

Drug-Drug Interaction Study of Ketoconazole and Ritonavir-Boosted Saquinavir[∇]

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Saquinavir, a potent human immunodeficiency virus protease inhibitor, is extensively metabolized by CYP3A4. Saquinavir is coadministered with ritonavir, a strong CYP3A4 inhibitor, to boost its exposure. Ketoconazole is a potent CYP3A inhibitor. The objectives of this study were to investigate the effect of ketoconazole on the pharmacokinetics of saquinavir/ritonavir and vice versa using the approved dosage regimens of saquinavir/ritonavir at 1,000/100 mg twice daily and ketoconazole at 200 mg once daily. This was an open-label, randomized two-arm, one-sequence, two-period crossover study in healthy subjects. In study arm 1, 20 subjects received saquinavir/ritonavir treatment alone for 14 days, followed in combination with ketoconazole treatment for 14 days. In arm 2, 12 subjects received ketoconazole treatment for 6 days, followed in combination with saquinavir/ritonavir treatment for 14 days. The pharmacokinetics were assessed on the last day of each treatment (days 14 and 28 in arm 1 and days 6 and 20 in arm 2). The exposures C_{max} and the area under the concentration-time curve from 0 to 12 h (AUC_{0-12}) of saquinavir and ritonavir with or without ketoconazole were not substantially altered after 2 weeks of concomitant dosing with ketoconazole. The C_{max} and AUC_{0-12} of ketoconazole, dosed at 200 mg once daily, were increased by 45% (90% confidence interval = 32 to 59%) and 168% (90% confidence interval = 146 to 193%), respectively, after 2 weeks of concomitant dosing with ritonavir-boosted saquinavir (1,000 mg of saquinavir/100 mg of ritonavir given twice daily). The greater exposure to ketoconazole when given in combination with saquinavir/ritonavir was not associated with unacceptable safety or tolerability. No dose adjustment for saquinavir/ritonavir (1,000/100 mg twice daily) is required when coadministered with 200 mg of ketoconazole once daily, and high doses of ketoconazole (>200 mg/day) are not recommended.

Saquinavir, a potent human immunodeficiency virus (HIV) protease inhibitor, is extensively metabolized by cytochrome P450 (CYP3A4; see the product information for Invirase capsules and tablets [Hoffman-La Roche, Inc.]). For the antiretroviral combination treatment of HIV-infected patients, saquinavir is coadministered with ritonavir, another HIV protease inhibitor and strong CYP3A4 inhibitor (see the product information for the Norvir 100-mg capsule [Abbott Laboratories]), to boost the exposure of saquinavir. The new dosage form of saquinavir (Invirase), a 500-mg film-coated tablet, was approved in 2004 in the United States and 2005 in Europe and allows a lower pill burden using the dosing regimen of saquinavir/ritonavir at 1,000/100 mg twice daily, which was approved in 2003. Saquinavir exhibits a pronounced food effect, and patients are advised to take saquinavir with ritonavir always with a full meal (according to the product information for Invirase capsules and tablets [Hoffman-La Roche, Inc.]). The imidazole antifungal compound ketoconazole is effective in the treatment of oropharyngeal candidiasis, the most common infection among persons infected with HIV (1). Ketoconazole is also known as a prototypic and strong CYP3A4 inhibitor (2) and is a recommended drug of choice for investigating the interaction

with CYP3A4 substrates such as saquinavir (5). It has already been shown that coadministration of ketoconazole at 200 or 400 mg once daily with saquinavir/ritonavir at 400/400 mg twice daily resulted in increased area under the drug plasma concentration-time curve (AUC) and peak plasma concentrations (C_{max}) for saquinavir and ritonavir, whereas the effect of saquinavir/ritonavir on the pharmacokinetics of ketoconazole was not evaluated (4). The present study was performed using the approved dosing regimens of saquinavir/ritonavir at 1,000/100 mg twice daily and ketoconazole at 200 mg once daily and documents the drug-drug interaction after 2 weeks of concomitant dosing in both directions, i.e., the effect of ketoconazole on the pharmacokinetics of saquinavir/ritonavir and that of saquinavir/ritonavir on ketoconazole. The objective of the study was to provide appropriate dosing guidelines to clinicians who treat HIV patients with saquinavir/ritonavir and ketoconazole.

