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Interaction Study of the Combined Use of Paroxetine and Fosamprenavir-Ritonavir in Healthy Subjects[▽]

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Human immunodeficiency virus-infected patients have an increased risk for depression. Despite the high potential for drug-drug interactions, limited data on the combined use of antidepressants and antiretrovirals are available. Theoretically, ritonavir-boosted protease inhibitors may inhibit CYP2D6-mediated metabolism of paroxetine. We wanted to determine the effect of fosamprenavir-ritonavir on paroxetine pharmacokinetics and vice versa and to evaluate the safety of the combination. Group A started with 20 mg paroxetine every day for 10 days; after a wash-out period of 16 days, subjects received paroxetine (20 mg every day) plus fosamprenavir-ritonavir (700/100 mg twice a day) from days 28 to 37. Group B received the regimens in reverse order. On days 10 and 37, pharmacokinetic curves were recorded. Twenty-six healthy subjects (18 females, 8 males) were included. Median (range) age and weight were 44.4 (18.2 to 64.3) years and 68.8 (51.0 to 89.4) kg. Three subjects were excluded (two because of adverse events; one for nonadherence). Addition of fosamprenavir-ritonavir to paroxetine resulted in a significant decrease in paroxetine exposure: the geometric mean ratios (90% confidence intervals) of paroxetine plus fosamprenavir-ritonavir to paroxetine alone were 0.45 (0.41 to 0.49) for the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}), 0.49 (0.45 to 0.53) for the maximum concentration of the drug in plasma (C_{max}), and 0.75 (0.71 to 0.80) for the apparent elimination half-life ($t_{1/2}$). The free fraction of paroxetine showed a median (interquartile range) increase of 30% (18 to 42%) after the addition of fosamprenavir-ritonavir. The AUC_{0-12}, C_{max}, C_{min}, and $t_{1/2}$ of amprenavir and ritonavir were similar to those of historical controls. No serious adverse events occurred. Fosamprenavir-ritonavir reduced total paroxetine exposure by 55%. This is partly explained by protein displacement of paroxetine. We think that this interaction is clinically relevant and that titration to a higher dose of paroxetine may be necessary to accomplish the needed antidepressant effect.

Human immunodeficiency virus (HIV)-infected patients may have an increased risk for the development of depression, due to social stigmatization, loss of friends or relatives to AIDS, and other factors. The lifetime prevalence of depression in HIV-infected patients has been estimated at 22 to 45% (reviewed in reference 22), which is higher than for the general, HIV-negative population (13 to 20%). Therefore, HIV-infected patients frequently use antidepressants. A recently published study showed that antiretroviral adherence in depressed HIV-infected patients was higher in patients on antidepressant therapy than in depressed HIV-infected patients that were not on antidepressant treatment (28). Thus, treatment of depression is important to improve adherence of antiretroviral agents.

Selective serotonin reuptake inhibitors (SSRIs) are often considered the first choice when antidepressant drugs are needed. They are better tolerated than tricyclic antidepressants. Paroxetine is frequently prescribed in HIV-infected patients with depression (3).

One of the protease inhibitors (PIs) used in the treatment of HIV/AIDS is fosamprenavir (Telzir/Lexiva), a prodrug of amprenavir. Fosamprenavir is given in combination with ritonavir to increase the plasma exposure of amprenavir. Fosamprenavir is a substrate for CYP3A4 and a mixed inhibitor/inducer of CYP3A4 (summary of product characteristics for Telzir [EMEA]). Ritonavir is a potent inhibitor of CYP_{450} and inhibits CYP3A4, CYP2D6, CYP2C9, CYP2C19, and others when given at a high dosage, about 600 mg twice a day (BID). Given at a low dose, 100 mg BID, ritonavir inhibits CYP3A4. Ritonavir is a substrate for CYP3A4 and a minor substrate for CYP2D6 (summary of product characteristics for Norvir [EMEA]). Paroxetine is metabolized by CYP2D6 (summary of product characteristics for Seroxat [CBG-MEB]). At the same time, paroxetine is a potent inhibitor of CYP2D6. Therefore, the combination of paroxetine and fosamprenavir-ritonavir can result in a potential drug interaction. The summary of product characteristics for Norvir (ritonavir; EMEA) states that concomitant use of CYP2D6 substrates (such as paroxetine) and ritonavir is not allowed unless the risk and benefit of this

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combination are evaluated. However, the effect of low-dose ritonavir (100 mg BID, given as a boosting agent) on paroxetine has not been established.

Based on the above information, the primary objective of this trial was to determine the effect of fosamprenavir-ritonavir on paroxetine pharmacokinetics. Furthermore, we wanted to determine the effect of paroxetine on the pharmacokinetics of fosamprenavir-ritonavir, the safety of the combination of fosamprenavir-ritonavir and paroxetine, and the effect of fosamprenavir-ritonavir on paroxetine pharmacodynamics.

MATERIALS AND METHODS

Study design and dosing of study drugs. This was an open-label, multiple-dose, two-arm, two-sequence, two-period, pharmacokinetic drug-drug interaction study in 26 healthy subjects. In group A, 13 subjects received 10 oral doses of 20 mg of paroxetine once daily (QD) (1 capsule of 20 mg of Seroxat taken at approximately 8:00 a.m. with a meal) during the first period of 10 days. After a wash-out period of 16 days (study days 11 to 27), all subjects in group A received 10 oral doses of 20 mg of paroxetine once daily, 20 oral doses of 700 mg of fosamprenavir twice daily (1 tablet of 700 mg of Telzir/Lexiva taken at approximately 8:00 a.m. and 8:00 p.m. with a meal), and 20 oral doses of ritonavir twice daily (1 capsule of Norvir 100 mg taken at approximately 8:00 a.m. and 8:00 p.m. with a meal) during the second phase of 10 days (study days 28 to 37). In group B, 13 other subjects received the regimens in reverse order.

