} of ritonavir for inhibition of acute HIV-1 cytopathogenicity in medium containing 10% fetal calf serum and 50% human serum was estimated to be approximately 2.1 μg/ml, which was selected as a target steady-state minimum concentration (8, 14, 20). Ritonavir and other drugs that inhibit HIV protease have become the most potent antiretroviral agents for the treatment of HIV-infected patients (7, 10, 27, 31).

After the administration of single oral doses under nonfasting conditions, plasma ritonavir concentrations increased more than dose proportionally, with the dose-normalized area under the plasma concentration-time curve (AUC/*D*) being an average of three- to fourfold higher for the 1,000-mg dose relative to that for the 100-mg dose (11). The rates of nonlinear increase diminished with increasing dose. The apparent terminal-phase elimination half-life ($t_{1/2\beta}$), which averaged 6.4 and 3.5 h for the 100- and 1,000-mg doses, respectively, was negatively correlated with the dose, but not invariably so. Renal clearance (CL_R) was a minor route of elimination, with less than 2% of the dose recovered in urine over a 48-h period. Ingestion of food before receipt of a 600-mg dose of the experimental formulation used in the single-dose study resulted in statistically significant increases in the area under the plasma concentration-time curve (AUC) (62%) and the amount recovered in the urine (101%). However, fasting and nonfasting conditions have minimal effects on the bioavailability of the commercially available liquid and capsule formulations (1). The objective of this study was to investigate the pharmacokinetics and safety of ritonavir in HIV-positive subjects after the administration of multiple oral doses of the experimental formulation under nonfasting conditions.

MATERIALS AND METHODS

Subject selection and screening. Ambulatory, HIV-positive male subjects ages 18 to 45 years with no AIDS-defining illnesses other than nonvisceral Kaposi's sarcoma were eligible for enrollment in this study. Other inclusion criteria included a documented positive enzyme-linked immunosorbent assay for HIV, a confirming positive HIV immunoblot, at least one $CD4^+$ lymphocyte count above 200/mm^3 during screening, no signs or symptoms of acute illness, negative results of a screen for drugs of abuse and alcohol, and the subject's agreement to discontinue all medications starting 2 weeks before the first dose and not to take any medication or alcohol during the study. Subjects were excluded from the study if they had recent known or suspected renal impairment, elevated liver function test results, and a history of substance abuse, psychiatric illness, seizure disorder, retinal abnormalities, or any other condition that could interfere with the observations in the study.

All subjects gave written informed consent to participate in this study.

Study design and dosing. This study used a phase I, randomized, multiple-dose, placebo-controlled, double-blind design. Sixty-four subjects were to be

* Corresponding author. Mailing address: Abbott Laboratories, D-4PK, AP13A, 100 Abbott Park Rd., Abbott Park, IL 60064-3500. Phone: (708) 937-2961. Fax: (708) 938-5193.

enrolled into four dosage groups (groups I, II, III, and IV) of 16 subjects each. Twelve subjects in each group were to receive ritonavir and four subjects in each group were to receive placebo. The subjects in the four groups received ritonavir at dosages of 200 mg every 12 h (q12h) (group I), 300 mg q12h (group II), 400 mg q12h (group III), and 500 mg q12h (group IV). To assess the effect of multiple dosing on the terminal-phase elimination rate constant (β), only the morning dose was given on days 1 and 17 (first and last days of dosing). No dose was given on day 2.

All subjects were confined to the research facility from approximately 24 h prior to administration of the first dose until all study procedures were completed on day 19. Subjects received a standard diet for meals and snacks. Water intake was not restricted. The formulation used was the same encapsulated liquid containing 200 mg of ritonavir per ml that was used in the initial single-dose study. Since a significant increase in AUC was shown with this formulation under nonfasting conditions (11), all doses were given within 10 min after completion of the scheduled meals (breakfast or dinner) with at least 200 ml of water. Breakfast, lunch, and dinner were served at approximately 0800, 1400, and 2000 h, respectively.

Blood and urine collection and assays. For analyses of ritonavir concentrations, numerous blood samples were collected predosing and at 1, 2, 3, 4, 6, 9, 12, 16, 20, 24, 32, 40, and 48 h after the morning doses on days 1 and 17 and predosing and at 1, 2, 3, 4, 6, 9, 12, 13, 14, 15, 16, 18, and 21 h after the morning doses on days 3, 8, and 16. Predose plasma ritonavir concentrations (hours 0 and 12) were also determined on days 4 to 7, 9, 10, and 12. Lunch was served after the blood collection at 6 h, and dinner was served after the blood collection at 12 h.

The concentrations of ritonavir in plasma and urine were measured by a validated reverse-phase high-performance liquid chromatography procedure with UV detection at 210 nm (11). Mean correlation coefficients for the low (0 to 0.5 μg/ml) and high (0.1 to 7.5 μg/ml) calibration curves for plasma were above 0.9996 and 0.9993, respectively. The between-day precision for the quality control plasma samples with concentrations of 0.025, 0.050, 0.25, 0.50, and 5.00 μg/ml ranged from 5.48 to 7.70%, with accuracy ranging from 96.2 to 103.1%. The lower limit of quantitation in plasma was 0.005 μg/ml.

Mean correlation coefficients for low (0.00 to 0.50 μg/ml) and high (0.10 to 7.50 μg/ml) urine calibration curves were above 0.9994 and 0.9996, respectively. The between-day precision for urine was within 8.17%, with accuracy ranging from 97.2 to 99.2%. The lower limit of quantitation in urine was 0.005 μg/ml.

Plasma protein binding. To determine whether the plasma protein binding of ritonavir changed after multiple dosings, the plasma protein binding of ritonavir was determined for plasma samples obtained at hours 6, 9, 12, and 16 on day 1 and for plasma samples obtained at hours 0, 2, 12, and 14 on days 8 and 16 for two group I subjects and two group IV subjects. In addition, the in vitro protein binding of ritonavir was determined in four plasma samples from healthy humans collected under fasting conditions and 2 h after ingestion of a high-fat meal. The protein binding was determined by an ultrafiltration technique with the Centrifree Micropartition system. Briefly, aliquots of ^{14}C-labeled ritonavir were added to the plasma samples, which were then centrifuged at 2,000 × *g* for 1 h at 22°C in a fixed-rotor centrifuge. Radioassay was performed for duplicate ultrafiltrations of each plasma sample. The precision of this method for the determination of ritonavir protein binding was determined to be ±0.2%.