MATERIALS AND METHODS

Study design and population. This was an open-label, randomized two-arm, one-sequence, two-period crossover study conducted in healthy male and female subjects. Subjects had to give written informed consent, and the study protocol was approved by the IRB Institutional Review Board (Comité Consultatif de Protections des Personnes dans la Recherche Biomédicale, Strasbourg, France). The study was conducted in full compliance with the principles of the Declaration of Helsinki III and performed according to the guidelines of Good Clinical Practice.

In order to enable stable pharmacokinetic conditions for saquinavir/ritonavir, the combination of these two drugs was administered for 2 weeks, ketoconazole

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alone was dosed for 6 days, and the combination of ritonavir/saquinavir with ketoconazole was administered in both study arms for another 2 weeks. In study arm 1, saquinavir/ritonavir was administered first (period 1), followed by the addition of ketoconazole (period 2), and in study arm 2, ketoconazole was dosed first (period 1), followed by the addition of saquinavir/ritonavir (period 2). The planned sample size for study arm 1 was $n = 20$, assuming a within-subject coefficient of variation (CV) of up to 30% for the saquinavir AUC and C_{max} , which was based on the observed within-subject CV of 27 and 24% for these parameters, respectively, from a previous study (BP17359 [Roche data on file]). For study arm 2, the sample size was set to $n = 12$, assuming a within-subject CV of up to 16% for the ketoconazole AUC and C_{max} based on the respective values of 16 and 14% observed in a previous study (WK14435 [Roche data on file]). These sample sizes would ensure that, with a probability of at least 80%, the two-sided 90% confidence interval (CI) for the geometric population mean of the individual parameter ratios (period 2/period 1) would be within 75 to 133% of the geometric population mean for saquinavir and within 80 to 125% of the geometric population mean for ketoconazole. Subjects were randomized to study arms 1 and 2 with a block size of eight (five to arm 1, three to arm 2).

Subjects underwent screening evaluations to determine eligibility within 28 days prior to study enrollment. Screening procedures included, among others, tests for HIV, tests for hepatitis B and C, and tests for pregnancy in women. Healthy male and female subjects, aged 18 to 65 years (inclusive) with body mass indexes between 18 to 30 kg/m² and being nonsmokers, were enrolled into the study. Intake of grapefruit or grapefruit juice was not allowed from 2 weeks prior to the first dose and during the study. In addition, the consumption of alcohol was not permitted during the study. Subjects were instructed to take the study drugs always with a meal. No concomitant medications were permitted during the study except to treat adverse events. Subjects who were on concomitant treatment with drugs known as CYP3A4 substrates, CYP3A4 inhibitors, or CYP3A4 inducers were excluded from the study. Women in the study had to be of non-child-bearing potential or under efficient nonhormonal contraception throughout the study and until at least 1 month thereafter.

In study arm 1, saquinavir/ritonavir at 1,000/100 mg was dosed twice daily for 28 days (excluding the evening dose on day 14), with ketoconazole at 200 mg once daily added from days 15 to 28 (period 1, days 1 to 14; period 2, days 15 to 28). In study arm 2, ketoconazole at 200 mg was dosed once daily for 20 days with saquinavir/ritonavir at 1,000/100 mg twice daily added from days 7 to 20 (period 1, day 1 to 6; period 2, day 7 to 20). In study arm 1 the pharmacokinetics were assessed for saquinavir/ritonavir over 12 h on days 14 and 28, and in arm 2 the pharmacokinetics were assessed for ketoconazole over 24 h on days 6 and 20. Study drugs were administered 30 min after the start of a standard high-fat, high-calorie breakfast (63 g of fat, 975 kcal) on days 14 and 28 for arm 1 or days 6 and 20 for arm 2. Plasma samples for pharmacokinetic assessments were collected predose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 h postdose (13 samples). In study arm 1, subjects received on days 14 and 28 saquinavir and ritonavir doses only in the morning. In study arm 1, plasma samples were analyzed for saquinavir and ritonavir, and in study arm 2 plasma samples were analyzed for ketoconazole. In both study arms additional predose concentration measurements were made on the last 2 to 4 days of periods 1 and 2 to document stable pharmacokinetic conditions. In study arms 1 and 2, during both pharmacokinetic assessment periods, subjects were confined to the study center. Safety parameters included medical history, physical examinations, standard safety laboratory assessments (hematology, blood chemistry, and urinalysis), vital signs, and serial electrocardiograms recorded at prespecified time points throughout the trial, as well as urine analyses for drugs of abuse, alcohol breath tests, and urine pregnancy tests (in females only). A medical follow-up examination was conducted 15 to 21 days after the last dose of study drugs. All subjects were observed for adverse events during the entire study period.

