The Effect of Garlic Supplements on the Pharmacokinetics of Saquinavir

Stephen C. Piscitelli,[1,a] Aaron H. Burstein,[1] Nada Welden,[2] Keith D. Gallicano,[3] and Judith Falloon[2]

[1]Pharmacy Department, Warren G. Magnuson Clinical Center, and [2]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and [3]Axelson Biopharma Research, Vancouver, Canada

Herbal therapies are widely used, but there are few data on their interactions with conventional medications. This study evaluated the effect of garlic supplements on the pharmacokinetics of saquinavir. Ten healthy volunteers received 10 doses of saquinavir (Fortovase) at a dosage of 1200 mg 3 times daily with meals for 4 days on study days 1–4, 22–25, and 36–39, and they received a total of 41 doses of garlic caplets taken 2 times daily on study days 5–25. Blood samples were obtained on study days 4, 25, and 39 for determination of saquinavir plasma pharmacokinetic parameters. In the presence of garlic, the mean saquinavir area under the curve (AUC) during the 8-h dosing interval decreased by 51%, trough levels at 8 h after dosing decreased by 49%, and the mean maximum concentrations (C_{max}) decreased by 54%. After the 10-day washout period, the AUC, trough, and C_{max} values returned to 60%–70% of their values at baseline. Patients should use caution when combining garlic supplements with saquinavir when it is used as a sole protease inhibitor.

We have been interested in the effects that complementary medicines can have on the pharmacokinetics of antiretroviral medicines, especially drugs metabolized by the cytochrome P_{450} (CYP450) system, because HIV-infected persons frequently use herbal remedies and dietary supplements along with conventional therapies [1, 2]. We have previously shown that St. John's wort, an inducer of CYP450, significantly affects the pharmacokinetics of indinavir, which is an inhibitor of the HIV type 1 (HIV-1) protease enzyme and a known CYP450 substrate [3]. Because garlic is one of the dietary supplements most commonly used by our HIV clinic population, we sought to investigate the effect of garlic on the pharmacokinetics of saquinavir, a protease inhibitor. The popularity of garlic supplements in our clinic population may be related to claims that garlic has anticholesterol activity, because hypercholesterolemia is a common side effect of antiretroviral therapy [4–6].

Although there are no data in the literature to suggest that garlic would alter the pharmacokinetics of protease inhibitors, there are conflicting in vitro assessments of its effect on CYP450 [7, 8]. We chose to examine the effect of garlic on saquinavir, rather than on protease inhibitors that are in more common clinical use, both because saquinavir is a known substrate of CYP450 and because it does not appreciably induce or inhibit drug metabolism. Because we did not know whether garlic might induce or inhibit CYP450, studying the effect of garlic on a protease inhibitor that itself affects CYP450 could have confounded the data. Moreover, any effects of garlic on the metabolism of saquinavir would be expected to markedly alter plasma concentrations of saquinavir because of saquinavir's low oral bioavailability; saquinavir is metabolized before systemic absorption by

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[a] Present affiliation: Virco Lab, Rockville, Maryland.

Reprints or correspondence: Dr. Judith Falloon, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Rm. 11C103, 10 Center Dr., MSC 1880, Bethesda, MD 20892-1880 (Jfalloon@nih.gov).

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the CYP3A4 isoform of the CYP450 enzyme system that is present in the villus tip enterocytes of the small intestine [9].

METHODS

Study design. We conducted a 2-treatment, 3-period, single-sequence, longitudinal study. Inclusion criteria included documented ELISA and Western blot results negative for HIV-1, age >18 years, and normal findings of laboratory and physical examinations. Subjects were excluded if they had smoked in the past year, had received any dietary supplements within 30 days, had a history of adverse reaction to garlic or saquinavir, were pregnant or lactating, were receiving concomitant medications metabolized via the CYP450 pathway, or had persistent diarrhea or a history of malabsorption.

The study design is summarized in figure 1. In period 1, subjects received saquinavir (Fortovase; Roche Laboratories) at a dosage of 1200 mg 3 times daily with meals for 3 days, receiving the last (10th) dose on the morning of study day 4. In period 2, garlic caplets (GarliPure, Maximum Allicin Formula; Natrol) from a single lot were administered 2 times daily on study days 5–24 with breakfast and dinner, and saquinavir from a commercial supply was administered with garlic on days 22–24. The final dose of garlic (the 41st) and saquinavir (the 10th) were administered simultaneously on the morning of day 25. Saquinavir and garlic were discontinued for 10 days, then saquinavir was again administered for 3 days with meals on days 36–38; subjects received the final dose of saquinavir on the morning of day 39 (period 3). In each period, serial blood samples were collected during the 8-h morning dosing interval for saquinavir in the absence (days 4 and 39) and presence (day 25) of garlic. Nine blood samples (7 mL each) were collected into Vacutainer tubes that contained heparin immediately before drug administration and at 0.5, 1, 2, 3, 4, 5, 6, and 8 h after the last (10th) dose of saquinavir. Blood was centrifuged to separate the plasma, and plasma aliquots were stored in polypropylene tubes and frozen at −80°C.