Data analysis. (i) Noncompartmental pharmacokinetic analysis. The pharmacokinetics of ritonavir were primarily determined by noncompartmental methods. The peak concentration in plasma (C_{max}), the time to reach C_{max} (T_{max}), the minimum concentration (C_{min}), and the predose concentration in the morning and evening (C_0 and C_{12}, respectively) were obtained directly from the observed data. With the exception of four occasions, the apparent terminal-phase β and $t_{1/2\beta}$ after administration of the doses on day 1 and day 17 were computed from the last three measurable concentrations in the apparent terminal log-linear phase. The clinically relevant (functional) $t_{1/2\beta}$ during multiple dosings was computed by using concentrations from T_{max} to the end of a dosing interval. The AUC from time zero to infinity after administration of the dose on day 1 ($AUC_{0-\infty}$) was calculated as $AUC_t + C_t/\beta$, where C_t was the concentration at the time of the last measurable concentration, and AUC_t was calculated by using the linear trapezoidal rule. The area under the concentration-time curve for a 24-h period (AUC_{24}) or over a dosing period ($AUC_{12a.m.}$ or $AUC_{12p.m.}$) was calculated by using the linear trapezoidal rule. The apparent total clearance from plasma (CL/*F*) was calculated as the dose divided by the $AUC_{0-\infty}$ or AUC_{24}. It should be noted that ritonavir has nonlinear pharmacokinetics; therefore, the reported CL/*F* values represent concentration-averaged CL values. Intergroup and interday statistical comparisons were conducted for AUC/*D*s and concentrations (e.g., C_{max}/D and C_{min}/D). CL_R was calculated as the amount of unchanged ritonavir recovered in the urine divided by the AUC_{48} for day 1 and by the AUC_{24} for day 16.

(ii) Compartmental pharmacokinetic analysis with NONMEM. All individual plasma concentration-time data from the four dosage groups were simultaneously fitted to a set of differential equations for a one-compartment open model with first-order absorption and Michaelis-Menten saturable metabolism [$CL/F = V_{max}/(K_m + C)$, where V_{max} is the maximum rate of metabolism] by using the nonlinear mixed-effects models (NONMEM) program developed by Sheiner and Beal (29). On the basis of the observed data, a fixed CL_R value (0.08 liter/h) was included in the model. The diurnal differences in pharmacokinetics were accounted for by assuming different absorptive rate constants (K_a) for the morning and evening doses. Although there was a clear trend of decreasing predose concentrations over the 2-week study period, suggesting autoinduction, the trend of decreasing AUC values over time was not statistically significant. Thus, models with or without enzyme induction were both evaluated. The induced enzyme activity was modeled through a separate enzyme compartment. The extent of induction was assumed to be concentration dependent according to a linear model, and the elimination of enzyme was assumed to be a first-order process (K_{elCYP}). Thus, $V_{max} = V_{max(initial)} + \Delta V_{max}$, in which ΔV_{max} corresponds to the (induced) enzyme expression from the enzyme compartment. Bioavailability was assumed to be unity for all four doses.

Eight pharmacokinetic parameters were estimated in the model: volume of distribution (V), absorptive rate constants for the morning doses (K_a) and evening doses ($K_a \cdot K_{af}$), absorption lag time (A_{lag}), initial maximum metabolism rate [$V_{max(initial)}$], K_m (ritonavir concentration at which metabolism rate is one-half of $V_{max(initial)}$], enzyme input rate due to induction (K_{oCYP} [where CYP is cytochrome P-450] per ritonavir concentration [in micrograms per milliliter]), and elimination rate constant of enzyme (k_{elCYP}). Interindividual random effects (ηs) were modeled for V_{max}, V, K_a, A_{lag}, assuming that the four parameters follow a log-normal distribution and that the ηs are independent with a mean of zero. Intraindividual residual errors were modeled with a mixed-proportional-and-additive-error model. The first-order approximation algorithm was used in the curve fitting.

Post hoc analysis was also performed for the data. Additional estimates of population means for those pharmacokinetic parameters were obtained by using individual post hoc estimates from NONMEM analyses. Within the NONMEM framework, the population mean parameters from individual post hoc estimates were defined as the geometric mean of the individual post hoc estimates. The interindividual random effect was estimated by calculating the standard deviations of the logarithms of the individual post hoc parameters. The 95% confidence intervals for the mean population parameters were obtained by exponentiation of the end point of the 95% confidence intervals.

Statistical analysis. Invariance with dose and day after multiple dosings was tested for pharmacokinetic parameters determined on days 8 and 16 by an analysis of variance (ANOVA) with effects for dose, subjects nested within dose, day (day 8 or 16), and day-by-dose interaction. The same test was also performed with single-dose and multiple-dose AUC/*D* (day 1 versus day 8 or 16). Since there appeared to be a trend of decreasing trough concentrations during the 2-week dosing period, the dose-normalized morning and evening trough concentrations were analyzed separately by using a multivariate repeated measures analysis of variance (6) with day as a within-subject factor (levels defined by the days of trough samples on day 4 and later), using dose as a between-subject factor, and including the day-by-dose interaction. Within the framework of this analysis, the mean for each day prior to day 16 was compared with the mean for day 16. A similar repeated-measures analysis was performed for dose-normalized C_{max} by using data from days 1, 3, 8, and 16, as well as for dose-normalized AUC for days 1, 3, 8, and 16. This same repeated-measures ANOVA model was also used to analyze the first-order disappearance rate constants (K) determined from concentrations at hours 9 and 12 after the morning and evening doses for days 3, 8, and 16. Diurnal effects were investigated by using ANOVA with effects for dose, subjects nested within dose, time of day (morning or evening), and the interaction of dose and time of day. Triglyceride levels were evaluated by an analysis of covariance, with the baseline level as the covariate and with all subjects receiving placebo considered a fifth treatment group. In this analysis, a linear contrast with the doses was determined to assess a trend with dose. Changes in triglyceride levels with time during multiple dosings were investigated by ANOVA with effects for dose (with the placebo considered a 0-mg dose), subjects nested within dose, time of evaluation, and the interaction of time and dose. For each time of triglyceride level evaluation, regressions were performed with baseline level and a pharmacokinetic variable (AUC, C_{max}, or C_{trough}) as the explanatory variables. A significance level of 0.05 was used for all tests; significance levels of between 0.05 and 0.1 were considered marginally significant.

RESULTS

Demographic characteristics and protocol deviations. Sixty-three subjects were enrolled into four dosage groups (groups I, II, III, and IV). In group IV, only 15 subjects entered the study and 11 received ritonavir. For group II, concentrations in plasma were evaluable for 11 of the 12 subjects receiving ritonavir. The average age of the 46 subjects receiving ritonavir throughout the study was 29 years (age range, 21 to 42 years), the average weight was 67.8 kg (weight range, 51 to 92 kg), the average height was 175.2 cm (height range, 162 to 190 cm), the average creatinine clearance (CL_{CR}) was 124 ml/min (CL_{CR} range, 42 to 192 ml/min), and the average α_1-acid glycoprotein concentration was 0.95 g/liter (α_1-acid glycoprotein concentration range, 0.53 to 1.7 g/liter). There was no statistically signif-

TABLE 1. Pharmacokinetic parameters for ritonavir after administration of single and multiple doses in regimens of q12h[a]