A crossover design with a wash-out period of 16 days was chosen to exclude period and carryover effects on the pharmacokinetics of amprenavir-ritonavir and paroxetine.

Study population. This trial was conducted with healthy males and females between 18 and 65 years of age at the day of first dosing. Subjects did not smoke more than 10 cigarettes, 2 cigars, or 2 pipes per day for at least 3 months prior to the first dosing, had a Quetelet Index (body mass index) of 18 to 30 kg/m^2, and were able and willing to sign the informed consent form prior to screening evaluations. Subjects had to be in good age-appropriate health, as established by medical history, physical examination, electrocardiography, biochemistry, hematology, and urinalysis testing within 3 weeks prior to the first dose. The main exclusion criteria were a history of psychiatric illness, poor and ultrarapid CYP2D6 metabolizer status as determined by genotyping, history of sensitivity/idiosyncrasy to fosamprenavir, ritonavir, paroxetine, or chemically related compounds or excipients, a positive HIV test, a positive hepatitis B or C test, or therapy with any drug for 2 weeks preceding dosing (except for acetaminophen, hormonal contraceptives, and loperamide). Other exclusion criteria were history of or current abuse of drugs, alcohol, or solvents and participation in a drug trial or donation of blood within 60 days prior to the first dose. Pregnant or breast-feeding females and female subjects of childbearing potential who were not willing to take precautions in order to prevent a pregnancy were also excluded.

CYP2D6 genotyping. DNA samples were analyzed at the Laboratory of Anthropogenetics, Radboud University Nijmegen Medical Centre. The genotyping of CYP2D6*3, CYP2D6*4, and CYP2D6*6 was performed with pyrosequencing technology (12). CYP2D6*5 and CYP2D6*xn alleles were detected with XL-PCRs (17, 24). Depending on the presence of the different alleles, poor (homozygote for alleles CYP2D6*3, *4, *5, and *6) and ultrarapid (at least one CYP2D6*xn allele and none of the other alleles) metabolizers were excluded.

Safety assessments and pharmacokinetic sampling. Blood samples for pharmacokinetics were collected throughout a 12-h period (0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h) after dosing on days 10 and 37 for amprenavir-ritonavir and throughout a 24-h period (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h) after dosing for paroxetine. Trough levels, just before intake of the drugs, were determined on days 1, 4, 8, 28, 31, and 35.

Serum biochemistry, hematology, and urinalysis were checked on days 1, 4, 8, 10, 28, 31, 35, and 37. Adverse events and physical condition of the subjects were assessed during the same visits. A pregnancy test for women and screening for drugs of abuse were conducted on days 1, 10, 28, and 37.

Pharmacodynamics. Serotonin is transported into blood platelets and central neurons by a similar active uptake transporter mechanism. It has been reported that changes in serotonin transport activities in platelets, induced by serotonin reuptake inhibitors, may be a potential surrogate marker of their effectiveness (serotonin reuptake inhibition) at the synaptosomal membrane in the brain (reviewed in reference 8). Therefore, serotonin concentrations in platelets were measured at the Laboratory of Pediatrics and Neurology at the Radboud University Nijmegen Medical Centre to determine the peripheral pharmacodynamic effect of paroxetine. For the determination of serotonin concentrations, venous whole blood was collected predose on days 1, 10, 28, and 37 of the trial. While the blood was clotting, platelets released serotonin, which was measured in serum.

Compliance. Study personnel supervised all medication intakes during the visits to the clinical trial unit on days 1, 4, 8, 10, 28, 31, 35, and 37. The exact times of dosing were recorded. Drug intakes by the subjects at home were monitored by the use of MEMS caps (Aardex Ltd., Zug, Switzerland), which record the opening of the medication bottle. Furthermore, subjects were asked to write down the exact times of medication intake in a booklet.

Bioanalysis of amprenavir-ritonavir and paroxetine (total and unbound) concentrations in plasma. Plasma samples were analyzed for amprenavir, ritonavir, and paroxetine (total concentrations) at the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre. The Department of Clinical Pharmacy has established a high-pressure liquid chromatography (HPLC) assay for amprenavir and ritonavir, derived from a reversed-phase HPLC method which was published previously (10). The method involves liquid-liquid extraction from plasma, followed by HPLC with an OmniSpher 3 C_{18} column (100 by 4.6 mm) and UV detection at 215 nm. For the determination of amprenavir and ritonavir in our trial, we used acetonitrile and 50 mM phosphate buffer (pH 5.60) as the mobile phase. The acetonitrile concentration was increased from 35% to 54% during a 21-min period; thereafter, it was returned to 35%. The accuracy for amprenavir was 102% at 0.15 mg/liter, 105% at 1.5 mg/liter, and 103% at 7.5 mg/liter. For ritonavir, the accuracy was 101%, 104% and 103%, respectively. The precision (within-day; coefficient of variation; $n = 15$) for amprenavir was 3.98%, 4.05%, and 2.55% at 0.15, 1.5 and 7.5 mg/liter, respectively. For ritonavir, the precision (within-day; coefficient of variation; $n = 15$) was 3.22%, 1.70%, and 0.89%, respectively. The precision (between-day; coefficient of variation; $n = 3$) for amprenavir was 5.04%, 2.67%, and 1.18% at 0.15, 1.5 and 7.5 mg/liter, respectively; for ritonavir it was 3.64%, 1.17%, and 1.10%, respectively. The calibration curves were linear over concentration ranges of 0.10 to 30 mg/liter for amprenavir and 0.045 to 30 mg/liter for ritonavir.

