

Koh-ichi Sugimoto · Nobutaka Araki ·  
Masami Ohmori · Ken-ichi Harada · Yimin Cui ·  
Shuichi Tsuruoka · Atsuhiko Kawaguchi ·  
Akio Fujimura

## Interaction between grapefruit juice and hypnotic drugs: comparison of triazolam and quazepam

Received: 17 October 2005 / Accepted: 14 November 2005 / Published online: 17 January 2006  
© Springer-Verlag 2006

**Abstract** *Objective:* Grapefruit juice (GFJ) inhibits cytochrome P450 (CYP) 3A4 in the gut wall and increases blood concentrations of CYP3A4 substrates by the enhancement of oral bioavailability. The effects of GFJ on two benzodiazepine hypnotics, triazolam (metabolized by CYP3A4) and quazepam (metabolized by CYP3A4 and CYP2C9), were determined in this study. *Methods:* Nine healthy subjects were administered 0.25 mg triazolam or 15 mg quazepam, with or without GFJ. Each trial was performed using an open, randomized, cross-over design with an interval of more than 2 weeks between trials. Blood samples were obtained during the 24-h period immediately following the administration of each dose. Pharmacodynamic effects were determined by the digit symbol substitution test (DSST) and utilizing a visual analog scale. *Results:* GFJ increased the plasma concentrations of both triazolam and quazepam and of the active metabolite of quazepam, 2-oxoquazepam. The area under the curve (AUC)(0–24) of triazolam significantly increased by 96% ( $p < 0.05$ ). The AUC(0–24) of quazepam (+38%) and 2-oxoquazepam (+28%) also increased; however, these increases were not significantly different from those of triazolam. GFJ deteriorated the performance of the subjects in the DSST after the triazolam dose (–11 digits at 2 h after the dose,  $p < 0.05$ ), but not after the quazepam dose. Triazolam and quazepam produced similar sedative-like effects, none of which were enhanced by GFJ. *Conclusion:* These results suggest that the effects of

GFJ on the pharmacodynamics of triazolam are greater than those on quazepam. These GFJ-related different effects are partly explained by the fact that triazolam is presystemically metabolized by CYP3A4, while quazepam is presystemically metabolized by CYP3A4 and CYP2C9.

**Keywords** Grapefruit juice · CYP3A4 · CYP2C9 · Triazolam · Quazepam

### Introduction

The ingestion of grapefruit juice (GFJ) increases the oral bioavailability of a number of drugs, including felodipine, terfenadine, cyclosporine and simvastatin [1–4]. The mechanism by which GFJ substantially reduces the oral clearance of these cytochrome P450 (CYP) 3A4 substrate drugs is by increasing their oral bioavailability through a post-transcriptional mechanism that decreases CYP3A4 protein content in the small intestine [5]; hepatic CYP3A4 activity is not blunted by GFJ [5]. Furanocoumarin-induced inhibition of CYP3A4 is thought to be involved in the drug-food interaction [6, 7].

Triazolam and quazepam are benzodiazepine derivatives which are frequently prescribed for the treatment of sleep disorders. In an in vitro study, von Moltke et al. demonstrated that triazolam is metabolized by CYP3A4 to  $\alpha$ -hydroxytriazolam and 4-hydroxytriazolam [8]. As expected, GFJ has been found to increase plasma triazolam concentration and enhance its pharmacodynamic effects in human subjects [9, 10]. However, because the metabolism of quazepam to 2-oxoquazepam is mediated by CYP3A4 and CYP2C9 [11], it is anticipated that the effects of GFJ on the pharmacokinetics and pharmacodynamics of triazolam may be greater than those on quazepam. This study was undertaken to examine this hypothesis. The effects of GFJ on the pharmacokinetics and pharmacodynamics of triazolam and quazepam were determined by an open, randomized, cross-over design in healthy subjects.

K.-i. Sugimoto · N. Araki · M. Ohmori · K.-i. Harada · Y. Cui ·  
S. Tsuruoka · A. Kawaguchi · A. Fujimura (✉)  
Division of Clinical Pharmacology,  
Department of Pharmacology,  
Jichi Medical School,  
3311-1 Minamikawachi,  
Tochigi, 329-0498, Japan  
e-mail: akiofuji@jichi.ac.jp  
Tel.: +81-285-587387  
Fax: +81-285-44-7562

## Methods

### Study design

The study was performed in an open, randomized, cross-over design with four phases. The interval between each phase (study day) was more than 2 weeks. The protocol was approved by the Ethics Committee of Jichi Medical School (Tochigi, Japan).