Group (no. of subjects)	$AUC_{0-\infty}$ (μg · h/ml), day 1[a]	AUC_{24} (μg · h/ml), day 16[a]	CL/*F* (liters/h)[a]		C_{max} (mg/ml)[a]		T_{max} (h)[a,b]		CL_R (liters/h)[a]		$t_{1/2}$ (h)[c]		C_{min} (μg/ml), day 16[a]
			Day 1	Day 16	Day 1	Day 16	Day 1	Day 16	Day 1	Day 16	Day 1	Day 17	
I (12)	18.7 ± 8.9	43.8 ± 13.0	13.1 ± 6.2	10.0 ± 3.2	2.0 ± 1.0	4.5 ± 1.3	4.3 ± 1.9	3.8 ± 1.0	0.07 ± 0.02	0.08 ± 0.03	8.9	8.5	0.6 ± 0.2
II (11)	33.4 ± 15.9	60.7 ± 23.2	11.8 ± 7.5	11.3 ± 4.4	4.4 ± 2.0	6.5 ± 2.4	3.9 ± 1.2	4.6 ± 2.0	0.10 ± 0.04	0.08 ± 0.05	9.7	8.0	0.7 ± 0.4
III (12)	68.9 ± 28.0	114.2 ± 37.1	7.7 ± 6.2	7.8 ± 2.9	9.0 ± 3.1	11.7 ± 4.0	3.6 ± 0.9	4.3 ± 0.8	0.08 ± 0.03	0.08 ± 0.06	5.6	8.1	1.1 ± 0.6
IV (11)	83.7 ± 32.1	170.3 ± 70.5	6.6 ± 2.0	6.8 ± 2.7	9.6 ± 2.3	14.2 ± 4.7	3.9 ± 0.3	4.3 ± 0.9	0.08 ± 0.04	0.06 ± 0.02	7.0	5.8	2.3 ± 2.3

[a] Data are means ± standard deviations.
[b] Average of morning and evening T_{max}.
[c] Data are harmonic means.

icant difference in demographic characteristics among the individuals in each of the dosage groups.

Pharmacokinetics. Table 1 summarizes the values for the pharmacokinetic parameters estimated from the concentrations in plasma and urine determined after the administration of a single dose on day 1 and after dosing q12h for two weeks (day 16). The observed C_{min}s for day 16 are also presented in Table 1. Figure 1 presents the mean plasma concentration-time profiles of ritonavir over the 2-week dosing period for the four dosage groups. In general, the pharmacokinetics of ritonavir appeared to be moderately dose dependent over the dosage range studied. A clear trend of decreasing predose ritonavir concentrations over the 2-week dosing period was observed; however, AUC values appeared to be relatively time invariant. In addition, there was also an apparent diurnal variation in the pharmacokinetics of ritonavir. These pharmacokinetic characteristics of ritonavir are discussed below.

(i) Effect of dose. Unlike the results observed after increasing the single doses administered, which showed a 3.6-fold decrease in CL/*F* for a 10-fold dose range (100 to 1,000 mg) (11), ritonavir pharmacokinetics after multiple dosings were only moderately nonlinear, with the CL/*F* for the highest dosage group averaging about 40% lower than that for the lowest dosage group. CL_R averaged 0.08 liter/h for all dosage groups throughout the 2-week dosing period, which is consistent with the 98 to 99% binding of ritonavir to plasma proteins. Although the plasma of both of the groups receiving drug at 400- and 500-mg q12h initially had minimum concentrations above the targeted protein binding-adjusted IC_{90} (2.1 μg/ml), only the group receiving a dosage of 500 mg q12h maintained a mean C_{min} value above the IC_{90} after dosing for 2 weeks.

Consistent with the decrease in CL/*F* with dose, analyses of the dose-normalized pharmacokinetic parameters on day 8 and day 16 showed that the increase of AUC/*D*, C_0/D, and C_{12}/D with dose were all statistically significant. A significant dosage effect was not observed for C_{max}/D. T_{max} values were similar among the dosage groups, generally occurring 3 to 4 h after the morning doses and 4 to 8 h after the evening doses. The increase in apparent terminal-phase β with dose was statistically significant, as reflected in the trend of decreasing $t_{1/2\beta}$ with dose. For drugs that show Michaelis-Menten kinetics in the dosage range studied, the estimation of a true $t_{1/2\beta}$ can only be obtained when concentrations are substantially below K_m. It should be noted that essentially all of the concentrations that were used to calculate the terminal-phase β were below 0.2 μg/ml, which is significantly lower than the estimated K_m, as discussed later. Since the AUC_{0-12} accounted for an average of 78% of the total single-dose AUC, the terminal phase $t_{1/2\beta}$ is not especially clinically relevant. To provide a measure of the fluctuation of the ritonavir concentration within one dosing interval, $t_{1/2}$ was also estimated on the basis of the mean peak and trough concentrations. The morning $t_{1/2}$ values ranged

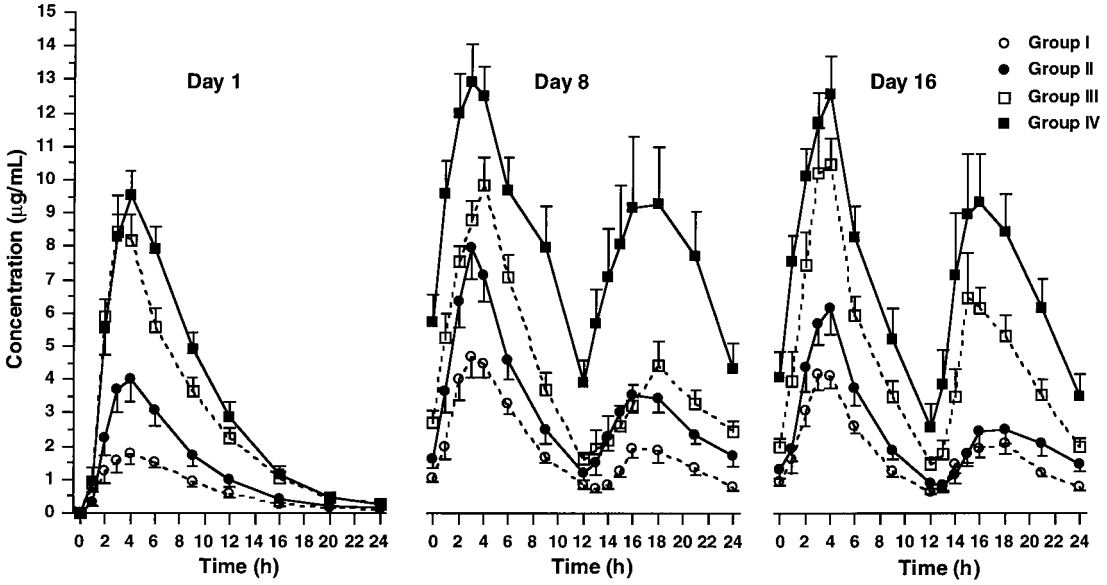

FIG. 1. Mean ± standard error of the mean plasma concentration-time profiles of ritonavir during dosings with multiple oral dosages of 200 mg q12h (group I; $n = 12$), 300 mg q12h (group II; $n = 11$), 400 mg q12h (group III; $n = 12$), and 500 mg q12h (group IV; $n = 11$) for 2 weeks under nonfasting conditions.