Total (bound plus unbound) plasma levels of paroxetine were analyzed by using a validated reversed-phase HPLC method. This method consists of a liquid-liquid extraction step followed by HPLC. Briefly, 0.5 ml plasma was vortexed and centrifuged with 50 μl internal standard (dibucaine in methanol-water), 0.5 ml 0.2 M NH_4OH, and 5 ml *tert*-butylmethylether. The organic layer was removed and dried. Then, 0.25 mM potassium hydrogen phosphate in acetonitrile (60/40) was added and vortexed. These samples were run with calibration curves and quality controls on a 3.5-μm SymmetryShield RP18 (150 by 4.6 mm; Waters) column with an in-line filter (Sure-guard) and acetonitrile–25 mM potassium hydrogen phosphate as the mobile phase. The acetonitrile concentration was 34% for 6 min, then increased to 60% for 2.5 min and returned to 34% for the final 4.5 min. The total run time was 13 min. Paroxetine and the internal standard were detected with fluorescence (extinction at 296 nm; emission at 350 nm). The accuracy for paroxetine was 95.9 to 104.1% over a concentration range of 0.0025 to 0.25 mg/liter. The overall precision (coefficient of variation) was 4.2% or less over a concentration range of 0.0025 to 0.25 mg/liter. The calibration curve was linear over a concentration range of 0.0025 to 0.25 mg/liter.

Unbound-paroxetine plasma concentrations were measured at Analytical Biochemical Laboratories, Assen, The Netherlands, to determine the effect of fosamprenavir-ritonavir on the free fraction of paroxetine. The percentage of unbound paroxetine in human plasma samples was determined via equilibrium dialysis using a Dianorm equilibrium dialyzer system. With this equilibrium dialyzer, free paroxetine was separated from the bound fraction using dialysis membranes with a molecular weight cutoff of 5,000. Human plasma samples were dialyzed against a buffer solution (pH 7.4) containing potassium biphosphate (1.9 g/liter), disodium phosphate (8.1 g/liter), sodium chloride (4.1 g/liter), and dextran (molecular weight, 64,000 to 76,000; 30 g/liter) for 4 h. After dialysis, the buffer compartment was diluted with blank human heparin plasma (1:1), and the plasma fraction was diluted with dialysis buffer solution (1:1). Both the free (i.e., unbound) and the total maximum concentration of paroxetine were analyzed by a validated LC-tandem mass spectrometry method. The samples were run on a 4-μm Synergi RP80A Fusion (75 by 4.6 mm; Phenomenex) column and ammonium formate–formic acid buffer–methanol as the mobile phase, which was applied as a gradient. Finally, the percent free paroxetine was calculated. We chose to determine maximum concentrations of the unbound paroxetine, because it was not possible to measure trough levels, which would be lower than the lower limit of quantification of paroxetine. The accuracy of the quality control samples for the free concentration of paroxetine was 107.5% at 0.150 ng/ml, 106.6% at 1.50 ng/ml, and 104.3% at 40.0 ng/ml. The overall precision (coefficient of variation) was 3.2%, 5.3%, and 4.3% at 0.150, 1.50 and 40.0 ng/ml, respec-

TABLE 1. Comparison of pharmacokinetic parameters of the combination of paroxetine and fosamprenavir/ritonavir and paroxetine alone (n = 23)

Period and regimen	Geometric mean (95% CI) [GMR, period 2/period 1 (90% CI)]		
	AUC_{0-24} (mg · h/liter)	C_{max} (mg/liter)	$t_{1/2}$ (h)
1. Paroxetine (20 mg QD)	0.59 (0.51–0.85)	0.034 (0.030–0.047)	21.1 (18.4–27.1)
2. Paroxetine (20 mg QD) + fAPV/r (700/100 mg BID)[a]	0.27 (0.24–0.36) [0.45 (0.41–0.49)]	0.017 (0.015–0.022) [0.49 (0.45–0.53)]	15.9 (13.4–20.9) [0.75 (0.71–0.80)]

[a] fAPV/r, fosamprenavir-ritonavir.

tively. The calibration curve was linear over the concentration range of 0.0500 to 50.0 ng/ml paroxetine in human plasma.

Pharmacokinetic analysis. Pharmacokinetic parameters for paroxetine, amprenavir, and ritonavir were calculated by noncompartmental methods by use of WinNonlin software (version 4.1; Pharsight Corporation, Mountain View, CA) and the log/linear trapezoidal rule. Based on the individual plasma concentration-time data, the following pharmacokinetic parameters were determined: the area under the plasma concentration-time curve from 0 to 24 h after intake for paroxetine (AUC_{0-24}) and from 0 to 12 h after intake for amprenavir and ritonavir (AUC_{0-12}; in milligram · hours per liter), the maximum concentration of the drugs in plasma (C_{max}; in milligrams per liter), the trough concentration in plasma (C_{min}; 12 h after intake for amprenavir-ritonavir; 24 h after intake for paroxetine; in milligrams per liter), the apparent elimination half-life ($t_{1/2}$; in hours) and the apparent oral clearance (C_L/F; in liters per hour).

