

## EFFECTS OF POMEGRANATE JUICE ON HUMAN CYTOCHROME P450 3A (CYP3A) AND CARBAMAZEPINE PHARMACOKINETICS IN RATS

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### ABSTRACT:

In this study, we investigated whether components of pomegranate could inhibit CYP3A-mediated drug metabolism. The ability of pomegranate to inhibit the carbamazepine 10,11-epoxidase activity of CYP3A was examined using human liver microsomes, and pomegranate juice was shown to be a potent inhibitor of human CYP3A. Addition of 25  $\mu$ l (5.0% v/v) of pomegranate juice resulted in almost complete inhibition of the carbamazepine 10,11-epoxidase activity of human CYP3A (1.8%). The inhibition potency of pomegranate juice was similar to that of grapefruit juice. In addition, we investigated the *in vivo* interaction between pomegranate juice and carbamazepine pharmacokinetics using rats. In comparison with water, the area under the concentration-time curve (AUC)

of carbamazepine was approximately 1.5-fold higher when pomegranate juice (2 ml) was orally injected 1 h before the oral administration of the carbamazepine (50 mg/kg). On the other hand, the elimination half-life of carbamazepine and the AUC ratio of carbamazepine 10,11-epoxide to carbamazepine were not altered by the injection of pomegranate juice. These data suggest that pomegranate juice component(s) impairs the function of enteric but not hepatic CYP3A. Thus, we discovered that a component(s) of pomegranate inhibits the human CYP3A-mediated metabolism of carbamazepine. Furthermore, pomegranate juice alters the carbamazepine pharmacokinetics in rats.

In the early 1990s, it was reported that coadministration of grapefruit juice with felodipine or nifedipine, which are calcium channel antagonists, resulted in a large increase in the oral bioavailability of these drugs and an enhancement of their pharmacodynamic effects (Bailey et al., 1989, 1991). Adverse experiences such as headaches, hypotension, facial flushing, and lightheadedness caused by these drugs were more frequently reported after the intake of grapefruit juice than after the intake of water (Bailey et al., 1991; Lundahl et al., 1995). Grapefruit juice interacts with drugs that undergo substantial presystemic metabolism mediated by cytochrome P450 (CYP) 3A4 (Bailey et al., 1998). The mechanism of action probably involves competitive or irreversible (mechanism-based) inhibition of CYP3A4 in the small intestine (Bailey et al., 2000). Furthermore, recent studies revealed that the furanocoumarin derivatives identified in grapefruit juice strongly inhibited the catalytic activity of CYP3A4 and resulted in a decrease in the first pass metabolism of orally administered therapeutic drugs that are catalyzed by CYP3A4 (Guo et al., 2000; Tassaneeyakul et al., 2000). On the other hand, it has been suggested that common orange juice does not inhibit the catalytic activity of CYP3A4 (Bailey et al., 1991). In recent years, there have been reports that citrus fruits as well as several other fruits have the potency to inhibit CYP3A activities in the liver and gut wall and thereby change the pharmacokinetics of certain drugs (Di Marco et al., 2002; Bailey

et al., 2003; Fujita et al., 2003; Ohnishi et al., 2003; Hidaka et al., 2004). The inhibitory effect of a fruit is believed to depend on the fruit species and to be due to differences in the components of the fruit (Bailey et al., 1991; Guo et al., 2000). These findings have led us to conduct further studies on the interaction between other food items and the drugs metabolized by CYP3A.

*Punica granatum* L. (Punicaceae), also referred to as pomegranate, is commonly eaten around the world, and it has been used in folk medicine for a wide variety of therapeutic purposes (Langley, 2000). Pomegranate is a rich source of crude fibers, pectin, sugars, and several tannins (Gil et al., 2000). In addition, it has been reported that pomegranate contains certain species of flavonoids and anthocyanins in its seed oil and juice, and it shows potent antioxidant activity, resulting in beneficial health effects such as inhibition of low-density lipoprotein oxidation and decrease in cardiovascular diseases (Gil et al., 2000; Aviram et al., 2002, 2004; Noda et al., 2002). Furthermore, adjuvant therapeutic properties of the fruit have been suggested for use in cases of breast cancer (Kim et al., 2002). Based on these findings, pomegranate has been increasingly popularized in Japan. Higher pomegranate consumption allows for an increased possibility of pomegranate-drug interaction. Therefore, it is important to assess the interaction between pomegranate and CYP3A-mediated drugs because there are few reports on the inhibition of CYP3A activities.