Adherence was assessed by patient interview at each study visit. Subjects were given dosing calendars as a compliance aid.

Informed consent was obtained from subjects and clinical research was conducted in accordance with guidelines for human experimentation as specified by the US Department of Health and Human Services. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board.

Drug analysis. Concentrations of saquinavir in plasma were measured by use of high-performance liquid chromatography (HPLC) with single-quadrupole mass spectrometric detection after a liquid-liquid extraction at basic pH with a mixture of 1-chlorobutane/acetone. Deuterated (d_5)–saquinavir internal standard solution (0.05 mL of 250 ng/mL in methanol)

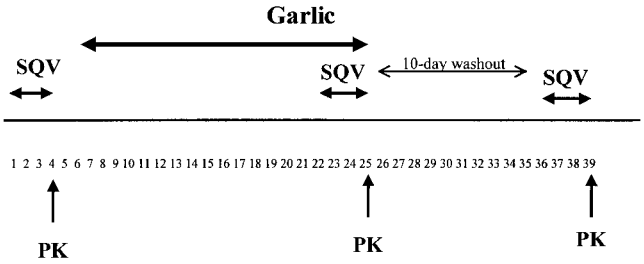

Figure 1. Design of the study to determine effect of garlic supplements on pharmacokinetics of saquinavir. *Horizontal arrows,* dosing intervals and washout periods. Garlic, garlic administration; PK, times when serial blood samples were obtained for pharmacokinetic analysis; SQV, saquinavir administration. Numbers indicate study days.

and 0.1 mol/L disodium tetraborate (0.1 mL) were added to plasma samples (1 mL). The resulting mixture (pH, 10) was extracted with 1-chlorobutane/acetone (8 mL; ratio, 75:25 [vol/vol]), and the organic extract was isolated and evaporated to dryness at 40°C. The residue was dissolved in acetonitrile plus 0.01 mol/L ammonium acetate (0.1 mL; ratio, 1:1 [vol/vol]), and the solution was transferred to glass inserts and centrifuged at 1500 *g* for 10 min at room temperature. An aliquot (0.010 mL) was analyzed by HPLC–mass spectrometry. The HPLC conditions were as follows: column, Zorbax C18 (Agilent Technologies); particle size, 3 μm; column dimensions, 5×47 mm; column temperature, 40°C; mobile phase composition, 0.01 mol/L ammonium acetate/acetonitrile (ratio, 40:60 [vol/vol]); flow rate, 0.7 mL/min. Analytes were detected in the mass spectrometer by use of atmospheric pressure electrospray ionization in the positive ion mode. Mass spectral data were obtained by single-ion monitoring at a mass-to-charge ratio of 676 for d_5-saquinavir and a mass-to-charge ratio of 671 for saquinavir. The lower limit of quantitation of the assay was 1 ng/mL for saquinavir. Duplicate or triplicate plasma quality-control samples were analyzed at low, medium, and high concentrations in each of 6 analytical batches. For data pooled over the batches, the 90% confidence limits around the mean assay biases were as follows ($n = 16$ samples per concentration level): at low concentration, 0.7%–17.9%; at medium concentration, −5.4%–8.3%, and at high concentration, −3.2%–6.8%. The within-batch coefficients of variation were 14.4% at low concentration, 2.1% at medium concentration, and 3.4% at high concentration; the between-batch coefficients of variation were 3.7% at low concentration, 8.0% at medium concentration, and 5.5% at high concentration.

Analytical reference standards of saquinavir mesylate and d_5-saquinavir mesylate were obtained from Roche Discovery Welwyn. Garlic caplets were analyzed for allicin and allin content by a contract laboratory (Research Triangle Park Laboratories; Raleigh, North Carolina) by use of US Pharmacopeia and National Formulary methods [10]. The allicin content was 4.64

Table 1. Mean saquinavir pharmacokinetic parameters, including paired comparison for saquinavir administered alone on 2 occasions (periods 1 and 3) and after coadministration with garlic (period 2) in 9 healthy volunteers.