Sample size and statistical analysis. The power analysis for proving equivalence in a crossover design according to the method of Hauschke et al. (16) was used for calculating the sample size for this trial. The sample size was determined to attain a power of 80% at an α level of 0.05 in the case of an equivalence range (0.8 to 1.25), an intraindividual coefficient of variation of 20%, and an intersubject coefficient of variation of 65% (4, 16). The calculated sample size was 20 (10 per group) subjects. We estimated a drop-out rate of 25% based on previous pharmacokinetic interaction studies in healthy volunteers carried out by our department. Therefore, a total of 26 subjects were included in this trial to ensure completed data from 20 subjects.

For the determination of a clinically relevant interaction, we used the bioequivalence approach (26). Geometric means were calculated for the $AUC_{0\text{-}12}$ (amprenavir-ritonavir), $AUC_{0\text{-}24}$ (paroxetine), C_{min}, C_{max}, $t_{1/2}$, and C_L/F. Geometric mean ratios (GMR) with 90% confidence intervals (CI) were calculated after log transformation of within-subject ratios for $AUC_{0\text{-}24}$, C_{max}, and $t_{1/2}$ for paroxetine. A GMR with 90% CI falling entirely within 0.80 to 1.25 was considered as bioequivalence.

Statistical evaluations were carried out using SPSS for Windows, version 12.0.1 [SPSS Inc, Chicago, IL].

RESULTS

Baseline characteristics. Twenty-six healthy subjects, 8 males and 18 females, with a median age of 44.4 years (range, 18.2 to 64.3), were included in this trial. Median (range) weight, height and body mass index were 68.8 kg (51.0 to 89.4), 1.71 m (1.58 to 1.83) and 24.0 kg/m^2 (17.5 to 29.7), respectively. Twenty-four subjects were Caucasians, one was Oriental, and one had a Mediterranean background.

Study completion. Compliance by 25 of the 26 subjects was good, as indicated by the subject's statements about the intake of the previous doses, the numbers of tablets and capsules in the returned vials, the booklets, and the MEMS caps (data not shown). One subject missed one dose of paroxetine on day 3 because of nausea; five subjects took paroxetine BID instead of QD for 1 or 2 days in the beginning of the trial period when paroxetine was combined with fosamprenavir-ritonavir, which had to be taken twice daily; all other subjects took all doses of paroxetine and fosamprenavir-ritonavir at the right time points.

One female subject was excluded for pharmacokinetic data analysis because of significant nonadherence (deviating plasma paroxetine levels and MEMS data indicating that medication bottles were not opened properly), discovered after the end of the study. Two subjects (one male and one female) discontinued the use of trial medication because of adverse events (as described below).

Pharmacokinetics. Pharmacokinetic parameters were calculated for 23 evaluable subjects. The geometric mean (95% CI) AUC_{0-24}, C_{max}, C_{min}, $t_{1/2}$, and C_L/F of paroxetine (total unbound and protein-bound paroxetine, given alone without fosamprenavir-ritonavir) were 0.59 mg · h/liter (0.51 to 0.85), 0.034 mg/liter (0.030 to 0.047), 0.019 mg/liter (0.017 to 0.030), 21 h (18 to 27), and 33.1 liters/h (29.1 to 46.9), respectively, which are similar to data from other studies with the same dosage (4, 14, 23). Table 1 shows the GMR of the $AUC_{0\text{-}24}$, C_{max}, and $t_{1/2}$ comparing paroxetine given alone and in combination with fosamprenavir-ritonavir. The $AUC_{0\text{-}24}$, C_{max}, and $t_{1/2}$ of paroxetine alone compared to paroxetine with fosamprenavir-ritonavir were considered not bioequivalent. The GMR for the $AUC_{0\text{-}24}$ of paroxetine was 0.45 (90% CI, 0.41 to 0.49), indicating that the $AUC_{0\text{-}24}$ of paroxetine (total of bound and unbound concentration) was significantly decreased by fosamprenavir-ritonavir. Figure 1 shows the pharmacokinetic 24-h curves of paroxetine, alone and in combination with fosamprenavir-ritonavir.

The free fraction (unbound paroxetine divided by total of unbound and bound paroxetine concentration) was increased in all subjects after the addition of fosamprenavir-ritonavir to paroxetine. The median (interquartile range) increase was 30% (18 to 42%), indicating that relatively more unbound paroxetine was present in combination with fosamprenavir-ritonavir and that protein displacement had occurred.

The free/unbound C_{max} of paroxetine in combination with fosamprenavir-ritonavir was lower than that of paroxetine given alone by 39.8% (median; interquartile range, 26.9 to 45.5%).

The pharmacokinetic parameters of amprenavir and ritonavir are shown in Table 2. The pharmacokinetic parameters of amprenavir and ritonavir were similar to the results of other trials (2, 7, 27; summaries of product characteristics for Norvir and Telzir [EMEA]), suggesting that paroxetine did not affect the pharmacokinetics of amprenavir-ritonavir. We did not compare the pharmacokinetics of fosamprenavir-ritonavir to a control group, which is a limitation of our trial. Figure 2 shows the median curve of amprenavir compared to historical controls (27).