Nine healthy Japanese men (age: 23–44 years old; weight: 56–80 kg) participated in this study after giving written informed consent. Genetic analysis [12] showed that all subjects were extensive metabolizers of CYP2C9 substrates. Four of the participants were smokers. The subjects were instructed not to take any medications, herbal dietary supplements and herbal tea throughout the study period. The consumption of GFJ was not allowed during the entire study period, except for when a trial was being conducted, while the consumption of caffeine-containing beverages, including coffee and green tea, and alcohol and smoking were prohibited from one night before the trial until a final blood sampling on the next morning. The subjects were given 250 ml of normal-strength GFJ (Tropicana, Kirin Beverage, Tokyo, Japan) three times daily for 3 days immediately prior to the trial according to a randomized schedule. The recommended dose for the treatment of sleep disorders in Japan is 0.25–0.5 mg triazolam and 15–30 mg quazepam. However, because 0.25 mg triazolam and 15 mg quazepam are most frequently used as initial doses, we chose these doses for the trials. On the day of the trial, subjects took a single oral dose of triazolam (0.25 mg; Halcion, Pharmacia & Upjohn, Tokyo, Japan) or quazepam (15 mg; Doral; Mitubishi Pharma, Tokyo, Japan) together with 250 ml of GFJ or water at 8:00 A.M. after an overnight fast. In the trials with GFJ, the subjects also took 250 ml of GFJ 4 and 12 h after the drug had been taken. The subjects had a light meal 4 h after taking either the triazolam or quazepam.

### Blood sampling

Blood samples (5 ml in each) for the analyses of triazolam and quazepam were collected in heparinized tubes immediately before and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the participants had taken the doses of triazolam or quazepam. Plasma samples were stored at  $-80^{\circ}\text{C}$  until the assay was carried out.

### Pharmacodynamic measurements

The pharmacodynamic effects of triazolam and quazepam were determined using the digit symbol substitution test (DSST) [13] and a visual analog scale (VAS) immediately before each blood sampling and from 0–12 h following the administration of the drug. In the DSST, the participants in the study substituted simple digit symbols using a pencil and paper. The number of digits correctly substituted in

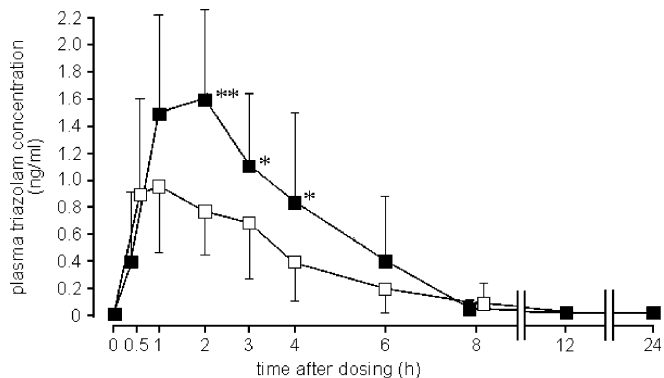
2 min was recorded. Subjective assessments were made on 100-mm long horizontal ungraded visual analogue scales. Sedative-like effects observed were the pairs of adjectives (in Japanese) such as drowsy/alert, calm/nervous, clumsy/skilled, mentally slow/quick-witted and discontented/contented. The subjects had been trained to perform the tests before the initiation of the study: prior to the trials they performed DSST several times after being given oral instructions; thereafter, they completed 12 h of baseline study (DSST and VAS) without medication on a different day.

### Assay of plasma triazolam

Plasma concentration of triazolam was measured by a high-performance liquid chromatography (HPLC) method developed in our laboratory. A 2-ml plasma sample was added to 4 ml of methanol, and the mixture was then shaken vigorously for 10 min, followed by centrifugation at 1,500 g for 10 min. The procedure was repeated three times, and the supernatant was evaporated to dryness under nitrogen stream. The residue was reconstituted with 100  $\mu\text{l}$  ethanol, and the whole amount was subjected by analysis using a two-step drug extraction process by HPLC.

The HPLC system consisting of a chromatography pump (PU-880; Jasco, Tokyo, Japan), an ultraviolet detector (Uvidec 875; Jasco) and a column (Fine Pak SilC18T5, 5  $\mu\text{m}$ , 250 $\times$ 4.6 mm; Jasco) was used to separate triazolam. The column temperature was set at  $40^{\circ}\text{C}$  with a column oven module. In the first process, the mobile phase consisted of methanol/ $\text{H}_2\text{O}$  (70:30, v/v). The fraction containing triazolam was separated from the interfering substances existing in the residue by collecting the effluent between 6.0 and 7.5 min after the injection. The collected effluent was evaporated to dryness and reconstituted with 100  $\mu\text{l}$  of a mobile phase consisting of methanol/ $\text{H}_2\text{O}$  (54.5:45.5, v/v), which was then injected into the identical HPLC system with the mobile phase. To reduce sample loss, the tube containing the residue obtained in the first process was again washed with the mobile phase, and this reconstituent (100  $\mu\text{l}$ ) was also applied to the second separation process. Triazolam was separated out by collecting effluent between 12.0 and 13.5 min. The mobile phase was pumped at a flow rate of 1.0 ml/min for the separation of triazolam. The absorbance of the effluent was monitored at 220 nm.

The effluent containing triazolam was evaporated to dryness and reconstituted with 50  $\mu\text{l}$  of internal standard (1  $\mu\text{g}/\text{ml}$  butyl *p*-hydroxybenzoate in methanol), and 20- $\mu\text{l}$  aliquots were applied to the HPLC system. The HPLC system was identical to that described above except for the mobile phase [methanol/ $\text{H}_2\text{O}$  (52.4:47.6, v/v)]. The recovery of 2.5 ng/ml triazolam was 88.7% ( $n=6$ ). The method was validated for the concentration range from 0.5 to 2.5 ng/ml. The intra-assay coefficient and accuracy were less than 8.6% and 85.1–108.4% ( $n=6$ ), respectively. The inter-assay coefficient and accuracy were less than 18.8% and 73.4–137.4% ( $n=6$ ), respectively. The lower detection limit was 0.15 ng/ml.