In the present study, using human liver microsomes, we first investigated whether the components of pomegranate could inhibit the CYP3A-mediated drug metabolism. Carbamazepine was used as a substrate for CYP3A, since this drug is metabolized to carbamazepine 10,11-epoxide by CYP3A (Kerr et al., 1994).

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**ABBREVIATIONS:** HPLC, high-performance liquid chromatography; AUC, area under the concentration-time curve; MRT, mean retention time.

It has been reported that grapefruit juice increased carbamazepine bioavailability by inhibiting CYP3A enzymes in the gut wall and liver in humans (Garg et al., 1998). If pomegranate inhibits CYP3A expressed in the gut wall and/or liver, as in the case of grapefruit juice, coadministration of pomegranate juice may alter carbamazepine pharmacokinetics. In the second experiment, we investigated the *in vivo* pharmacokinetic interaction of pomegranate juice with carbamazepine in rats.

### Materials and Methods

**Chemicals.** Carbamazepine and carbamazepine 10,11-epoxide were purchased from Sigma-Aldrich (St. Louis, MO). Pooled human liver microsomes were obtained from Daiichi Pure Chemicals (Tokyo, Japan). All chemicals and solvents were of the highest grade that was commercially available.

**Fruit Samples.** Pomegranate (California) and grapefruit (white) (*Citrus paradise*) (Florida) were obtained from local commercial sources. The fruit samples were stored at 4°C until use. Pomegranate juice was obtained by squeezing the edible portion of the fruit. All samples were tested soon after they were squeezed and filtered.

**Assay of Carbamazepine 10,11-Epoxidation Activity of Human CYP3A.** Assay of the carbamazepine 10,11-epoxidation activity of human CYP3A was performed according to the method of Nakamura et al. (2002), with minor modifications. Briefly, a typical incubation mixture consisted of 100 mM sodium potassium phosphate buffer (pH 7.4), 50  $\mu$ M EDTA disodium salt, an NADPH-generating system (0.5 mM NADP<sup>+</sup>, 5 mM MgCl<sub>2</sub>, 5 mM glucose 6-phosphate, and 1 unit/ml glucose-6-phosphate dehydrogenase), and a microsomal fraction of human liver in a final volume of 0.5 ml. The carbamazepine concentration was 100  $\mu$ M. The protein concentration and reaction time were predetermined based on the linearity between the microsomal protein concentration (up to 0.2 mg/ml) and reaction time (up to 60 min) versus metabolite formation rate. Based on the results obtained, the protein concentration and the reaction time were determined to be 0.2 mg/ml and 60 min, respectively. Reactions were initiated by the addition of carbamazepine and stopped by the addition of 5 ml of ethyl acetate. Clonazepam (0.5 nM) was added as an internal standard. Following centrifugation (3000 rpm, 10 min), the organic phase was evaporated at 40°C. The residue was dissolved in 100  $\mu$ l of HPLC mobile phase, and 20  $\mu$ l of the resultant mixture was injected into an HPLC apparatus. The mobile phase consisted of 50 mM acetate buffer (pH 6.4) and acetonitrile (5.5:4.5 v/v).

**HPLC Conditions.** The HPLC system consisted of an LC-10ADvp pump (Shimadzu, Kyoto, Japan), a Shimadzu L-4200 UV absorbance detector, a Shimadzu SIL-10ADvp auto injector, and a Shimadzu SCL-10Avp system controller. The system was equipped with a Cadenza CD-C18 column (3  $\mu$ m, 4.6  $\times$  250 mm; Intact, Kyoto, Japan) preceded by a precolumn (5  $\mu$ m, 2  $\times$  5 mm). The mobile phase was delivered at a flow rate of 0.7 ml/min at 40°C. Quantification was performed by determining the HPLC peak areas monitored at 245 nm and comparing the peak area of the internal standard with those of the chemicals. Under these conditions, the retention times of carbamazepine 10,11-epoxide, carbamazepine, and the internal standard were 6.2, 8.7, and 11.3 min, respectively.