Paroxetine levels in the two groups were compared to exclude a period effect or a carryover effect on the pharmacoki-

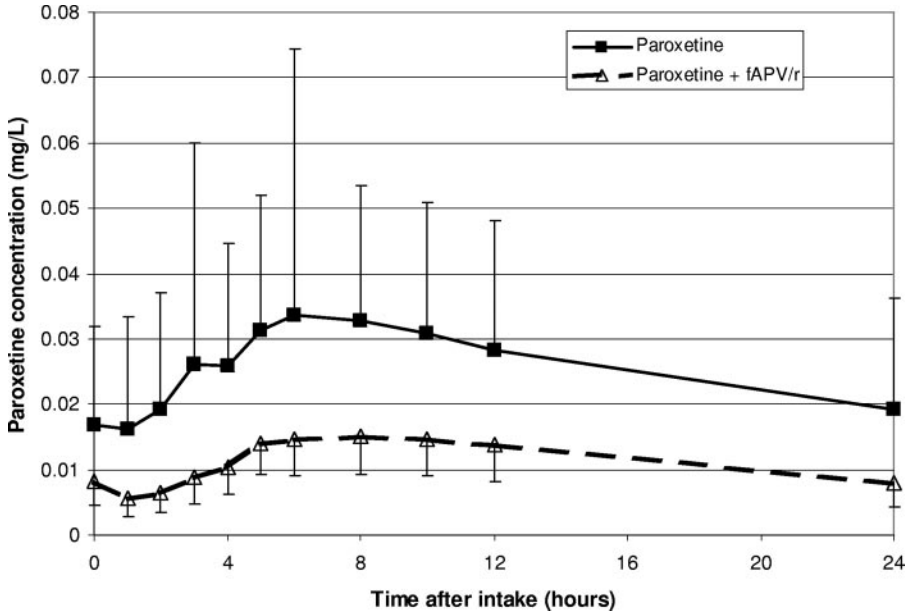

FIG. 1. Pharmacokinetic curves (geometric mean and standard deviation) of paroxetine alone and in combination with fosamprenavir-ritonavir (fAPV/r) (n = 23).

netic parameters. Paroxetine concentration ratios between groups A and B showed no difference (independent-samples t test, $P = 0.238$).

Pharmacodynamics. Paired serotonin concentrations in platelets could be determined for only 17 subjects, because at least one of the whole-blood samples of the other subjects was hemolytic. The median decrease in serotonin concentration in platelets after a 10-day use of paroxetine alone was 87% compared to baseline. The median decrease of serotonin concentrations after a 10-day use of paroxetine in combination with fosamprenavir-ritonavir was 81% compared to baseline serotonin concentrations. There was no significant difference in change in serotonin concentration with paroxetine alone versus paroxetine in combination with fosamprenavir-ritonavir (Wilcoxon signed-ranks test, $P = 0.554$).

Adverse events and safety assessments. Table 3 shows the most frequently occurring adverse events (defined as any adverse event experienced by two or more persons) during the different periods of the trial (paroxetine alone and paroxetine in combination with fosamprenavir-ritonavir). No serious adverse events were reported. Two subjects withdrew because of adverse events: one female subject experienced grade III diarrhea, and another male subject had grade II nausea; both subjects were using paroxetine and fosamprenavir-ritonavir when they withdrew. Eight subjects (two males and six females) experienced rashes at the end of the period in which they received paroxetine combined with fosamprenavir-ritonavir; one of these subjects had a grade III rash. Four of the subjects experiencing rashes received cetirizine. The subject with the severe rash also received clemastine and hydrocortisone (once, subcutaneously). The other adverse events were mild. None of the subjects experienced permanent adverse effects due to the use of trial medication.

As shown in Table 3, seven subjects experienced diarrhea when paroxetine was combined with fosamprenavir-ritonavir. These subjects had a significantly smaller difference in paroxetine AUC and C_{max} (total concentrations) between the period with paroxetine alone and the period in which the combination of paroxetine and fosamprenavir-ritonavir was used than subjects who did not experience diarrhea ($P = 0.040$ and $P = 0.048$, respectively; independent-samples t test). So, fosamprenavir-ritonavir-associated diarrhea did not cause a reduced

TABLE 2. Pharmacokinetic parameters of amprenavir and ritonavir (n = 23) compared to population data

Drug and data source	Geometric mean (95% CI)			
	AUC_{0-12} (mg · h/liter)	C_{min} (mg/liter)	C_{max} (mg/liter)	$t_{1/2}$ (h)
Amprenavir				
This study	44.1 (40.5–50.2)	2.7 (2.4–3.2)	5.8 (5.4–6.6)	11.5 (10.1–15.2)
Population data[a]	39.6 (34.5–45.3)	2.1 (1.8–2.5)	6.1 (5.4–6.9)	15–23
Ritonavir				
This study	5.1 (4.5–6.2)	0.18 (0.16–0.23)	0.92 (0.81–1.3)	4.1 (3.6–5.0)
Population data[b]	5.8 (4.8–7.0)	0.15 (0.11–0.22)	1.2 (0.98–1.5)	3–5

[a] Data from references 7 and 27 and the summary of product characteristics for Telzir (EMEA).
[b] Data from reference 27 and the summary of product characteristics for Norvir (EMEA).