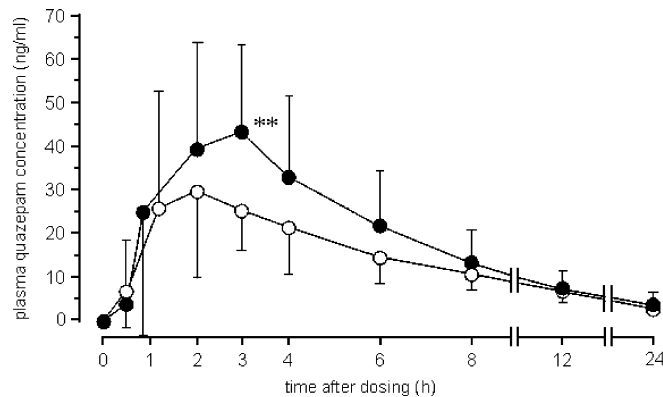


**Fig. 1** Plasma concentrations of triazolam after a single oral dose of 0.25 mg triazolam with grapefruit juice (black squares) or water (open squares). Points represent the mean ( $n=9$ )  $\pm$  SD. \* $p<0.05$ , \*\* $p<0.01$  versus the control (water) values

#### Assay of plasma quazepam and its metabolites, 2-oxoquazepam and *N*-desmethyl-2-oxoquazepam

Plasma concentrations of quazepam and its metabolites were measured by a column-switching HPLC analysis. An aliquot of each plasma sample (1.5 ml), to which 0.1 ml of cisapride (800 ng/ml) was added as an internal standard, was first alkalized by adding 500  $\mu$ l 0.5 M NaOH followed by the addition of 0.4 ml H<sub>2</sub>O and 5 ml of toluene/chloroform (85:15, v/v). The mixture was shaken vigorously for 15 min and then centrifuged at 2,000 g for 10 min. A 4.5-ml portion of the organic layer was evaporated to dryness in vacuo at 45°C. The residue was reconstituted with 0.8 ml of eluent A (see below) and used as an extract.

A 0.5-ml aliquot of the extract was injected onto the column-switching HPLC system which consisted of a chromatography pump (LC-10A; Shimadzu, Tokyo, Japan), an autoinjector (AS-8020; Tosoh, Tokyo, Japan) and an ultraviolet detector (SPD-10A; Shimadzu). Column I (TSK-BSA-C8, 5  $\mu$ m, 10 $\times$ 4.6 mm; Tosoh) was used for pretreatment and column II (STR-ODS II, 5  $\mu$ m, 150 $\times$ 4.6 mm; Shimadzu) for the separation of quazepam and 2-oxoquazepam. The column temperature was set at 30°C with a column oven module. Between 0 and 13.0 min after the injection of a sample, cisapride was separated from the interfering substances present in the extract on column I by means of a mobile phase solvent (eluent A) consisting of acetonitrile/0.02 mol/l KH<sub>2</sub>PO<sub>4</sub> (13:87, v/v).



**Fig. 2** Plasma concentrations of quazepam after a single oral dose of 15 mg quazepam with grapefruit juice (black circles) or water (open circles). Points represent the mean ( $n=9$ )  $\pm$  SD. \*\* $p<0.01$  versus the control (water) values

Between 13.0 and 20.0 min after the injection, quazepam and its metabolites, which had been retained on column I were eluted with a mobile phase (eluent B) consisting of acetonitrile/perchloric acid/0.02 mol/l KH<sub>2</sub>PO<sub>4</sub> (41.00:0.05: 58.95, v/v/v), and the effluent from column I was switched to column II. Then *N*-desmethyl-2-oxoquazepam was separated on column II by eluting with eluent B (between 20.0 and 32.0 min). Quazepam and 2-oxoquazepam were separated on column II by eluting with a mobile phase solvent (eluent C) consisting of acetonitrile/0.02 mol/l KH<sub>2</sub>PO<sub>4</sub> (62.5:37.5, v/v) between 32.0 and 46.5 min. The mobile phase was pumped at a flow rate of 0.6 ml/min. The absorbance of the effluent from column II was monitored at 254 nm for the metabolites and 286 nm for quazepam.

The lower detection limits were 0.8 ng/ml, with a linear calibration range from 1 to 100 ng/ml of quazepam and its metabolites. The recoveries of quazepam and its metabolites was more than 93.2% at 10 ng/ml of the concentrations ( $n=6$ ). The inter- and intra-assay coefficient of variations were less than 4.6% at 10 ng/ml of these compounds.

