Effect of short-term administration of garlic supplements on single-dose ritonavir pharmacokinetics in healthy volunteers

Keith Gallicano,^{1,2} Brian Foster³ & Shurjeel Choudhri^{4,5}

¹Clinical Investigation Unit and ²Ottawa Hospital Research Institute, The Ottawa Hospital-General Campus, and ³Therapeutics Products Directorate, Health Canada, Ottawa, Ontario, ⁴Departments of Medicine and Microbiology, University of Manitoba, and ⁵St. Boniface General Hospital, Winnipeg, Manitoba, Canada

Aims To evaluate the effect of acute dosing of garlic supplements on the single-dose pharmacokinetics of ritonavir.

Methods Ten healthy volunteers (five male, five female) were equally randomized in a crossover design to receive 400 mg of a single dose of ritonavir within 10 min after eating breakfast either alone or with 10 mg of Natural Source Odourless Garlic. They received a total of eight doses of garlic extract (2 × 5 mg capsules) taken twice daily for 4 days. Ritonavir and the seventh garlic dose were administered simultaneously.

Results Coadministration of garlic nonsignificantly decreased area under the plasma concentration-time curve (AUC(0,∞)) by -17% (90% confidence interval (CI), -31% to 0%; range -46% to 68%) and peak plasma concentration of ritonavir by -1% (90% CI, -25% to 31%; range -51% to 136%).

Conclusions Acute dosing of the garlic capsules over 4 days did not significantly alter the single-dose pharmacokinetics of ritonavir in healthy volunteers. Given the complex effects of both ritonavir and garlic on drug metabolism, the results of our study should not be extrapolated to steady-state conditions, where the possibility of an interaction still needs to be evaluated.

Keywords: garlic supplements, pharmacokinetic interaction, ritonavir

Introduction

In patients infected with HIV, garlic has been used as a dietary supplement to improve health and as a herbal complementary medicine to treat some opportunistic infections. Garlic cloves, commercially available garlic products, and several organic sulphur compounds derived from garlic have been shown to alter the activity of cytochrome P450 (CYP) enzymes *in vitro* and *in vivo* [1–3]. The effects are dependent on time and dose. In an *in vitro* study, raw garlic and garlic products inhibited the activities of P-glycoprotein and human CYP3A4 [1]. In the rat, a single dose of garlic oil significantly decreased hepatic CYP activity, but daily administration for 5 days significantly increased hepatic CYP activity [2]. In a study in healthy volunteers, a 3-week administration of a garlic supplement decreased plasma concentrations of the protease inhibitor saquinavir by about 50% [4].

We identified two HIV-infected persons taking garlic or garlic supplements for >2 weeks who developed severe gastrointestinal (GI) toxicity after beginning ritonavir-containing antiretroviral therapy (400 or 600 mg twice daily) [5]. The symptoms resolved after discontinuing garlic or ritonavir. These cases suggest a possible interaction between ritonavir and garlic, as high ritonavir concentrations or ingestion of large amounts of garlic can cause GI symptoms. Ritonavir is a protease inhibitor that is mainly metabolized by CYP3A4 and has a high binding affinity to P-glycoprotein [6]. It simultaneously inhibits and induces CYP3A4 and predominantly induces other drug-metabolizing enzymes [6]. Therefore, the observed GI toxicity could have resulted from either ritonavir inhibiting or inducing the metabolism of garlic constituents leading to toxic concentrations of compounds derived from garlic, or the latter inhibiting the CYP-mediated metabolism or P-glycoprotein-mediated transport of ritonavir leading to elevated levels of the drug. To determine if ritonavir concentrations were increased in the presence of garlic we evaluated the effect of acute dosing of garlic supplements on the single-dose pharmacokinetics of the drug.

Correspondence: Keith Gallicano PhD, Axelson Biopharma Research, Inc., 201-4606 Canada Way, Burnaby, BC, Canada V5G 1K5. Tel.: +1 604 222 4184; Fax: +1 604 222 3141; E-mail: kgallicano@axelson.net

Received 18 June 2002, accepted 6 September 2002.

Methods

Participants

Ten nonsmoking Caucasian volunteers (five men and five women) who were HIV seronegative provided informed consent and participated in the study. Their mean $\pm$ s.d. weight was 75.2 ± 15.0 kg (range 49.9–104 kg). The University of Manitoba Faculty Committee on the Use of Human Subjects in Research approved the protocol for this study.