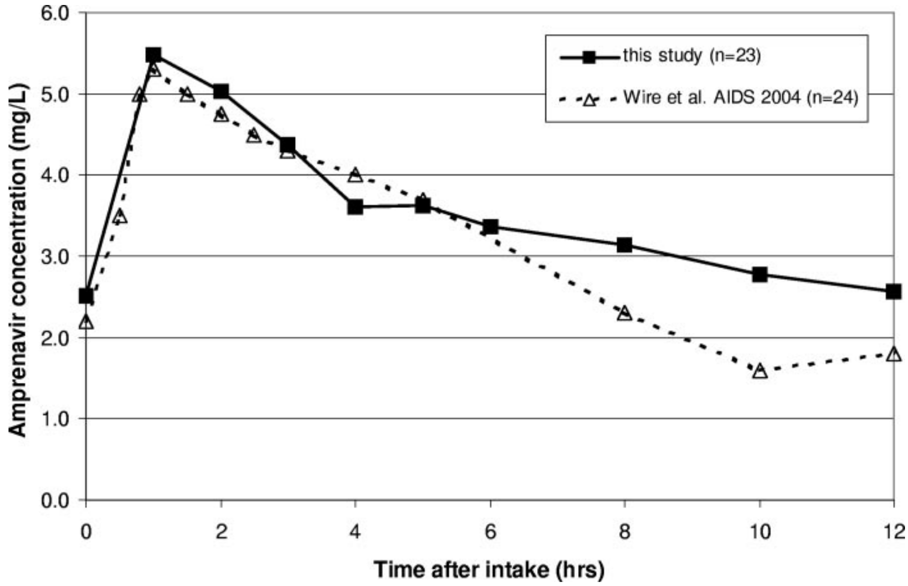

FIG. 2. Median pharmacokinetic curve of amprenavir (n = 23) compared to historical controls (27).

absorption of paroxetine, because if it had, we would have expected to find a greater difference in AUC and C_{max} between the two trial periods in patients experiencing diarrhea.

DISCUSSION

The decrease of 55% in paroxetine plasma exposure when paroxetine was combined with fosamprenavir-ritonavir was unexpected. Based on the fact that ritonavir and paroxetine are both inhibitors of CYP2D6 (summaries of product characteristics for Seroxat [CBG-MEB] and Norvir [EMEA]), we expected that either ritonavir would inhibit the metabolism of paroxetine, which would have caused higher paroxetine levels, or paroxetine would inhibit ritonavir metabolism and that the associated increase in ritonavir levels would have had a greater booster effect and increased amprenavir levels.

TABLE 3. Numbers of subjects experiencing adverse events[a]

System	Adverse event	No. of subjects in period: Paroxetine alone (n = 25)	Paroxetine with fosamprenavir-ritonavir (n = 26)
Gastrointestinal	Diarrhea	2	7
	Nausea	4	13
Musculoskeletal and connective tissue	Stiff jaws	3	
Nervous	Headache	3	4
	Flat emotions	4	
	Tiredness	8	8
	Dizziness		4
Skin and subcutaneous	Rash		8
Overall		37	62

[a] Adverse events reported by two or more subjects per group.

We could think of four possible explanations for the decrease in paroxetine plasma levels: (i) displacement of protein binding of paroxetine by fosamprenavir and/or ritonavir; (ii) CYP3A acts as a secondary metabolic pathway for paroxetine, induced by fosamprenavir; (iii) induction of CYP2D6-mediated metabolism of paroxetine by fosamprenavir and/or ritonavir; (iv) decreased absorption of paroxetine by fosamprenavir and/or ritonavir.

The first and most likely explanation (displacement of protein binding) is possible because paroxetine (95% [summary of characteristics; CBG-MEB]), amprenavir (90% [summary of characteristics; EMEA]) and ritonavir (98 to 99% [summary of characteristics; EMEA]) are all highly bound to the same plasma proteins (alpha-1 acid glycoprotein and albumin). The Food and Drug Administration's prescribing information for Prezista (darunavir), a novel PI, describes the same effect on total paroxetine concentrations when paroxetine is combined with darunavir-ritonavir. In our trial we found a median increase of 30% in the paroxetine-free fraction, which is indicative of protein displacement. An interaction caused by protein displacement is usually not clinically relevant, because after establishment of a new equilibrium, the free (i.e., effective) concentration of a drug is not changed. However, in this trial the free C_{max} of paroxetine decreased by 40%, so the interaction can be only partly explained by protein displacement. In our trial we did not find a significant difference in change in serotonin concentration in platelets using paroxetine alone versus paroxetine in combination with fosamprenavir-ritonavir (Wilcoxon signed-ranks test: P = 0.554). A possible explanation for a lack of a pharmacodynamic effect could be that the reuptake of serotonin is already saturated with a low paroxetine concentration. Furthermore, whole-blood serotonin levels are indicative of serotonin reuptake in plasma and most likely also reflect the activity taking place in the central neurons, but depletion of platelet serotonin is not a reliable index of antidepressant efficacy. Previously, no correlation was found between changes in platelet levels and the Hamilton depression rating scale scores (8).

The second explanation (induction of metabolism) is based on data that (fos)amprenavir can induce CYP3A4 (summary of characteristics, EMEA). A trial combining phenytoin (a CYP3A4 inducer) with paroxetine showed decreased paroxetine levels (data on file; GlaxoSmithKline). These data are, however, in contrast with those of other trials that showed no interactions between paroxetine and two well-known CYP3A4 substrates: alprazolam (6) and terfenadine (20). Another factor which makes this explanation less likely is that ritonavir is a very strong inhibitor of CYP3A4 (summary of characteristics, EMEA) and would increase paroxetine levels if paroxetine was metabolized through CYP3A4.

The third explanation (induction of CYP2D6-mediated paroxetine metabolism) is not likely, as no data about induction of CYP2D6 by fosamprenavir-ritonavir have been reported so far. Furthermore, it is known that CYP2D6 is not easily induced. Rifampin, which is a strong inducer, decreases plasma levels of CYP2D6 substrates by only approximately 25% (5, 11). Our trial showed a decrease in paroxetine AUC_{0-24} of 55%. Moreover, in a previous study, our group found a modest inhibitory effect of 100 mg ritonavir BID on the activity of CYP2D6 (1).