Bioanalysis. For the determination of saquinavir and ritonavir pharmacokinetics, blood samples of 5.5 ml were collected by peripheral venous catheter or venipuncture into tubes containing lithium heparin as the anticoagulant, and for the pharmacokinetics of ketoconazole, blood samples of 2.6 ml were collected into EDTA-containing tubes. Plasma was separated by centrifugation for 10 min at $1,500 \times g$ and 4°C. The total plasma concentrations of saquinavir and ritonavir were analyzed by PRA International–Early Development Services (formerly Pharma Bio-Research Group B.V, Assen, The Netherlands) using a validated high-pressure liquid chromatography–tandem mass spectrometry method with two linear concentration ranges. The low calibration range was 1.00 to 100 ng/ml for both analytes, using aliquots of 200 μ l of plasma. The high calibration range was 10 to 10,000 ng/ml for both analytes, using aliquots of 100 μ l of plasma. The precision of the low concentration assay ranged from 0.7 to 7.0% for saquinavir and from 3.6 to 7.8% for ritonavir. The accuracy ranged from 93.1 to 100.2% and from 94.2 to 95.8% for saquinavir and ritonavir, respectively. The precision of the

TABLE 1. Summary of demographic characteristics of the study populations

Characteristic	Study arm 1 ($n = 29$)	Study arm 2 ($n = 13$)
No. of males/no. of females	27/2	13/0
Black/Caucasian	1/28	0/13
Mean age in yr (range)	33.0 (19–62)	30.5 (19–58)
Mean body wt in kg (range)	76.7 (45.0–107.8)	80.4 (64.7–100.0)
Mean body mass index in kg/m ² (range)	24.5 (18.0–29.5)	25.1 (20.1–29.2)

high concentration assay ranged from 3.5 to 6.8% for saquinavir and from 4.2 to 6.1% for ritonavir. The accuracy ranged from 98.9 to 101.4% and from 95.0 to 103.5% for saquinavir and ritonavir, respectively. Total ketoconazole plasma concentrations were analyzed by AAI Pharma Deutschland GmbH & Co. KG (formerly AAI Deutschland GmbH & Co. KG, Neu-Ulm, Germany) using a validated high-pressure liquid chromatography–fluorescence method with a calibration range from 25.0 to 2,500 ng/ml, using aliquots of 100 μ l of plasma. The precision ranged from 2.2 to 6.0%, and the accuracy ranged from 97.8 to 101.0%.

Pharmacokinetic evaluation. Pharmacokinetic parameters were estimated using standard noncompartmental methods (Software WinNonlin Professional, version 5.2; Pharsight Corp., Mountain View, CA) and actual sampling times. The following pharmacokinetic parameters were directly obtained from the observed concentration-versus-time data: the maximum plasma concentration (C_{max}), the time to C_{max} (T_{max}), and the drug concentration at 12 h after administration (C_{12}) for saquinavir/ritonavir or at 24 h after administration (C_{24}) for ketoconazole. The area under the drug plasma concentration–time curve from time zero until 12 h (AUC_{0-12}) for saquinavir/ritonavir or until 24 h (AUC_{0-24}) for ketoconazole was calculated by applying the linear trapezoidal rule. The terminal elimination half-life ($t_{1/2}$) was estimated by $\ln 2/k_{el}$, where k_{el} is the terminal elimination rate constant determined by linear regression of the last four natural log-transformed concentration time points with a maximum exclusion of one intermediate concentration time point and fitting with an adjusted residuals squared value that is ≥ 0.90 . The apparent oral plasma clearance at steady-state (CL_{ss}/F) was estimated by calculating the dose/ AUC_{0-12} for saquinavir and ritonavir and dose/ AUC_{0-24} for ketoconazole.