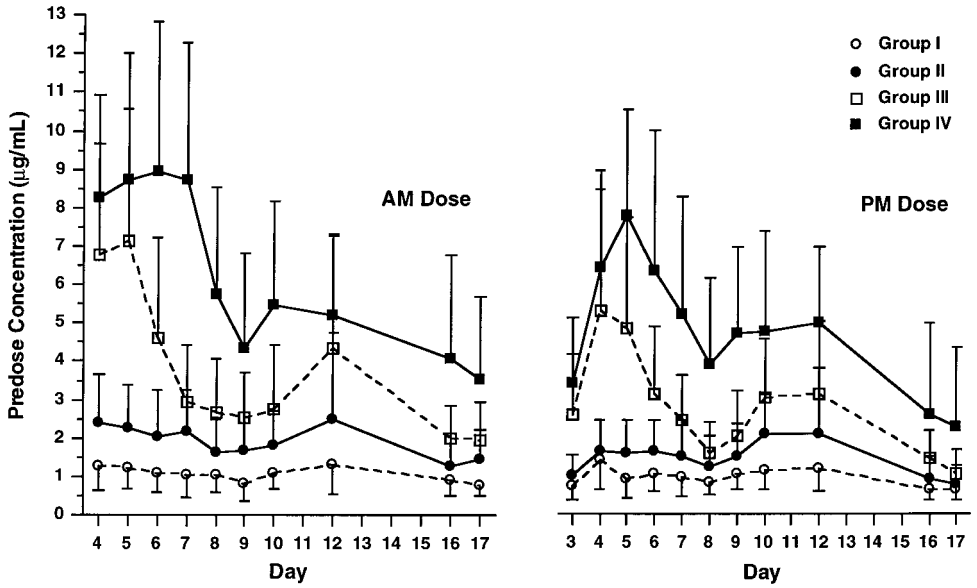

FIG. 2. Mean ± standard deviation predose ritonavir concentrations during dosings with multiple oral dosages of 200 mg q12h (group I; $n = 12$), 300 mg q12h (group II; $n = 11$), 400 mg q12h (group III; $n = 12$), and 500 mg q12h (group IV; $n = 11$) for 2 weeks under nonfasting conditions.

from 3 to 5 h, and those for the evening doses ranged from 5 to 7 h, probably due to the protracted absorption associated with a longer gastric residence time after the evening meal.

(ii) Effect of multiple dosings (time effect). As shown in Fig. 2, predose ritonavir concentrations decreased over the 2-week dosing period. However, AUC values appeared to remain relatively time invariant (Table 1). The statistical analyses revealed that the mean AUC/*D* for day 1 was not statistically significantly different from that for day 8, 16, or 17 ($P > 0.25$ for all comparisons). The mean terminal-phase β values were also not statistically significantly different between day 1 and day 17. However, the mean AUC/*D* for day 8 was marginally statistically significantly higher (11%) than that for day 16. The mean values of $C_{maxa.m.}$, C_0, and C_{12} on day 16 were also significantly or marginally significantly lower than those on day 8.

For predose concentrations, slightly greater decreases were generally associated with the higher doses. The mean changes in C_0s from day 5 to day 16 for dosage groups I to IV were 27, 45, 72, and 54%, respectively, and those for C_{12}s were 31, 44, 70, and 67%, respectively. The results of ANOVAs for multivariate repeated measures for the C_0/D and C_{12}/D values over time showed that the multiple-dose effect was statistically significant for both parameters. The results of the contrast tests showed that the mean predose concentration on day 16 was significantly lower than those determined before day 16, with the exception of C_0 determined on day 9. However, the day 17 C_0/D was similar to the day 16 C_0/D and the day 17 C_{12}/D was slightly higher than the day 16 C_{12}/D, suggesting that the trough concentrations may be stabilized at the end of the 2-week dosing period. Within a dosage group, the highest trough concentration generally was attained after dosing for 2 days.

Similar analyses were performed for the C_{max}/D values determined on days 1, 3, 8, and 16. The least-squares means for C_{max}/D values on day 1 and day 3 averaged across all doses were significantly lower on both days (34.8 and 21.2%, respectively) than that on day 16, while the C_{max}/D values were similar between day 8 and day 16.

(iii) Diurnal variation and fluctuation index. Multiple-dose pharmacokinetic parameters for ritonavir as a function of time of dosing (morning and evening) on day 16 are provided in Table 2. Relative to the respective mean values in the morning, the evening T_{max} was an average of 1.7 h later, the evening C_{max} was an average of 40% lower, and the evening AUC was an average of 32% lower. The C_{12} was an average of 32% lower than the C_0. These differences were all statistically significant. The degree of fluctuation, computed as $(C_{max} - C_{min})/C_{avg}$, ranged from 1.33 (500 mg) to 1.86 (300 mg) during the morning intervals and from 1.24 (500 mg) to 1.36 (400 mg) for the evening intervals. The diurnal difference in T_{max} and C_{max} tended to be less for the high-dose groups than for the lower-dose groups. For predose concentrations, there was not a clear trend with dose.

TABLE 2. Pharmacokinetic parameters for ritonavir on day 16 after administration of morning and evening doses

Group	AUC_{24} (μg · h/ml)[a]		C_{max} (μg/ml)[a]		Predose concn (μg/ml)[a]		T_{max} (h)[a]		$t_{1/2}$(h)[b]	
	Morning	Evening	Morning	Evening	C_0	C_{12}	Morning	Evening	Morning	Evening
I	26.7 ± 8.7	17.1 ± 5.9	4.5 ± 1.3	2.3 ± 0.9	0.9 ± 0.4	0.6 ± 0.3	3.5 ± 1.0	4.1 ± 1.6	3.1	4.1
II	38.1 ± 14.5	22.6 ± 10.7	6.5 ± 2.4	3.2 ± 1.3	1.3 ± 0.9	0.9 ± 0.6	3.2 ± 0.9	6.9 ± 2.8	2.9	7.7
III	65.8 ± 21.0	48.4 ± 19.1	11.6 ± 4.1	7.4 ± 4.0	2.0 ± 0.9	1.5 ± 0.7	3.7 ± 0.7	4.8 ± 1.8	2.9	5.3
IV	90.4 ± 32.4	79.9 ± 39.5	13.0 ± 3.8	11.5 ± 6.3	4.1 ± 2.7	2.6 ± 2.4	3.7 ± 0.5	4.8 ± 2.0	3.6	5.7

[a] Values are means ± standard deviations.
[b] Harmonic mean $t_{1/2}$ based on peak and trough concentrations.

TABLE 3. Pharmacokinetic parameters for ritonavir estimated by curve fitting using NONMEM[a]

Model	OBJ	Estimate (% CV)							
		k_a (h^{-1})	K_{af}	A_{lag} (h)	V (liters)	V_{max} (mg/h)	K_{oCYP} (mg/h)	K_m (μg/ml)	k_{elCYP} (h^{-1})
With autoinduction	3872	1.65 (6)	0.317 (10)	0.616 (10)	49.7 (4)	46.9 (19)	0.0247 (53)	3.43 (25)	0.0082 (72)
Without autoinduction	6068	1.25 (7)	0.377 (8)	0.681 (7)	27.5 (40)	72.2 (16)	NA (NA)	11.9 (36)	NA (NA)

[a] OBJ, objective function; NA, not applicable.

(iv) Intersubject variability. The intersubject variability in CL/*F*, estimated from values obtained on days 1, 8, and 16, was comparable to the variability obtained in the single-dose study, averaging 37 to 52%. Regression analyses found no statistically significant correlation between ritonavir pharmacokinetic parameters with body weight, CL_{CR}, or α_1-acid glycoprotein concentration.