Study design

This was an open, nonfasting, two-treatment, two-period, two-sequence crossover study. Participants were equally randomized to receive 400 mg of a single dose of ritonavir (Norvir, Abbott Laboratories; four 100-mg capsules) either alone in one group or with 10 mg of garlic extract (two 5-mg liquid-filled soft gelatin capsules of Natural Source Odourless Garlic Life Brand, equivalent to 1 g of fresh garlic) in another group. Ritonavir was administered with 150 ml of water within 10 min after the subject finished a standard full breakfast. Subjects received a total of eight doses of garlic extract taken twice daily for 4 days. Ritonavir and the seventh garlic dose were administered simultaneously under observation. Study medications were stopped for 7 days between treatment periods. The formulation was chosen because it was used by one of the two patients with symptoms of GI toxicity during coadministration of garlic and ritonavir [5].

Blood sampling procedure

Fourteen heparinized blood samples (5 ml each) were collected for ritonavir measurements before drug administration and at 0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24 and 30 h after the dose. Blood was equilibrated at 4°C for 10 min and then centrifuged (1500 g at 4°C for 10 min) to separate plasma.

Drug analysis

Concentrations of ritonavir in plasma were measured by high-performance liquid chromatography with u.v. detection [7]. The 90% confidence limits around the mean assay biases of duplicate quality-control samples at low and high concentrations pooled over ten batches ranged from 4.6% to 14.1% and from 4.6% to 11.6%, respectively.

The garlic product contained garlic powder suspended in liquid and encapsulated inside a soft gelatin capsule. Because the product contained no details of its allicin content, the capsules were assayed by a contract laboratory (University of Guelph Laboratory Services, Guelph,

Ontario, Canada) using the method of Lawson *et al.* [8]. The allicin content was below the lower limit of quantification of the assay ($50 \mu\text{g g}^{-1}$ extract).

Pharmacostatistical analysis

The following ritonavir plasma pharmacokinetic parameters were analysed using noncompartmental methods: highest observed concentration (C_{max}), time to reach C_{max} (t_{max}), apparent terminal disposition half-life ($t_{1/2,z}$), and area under the concentration-time curve from time of dosing to infinity $\text{AUC}(0,\infty)$.

Differences in mean logarithmically transformed pharmacokinetic parameters between treatments were analysed by analysis of variance (ANOVA) appropriate for a crossover study. The ANOVA model included the effects of sequence, sex, subject nested within sequence and sex, period, treatment, and sex-by-treatment interaction. All effects except sequence and sex were tested with the mean-square residual (MSR); effects of sequence and sex were tested with the subject mean-square (MS) term.

Results

All participants completed the study without incidence of adverse events. Pharmacokinetic data for ritonavir are summarized in Table 1 and individual $\text{AUC}(0,\infty)$ values are shown in Figure 1.

After 4 days of garlic supplementation there was a nonsignificant decrease in $\text{AUC}(0,\infty)$ and C_{max} values by -17% (range -46% to 68%) and -1% (range -51% to 136%), respectively. Half-life remained unchanged at 5.2 h.

The ANOVA indicated no significant sex-related differences in ritonavir pharmacokinetics or differential effects of garlic on change in ritonavir pharmacokinetics in males compared with females, although women tended to have higher peak concentrations over the two periods (5886 vs. 4376 ng mL^{-1} , $P = 0.06$) and larger decreases in $\text{AUC}(0,\infty)$ values with garlic ($P = 0.07$).

Discussion

Acute dosing of odourless garlic extract over 4 days did not significantly alter the single-dose pharmacokinetics of ritonavir in healthy volunteers, although there was a trend for a decrease in ritonavir concentrations. The pharmacokinetic results are specific to this odourless garlic supplement and cannot be extrapolated to other garlic products. The garlic formulation used in our study had been shown to inhibit human CYP3A4-mediated metabolism and P-glycoprotein-mediated transport *in vitro* [1]. Furthermore, garlic induces CYP metabolism in the rat [2, 3]. Therefore, the apparent lack of detectable phar-

Table 1 Mean single-dose plasma pharmacokinetic parameters for ritonavir administered alone and after coadministration with 4 days of garlic supplement.

Parameter	Treatment*		ANOVA for crossover design†			
	Ritonavir and garlic	Ritonavir alone	Point estimate (90% CI, %)	P-value	Pooled intra-subject CV (%)	Pooled inter-subject CV (%)
C_{max} (ng mL ⁻¹)	5045 (5396 ± 1728)	5104 (5444 ± 1740)	98.8 (74.8–131)	0.939	33.5	ne
t_{max} (h)	2.9 (0.9, 5.9)	2.7 (0.9, 7.9)	0 (–1.8–1.5)	>0.05	60.8	50.6
$t_{1/2,z}$ (h)‡	5.0 (5.2 ± 2.1)	5.1 (5.2 ± 1.8)	98.7 (90.9–107)	0.759	9.5	36.4
AUC(0–∞) (µg mL ⁻¹ h)	44.5 (47.6 ± 15.6)	53.7 (58.0 ± 21.6)	82.8 (68.7–99.8)	0.094	22.1	31.5