#### Pharmacokinetic calculations

Pharmacokinetics were characterized by maximum plasma concentration  $C_{max}$ , time to maximum plasma concentra-

**Table 1** Pharmacokinetic parameters<sup>a</sup> of triazolam, quazepam and 2-oxoquazepam after a single oral dose of the drug (0.25 mg triazolam or 15 mg quazepam) with grapefruit juice (GFJ) or water in nine healthy Japanese male volunteers

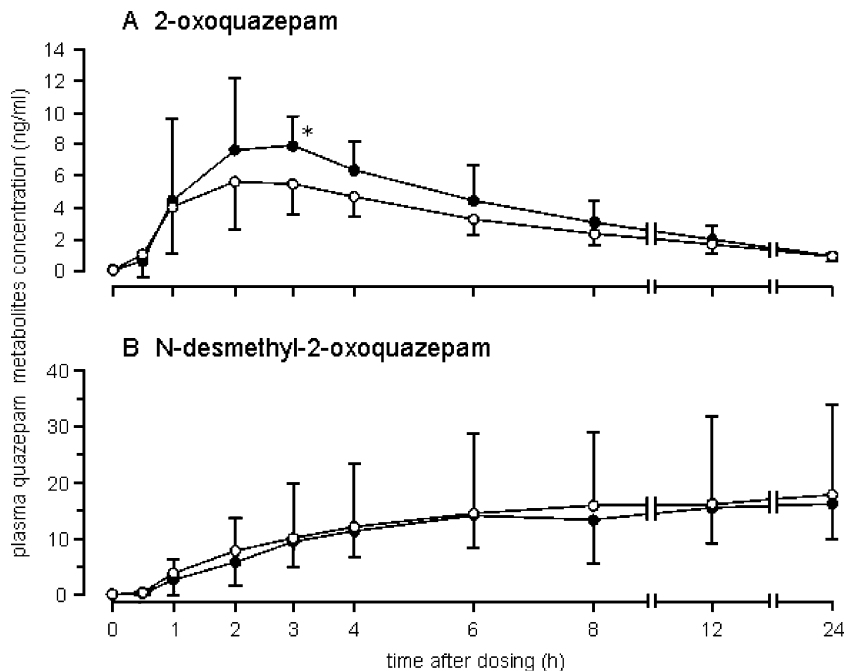
	$C_{max}$ (ng/ml)			$t_{max}$ (h)		AUC(0–24) (ng h/ml)		
	Water	GFJ	Ratio	Water	GFJ	Water	GFJ	Ratio
Triazolam	1.3 $\pm$ 0.4 <sup>b</sup>	1.9 $\pm$ 0.6*	1.55 $\pm$ 0.46 (1.27; 1.84)	1.4 $\pm$ 1.0	1.6 $\pm$ 0.5	6.3 $\pm$ 2.7	10.9 $\pm$ 4.0*	1.96 $\pm$ 1.02 (1.34; 2.59)
Quazepam	36.9 $\pm$ 21.7	52.0 $\pm$ 20.8*	1.62 $\pm$ 0.59 (1.25; 2.00)	2.2 $\pm$ 1.0	2.6 $\pm$ 0.7	247.5 $\pm$ 110.2	324.2 $\pm$ 150.1	1.38 $\pm$ 0.43 (1.12; 1.64)
2-Oxoquazepam	6.1 $\pm$ 1.4	9.7 $\pm$ 3.5*	1.57 $\pm$ 0.36 (1.35; 1.79)	2.4 $\pm$ 1.0	2.4 $\pm$ 0.9	53.5 $\pm$ 14.8	67.6 $\pm$ 24.5	1.28 $\pm$ 0.34 (1.07; 1.50)

\* $p<0.05$  versus water

<sup>a</sup> $C_{max}$ , Maximum plasma concentration;  $t_{max}$ , time to maximum concentration; AUC(0–24), area under the plasma concentration time curve from 0 to 24 h after administration of the dose

<sup>b</sup>Values are the mean  $\pm$  SD (90% confidence interval)

**Fig. 3** Plasma concentrations of quazepam metabolites, 2-oxoquazepam (a) and *N*-desmethyl-2-oxoquazepam (b), after a single oral dose of 15 mg quazepam with grapefruit juice (black circles) or water (open circles). Points represent the mean ( $n=9$ )  $\pm$  SD. \* $p<0.05$  versus the control (water) values



tion ( $t_{max}$ ), elimination half-life ( $t_{1/2}$ ) and area under the plasma concentration-time curve from 0–24 h post-administration of the drug [AUC(0–24)]. The elimination rate constant ( $K_e$ ) was determined using last three points of a linear regression analysis of a log-linear phase of the plasma drug concentration-time curve. Elimination half-life ( $t_{1/2}$ ) was calculated as follows:

$$t_{1/2} = \ln 2 / K_e$$

The AUC(0–24) was calculated by the trapezoidal rule.

### Statistical analysis

Data are expressed as the mean  $\pm$  standard deviation (SD). Data were analyzed by paired Student's *t*-test or analysis of variance (ANOVA) using the statistical program *Statview* for Windows, version 5.0 (SAS Institute, Cary, N.C.). A correlation between plasma drug concentrations and the decrease in the number of digit substitutions was analyzed by the Pearson's correlation coefficient. Differences were regarded as statistically significant when the *p* value was less than 0.05.