**Inhibitory Effects of Pomegranate on CYP3A Activity.** The inhibitory effects of pomegranate on CYP3A activity were evaluated by the method of Guo et al. (2000) with minor modifications. Briefly, an appropriate amount of fruit juice was dried in a concentrator. The reaction mixture described above (prior to the addition of carbamazepine) was added, and the fruit juice sample was resuspended using a vortex mixer at full power for 2 s. The substrate, carbamazepine, was added after preincubation of the mixture at 37°C for 5 min. The reaction was performed as described above. The inhibitory effects of pomegranate juice or grapefruit juice on carbamazepine 10,11-epoxidation were expressed as a percentage of the residual activity in comparison with the control in the absence of fruit juice. Each assay was performed in duplicate.

**Effects of Preincubation of Pomegranate Juice on Human CYP3A Activity.** As an index of mechanism-based inhibition, pomegranate juice was preincubated at 37°C for 0, 5, 10, 15, or 30 min in the reaction mixture, according to the method described above.

**Animals.** Male Wistar rats (Kiwa Animal Lab Service Co., Ltd., Wakayama, Japan), weighing 280 to 300 g and maintained at the Department

of Bio-resources, Division of Biotechnology, Frontier Science Research Center, Miyazaki University, were used throughout the study. The rats were housed in stainless steel cages with three animals per cage in a temperature-controlled (22–24°C) room with a 12-h light/dark cycle. The rats were allowed free access to standard rat chow (Sankyo) and water for 1 week before the experiments and were fasted overnight before the experiments. The Ethics Review Committee for Animal Research of Miyazaki University approved the experimental protocol. The experiments were carried out according to the Guideline for Animal Experiments in Miyazaki University.

**Pharmacokinetic Experiments.** Each animal was anesthetized with pentobarbital (50 mg/kg intraperitoneally), and the carotid artery was cannulated with polyethylene tubing (PE-50; Clay Adams, Parsippany, NJ) to collect blood samples. The tube was filled with a heparin lock to prevent blood clotting. The solution consisted of 100 U/ml heparin in saline.

During the experiment, the body temperature was maintained at 37  $\pm$  0.5°C to prevent hypothermic alteration of blood circulation. The carbamazepine solution for injection was prepared by dissolving 50 mg of carbamazepine in a mixture of polyethylene glycol 400 (5 ml), ethanol (2 ml), and saline (13 ml). Two milliliters of pomegranate juice, grapefruit juice, or water was orally administered to the rats. Carbamazepine was orally administered at a dose of 50 mg/kg through gastric intubation at 1, 24, 48, or 72 h after the pretreatment. Blood samples (approximately 0.2 ml) were collected through the carotid artery at 0, 15, and 30 min and 1, 2, 4, 6, 8, 10, 12, and 24 h after the oral administration of carbamazepine. The samples were immediately centrifuged at 16,000g and 4°C for 5 min, and the plasma was separated. The plasma samples that were collected were stored at –80°C until their analysis.

**Pharmacokinetic Analysis.** The peak plasma concentrations ( $C_{max}$ ) and the time required to reach  $C_{max}$  ( $T_{max}$ ) of carbamazepine and carbamazepine 10,11-epoxide were obtained from the actual data recorded after oral administration. The plasma concentration-time profile (0–24 h) of each rat was analyzed by a model-independent method using the MULTI computer program (Yamaoka and Nakagawa, 1983). The area under the concentration-time curve (AUC) was calculated from the values obtained (0–24 h) using the trapezoidal rule. Mean retention time (MRT) was estimated by moment analysis (Yamaoka et al., 1978). The half-life ( $t_{1/2}$ ) was calculated by dividing the natural logarithm of 2 by  $K_{el}$ , which is the apparent elimination rate constant that is obtained from the elimination phase gradient.