Parameter	Mean value[a]			Ratio of means, % (90% CI)[b]		Pooled intrasubject CV, %
	Period 1	Period 2	Period 3	Period 2 to period 1	Period 3 to period 1	
C_{max}, ng/mL	1190 (1245 ± 388)	542 (673 ± 397)	722 (781 ± 347)	45.6[c] (27.3–76.1)	60.6 (36.3–101)	56.7
t_{max}, h	2.0 (2.0, 5.0)	1.0 (1.0, 2.0)	2.0 (1.0, 5.0)	−1.0[d] (−2.0 to −1.0)	NC	NC
AUC, h·ng/mL	3382 (3548 ± 1139)	1673 (1961 ± 1081)	2184 (2474 ± 1603)	49.5[c] (30.8–79.3)	64.6 (40.3–104)	51.8
C_0, ng/mL	99.0 (109 ± 48.8)	62.8 (76.8 ± 50.4)	67.3 (73.9 ± 37.7)	63.4 (40.0–101)	68.0 (42.8–108)	50.5
C_8, ng/mL	108 (122 ± 62.5)	55.3 (62.4 ± 30.0)	75.6 (89.5 ± 67.1)	51.3[c] (35.4–74.2)	70.0 (48.4–101)	39.5

NOTE. AUC_8, area under plasma concentration plotted against time curve for the 8-h dosing interval; C_0, observed predose plasma concentration; C_8, observed concentration at hour 8, the end of the dosing interval; C_{max}, highest observed plasma concentration; CV, coefficient of variation; NC, not calculated; t_{max}, time of greatest observed plasma concentration.

[a] Geometric mean (arithmetic mean ± SD), except for t_{max} values, which are binomial median (minimum, maximum).

[b] Ratios of means (i.e., differences in means) are percentage ratio of least-squares geometric treatment means. They were calculated using geometric means that were not rounded off.

[c] $P < .028$; Dunnett's test.

[d] $P < .025$; Wilcoxon signed rank test.

mg/caplet and the allin content was 11.2 mg/caplet. Both values were in excess of the labeled amounts of 3.6 mg/caplet and 4.8 mg/caplet, respectively. Thus, the garlic dose was roughly equivalent to two 4-g cloves of garlic daily.

Pharmacokinetic analysis. The plasma concentration (C) versus time (t) data for saquinavir were analyzed by noncompartmental methods. The highest concentration (C_{max}), the hour 0 concentration (predose C_0), the hour 8 concentration (postdose C_8), and the time to achieve C_{max} (t_{max}) were determined directly from the observed data. The area under the plasma concentration-time curve during the 8-h dosing interval (AUC_8) was calculated by the linear trapezoidal method.

Differences in mean pharmacokinetic parameters for saquinavir between treatment periods were analyzed by analysis of variance (ANOVA) appropriate for a longitudinal study, followed by Dunnett's test for multiple comparisons of the treatments in periods 2 and 3 against the reference in period 1. Dunnett's test was used to maintain the experiment-wise error rate at 5% ($\alpha = .05$) for the 2 pairwise comparisons; $P < .028$ was considered statistically significant at the 5% level. The ANOVA model included the effects of subject and period. All parameters except t_{max} were logarithmically (ln) transformed before analysis, and ANOVA summary statistics were based on least-squares geometric means. Median t_{max} values were compared by the Wilcoxon signed rank test for the 2 pairwise comparisons. The significance level for each comparison of t_{max} was set at .025 from the Bonferroni-adjusted P value. The 90% confidence limits around the absolute difference in Wilcoxon medians or around the ratio of geometric means were calculated relative to the reference drug-alone treatment in period 1. Confidence limits around ratios were calculated with use of the critical t value from the Dunnett's test. For logarithmically transformed data, intraindividual coefficient of variation (subject-by-treatment interaction) was calculated as $100\% \times (e^{MSR} - 1)^{1/2}$, where MSR is the mean-square residual in the ANOVA model. Statistical computations were done with SAS for Windows, version 8.0 (SAS Institute).

RESULTS

Subjects. Ten subjects were enrolled: 4 were men and 6 were women. Their mean age (± SD) was 38 ± 7.8 years. Nine subjects were included in the analysis. One subject was excluded because her baseline AUC was found to be 1207 h·ng/mL, which was 58% of the AUC of the subject with the next higher value, and she admitted to noncompliance with the dosing regimen. The decision to exclude her data was confirmed by a statistical outlier test [11]. Only 1 other patient reported missing any doses of drug, and this subject reported missing 1 dose on the first day of the second saquinavir period (3 days before blood samples were obtained).