Statistical analysis. In both study arms, predose concentrations measured on the last 2 to 4 days of periods 1 and 2 were summarized per study arm and study day. Pharmacokinetic parameters were summarized per study arm and treatment for all subjects who completed the trial. For the assessment of the drug–drug interaction, the study variables were AUC_{0-12} and C_{max} for saquinavir/ritonavir and AUC_{0-24} and C_{max} for ketoconazole. Natural log-transformed values of these parameters were used, and the exposure ratios were determined in arm 1 for saquinavir and ritonavir for day 28 to day 14 and in arm 2 for ketoconazole for day 20 to day 6. The geometric means of the individual exposure ratios, together with the corresponding two-sided 90% CIs, were calculated. No formal confirmatory hypothesis testing was planned, and P values were interpreted in an exploratory manner. The statistical analysis was performed using software SAS v8.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Demographics. A total of 42 healthy subjects were enrolled in the present study. A total of 29 subjects (27 males and 2 females) were randomly assigned to study arm 1 assessing the effect of ketoconazole on the plasma concentrations of saquinavir/ritonavir, and 13 subjects (all males) were randomly assigned to study arm 2 assessing the effect of saquinavir/ritonavir and the plasma concentrations of ketoconazole. Each of the 42 subjects received at least one dose of study drug(s), and 32 subjects (20 in arm 1, 12 in arm 2) completed the study as planned. The demographic characteristics of the study population are shown in Table 1.

TABLE 2. Geometric mean predose plasma concentrations of saquinavir/ritonavir in study arm 1 ($n \leq 29$)

Agent	Mean concn ($\mu\text{g/ml}$) ^a							
	Study arm 1, period 1, at day:				Study arm 1, period 2, at day:			
	11	12	13	14	25	26	27	28
Saquinavir	0.84 (120)	0.97 (71)	0.99 (74)	1.3 (84)	0.67 (90)	0.84 (120)	1.0 (100)	1.3 (66)
Ritonavir	0.29 (130)	0.33 (76)	0.38 (40)	0.38 (74)	0.32 (89)	0.30 (84)	0.40 (75)	0.45 (59)
Ketoconazole					0.93 (44)	1.0 (38)	1.0 (44)	1.1 (36)

^a The percent CV is indicated in parentheses.

Evaluation of predose concentrations. Serial predose measurements of the last 2 to 4 days of each treatment period are summarized per study arm and treatment day in Tables 2 and 3. In study arm 1, the daily saquinavir and ritonavir predose concentrations were of similar magnitude within each measured period, with pronounced but comparable interindividual variabilities expressed as the %CV in both periods (Table 2). In addition, the daily saquinavir and ritonavir predose concentrations were of similar dimensions in the absence or presence of ketoconazole coadministration. Likewise, in arm 2, the daily ketoconazole predose concentrations were stable within each measured period but were ~17-fold higher in the presence of saquinavir/ritonavir coadministration compared to ketoconazole treatment alone (Table 3). Also, in study arm 2 period 2, the daily saquinavir and ritonavir predose concentrations were not dissimilar from those seen for saquinavir/ritonavir throughout study arm 1.

Assessment of drug-drug interactions. The pharmacokinetic interaction was evaluated in the 20 subjects in arm 1 and the 12 subjects in arm 2, who completed the entire study. Figure 1 shows the 12-h plasma log-transformed concentration-versus-time profiles of saquinavir/ritonavir in the absence (day 14) or presence of ketoconazole coadministration (day 28) in study arm 1, and Fig. 2 shows the respective 24 h profiles of ketoconazole in the absence (day 6) or presence of saquinavir/ritonavir coadministration (day 20) in study arm 2. Summaries of the pharmacokinetic parameters for saquinavir/ritonavir (study arm 1) with or without ketoconazole coadministration are presented in Table 4, and those for ketoconazole with or without saquinavir/ritonavir coadministration are presented in Table 5.