(v) Compartmental analysis by NONMEM. As summarized in Table 3, the model with enzyme induction was substantially superior, with an improvement in goodness of fit, measured by reduction in the objective function, by 774 units. The post hoc estimated plasma concentration-time profiles relative to the observed data are presented in Fig. 3. With the exception of K_{oCYP} and k_{elCYP}, which had coefficients of variation (CV; which is standard error of estimate/parameter estimate) of 53 and 72%, respectively, the CVs for all other parameter estimates were less than 25%, indicating that the precision of the estimation was good. The typical A_{lag} time was estimated to be 0.616 ± 0.062 h, the K_a for the evening dose was estimated to be 68% slower than the K_a for the morning dose (1.65 ± 0.0.095 h^{-1}), the typical apparent V was estimated to be 49.7 ± 1.84 liters, the typical K_m and initial V_{max} were estimated to be 3.43 ± 0.849 μg/ml and 46.9 ± 8.88 mg/h, respectively, the $t_{1/2}$ for CYP3A was estimated to be 3.5 days (elimination rate constant, 0.0082 ± 0.0059 h^{-1}), and the induction rate of enzyme was 0.0247 ± 0.0131 mg/h per ritonavir concentration (in micrograms per milliliter) (Table 3). The interindividual variability (ω^2) estimated for K_a, A_{lag}, V, and V_{max} were 0.936, 0.0315, 0.0888, and 0.0693, respectively, and the standard errors for the corresponding ω^2 estimates were 0.191, 0.0304, 0.0173, and 0.0159, respectively. The residual errors (σ^2) were estimated to be 0.206 and 0.00357 for the proportional error and the additive error, respectively, and the standard errors for the corresponding σ^2 estimates were 0.025 and 0.000995, respectively.

These population means estimated from individual post hoc estimates from NONMEM analyses are expected to represent true population means and to be close to the ones estimated directly from NONMEM since many data were available for each individual. Table 4 indicates that the population mean of K_a directly from NONMEM was slightly higher than the one from individual post hoc estimates, while the population means of V_{max}, V, and A_{lag} directly from NONMEM were matched with the corresponding mean from individual post hoc estimates. In addition, the estimates for interindividual variability for V_{max}, V, and A_{lag} are comparable between NONMEM and post hoc analyses. However, NONMEM estimated a larger intersubject variability for K_a (96.8 versus 40.2% by NONMEM and post hoc analysis, respectively). Overall, the small standard errors associated with parameter estimates and the good agreement between NONMEM and post hoc estimates suggest that the first-order approximation method was adequate in the NONMEM analysis of our dense data set. As discussed later, pharmacokinetic parameters obtained from a phase II outpatient study with a clinical dosage of 600 mg q12h agree well with the values obtained by simulation with these NONMEM parameters.

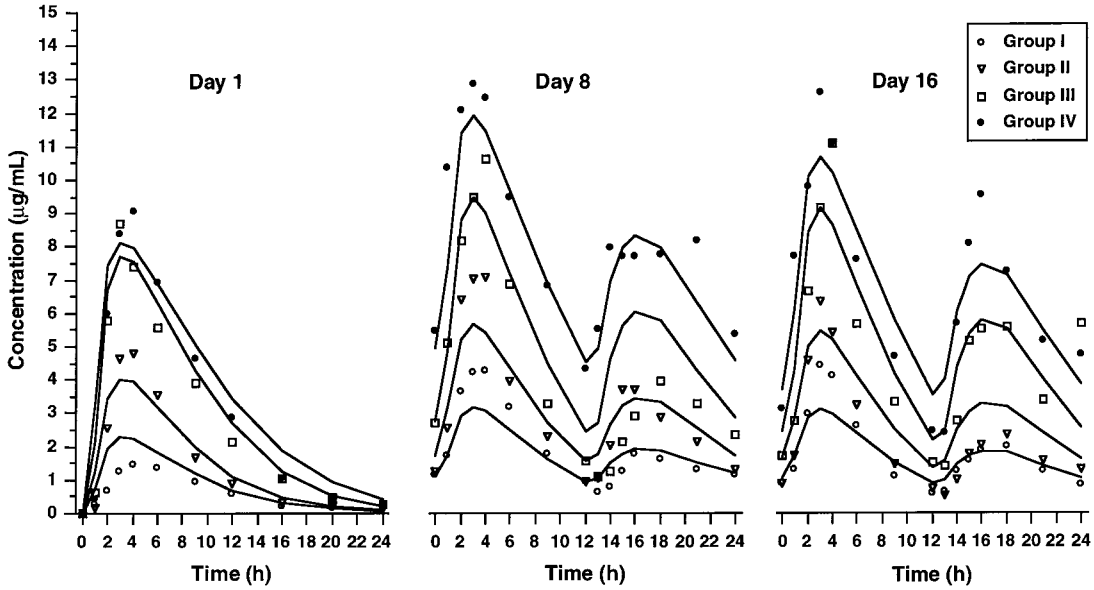

FIG. 3. Mean fitted (post hoc estimate) versus mean observed plasma ritonavir concentration-time profiles during dosings with multiple oral doses.

TABLE 4. Comparison of post hoc and NONMEM in the estimation of population pharmacokinetic parameters for ritonavir[a]

Method and variable	K_a (h^{-1})	A_{lag} (h)	V (liters)	V_{max} (mg/h)
Post hoc				
Estimate	0.967	0.711	42.4	46.5
95% CI	0.884–1.057	0.682–0.731	39.7–45.2	43.2–50.1
% CV	40.2	12.1	25.8	21.8
95% CI	NA	NA	NA	NA
NONMEM				
Estimate	1.65	0.616	49.7	47.1
95% CI	1.46–1.84	0.491 ± 0.741	29.3–64.9	45.9–53.5
% CV	96.8	17.8	29.8	26.3
95% CI	74.4–115	0–34.7	23.3–35.1	19.4–31.8

[a] Post hoc population parameter was calculated as the geometric mean of individual parameters estimated in the NONMEM program. CV denotes interindividual variability (standard deviation/arithmetic mean for post hoc analysis = $100 \cdot \omega$ for NONMEM). NA, not available.

(vi) Elevation of triglyceride levels. As illustrated in Fig. 4, plasma triglyceride levels increased from the baseline levels during the 2-week dosing period for all dosage groups, including subjects who received placebo. With the pretreatment measurement as the baseline and covariate, the increase in triglyceride levels was found to be dose dependent, showing statistically significant trends with dose for the determinations throughout the 2-week period. Relative to the placebo group, the elevation was statistically significant for the group receiving drug at 500 mg q12h after multiple dosings for approximately 3 days; for the groups receiving ritonavir at 400 and 300 mg q12h, it was significant after dosing for approximately 7 days and 2 weeks, respectively, suggesting that the effect is dose dependent.

On the basis of the results of linear regression, including baseline triglyceride level as an additional variable, the indices of determination (R^2) between triglyceride levels and the values of the pharmacokinetic parameters for ritonavir (AUC, C_{max}, or C_0) were similar for all three parameters, ranging from approximately 28 to 45%.