Pharmacokinetic parameters. Pharmacokinetic parameters of saquinavir during the 3 study phases are shown in table 1. In the presence of garlic supplementation, the mean saquinavir AUC decreased by 51%, from 3382 to 1673 h·ng/mL (range, −84% to 12%; $P = .007$). The mean C_8 decreased by 49%, from 108 to 55 ng/mL (range, −82% to 33%; $P = .002$), and the mean C_{max} decreased by 54%, from 1190 to 542 ng/mL (range, −88% to 30%; $P = .006$). After the 10-day washout period, pharmacokinetic parameters did not return to baseline values, as shown in table 1. The mean AUC returned to 65% of the baseline value, the C_{max} to 61% of baseline, and the C_8 to 70% of baseline.

Further examination of individual concentration-time profiles demonstrated a dichotomous response to garlic supplementation. Figure 2 shows 2 separate dispositions of saquinavir

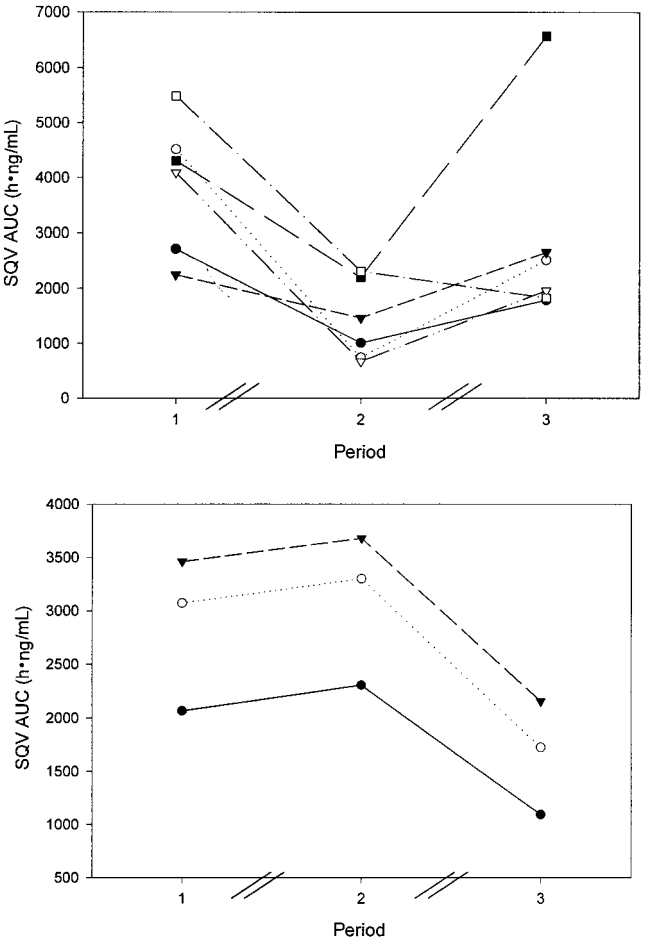

Figure 2. Individual AUC_8 values (area under plasma concentration plotted against time curve during the 8-h dosing interval) of saquinavir (SQV) at baseline (period 1), after treatment with garlic (period 2), and after the 10-day washout period (period 3) for individual healthy volunteers. *Top,* data from 6 subjects with decreased SQV exposure after garlic whose values returned to near baseline after washout. *Bottom,* data from 3 subjects with no change in SQV exposure after garlic but subsequent decrease after washout period.

pharmacokinetics in response to garlic therapy. Six subjects demonstrated a large and consistent decrease in the saquinavir AUC with garlic (figure 2, *top*). With 1 exception, these 6 subjects demonstrated an AUC after the washout period that was less than their baseline AUC. Three other subjects showed a different, but also consistent, effect of garlic therapy (figure 2, *bottom*). These subjects had no significant change in saquinavir AUC with garlic but then demonstrated a substantial drop in AUC after the 10-day washout period.

Adverse effects. No subject discontinued the study because of an adverse effect, and adverse effects were similar among the 3 sets of saquinavir treatments. The most commonly seen adverse effects were associated with saquinavir dosing and included abdominal discomfort (10 episodes), diarrhea (9 episodes), and mild nausea (6 episodes). Subjects considered these adverse effects to be mild or moderate in severity and none required intervention. Two patients reported fatigue that lessened in severity throughout the study. Side effects that occurred less often were dyspepsia, headache, and an unpleasant taste in the mouth. No adverse effects were reported when garlic was administered alone.

Two subjects had elevated hepatic transaminase levels ($\leq$3 times the upper limit of normal) on the last day of study. These levels returned to normal within 2 weeks after the study. One subject had transient 1^+ proteinuria and mild elevation in blood urea nitrogen level (25 mg/dL) at the end of saquinavir dosing in period 3, and 1 subject had asymptomatic eosinophilia (1974 cells/μL) at the end of dosing in period 3 that returned to normal within 5 days.