In study arm 1, differences in the plasma concentration-versus-time profiles for saquinavir and ritonavir during coadministration with or without ketoconazole were small and not

clinically meaningful. Mean values for all pharmacokinetic parameters of saquinavir and ritonavir were similar in the absence or presence of ketoconazole coadministration, and the interindividual variability (as expressed as the %CV) was similar for both compounds and in both of the treatment periods. The geometric mean ratio estimates for the AUC_{0-12} and C_{max} of saquinavir and ritonavir were close to 1, and all four 90% CIs were within the range of 0.86 to 1.26 (Table 4). Based on these results, it can be concluded that the addition of ketoconazole at a dose of 200 mg once daily to the approved therapeutic regimen of saquinavir/ritonavir at 1,000/100 mg twice daily for 14 days did not have a clinically relevant effect on the pharmacokinetic exposures of saquinavir or ritonavir.

In study arm 2, the plasma concentration-versus-time profiles showed a considerable increase in ketoconazole exposure during coadministration with saquinavir/ritonavir. The terminal elimination of ketoconazole was prolonged after 14 days of coadministration with saquinavir/ritonavir, as indicated by the flatter decline in the log-transformed concentration versus time profile. The absorption of ketoconazole was minimally prolonged during saquinavir/ritonavir coadministration, as expressed by a 1-h delay in the median T_{max} , from 2.5 h in period 1 to 3.5 h in period 2. The median $t_{1/2}$ was also prolonged from 4.3 h in period 1 to 10.7 h in period 2, and the geometric mean

TABLE 3. Geometric mean predose plasma concentrations of ketoconazole and saquinavir/ritonavir in study arm 2 ($n \leq 13$)

Agent	Mean concn ($\mu\text{g/ml}$) ^a					
	Study arm 2, period 1, at day:		Study arm 2, period 2, at day:			
	5	6	17	18	19	20
Ketoconazole	0.072 (51)	0.070 (60)	1.2 (54)	1.3 (54)	1.2 (47)	1.2 (61)
Saquinavir			1.1 (42)	1.1 (57)	0.83 (62)	1.3 (72)
Ritonavir			0.48 (36)	0.40 (45)	0.42 (70)	0.51 (57)

^a The percent CV is indicated in parentheses.

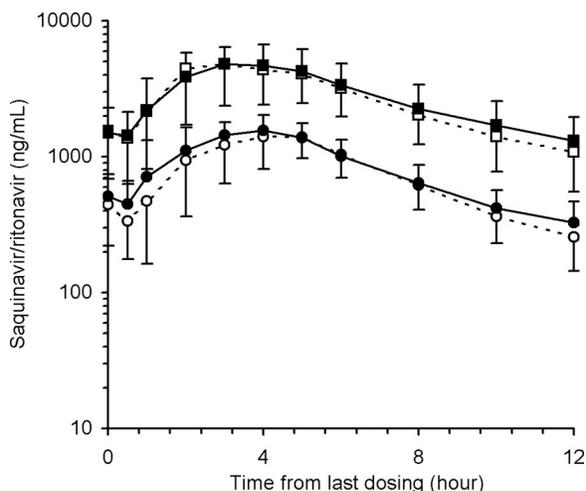


FIG. 1. Mean plasma concentration-time profiles of saquinavir and ritonavir (circles) after 14 days of saquinavir/ritonavir at 1,000/100 mg given twice daily (open symbols, dotted line) and after 14 days of coadministration of saquinavir/ritonavir with ketoconazole at 200 mg once daily (filled symbols, solid line). The standard deviations are shown as error bars.

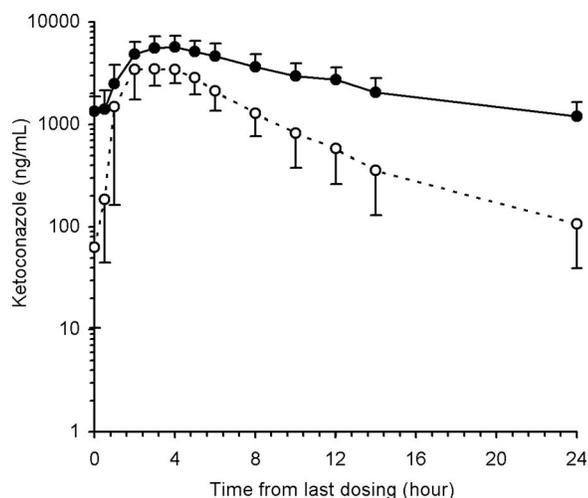


FIG. 2. Mean plasma concentration-time profiles of ketoconazole after 6 days of ketoconazole at 200 mg given once daily (open symbols, dotted line) and after 14 days of coadministration of ketoconazole with saquinavir/ritonavir at 1,000/100 mg twice daily (filled symbols, solid line). The standard deviations are shown as error bars.