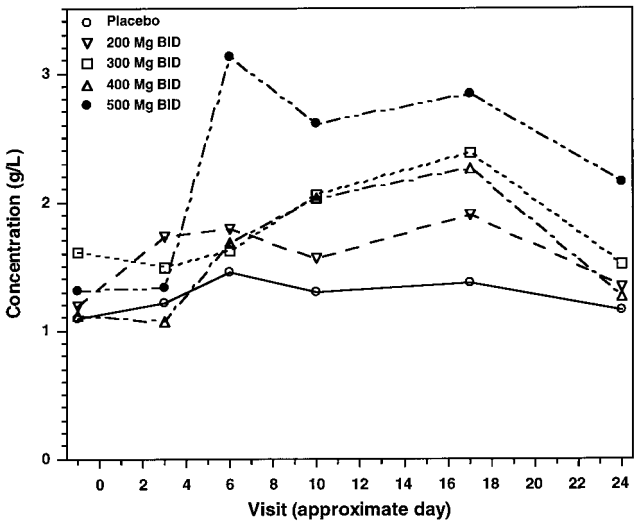

FIG. 4. Least-squares mean triglyceride levels from ANOVA (see Statistical Analysis section in text) for subjects receiving multiple oral dosages of 200 mg q12h (group I), 300 mg q12h (group II), 400 mg q12h (group III), and 500 mg q12h (group IV) for 2 weeks under nonfasting conditions. Measurements are not available for all subjects for all days. The placebo curve represents data from subjects receiving placebo across four study groups. BID, twice daily (q12h).

(vii) Plasma protein binding. The mean plasma protein binding of ritonavir determined for the four subjects decreased from 98.13% ± 0.20% on day 1 to 98.09% ± 0.06% on day 8 and 97.82% ± 0.15% on day 16. Relative to the binding on day 1, the plasma protein binding on day 16 was lower for all four subjects; however, the day 8 plasma protein binding was similar to that on day 1. The study that evaluated the effects of meals on the plasma protein binding of ritonavir showed that the free drug concentrations increased 44.7% ± 20.51% at 1 μg/ml and 53.25% ± 28.91% at 15 μg/ml in the postprandial plasma.

DISCUSSION

The more than dose-proportional increase in ritonavir concentrations with dose observed in the present study generally agrees with that observed in a prior study assessing single doses of ritonavir (11). In vitro studies with human liver microsomes demonstrated that CYP3A is the major isoform involved in ritonavir metabolism, with CYP2D6 also contributing to the formation of one of the major metabolites (17). The nonlinear increase in the concentrations in plasma with dose is probably due to saturable metabolism. The K_m values estimated in cDNA-transfected CYP3A4 B-lymphoblastoid microsomes ranged from 0.08 to 0.71 μM (0.056 to 0.50 μg/ml) for the three major metabolites observed in humans (17). The K_m estimated in the present study was 3.43 μg/ml, which appears to be consistent with the protein binding-corrected in vitro K_m. The initial V_{max} was 46.9 mg/h, indicating that the initial CL would be 13.7 liters/h after the administration of a low dose. After dosing for 2 weeks, the V_{max} was estimated to be 68 and 52 mg/h for the dosage regimens of 500 and 200 mg q12h, respectively. The significantly less pronounced nonlinearity observed in this multiple-dose study compared to that observed in the single-dose study is partly due to the narrower dosage range used in the present study and could also be partly due to a greater extent of induction at higher dosages than at lower dosages. According to the concentration-dependent induction model, the ritonavir V_{max} estimate predicts a low probability of accumulation for dosages at least up to 800 mg q12h. No individual in this study showed evidence of nonlinear accumulation of ritonavir. The low observed steady-state CL of ritonavir (from 6.8 to 11.3 liters/h) indicates minimal hepatic first-pass metabolism and agrees well with the high oral bioavailability of the drug (14). On the basis of the well-stirred model of hepatic metabolism, the extent of first-pass metabolism, expressed as percent extraction = $100 \cdot CL_p/(CL_p + Q_h)$, where CL_p is the CL of ritonavir from plasma and Q_h is the hepatic plasma flow rate at 50 liters/h, is computed to be 12 to 18% for CL ranging from 6.8 to 11.3 liters/h. On the basis of CL from blood ($C_{rbc}/C_{plasma} \simeq 0.1$ for ritonavir, where C_{rbc} and C_{plasma} are concentrations in erythrocytes and plasma, respectively) the extent of first-pass extraction would range from 11 to 17%. Since CYP3A4 is present in gastrointestinal tissue, first-pass loss during absorption of lower ritonavir doses may also explain part of the nonlinearity.

Since the predose concentrations decreased over the 2-week dosing interval while the AUC values appeared to be substantially less affected, various mechanisms such as changes in protein binding, changes in absorption, or enzyme induction were considered to be potential causes for the enigmatic time-dependent changes in ritonavir pharmacokinetics. Since the C_{max} values (those on day 8 versus those on day 16) were relatively time invariant and since no statistically significant change in T_{max} was observed, it is unlikely that the time-

dependent decrease in trough ritonavir concentrations was solely due to faster but not reduced absorption.

For a highly protein-bound drug with a low extraction ratio, a decrease in total drug concentration (bound plus free drug) can result from an increase in the concentration of the free fraction, assuming the CL of the free drug remains constant. Although the free fraction in the plasma of a subset of four subjects from the present study increased slightly (17%) after multiple dosings, the extent of decrease in protein binding was too small to account for the extent of change in trough concentrations. Thus, the apparent time-related change in total concentration slightly overestimates the change in the therapeutically relevant free concentration. In a separate experiment, we also found that the free fraction of ritonavir (1 and 15 μg/ml) in postprandial plasma was an average of 18 to 92% higher than that obtained after an overnight fast. This indicates that changes in the plasma lipid composition may have some effects on the plasma protein binding of ritonavir and may partially account for the diurnal variation in the pharmacokinetics.

Ritonavir is extensively metabolized. In vitro and in vivo studies have shown that it is a potent inhibitor of CYP3A (IC_{50}s, 0.05 to 1.4 μg/ml) and also is a significant inhibitor of CYP2D6 (IC_{50}, 1.75 μg/ml). This has been verified in vivo with clarithromycin, rifabutin, and desipramine as probe substrates (3, 4, 25). Examples of other drugs that inhibit metabolism and that also increase CYP450 enzyme activities include triacetyloleandomycin, erythromycin, and ketoconazole (28). This inhibition-associated induction may be responsible for the nonlinear and nonstationary ritonavir pharmacokinetics in which the overall level of CYP is increased due to either lower posttranslational turnover or increased transcription (2, 24). The increased synthesis might be viewed as a feedback-driven homeostatic response to the effects of inhibition, although the mechanism of CYP3A induction has not been fully elucidated.