Decreased absorption of paroxetine is our fourth and final explanation. So far, no effect of fosamprenavir-ritonavir on absorption of other drugs has been described. We thought that a decrease in absorption could have been indicated by the fact that diarrhea occurred more frequently when fosamprenavir-ritonavir was combined with paroxetine than when paroxetine was given alone. However, the subjects with diarrhea had a significantly lower difference in AUC and C_{max} between the two trial periods (with or without fosamprenavir-ritonavir) than patients without diarrhea.

We found that the half-life of paroxetine was decreased by 25% in combination with fosamprenavir-ritonavir. It has been reported that a decrease in protein binding results in a shorter half-life. However, this has been described mostly for drugs with a relatively small apparent volume of distribution (<0.25 liter/kg) (13) and the apparent volume of distribution of paroxetine is larger (about 8.7 liters/kg) (19); paroxetine is extensively distributed into tissues (19; summary of characteristics; CBG-MEB). Furthermore, the $t_{1/2}$ of paroxetine is approximately 1 day according to the literature. The $t_{1/2}$ was calculated during steady state with a dosing interval of 24 h and the last two blood samples taken at 12 and 24 h after intake, so there is a large uncertainty under these conditions. Therefore, one should be very cautious with the interpretation of changes in $t_{1/2}$.

No serious adverse events were reported during the trial. Surprisingly, rashes occurred in eight subjects when paroxetine was combined with fosamprenavir-ritonavir. We expect the rash to be an adverse event of fosamprenavir, as it is described as "common" (i.e., occurring in $\geq$1/100 and $\leq$1/10 subjects) in the summary of product characteristics of fosamprenavir (EMEA), but we cannot explain the higher incidence in our trial than mentioned in the summary of product characteristics. The combination of fosamprenavir-ritonavir with paroxetine seems safe, but larger studies are needed to confirm our observation, as the sample size of our trial was too small to draw definite conclusions about safety.

Only a few interactions between antiretroviral drugs and antidepressants have been investigated so far (25). As mentioned above, Aarnoutse et al. found a modest inhibitory effect of ritonavir (100 mg BID) on the activity of CYP2D6 in healthy extensive metabolizers (1), which resulted in a 26% increase in the geometric mean AUC of desipramine (a tricyclic antidepressant). Furthermore, coadministration of 30 mg fluoxetine (SSRI; inhibitor of CYP2D6) BID and ritonavir as a single dose in 16 healthy subjects resulted in a 19% increase in ritonavir AUC. Fluoxetine concentrations were not measured. However, postmarketing experience has revealed reports of cardiac and neurologic events when ritonavir and fluoxetine have been combined (21; summary of characteristics [EMEA]), and several cases of the serotonin syndrome in HIV-infected patients receiving antiretroviral therapy and fluoxetine have been reported (9). Moreover, no pharmacokinetic interaction between escitalopram (SSRI; substrate for CYP3A4, CYP2C19, and CYP2D6) and ritonavir was observed. The fourth study we found was an in vitro study with bupropion (an antidepressant and smoking cessation aid) and ritonavir which showed that ritonavir has a low 50% inhibitory concentration for inhibition of bupropion hydroxylation through CYP2B6, indicating the possibility of a clinically important CYP2B6 inhibition in vivo (18). No study combining bupropion with ritonavir in vivo has been performed yet. Finally, short-term low-dose administration of ritonavir (four doses of 200 mg) showed a decreased oral clearance of trazodone (CYP3A substrate) and increases in AUC and adverse reactions (15). We think that our trial contributes to the limited data on interactions between antidepressants and antiretroviral agents.

In conclusion, our data show an interaction between paroxetine and fosamprenavir-ritonavir. Fosamprenavir-ritonavir decreases the AUC_{0-24} of paroxetine (total concentration) by 55%. The C_{max} of the unbound concentrations was decreased by 40%. We think that this interaction is clinically relevant and that titration to a higher dose of paroxetine may be necessary to accomplish the needed antidepressant effect. More research is necessary to fully elucidate the mechanism behind this interaction. It appears that paroxetine does not have an effect on the pharmacokinetics of amprenavir-ritonavir.

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REFERENCES

1. **Aarnoutse, R. E., J. Kleinnijenhuis, P. P. Koopmans, D. J. Touw, J. Wieling, Y. A. Hekster, and D. M. Burger.** 2005. Effect of low-dose ritonavir (100 mg twice daily) on the activity of cytochrome P450 2D6 in healthy volunteers. Clin. Pharmacol. Ther. **78:**664–674.
2. **Arvieux, C., and O. Tribut.** 2005. Amprenavir or fosamprenavir plus ritona-