CL_{ss}/F value was decreased by more than 50% with saquinavir/ritonavir coadministration, from 8.22 to 3.07 liters/h. Increases in the mean C_{24} values for ketoconazole of >10-fold were seen in period 2 relative to period 1. The intersubject variabilities (as expressed by the %CV) were comparable in periods 1 and 2 for ketoconazole. The geometric mean ratio estimates for AUC_{0-24} and C_{max} of ketoconazole were substantially larger than 1 (Table 5). After 14 days of coadministration of saquinavir/ritonavir, the mean ketoconazole AUC_{0-24} and C_{max} were increased by 2.68- and 1.45-fold, respectively, compared to the administration of ketoconazole alone.

Safety results. The study medications were generally well tolerated by healthy subjects. There were 10 early discontinuations from the study: 9 in study arm 1 and 1 in study arm 2. The reasons for study withdrawals were adverse events in six subjects in arm 1, elevated safety laboratory parameters (high triglycerides and low-density lipoprotein cholesterol, respectively) in two subjects in arm 1, and other reasons in the remaining one subject in arm 1 and the one subject in arm 2. In study arm 1, the adverse events reporting rates were similar in period 1 (days 1 to 14) and period 2 (days 15 to 28), with 62

TABLE 5. Summary of pharmacokinetic parameters for ketoconazole in study arm 2 ($n = 12$)

Parameter	GM (%CV) ^a		GMR (90% CI) ^b
	Period 1 (ketoconazole alone)	Period 2 (ketoconazole + saquinavir + ritonavir)	
C_{max} ($\mu\text{g/ml}$)	3.86 (28.9)	5.59 (29.1)	1.45 (1.32–1.59)
AUC_{0-24} ($\mu\text{g} \cdot \text{h/ml}$)	24.3 (36.4)	65.2 (32.1)	2.68 (2.46–2.93)
C_{24} (ng/ml)	87.4 (79.7)	1,100 (47.8)	NA
T_{max} (h)	2.5 (2.0–5.0)	3.5 (2.0–4.0)	NA
$t_{1/2}$ (h)	4.3 (2.1–7.0)	10.7 (6.7–18.5)	NA
CL_{ss}/F (liter/h)	8.22 (36.4)	3.07 (32.1)	NA

^a GM, geometric mean.

^b GMR, geometric mean ratio of period 2/period 1. NA, not assessed.

and 58% of subjects, respectively. Gastrointestinal disorders were the most frequently adverse events reported by 41 and 25% of subjects in periods 1 and 2, respectively, followed by events related to infections and infestations, and nervous system disorders reported by 17% of subjects each during saquinavir/ritonavir treatment in period 1. In study arm 2, the reporting rate for adverse events was lower during period 1 (days 1 to 6) with 23% of subjects compared to that of 92% of subjects in period 2 (days 7 to 20). Again, gastrointestinal disorders were the most prominent adverse events reported by 54% of subjects during triple combination therapy in period 2. The majority of the adverse events were mild to moderate in intensity. All adverse events were resolved without sequelae. The greater exposure to ketoconazole when given in combination with saquinavir/ritonavir was not associated with unacceptable safety or tolerability in the present study.