Modeling of the time course of ritonavir concentrations in the present study indicated that use of an enzyme induction model significantly improved the quality of fit. The extent of induction was estimated to be relatively small for low dosages such as 200 mg q12h (12%), but would be 45% for a dosage of 500 mg q12h. The estimated time course of enzyme induction ($t_{1/2}$ of 3.5 days) agrees well with the observation that trough concentrations appeared to stabilize at the end of the 2-week dosing (Fig. 2). Consistent with the induction time course, the day 8 C_{min} (dosing q12h for 6 days) and the day 16 C_{min} (dosing q12h for 14 days) were not substantially different. The $t_{1/2}$ of carbamazepine induction of clonazepam metabolism was also estimated to be about 3 days (18, 19). For most inducers, the course of enzyme induction is attained within 1 to 4 weeks (2, 23, 32). It must be recognized, however, that the single-dose pharmacokinetics of ritonavir are nonlinear; thus, standard expectations based on linear pharmacokinetics are misleading. In such cases, comparison of single-dose CL or AUC to multiple-dose values is inappropriate. On the basis of the model fit, with K_m being 3.4 μg/ml, the regimen of 500 mg q12h without autoinduction would produce AUC values on day 14 that would be threefold higher than those observed after a single dose. With the autoinduction phenomenon, the predicted AUC on day 14 is similar to those for the first dose and for day 8, yet the model correctly predicts that C_{min} should decline by more than a factor of 2 from its highest value at about day 4. From our assessment of the results from this study, the presence of Michaelis-Menten kinetics with autoinduction is the most parsimonious model that can account for declining minima, with no apparent effect on T_{max} and with only modest changes in protein binding.

Since this study was the first multiple-dose phase I study of ritonavir, without a prior knowledge of autoinduction, the study did not investigate the recommended clinical dosage of 600 mg q12h. On the basis of the estimated population parameters, assuming no changes in the fraction of dose absorbed, the steady-state AUC, C_{max}, and C_0 for a regimen of 600 mg q12h are simulated to be 201 μg · h/ml, 14 μg/ml, and 4.8 μg/ml, respectively. The mean steady-state AUC, C_{max}, and C_0 observed in 10 HIV-infected patients after receiving ritonavir at 600 mg q12h for 3 to 4 weeks in a phase II outpatient study were 155 μg · h/ml, 11 μg/ml, and 3.7 μg/ml (averages of week 3 and 4 data), respectively (22). The good agreement between predicted and observed values indicates that the pharmacokinetics of ritonavir are reasonably described by the proposed Michaelis-Menten autoinduction model. It should be noted that subsequent studies have substantially strengthened the case for autoinduction, with statistically significant decreases in AUC observed at higher doses, as well as demonstrable inductive effects of CYP1A2 and glucuronosyltransferase (1, 5, 12, 26).

Several of the inducers of CYP such as phenobarbital or rifampin also produce increases in γ-glutamyltransferase (GGT) and alterations in lipid metabolism (2, 21, 22). The enzymes that catalyze triglycerol and GGT syntheses are localized in smooth endoplasmic reticulum, where the synthesis of various enzymes can be induced by a variety of compounds (9, 13). In this regard, while GGT levels were not monitored in the present study, parallel increases in triglyceride and GGT levels have been observed in patients receiving ritonavir in phase II and phase III studies (1, 8, 20). At present, the mechanism for the ritonavir-associated increase in triglyceride level has not been established, but it is known that ritonavir does not affect lipoprotein lipase activity (unpublished data). Consistent with the time course of the multiple-dose pharmacokinetics of ritonavir, triglyceride and GGT levels in patients receiving ritonavir treatment generally reached steady state after dosing for approximately 2 weeks (8).

Since ritonavir showed significant diurnal variation in pharmacokinetics, it may be asked whether this variation has an effect on the antiviral activity of ritonavir, particularly in light of the recent demonstration of the rapid turnover of virions in the plasma of subjects with HIV-1 infection (10). It should be noted that the evening plasma concentration-time profile is substantially flatter than the morning profile, as can be seen from the T_{max} that is 1.7 h later in the evening and the apparent $t_{1/2}$ that is 5 to 7 h in the evening (on the basis of the peak trough concentration) and 3 to 5 h in the morning. Consistently, C_0 is higher than C_{12}. Therefore, the lowest concentration over a 24-h period generally appears to be a brief period immediately after the evening dose was administered. Using literature values of viral dynamics, we have simulated the intraday fluctuation in ritonavir concentrations and find that it has little effect on the suppression of viral activity. This is most easily understood in the context of an E_{max} model. Minimum concentrations for the recommended regimen of 600 mg q12h are near the in vitro protein binding-corrected IC_{90} for HIV; thus, substantial inhibition is maintained throughout the dosing interval.

In summary, under nonfasting conditions, the multiple-dose pharmacokinetics of ritonavir are moderately dose dependent, with the CL/*F* for the group receiving drug at 500 mg q12h averaging about 40% lower than that for the group receiving drug at 200 mg q12h, likely due to saturable metabolism. The low CL of ritonavir indicates minimal hepatic first-pass metabolism. Ritonavir concentrations showed moderate diurnal fluctuation, with morning AUC and C_{max} values being greater than

the evening values by about 30 to 40% and C_{12} being 32% lower than C_0. The AUC_{24} and terminal-phase β values remained relatively time invariant over the 2-week study period, but trough concentrations decreased 30 to 70% across the dosage groups. While the mean trough concentrations from the regimen of 500 mg q12h were near the target IC_{90} of 2.1 μg/ml, there was considerable variability across subjects, probably explaining the clinical observation that a dosage of 600 mg q12h provided a greater and more sustained antiviral response than a dosage of 500 mg q12h (8).

ACKNOWLEDGMENTS

We are grateful for helpful discussions with Stuart Beal (University of California at San Francisco) and Guoliang Cao (Clinical Statistics, Abbott Laboratories) with regard to the results of NONMEM analysis.