vir in HIV infection: pharmacology, efficacy and tolerability profile. Drugs **65:**633–659.
3. **Basu, S., L. A. Chwastiak, and R. D. Bruce.** 2005. Clinical management of depression and anxiety in HIV-infected adults. AIDS **19:**2057–2067.
4. **Belle, D. J., C. S. Ernest, J. M. Sauer, B. P. Smith, H. R. Thomasson, and J. W. Witcher.** 2002. Effect of potent CYP2D6 inhibition by paroxetine on atomoxetine pharmacokinetics. J. Clin. Pharmacol. **42:**1219–1227.
5. **Branch, R. A., A. Adedoyin, R. F. Frye, J. W. Wilson, and M. Romkes.** 2000. In vivo modulation of CYP enzymes by quinidine and rifampin. Clin. Pharmacol. Ther. **68:**401–411.
6. **Calvo, G., C. Garcia-Gea, A. Luque, A. Morte, R. Dal-Re, and M. Barbanoj.** 2004. Lack of pharmacologic interaction between paroxetine and alprazolam at steady state in healthy volunteers. J. Clin. Psychopharmacol. **24:**268–276.
7. **Chapman, T. M., G. L. Plosker, and C. M. Perry.** 2004. Fosamprenavir: a review of its use in the management of antiretroviral therapy-naive patients with HIV infection. Drugs **64:**2101–2124.
8. **Dechant, K. L., and S. P. Clissold.** 1991. Paroxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in depressive illness. Drugs **41:**225–253.
9. **DeSilva, K. E., D. B. Le Flore, B. J. Marston, and D. Rimland.** 2001. Serotonin syndrome in HIV-infected individuals receiving antiretroviral therapy and fluoxetine. AIDS **15:**1281–1285.
10. **Droste, J. A. H., C. P. W. G. M. Verweij-van Wissen, and D. M. Burger.** 2003. Simultaneous determination of the HIV drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8 and nevirapine in human plasma by reversed phase high performance liquid chromatography. Ther. Drug Monitoring **25:**393–399.
11. **Eichelbaum, M., S. Mineshita, E. E. Ohnhaus, and C. Zekorn.** 1986. The influence of enzyme induction on polymorphic sparteine oxidation. Br. J. Clin. Pharmacol. **22:**49–53.
12. **Eriksson, S., L. M. Berg, M. Wadelius, and A. Alderborn.** 2002. Cytochrome p450 genotyping by multiplexed real-time DNA sequencing with pyrosequencing technology. Assay. Drug Dev. Technol. **1:**49–59.
13. **Gibaldi, M.** 1991. Drug disposition-distribution, p. 195–202. *In* Biopharmaceutics and clinical pharmacokinetics. Lea and Febiger, Philadelphia, PA.
14. **Goldberg, M. R., R. C. Lowry, D. G. Musson, K. L. Birk, A. Fisher, M. E. De Puy, and C. R. Shadle.** 1999. Lack of pharmacokinetic and pharmacodynamic interaction between rizatriptan and paroxetine. J. Clin. Pharmacol. **39:**192–199.
15. **Greenblatt, D. J., L. L. von Moltke, J. S. Harmatz, S. M. Fogelman, G. Chen, J. A. Graf, P. Mertzanis, S. Byron, K. E. Culm, B. W. Granda, J. P. Daily, and R. I. Shader.** 2003. Short-term exposure to low-dose ritonavir impairs clearance and enhances adverse effects of trazodone. J. Clin. Pharmacol. **43:**414–422.
16. **Hauschke, D., M. Kieser, E. Diletti, and M. Burke.** 1999. Sample size determination for proving equivalence based on the ratio of two means for normally distributed data. Stat. Med. **18:**93–105.
17. **Hersberger, M., J. Marti-Jaun, K. Rentsch, and E. Hanseler.** 2000. Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. Clin. Chem. **46:**1072–1077.
18. **Hesse, L. M., L. L. von Moltke, R. I. Shader, and D. J. Greenblatt.** 2001. Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity in vitro: potential drug interactions with bupropion. Drug Metab. Dispos. **29:**100–102.
19. **Kaye, C. M., R. E. Haddock, P. F. Langley, G. Mellows, T. C. Tasker, B. D. Zussman, and W. H. Greb.** 1989. A review of the metabolism and pharmacokinetics of paroxetine in man. Acta Psychiatr. Scand. Suppl. **350:**60–75.
20. **Martin, D. E., B. D. Zussman, D. E. Everitt, L. J. Benincosa, R. C. Etheredge, and D. K. Jorkasky.** 1997. Paroxetine does not affect the cardiac safety and pharmacokinetics of terfenadine in healthy adult men. J. Clin. Psychopharmacol. **17:**451–459.
21. **Ouellet, D., A. Hsu, J. Qian, J. E. Lamm, J. H. Cavanaugh, J. M. Leonard, and G. R. Granneman.** 1998. Effect of fluoxetine on pharmacokinetics of ritonavir. Antimicrob. Agents Chemother. **42:**3107–3112.
22. **Penzak, S. R., Y. S. Reddy, and S. R. Grimsley.** 2000. Depression in patients with HIV infection. Am. J. Health Syst. Pharm. **57:**376–386.
23. **Ruwe, F. J., R. A. Smulders, H. J. Kleijn, H. L. Hartmans, and J. M. Sitsen.** 2001. Mirtazapine and paroxetine: a drug-drug interaction study in healthy subjects. Hum. Psychopharmacol. **16:**449–459.
24. **Steijns, L. S., and W. J. Van Der.** 1998. Ultrarapid drug metabolism: PCR-based detection of CYP2D6 gene duplication. Clin. Chem. **44:**914–917.
25. **Tseng, A. L., and M. M. Foisy.** 1999. Significant interactions with new antiretrovirals and psychotropic drugs. Annals Pharmacother. **33:**461–473.
26. **U.S. Department of Health and Human Services, Center for Drug Evaluation and Research, and Food and Drug Administration.** 2001. Guidance for industry. Statistical approaches to establishing bioequivalence. www.fda.gov/cder/guidance/3616fnl.htm
27. **Wire, M. B., C. Ballow, S. L. Preston, C. W. Hendrix, P. J. Piliero, Y. Lou, and D. S. Stein.** 2004. Pharmacokinetics and safety of GW433908 and ritonavir, with and without efavirenz, in healthy volunteers. AIDS **18:**897–907.
28. **Yun, L. W., M. Maravi, J. S. Kobayashi, P. L. Barton, and A. J. Davidson.** 2005. Antidepressant treatment improves adherence to antiretroviral therapy among depressed HIV-infected patients. J. Acquir. Immune. Defic. Syndr. **38:**432–438.