## Results

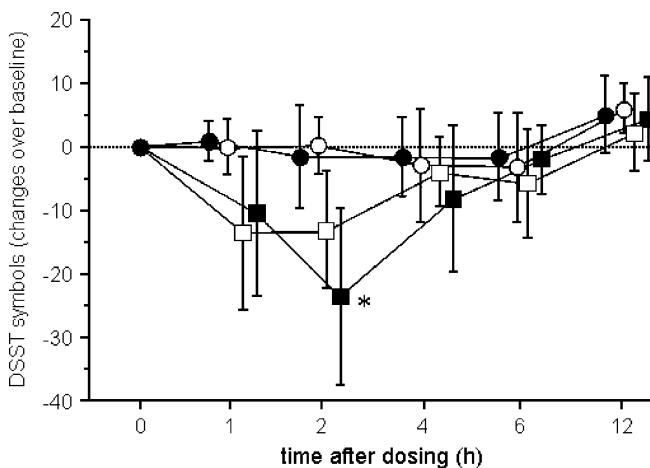
### Plasma concentration of triazolam

The plasma concentration of triazolam increased after the 0.25 dose had been given with GFJ relative to the same dose given with water (Fig. 1). The  $C_{max}$  and AUC(0–24) of the agent were significantly greater with GFJ than with water (Table 1). No significant difference was observed

in the  $t_{1/2}$  (in hours) between the two groups (water: 3.6, GFJ: 3.4).

### Plasma concentrations of quazepam and 2-oxoquazepam

Plasma concentrations of quazepam and 2-oxoquazepam increased after a dose of quazepam was given with GFJ (Figs. 2 and 3a). The values of  $C_{max}$  of these agents in the trial with GFJ were significantly greater than those in the trial with water (Table 1). Their AUC(0–24) were also greater in the trial with GFJ (Table 1), but they did not



**Fig. 4** Digit symbol substitution test (DSST, expressed as changes over the predose baseline) after a single oral dose of 0.25 mg triazolam or 15 mg quazepam with grapefruit juice or water. Open circle quazepam with water, black circle quazepam with grapefruit juice, open square triazolam with water, closed square triazolam with grapefruit juice. Points represent the mean ( $n=9$ )  $\pm$  SD. \* $p<0.05$  versus the control (water) values

**Table 2** Pharmacodynamic parameters<sup>a</sup> of quazepam following the administration of a single oral administration with grape fruit juice (GFJ) or water

	DSST (digits/h)		Drowsiness (VAS mm/h)		Mental slowness (VAS mm/h)	
	AUC(0–6)	AUC(0–12)	AUC(0–6)	AUC(0–12)	AUC(0–6)	AUC(0–12)
Triazolam + water	-49 (-68, -26)	-57 (-100, -15)	160 (42, 277)	200 (-13, 412)	126 (59, 192)	212 (64, 360)
Triazolam + GFJ	-64 (-98, -31)	-58 (-102, -14)	147 (33, 261)	136 (-88, 360)	126 (55, 198)	146 (-12, 305)
Quazepam + water	-9 (-30, 12) <sub>a,b</sub>	0 (-40, 39)	187 (81, 292)	343 (116, 569)	100 (25, 175)	191 (19, 363)
Quazepam + GFJ	-6 (-22, 11) <sub>a,b</sub>	5 (-28, 37) <sub>a,b</sub>	162 (114, 210)	283 (196, 370)	88 (33, 143)	188 (85, 292)

<sup>a</sup>DSST, Digit substitution symbol test; VAS, visual analog scale; AUC(0-*n*), area under the symbol number or analog scale-time curve between time zero and *n* hours after administration

<sup>b</sup>Data are mean values (90% confidence interval); values followed by 'a',  $p < 0.05$  versus triazolam + water; values followed by 'b',  $p < 0.05$  versus triazolam + GFJ

reach statistical significance. The concentration of *N*-desmethyl-2-oxoquazepam in the plasma rose up to 24 h following the administration of quazepam; consequently, the  $C_{\max}$  of this metabolite could not be determined in this study. However, GFJ did not affect the plasma concentrations of this metabolite during the period we examined (Fig. 3b). No significant difference in the GFJ-related increase in AUC(0–24) was observed between triazolam and quazepam (difference: 58%). There was also no significant difference in the increase in  $C_{\max}$  between triazolam (+55%) and quazepam (+62%). The increase in the AUC(0–24) or  $C_{\max}$  of 2-oxoquazepam did not significantly differ from that of triazolam. The ratio of 2-oxoquazepam to quazepam in the  $C_{\max}$  or AUC(0–24) did not differ between two trials  $C_{\max}$ : water, 0.21;  $C_{\max}$ : GFJ, 0.19; AUC(0–24): water, 0.24; AUC(0–24): GFJ, 0.22). The  $t_{1/2}$  (in hours) of these agents did not significantly differ between the water and GFJ trials (quazepam: water, 5.9; quazepam: GFJ, 4.5; 2-oxoquazepam: water, 6.3; 2-oxoquazepam: GFJ, 5.8).