**Data Analysis.** Data from the *in vitro* experiments are expressed as mean  $\pm$  S.D. and those from the *in vivo* experiments are expressed as mean  $\pm$  S.E.M. Unpaired Student's *t* test and one-way analysis of variance, followed by least-significant difference analysis, were used to test for significant differences in mean values. The significance level was set at  $p < 0.05$ .

## Results

**Inhibition of Microsomal Human CYP3A Activity.** The results of the inhibition of carbamazepine 10,11-epoxidase activity of human CYP3A by pomegranate juice or grapefruit juice are shown in Fig. 1.

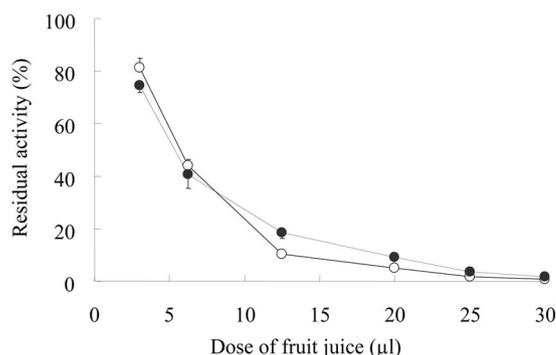


FIG. 1. Inhibition of human CYP3A by pomegranate juice or grapefruit juice. The amount of fruit juice added to the incubation mixture was 3.0, 6.25, 12.5, 20, 25, and 30  $\mu$ l, respectively. The control activity of carbamazepine 10,11-epoxidation by human liver microsomes determined in the absence of fruit juice was 0.208 nmol/min/mg protein.  $\circ$ , pomegranate juice;  $\bullet$ , grapefruit juice. Each point and bar represents the mean and S.D. of three independent assays.

Addition of 25  $\mu\text{l}$  (5.0% v/v) of pomegranate juice resulted in almost complete inhibition of carbamazepine 10,11-epoxidase activity of human CYP3A (1.8%). The inhibition potency of pomegranate juice was similar to that of grapefruit juice, and it depended on the amount of juice added to the reaction mixture (0.6% to 6.0% v/v).

We examined whether the component(s) of pomegranate juice inhibited human CYP3A in a mechanism-based manner. The effect of the length of the preincubation period on the inhibition of carbamazepine 10,11-epoxidase activity by pomegranate juice or grapefruit juice was tested, and the results are shown in Fig. 2. The inhibition potency of pomegranate juice was altered by the elongation of the preincubation period, and this was similar to the observation made in the case of grapefruit juice. The mean residual CYP3A activities observed with pomegranate juice at the preincubation times of 0, 5, 10, 15, and 30 min were 90.0%, 81.4%, 75.0%, 64.7%, and 45.7%, respectively. The residual activities observed with grapefruit juice were 92.2%, 74.5%, 58.2%, 53.6%, and 38.3%, respectively. These results suggest that pomegranate juice contains a mechanism-based inhibitor(s) as in the case of grapefruit juice. Thus, we discovered that the component(s) present in the pomegranate juice reversibly and/or irreversibly inhibits the human CYP3A-mediated metabolism of carbamazepine.

**Effects of Pomegranate Juice on Carbamazepine Pharmacokinetics in Rats.** Since pomegranate juice inhibits CYP3A, we considered the possibility that coadministration of pomegranate juice might alter carbamazepine pharmacokinetics. Therefore, in the present study, the effects of pomegranate juice on carbamazepine pharmacokinetics were examined using rats. The plasma carbamazepine and carbamazepine 10,11-epoxide concentration-time profiles were investigated after oral administration of carbamazepine at a dose of 50 mg/kg to rats treated with pomegranate juice, grapefruit juice, or water (control). The results are shown in Fig. 3. The plasma concentrations of carbamazepine and carbamazepine 10,11-epoxide were significantly higher in rats treated with pomegranate juice than in rats treated with water. The pharmacokinetic parameters are summarized in Table 1. The mean AUCs of carbamazepine and carbamazepine 10,11-epoxide observed in rats treated with pomegranate juice were approximately 1.45 and 1.44 times larger, respectively, than the values obtained in rats treated with water. The concentration-time profiles observed in rats treated with pomegranate juice were similar to those observed in rats treated with grapefruit juice. On the other