REFERENCES

1. **Abbott Laboratories.** 1996. NORVIR™ pro duct information. Abbott Laboratories, Abbott Park, Ill.
2. **Barry, M., and J. Feely.** 1990. Enzyme induction and inhibition. Pharmacol. Ther. **48:**71–94.
3. **Bertz, R. J., G. Cao, J. H. Cavanaugh, A. Hsu, G. R. Granneman, and J. M. Leonard.** 1996. Effect of ritonavir on the pharmacokinetic of desipramine, abstr. 2768. *In* Program and abstracts of the XI International Conference on AIDS.
4. **Cato, A., J. H. Cavanaugh, H. Shi, A. Hsu, G. R. Granneman, and J. M. Leonard.** 1996. Assessment of multiple doses of ritonavir on the pharmacokinetic of rifabutin, abstr. 2766. *In* Program and abstracts of the XI International Conference on AIDS.
5. **Cato, A., A. Hsu, R. Granneman, J. Leonard, G. Carlson, L. Carothers, and C. Locke.** 1995. Assessment of the pharmacokinetic interaction between the HIV-1 protease inhibitor ABT-538 and zidovudine, abstr. I33, p. 211. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Cole, J. W. L., and J. E. Grizzle.** 1966. Applications of multivariate analysis of variance to repeated measures experiments. Biometrics **22:**810–828.
7. **Craig, J. C., I. B. Duncan, D. Hockley, C. Grief, N. A. Roberts, and J. S. Mills.** 1991. Antiviral properties of Ro 31-8959, an inhibitor of human immunodeficiency virus (HIV) proteinase. Antiviral Res. **16:**295–305.
8. **Danner, S. A., A. Carr, J. M. Leonard, L. M. Lehman, F. Gudiol, J. Gonzales, A. Raventos, R. Rubio, E. Bouza, V. Pintade, A. Gil Aguado, J. Garcia de Lomas, R. Delgado, J. C. C. Borleffs, A. Hsu, J. M. Valdes, C. A. B. Boucher, and D. A. Cooper.** 1995. Safety, pharmacokinetics, and preliminary efficacy of ritonavir, an inhibitor of HIV-1 protease. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. N. Engl. J. Med. **333:**1528–1533.
9. **Goldberg, D. M., M. W. Roomi, A. Yu, and D. A. K. Roncari.** 1980. Effects of phenobarbital upon triacylglycerol metabolism in the rabbit. Biochem. J. **192:**165–175.
10. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD-4 lymphocytes in HIV-1 infection. Nature **373:**123–126.
11. **Hsu, A., G. R. Granneman, K. Marsh, G. Witt, J. Valdes, K. Meyer, K. Erdman, J. F. Denissen, J. P. DeCourt, and J. Leonard.** Single-dose pharmacokinetics of ritonavir, an HIV-protease inhibitor. Submitted for publication.
12. **Hsu, A., G. R. Granneman, G. Witt, J. H. Cavanaugh, and J. M. Leonard.** 1996. Assessment of multiple doses of ritonavir on the pharmacokinetics of theophylline, abstr. 2767. *In* Program and abstracts of the XI International Conference on AIDS.
13. **Hubscher, G.** Glyceride metabolism, p. 280–370. *In* S. J. Wakil (ed.), Lipid metabolism. Academic Press, London, United Kingdom.
14. **Kempf, D. J., K. C. Marsh, J. F. Denissen, E. McDonald, S. Vasavanonda, C. A. Flentge, B. E. Green, L. Fino, C. H. Park, X.-P. Kong, N. E. Wideburg, A. Saldivar, L. Ruiz, W. M. Kati, H. L. Sham, T. Robins, K. D. Stewart, A. Hsu, J. J. Platner, J. M. Leonard, and D. W. Norbeck.** 1995. ABT-538 is a potent inhibitor of human immunodeficiency virus protease and has high oral bioavailability in humans. Proc. Natl. Acad. Sci. USA **92:**2484–2488.
15. **Kempf, D. J., D. W. Norbeck, L. Codacovi, X. C. Wang, W. E. Kohlbrenner, N. E. Wideburg, D. A. Paul, M. F. Knigge, S. Vasavanonda, A. Craig-Kennard, A. Saldivar, W. Rosenbrook, J. J. Clement, J. J. Plattner, and J. Erickson.** 1990. Structure-based C_2 symmetric inhibitors of HIV protease. J. Med. Chem. **33:**2687–2689.
16. **Kohl, N. E., E. A. Emini, W. A. Schleif, L. J. Davis, J. C. Heimbach, R. A. F. Dixon, E. M. Scolnick, and I. S. Sigal.** 1988. Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA **85:**4686–4690.
17. **Kumar, G. I., A. D. Rodrigues, A. M. Buko, and J. F. Denissen.** 1996. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. J. Pharmacol. Exp. Ther. **277:**423–431.
18. **Lai, A. A., R. H. Levy, and R. E. Cutler.** 1979. Time-course of interaction between carbamazepine and clonazepam in normal man. Clin. Pharmacol. Ther. **24:**316–323.
19. **Levy, R. H., A. A. Lai, and M. S. Dumain.** 1979. Time-dependent kinetic IV: pharmacokinetic theory of enzyme induction. J. Pharm. Sci. **68:**398–399.
20. **Markowitz, M., M. Saag, W. Powderly, A. M. Hurley, A. Hsu, J. M. Valdes, D. Henry, F. Sattler, A. LaMarca, and J. M. Leonard.** 1995. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. 1995. N. Engl. J. Med. **333:**1534–1539.
21. **Martin, J. V., P. J. Martin, and D. M. Goldberg.** 1976. Enzyme induction as a possible cause of increased serum-triglycerides after oral contraceptives. Lancet **i:**1107–1108.
22. **Martin, P. J., R. V. Hague, J. V. Martin, D. R. Cullen, and D. M. Goldberg.** 1976. The association between serum triglycerides and gamma glutamyl transpeptidase activity in diabetes mellitus. Clin. Biochem. **9:**208–211.
23. **Ohnhaus, E. E., A. M. Breckenridge, and B. K. Park.** 1989. Urinary excretion of 6-β-hydroxycortisol and the time course measurement of enzyme induction in man. Eur. J. Clin. Pharmacol. **36:**39–46.
24. **Okey, A. B.** 1990. Enzyme induction in the cytochrome P-450 system. Pharmacol. Ther. **45:**241–298.
25. **Ouellet, D., A. Hsu, G. R. Granneman, G. Carlson, H. Guenther, D. Mukherjee, C. Locke, and J. M. Leonard.** 1996. Assessment of the pharmacokinetic interaction between ritonavir (R) and clarithromycin (C). Clin. Pharmacol. Ther. **59:**143.
26. **Ouellet, D., A. Hsu, J. Qian, J. H. Cavanaugh, J. M. Leonard, and G. R. Granneman.** 1996. Effect of ritonavir on the pharmacokinetic of ethinyl estradiol in healthy female volunteers, abstr. 2765. *In* Program and abstracts of the XI International Conference on AIDS.
27. **Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho.** 1996. HIV-1 dynamics *in vivo*: virion clearance rate, infected cell lifespan, and viral generations. Science **271:**1582–1586.
28. **Pichard, L., I. Fabre, G. Fabre, J. Domergue, B. Saint Auber, G. Mourad, and P. Maurel.** 1990. Cyclosporine A drug interactions: screening for inducers of cytochrome P-450 (cyclosporine A oxidase) in primary cultures of human hepatocytes and in liver microsomes. Drug Metab. Dispos. **18:**595–606.
29. **Sheiner, L. B., and S. Beal.** 1980. Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J. Pharmacokinet. Biopharm. **8:**553–571.
30. **Tomasselli, A. G., W. J. Howe, T. K. Sawyer, A. Wlodawer, and R. L. Heinrikson.** 1991. The complexities of AIDS: an assessment of the HIV protease as a therapeutic target. Chim. Oggi **1991:**6–27.
31. **Vacca, J. P., B. D. Dorsey, W. A. Schlief, R. B. Levin, S. L. McDaniel, P. L. Darke, J. Zugay, J. C. Quintero, O. M. Blahy, E. Roth, V. V. Sardana, A. J. Schlabach, P. I. Graham, J. H. Condra, L. Gotlib, M. K. Holloway, J. Lin, I.-W. Chen, K. Vastag, D. Ostovic, P. S. Anderson, E. A. Emini, and J. R. Huff.** 1994. L-735-524: an orally bioavailable human immunodeficiency virus type I protease inhibitor. Proc. Natl. Acad. Sci. USA **91:**4096–4100.
32. **Venkatesan, K.** 1992. Pharmacokinetic drug interactions with rifampicin. Clin. Pharmacokinet. **22:**47–65.