DISCUSSION

In order to assess the impact of drug-drug interaction at the CYP3A4 metabolic pathway, strict exclusion criteria were set in the present study with regard to concomitant use of CYP3A4 substrates, inhibitors, or inducers. Since hormonal contraceptives, being CYP3A4 substrates, were not allowed, women had to be of non-child-bearing potential or under efficient nonhormonal contraceptive protection. With these requirements, only two women could be recruited into the

TABLE 4. Summary of pharmacokinetic parameters for saquinavir and ritonavir in study arm 1 ($n = 20$)^a

Parameter	Saquinavir			Ritonavir		
	GM (%CV)		GMR (90% CI)	GM (%CV)		GMR (90% CI)
	Period 1 (+ ritonavir)	Period 2 (+ ritonavir + ketoconazole)		Period 1 (+ saquinavir)	Period 2 (+ saquinavir + ketoconazole)	
C_{max} ($\mu\text{g/ml}$)	5.01 (51.5)	5.10 (36.3)	1.02 (0.86–1.20)	1.53 (39.4)	1.66 (26.4)	1.08 (0.96–1.21)
AUC_{0-12} ($\mu\text{g} \cdot \text{h/ml}$)	30.0 (53.3)	32.2 (40.3)	1.07 (0.92–1.26)	8.9 (36.3)	9.95 (30.3)	1.12 (1.03–1.22)
C_{12} ($\mu\text{g/ml}$)	0.956 (56.3)	1.13 (62.4)	NA	0.230 (57.1)	0.292 (57.7)	NA
T_{max} (h)	3.0 (2.0–6.0)	3.0 (2.0–5.0)	NA	4.0 (2.0–5.0)	4.0 (1.0–5.0)	NA
$t_{1/2}$ (h)	4.9 (4.1–5.9)	5.2 (4.5–6.8)	NA	3.7 (3.1–5.6)	4.2 (3.5–5.5)	NA
CL_{ss}/F (liter/h)	33.4 (53.3)	25.6 (43.3)	NA	11.2 (36.3)	8.7 (32.8)	NA

^a GMR, geometric mean ratio of period 2/period 1. GM, geometric mean. NA, not assessed.

present study. Both were randomized to study arm 1 and completed all study procedures. Knowing that for saquinavir statistically significant greater exposures have been observed in women than in men (study BP17359 with 87 males and 7 females [Roche data on file]), the exposures of the two women to saquinavir ($AUC_{0-12} = 47.7$ and $43.4 \mu\text{g h/ml}$ in period 1 and 29.8 and $66.0 \mu\text{g h/ml}$ in period 2) in the present study in both periods were similar to those seen in the remaining 18 men within 40 to 53% variability (Table 4), and therefore the conclusions made for the whole population in this study arm may also apply to female patients. On the other hand, the effect of saquinavir and ritonavir on the exposure of ketoconazole (arm 2) was only studied in men. However, lacking data of female subjects in this study arm, and considering also the related safety aspects, it cannot be assumed that this increase in ketoconazole exposure during concomitant saquinavir/ritonavir at a 1,000/100-mg twice-daily treatment would be of different magnitude in women.

In this study in healthy volunteers, the addition of ketoconazole at a dose of 200 mg once daily to the approved therapeutic dosing regimen of saquinavir/ritonavir at 1,000/100 mg twice daily for 2 weeks did not have a clinically relevant effect on the pharmacokinetic exposures of saquinavir and ritonavir. For both compounds, the 90% CIs surrounding the geometric mean ratio estimates for the AUC_{0-12} and C_{max} were within or only 1% exceeding the upper limit of the no-effect boundary (0.80 to 1.25) as defined in the U.S. Food and Drug Administration guideline for the industry for in vivo drug interaction studies (5).

By comparison, after 6 days of pretreatment with ketoconazole at 200 mg once daily, the addition of saquinavir/ritonavir at 1,000/100 mg twice daily for 2 weeks increased the exposure of ketoconazole by 2.68 (2.46 to 2.93)-fold for AUC_{0-24} and by 1.45 (1.32 to 1.59)-fold for C_{max} . The median elimination half-life for ketoconazole was also more than doubled, from 4.3 h after treatment with ketoconazole alone to 10.7 h after coadministration with saquinavir/ritonavir. The reduced clearance of ketoconazole resulted in predose concentrations of ketoconazole that were >10-fold higher in all subjects after coadministration with saquinavir/ritonavir compared to values seen after 6 days of treatment with ketoconazole alone.