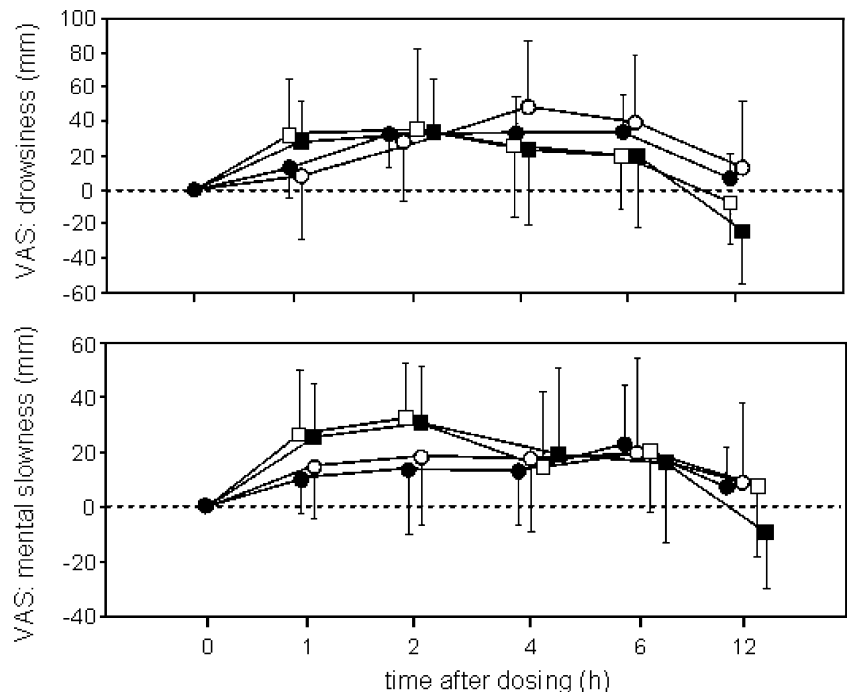
## Pharmacodynamics

Triazolam remarkably reduced the number of digit substitutions (Fig. 4, Table 2). On the other hand, the effect of quazepam on the objective performance in the DSST was small. Triazolam with GFJ caused an additional decrease in digit substitution, especially at 2 h following administration of the dose (Fig. 4). However, GFJ did not deteriorate the performance in the DSST after the quazepam dose.

Triazolam caused significant ( $p < 0.0001$ ) drowsiness and mental slowness, with a peak at 2 h after administration of the dose (Fig. 5). Time courses of other subjective effects were similar (data not shown). Quazepam also produced similar sedative-like drug effects but its peak was slightly later – at 4–6 h after administration of the dose (Fig. 5). GFJ did not significantly enhance these triazolam- or quazepam-induced subjective effects (Table 2).

A significant negative correlation was noted between triazolam concentrations in the plasma and the decrease in

**Fig. 5** Sedative-like self-rated drug effects [based on scores from the 100-mm visual analog scale (VAS), expressed as changes over the predose baseline] after a single oral dose of 0.25 mg triazolam or 15 mg quazepam with grapefruit juice or water. *Open circle* Quazepam with water, *black circle* quazepam with grapefruit juice, *open square* triazolam with water, *black square* triazolam with grapefruit juice. Points represent the mean ( $n=9$ )  $\pm$  SD



the number of digit substitutions at 2 h after the dose had been given ( $R=-0.539$ ,  $p<0.05$ ,  $n=18$ ). There were no significant correlations between the plasma concentrations of quazepam or 2-oxoquazepam and any pharmacodynamic parameters.

## Discussion

In this study, the  $C_{\max}$  of triazolam, quazepam and 2-oxoquazepam significantly increased during the trials with GFJ. The oral bioavailability of triazolam was about 60% due to presystemic elimination. Triazolam is mainly metabolized by CYP3A4 [8], and the elimination  $t_{1/2}$  is 2–4 h. The bioavailability of quazepam is assumed to be 29–35% based on a calculation of the ratio of the dose-normalized cumulative amount excreted in Japanese subjects (personal communication, Mitsubishi Pharma, Tokyo, Japan). Quazepam is metabolized by CYP3A4 and CYP2C9 [11], and it has been reported that in plasma the elimination  $t_{1/2}$  of quazepam and an active metabolite, 2-oxoquazepam, are 25–40 h and that of *N*-desmethyl-2-oxoquazepam is 70–75 h [14, 15]. The calculated elimination  $t_{1/2}$  of quazepam and 2-oxoquazepam in the present study were shorter than those reported in the previous studies because the length of sampling period was not enough to estimate terminal half-lives of these compounds. In the present study, the effect of GFJ on the pharmacokinetics of quazepam and its metabolites were examined for 24 hours after administration of the dose; however, a longer sampling period post-dose administration and an analysis system with a high sensitivity are required to correctly estimate the half-lives of quazepam, 2-oxoquazepam and in particular *N*-desmethyl-2-oxoquazepam. In fact, we were unable to determine even the  $C_{\max}$  for *N*-desmethyl-2-oxoquazepam.