CI, Confidence interval; CV, coefficient of variation; ne, not estimable. *Values of treatment means are expressed as geometric mean with arithmetic mean ± SD given in parentheses ($n=10$); t_{max} values are expressed as binomial medians with minimum and maximum given in parentheses. †Percentage ratio of the least-squares geometric treatment mean of the coadministered treatment relative to that of the ritonavir-alone treatment. The value for t_{max} is the point estimate of the absolute difference of expected medians, relative to the ritonavir-alone treatment as analysed by the nonparametric Wilcoxon rank-sum test. The level of significance was set at 0.05. For logarithmically transformed data, intrasubject CV was calculated as $100\% \times (e^{MSR} - 1)^{1/2}$ and intersubject CV was estimated as $100\% \times (e^{(MS - MSR)/2} - 1)^{1/2}$. ‡Harmonic means were 4.8 h (without garlic) and 4.7 h (with garlic).

Figure 1 Individual AUC(0,∞) values for ritonavir after treatment with a 400-mg single dose of drug alone (A) and with garlic after the seventh dose of garlic (B). Vertical bars represent mean AUC values for ten participants.

macokinetic interaction could have resulted from a transitory effect of induction and inhibition on the various drug disposition pathways of ritonavir.

A longer duration of garlic therapy may be required to observe a significant decrease in ritonavir plasma concentrations, as suggested by the effect of 3 weeks of garlic on saquinavir pharmacokinetics [4]. Although our results are not predictive of steady-state ritonavir conditions, the

effect of garlic may be less after multiple doses than after a single dose of ritonavir, because ritonavir undergoes autoinduction during the first 2 weeks of therapy [6], potentially minimizing the effects of further induction.

The lack of measurable allicin content in the formulation was not unusual, as undetectable amounts of allicin and allicin degradation products are typical of products containing garlic powder suspended in a gelatin capsule [8]. Garlic contains numerous chemicals in addition to allicin and allicin-related compounds that could contribute to drug interactions. However, their role remains to be investigated in clinical studies.

Our pharmacokinetic results suggest that the GI toxicity observed in the two individuals [5] was not likely to be related to increased systemic concentrations of ritonavir. This is supported by the observation that GI symptoms recurred during rechallenge with low-dose ritonavir (100 mg twice daily) in the presence of garlic [5].

In conclusion, previous *in vitro* and animal studies have shown that garlic can act as an enzyme inhibitor during acute dosing, and an enzyme inducer during chronic dosing. However, in the present study performed under acute dosing nonsteady-state conditions, there was no significant effect of the tested garlic product on the single-dose pharmacokinetics of ritonavir in healthy volunteers. Given the complex effects of both ritonavir and garlic on enzyme inhibition and enzyme induction, the results of our study should not be extrapolated to steady-state conditions, where the possibility of an interaction still needs to be evaluated.

We thank Tracy Leclaire for conducting the clinical trial and Linda Oliveras for performing the ritonavir measurements. This work was supported by a grant from the Canadian Foundation for AIDS Research.

References

- 1 Foster BC, Foster MS, Vandenhoeck S *et al.* An *in vitro* evaluation of human cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. *J Pharm Pharmaceut Sci* 2001; **4**: 176–184.
- 2 Dalvi RR. Alterations in hepatic phase I and phase II biotransformation enzymes by garlic oil in rats. *Toxicol Lett* 1992; **60**: 299–305.
- 3 Haber D, Siess MH, de Waziers I, Beaune P, Suschetet M. Modification of hepatic drug-metabolizing enzymes in rat fed naturally occurring allyl sulphides. *Xenobiotica* 1994; **24**: 169–182.
- 4 Piscitelli SC, Burstein AH, Welden N, Gallicano KD, Falloon J. The effect of garlic supplements on the pharmacokinetics of saquinavir. *Clin Infect Dis* 2002; **34**: 234–238.
- 5 Laroche M, Choudhri S, Gallicano K, Foster B. Severe gastrointestinal toxicity with concomitant ingestion of ritonavir and garlic. *Can J Infect Dis* 1998; **9** (Suppl A): 471P. (Abstr.).
- 6 Hsu A, Granneman GR, Bertz RJ. Ritonavir: clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet* 1998; **35**: 275–291.
- 7 Khaliq Y, Gallicano K, Venance S, Kravcik S, Cameron DW. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. *Clin Pharmacol Ther* 2000; **68**: 637–646.
- 8 Lawson LD, Wang Z-YJ, Hughes BG. Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfates in commercial garlic products. *Planta Med* 1991; **57**: 363–370.