The 2-week treatment phases of saquinavir/ritonavir and the concomitant triple-drug treatment with ketoconazole was considered sufficient in order to achieve stable pharmacokinetic conditions for all three medications involved. This treatment duration, although longer than required based on the half-lives of ritonavir and saquinavir, was selected based on the fact that ritonavir not only inhibits the metabolism of CYP3A4 but also increases the enzyme activity of CYP3A4 (inhibition-associated induction). Due to this autoinduction, plasma concentrations of saquinavir/ritonavir generally reach steady state 2 weeks after the start of ritonavir administration (3).

Studies have already been performed investigating the drug-drug interaction between ketoconazole and saquinavir, or ritonavir, or saquinavir/ritonavir using several dosing regimens. In these studies, the saquinavir and ritonavir doses used were different from the approved dosing regimen for saquinavir/ritonavir of 1,000/100 mg twice daily. Coadministration of ketoconazole at 400 mg once daily with saquinavir at 1,200 mg three times daily increased the AUC of saquinavir by 190%,

but saquinavir did not change the AUC of ketoconazole (see the product information for Invirase capsules and tablets [Hoffman-La Roche, Inc.]). Coadministration of ritonavir at 500 mg twice daily with ketoconazole at 200 mg once daily resulted in a 3.4-fold increase in the ketoconazole AUC and an 18% increase in ritonavir AUC (see the product information for the Norvir 100-mg capsule [Abbott Laboratories]). Coadministration of saquinavir/ritonavir at 400/400 mg twice daily with ketoconazole at 200 or 400 mg once daily yielded increases in the saquinavir AUC by 37% and the ritonavir AUC by 29% (4). The results of the present study are closest to those obtained with the ritonavir and ketoconazole combination, although the increase in ketoconazole exposure was somewhat less for the AUC in the present study (2.68-fold versus 3.4-fold) and C_{max} (45% in present study versus 55% in the product information for the Norvir 100-mg capsule [Abbott Laboratories]). The metabolism and elimination of ketoconazole are clearly affected by the CYP3A4 inhibitory effect of ritonavir, whereas the influence of ketoconazole, an inhibitor for both CYP3A4 and P-glycoprotein, on the saquinavir/ritonavir combination is small, as shown by the clinically irrelevant increases in plasma exposures of these two compounds. In the present study, the CYP3A4 inhibitory effect of ketoconazole on saquinavir, as seen in the above saquinavir ketoconazole interaction studies, is, for the most part, superimposed by the CYP3A4 inhibitory effect of ritonavir on saquinavir. The increase in the saquinavir AUC by 37% and the ritonavir AUC by 29% when combined with ketoconazole, as observed in an earlier study (4), may have been due to the higher ketoconazole dose (400 mg) and/or different saquinavir/ritonavir doses (400/400 mg) used compared to those in the present study. A full CYP3A inhibitory effect of ketoconazole may not have been reached with the ketoconazole standard dose of 200 mg daily in the present study. However, based on the existing data, and for safety reasons, a ketoconazole multiple-dose regimen of 400 mg daily was not considered acceptable for this study in healthy volunteers due to the hepatic toxicity liability of ketoconazole (see Drug Information Online [<http://www.drugs.com/mmx/ketoconazole.html>]) and as a result of the maximum 200-mg daily ketoconazole dose recommendations in combination with ritonavir (product information for the Norvir 100-mg capsule [Abbott Laboratories]). With a 400-mg daily dose of ketoconazole in the present study, further increases of ketoconazole plasma concentrations would have been most likely.

The safety results as recorded by adverse events and laboratory safety measurements were consistent with those indicated in the respective product information for these three drugs (see also the product information for the Norvir 100-mg capsule [Abbott Laboratories], for Invirase capsules and tablets [Hoffman-La Roche, Inc.], and for the Nizoral 200-mg tablet [Janssen-Cilag, Ltd.]). There were no vital-sign or ECG abnormalities of clinical relevance recorded during this study.

Conclusions. The present two-arm drug-drug interaction study involving ketoconazole at 200 mg/day and ritonavir-boosted saquinavir (1000 mg of saquinavir and 100 mg of ritonavir twice daily) indicated that the C_{max} and AUC_{0-12} of both saquinavir and ritonavir are not substantially altered by the addition of ketoconazole for the duration of 2 weeks, but that, on the other hand, the C_{max} and AUC_{0-24} of ketoconazole are increased by 45 and 168%, respectively, after the addition

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