The metabolism of quazepam and 2-oxoquazepam is mediated by CYP3A4 and CYP2C9, and the contribution of CYPs to the metabolic process is presumed to be 50:50 based on an inhibition study using the cDNA-expressed human CYPs and antiserum for CYP3A4 and CYP2C [11]. These findings led us to speculate that the inhibition of CYP3A4 activity by GFJ has a greater influence on the pharmacokinetics of triazolam than on those of quazepam. The AUC(0–24) of triazolam was significantly increased by GFJ, while the elevation in this parameter for quazepam or 2-oxoquazepam by GFJ did not reach statistical significance. However, the GFJ-related changes of AUC(0–24) or  $C_{\max}$  for triazolam did not significantly differ from those for quazepam or 2-oxoquazepam. It therefore appears that the inhibition of intestinal CYP3A4 activities by GFJ enhances the oral bioavailability of both quazepam and triazolam. The ratio of 2-oxoquazepam to quazepam in the  $C_{\max}$  or AUC(0–24) did not differ significantly between the trials with and without GFJ. GFJ elevated the plasma concentrations of quazepam and 2-oxoquazepam, but not of *N*-desmethyl-2-oxoquazepam. The quazepam metabolite 2-oxoquazepam is further metabolized to *N*-desmethyl-2-oxoquazepam by CYP3A4 and CYP2C9 and to another metabolite by CYP3A4 [11]. Consequently, it is likely that

in the present study the effect of GFJ diminished with the formation of *N*-desmethyl-2-oxoquazepam. In addition, GFJ did not prolong the  $t_{1/2}$  of triazolam, quazepam and 2-oxoquazepam. These pharmacokinetic alterations may be caused by the GFJ-induced inhibition of CYP3A4 activity in the intestine, but not in the liver [5]. Lilja et al. [10] been reported that repeated consumption of GFJ prolonged the  $t_{1/2}$  of triazolam, suggesting the inhibition of hepatic CYP3A4 by GFJ. In their study, subjects were given 200 ml of double-strength GFJ three times a day for 3 days, while we gave 250 ml of normal-strength GFJ three times a day for 4 days to our participants. Not only the length of the treatment period but the strength of GFJ may be a determinant of the GFJ-induced inhibition of hepatic CYP3A4, if this inhibition is truly caused by GFJ.

GFJ increases plasma triazolam concentration [9, 10], which is consistent with the present results. A significant increase in the pharmacodynamic effects of triazolam has been observed following multiple doses of GFJ [10], but not following a single dose [9, 16]. Hukkinen et al. [9] found that psychomotor function, as assessed by DSST, was not further impaired by a single dose of 250 ml GFJ despite an increase in plasma triazolam concentration [9]. In their study, triazolam  $C_{\max}$  increased by only 30% following a single dose of GFJ. Similarly, Vanakoski et al. [16] observed that a single, concomitant ingestion of 300 ml GFJ did not influence the triazolam-induced objective effects, including DSST. In the present study in which multiple-doses of GFJ were given,  $C_{\max}$  increased by 55% on average; furthermore, the maximum reduction in DSST performance showed a weak – but significant – correlation with the plasma concentration of triazolam. The present results suggest that pharmacodynamic alteration by the GFJ-triazolam interaction may be caused by the increase in plasma triazolam concentration. Taken together, a single dose of GFJ may not enough to cause the interaction with triazolam on psychomotor function, probably because of an insufficient increase in plasma triazolam concentration.

GFJ increased plasma quazepam and 2-oxoquazepam concentrations, but it did not alter the pharmacodynamic effects. The major metabolites of quazepam, 2-oxoquazepam and *N*-desmethyl-2-oxoquazepam are reported to be pharmacologically active, although the involvement of these metabolites has not been proven to date. Roth et al. [17] suggested that the hypnotic effect of quazepam is caused by the metabolite *N*-desmethyl-2-oxoquazepam [17]. In their study, a repeated dose of quazepam, however, did not increase the incidence or the duration of daytime napping; similarly, the hypnotic and sedative effect of quazepam disappeared 12 h after the dose despite a high plasma concentration of *N*-desmethyl-2-oxoquazepam. If the effect of quazepam is solely caused by *N*-desmethyl-2-oxoquazepam, the sedative-like subject-rated effects of quazepam should have been evident even 12 h after administration of the dose of quazepam. However, we observed the maximum sedative-like subject-rated effects by quazepam 4–6 h after the dose. It therefore appears that the sedative effect observed in the present study was caused

by quazepam itself. A contribution by 2-oxoquazepam to the effects of quazepam is possible, but it is not obvious based on our results, and the contribution of *N*-desmethyl-2-oxoquazepam was small, if any. The effect of quazepam on psychomotor function as assessed by DSST was negligible, a result similar to that found by Nikaido et al. [18]. Our results suggest that the detrimental effects of 15 mg quazepam on psychomotor functions may be small even if the drug is taken with GFJ. However, 0.25 mg triazolam and 15 mg quazepam produced significant sedative-like, subject-rated effects in this study. The effect of GFJ on plasma quazepam or 2-oxoquazepam concentration was diminished at 4–6 h after the dose had been given, which is the time span when the sedative effect was at its maximum; consequently, the subject-rated effect of quazepam may not be enhanced by GFJ.