DISCUSSION

Our study is the first to demonstrate that garlic supplements, which are readily available and widely used, might have a detrimental effect on concomitant medications. In our study, long-term use of garlic caplets led to a significant decline in the plasma concentrations of saquinavir, an HIV-1 protease inhibitor. The mean AUC for saquinavir at baseline in our study was lower than that expected for HIV-infected patients, which is consistent with previous studies showing that AUC values for healthy volunteers are approximately one-half of AUC values for HIV-infected patients [12]. Even given the higher concentrations expected in HIV infection, however, a decrease of the magnitude we observed would be expected to be clinically important [13, 14].

Our study design was unable to define the mechanism for the alterations in saquinavir levels, but the similarity in the magnitude of the decreases in AUC, C_{max}, and C_8 suggests that garlic affected the bioavailability of saquinavir rather than its systemic clearance. Because the AUC for saquinavir did not return to baseline values after the washout period, it seems likely that this effect is not caused by impairment of absorption in the gastrointestinal tract. The effect may be caused by induction of CYP450 in the gut mucosa that results in diminished systemic levels; however, because saquinavir is a known substrate of P-glycoprotein, we cannot exclude contributions from induction of P-glycoprotein [15].

The mechanism for the prolonged effects of garlic on saquinavir levels after a washout period is unknown but may involve the formation of a component of garlic that has a long half-life and enzyme-inducing properties. However, we did not measure plasma concentrations of garlic components, so we could not evaluate this hypothesis. Alternatively, long-term use of garlic may lead to the production and slow accumulation of a saquinavir metabolite that induces saquinavir metabolism. This may explain why some patients demonstrated a delay in

the reduction of their saquinavir concentrations. Decreases in exposure to saquinavir over time have been demonstrated in HIV-infected patients [16].

Several studies suggest that short-term exposure to garlic can alter the CYP3A4 isoform of the CYP450 enzyme system, the isozyme through which saquinavir is metabolized. An in vitro study demonstrated that fresh and aged cloves of garlic and a commercial garlic product inhibited CYP3A4-mediated metabolism in a human microsome model [7]. In another study in rats, a single dose of garlic oil resulted in a significant depression of hepatic CYP450, aminopyrine *N*-demethylase, and aniline hydroxylase activity, but administration of garlic for 5 days led to a significant increase in hepatic CYP450 activity [8]. Therefore, in our study, we chose to administer garlic for a full 3 weeks to simulate long-term administration and to avoid the contradictory observations that can occur when only short-term administration is studied.

One previous study evaluated the effect of garlic on single doses of ritonavir, another HIV-1 protease inhibitor that is metabolized by CYP3A4 [17]. In this study, ritonavir plasma concentrations were determined after a single 400-mg dose and then again after receiving a commercial garlic capsule for 4 days. After garlic use, investigators observed an insignificant (17%) decrease in the ritonavir AUC. These negative results are most likely explained by the short duration of garlic administration. The fact that ritonavir is both an inhibitor and inducer of CYP450 isozymes, so that single doses do not reflect concentrations at steady state, may also have affected the results.

The bimodal distribution of the effect of garlic remains unexplained and requires further investigation. We could not identify any patient demographic characteristic that would explain these results. Although our findings may simply be interpatient variability, the concentration-time profiles are very consistent within the 2 groups, which suggests a true but dichotomous effect of garlic on the disposition of saquinavir. The effect may be related to differences in CYP3A4 content, differences in the metabolism or absorption of garlic, or some genetic variable that we have not identified. Although such data increase the intrasubject variability, we were still able to detect a significant difference in saquinavir levels with garlic, despite intrasubject variabilities of 40%–52%, because of the marked effect in those patients whose concentrations decreased after garlic therapy.

The implications of the effect of garlic on saquinavir concentrations are wide-ranging. Not only should physicians and their patients be concerned about the use of garlic in patients receiving saquinavir, but there may be concerns about other drugs that are CYP450 substrates as well, especially those metabolized by the CYP3A4 isoform. Patients receiving saquinavir as their sole protease inhibitor should avoid using garlic supplements. Whether pharmacokinetic enhancement by inhibitors of CYP450, such as ritonavir, can prevent garlic-induced alterations in saquinavir concentrations remains to be determined.

Clinicians and patients should not assume that dietary supplements are benign therapies. Some of these products may have potent pharmacological actions and may alter the blood levels of concomitant medications. As more studies are conducted with alternative and conventional medicines, health care professionals can expect the identification of additional herb-drug interactions.

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