CYP2C9 metabolizes many clinically important drugs including the *S*-enantiomer of warfarin and tolbutamide [19]. The enzyme is also involved in the metabolism of quazepam [11]. Two common polymorphisms of CYP2C9 have been reported; the \*2 allele (Arg144Cys) and the \*3 allele (Ile359Leu) [20]. CYP2C9\*2 and CYP2C9\*3 diminish the clearance of *S*-warfarin and tolbutamide, respectively [19]. The frequencies of these alleles are as follows: CYP2C9\*2, 8% in Caucasians and 0% in Japanese; CYP2C9\*3: 6% in Caucasians and 2% in Japanese [12, 21]. Therefore, when quazepam is given to patients with one or these CYP2C9 polymorphisms, the effects of GFJ on the pharmacokinetics and pharmacodynamics of the drug may be exaggerated. Further study is needed to address the issue.

In summary, the results of this study demonstrate that the effects of GFJ on the pharmacodynamics of triazolam are greater than on those of quazepam. These GFJ-induced different effects are partly explained by the fact that triazolam is mainly metabolized by CYP3A4 while quazepam is metabolized by CYP3A4 and CYP2C9.

**Acknowledgements** We thank Ms. Junko Koyano, Hiroko Susuki and Mariko Hojo for their technical assistance.

## References

- Edgar B, Bailey D, Bergstrand R, Johnsson G, Regardh CG (1992) Acute effects of drinking grapefruit juice on the pharmacokinetics and dynamics of felodipine and its potential clinical relevance. *Eur J Clin Pharmacol* 42:313–317
- Benton RE, Honig PK, Zamani K, Cantilena LR, Woosley RL (1996) Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. *Clin Pharmacol Ther* 59:383–388
- Ducharme MP, Warbasse LH, Edwards DJ (1995) Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin Pharmacol Ther* 57:485–491
- Lilja JJ, Kivistö KT, Neuvonen PJ (1998) Grapefruit juice-simvastatin interaction: Effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* 64:477–483
- Lown KS, Bailey DG, Fontana RJ, Janardan JK, Adair CH, Fortlage LA, Brown MB, Guo W, Watkins PB (1997) Grapefruit juice increases felodipine oral bioavailability in human by decreasing intestinal CYP3A protein expression. *J Clin Invest* 99:2545–2553
- Fukuda K, Ohta T, Oshima Y, Ohashi N, Yoshikawa M, Yamazoe Y (1997) Specific CYP3A4 inhibitors in grapefruit juice: furanocoumarin dimers as components of drug inhibition. *Pharmacogenetics* 7:391–396
- Paine MF, Criss AB, Watkins PB (2004) Two major grapefruit juice components differ in intestinal CYP3A4 inhibition kinetics and binding properties. *Drug Metab Dispos* 32:1146–1153
- Moltke LL von, Greenblatt DJ, Harmatz JS, Duan SX, Harrel JM, Catreau-Bibbo NM, Pritchard GA, Wright CE, Shader RI (1996) Triazolam biotransformation by human liver microsomes in vitro: effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *J Pharmacol Exp Ther* 276:370–379
- Hukkinen SK, Varhe A, Olkkola KT, Neuvonen PJ (1995) Plasma concentrations of triazolam are increased by concomitant ingestion of grapefruit juice. *Clin Pharmacol Ther* 58:127–131
- Lilja JJ, Kivistö KT, Backman JT, Neuvonen PJ (2000) Effect of grapefruit juice dose on grapefruit juice-triazolam interaction: repeated consumption prolongs triazolam half-life. *Eur J Clin Pharmacol* 56:411–415
- Fujisaki H, Hirotsu K, Ogawa T, Mizuki K, Mizuta H, Arima N (2001) Metabolism of quazepam and its metabolites in humans: Identification of metabolic enzymes and evaluation of drug interaction in vitro (in Japanese with English abstract). *Yakubutsudoutai (Xenobio Metabol Dispos)* 16:558–568
- Nasu K, Kubota T, Ishizaki T (1997) Genetic analysis of CYP2C9 polymorphism in a Japanese population. *Pharmacogenetics* 7:405–409
- Sostmann HJ, Sostmann H, Crevoisier C, Bircher J (1989) Dose equivalence of midazolam and triazolam: A psychometric study based on flicker sensitivity, reaction time and digit symbol substitution test. *Eur J Clin Pharmacol* 36:181–187
- Chung M, Hilbert JM, Gural RP, Radwaniski E, Symchowicz S, Zampaglinone N (1984) Multiple-dose quazepam kinetics. *Clin Pharmacol Ther* 35:520–524
- Hilbert JM, Chung M, Maier G, Grural R, Symchowicz S, Zampaglinone N (1984) Effect of sleep on quazepam kinetics. *Clin Pharmacol Ther* 36:99–104
- Vanakoski J, Mattila MJ, Seppälä T (1996) Grapefruit juice does not enhance the effects of midazolam and triazolam in man. *Eur J Clin Pharmacol* 50:501–508
- Roth TG, Roehrs TA, Koshorek GL, Greenblatt DJ, Rosenthal LD (1997) Hypnotic effects of low doses of quazepam in older insomniacs. *J Clin Psychopharmacol* 17:401–406
- Nikaido AM, Ellinwood EH Jr (1987) Comparison of the effects of quazepam and triazolam on cognitive-neuromotor performance. *Psychopharmacology* 92:459–464
- Goldstein JA, de Morais SM (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* 4:285–299
- Goldstein JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol* 52:349–355
- Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, Miners JO, Birkett DJ, Goldstein JA (1996) The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 6:341–349