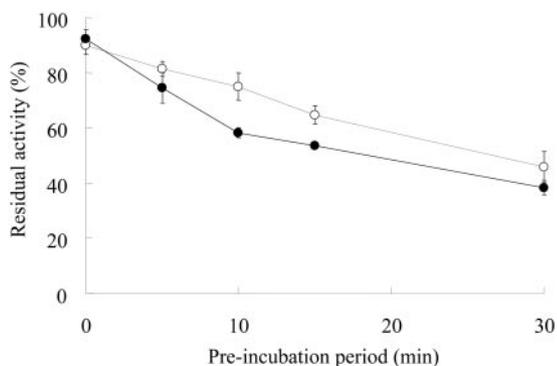


FIG. 2. Effect of preincubation period on the inhibition of carbamazepine 10,11-epoxidase activity by pomegranate juice or grapefruit juice. The amount of fruit juice added to the incubation mixture was 3.0  $\mu\text{l}$ . The concentration of carbamazepine was 100  $\mu\text{M}$ . Fruit juice was added to the reaction mixture and incubated for the indicated period before the start of the reaction by the addition of a substrate. The control activity of carbamazepine 10,11-epoxidation by human liver microsomes determined in the absence of fruit juice was 0.213 nmol/min/mg protein.  $\circ$ , pomegranate juice;  $\bullet$ , grapefruit juice. Each point and bar represents the mean and S.D. of three independent assays.

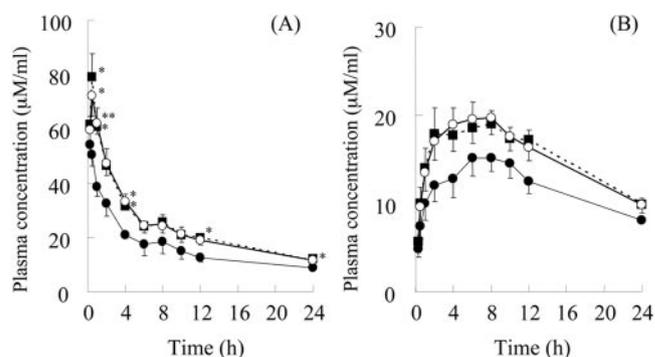


FIG. 3. Plasma concentration-time profiles of rats treated with 50 mg/kg carbamazepine at 1 h after a single exposure to pomegranate juice, grapefruit juice, or water (2 ml p.o., each). A, carbamazepine; B, carbamazepine 10,11-epoxide.  $\bullet$ , control;  $\circ$ , pomegranate juice;  $\blacksquare$ , grapefruit juice. Each point and bar represents the mean and S.E.M. of five or six rats. \*,  $p < 0.05$ , \*\*,  $p < 0.01$  versus control values.

TABLE 1

Pharmacokinetic parameters of carbamazepine and carbamazepine 10,11-epoxide after administration of carbamazepine in 1 h after a single exposure to pomegranate juice, grapefruit juice, or water in rats

Data are presented as mean  $\pm$  S.E.M. of five or six rats. Comparisons are between the treatments with juice and water.

Parameters	Control	Pomegranate Juice	Grapefruit Juice
	Average S.E.M.	Average S.E.M.	Average S.E.M.
<b>Carbamazepine</b>			
$C_{\max}$ ( $\mu\text{mol/ml}$ )	53.6 $\pm$ 4.5	74.5 $\pm$ 6.6*	79.9 $\pm$ 8.3*
$T_{\max}$ (h)	0.38 $\pm$ 0.06	0.50 $\pm$ 0.11	0.45 $\pm$ 0.05
AUC ( $\mu\text{mol} \cdot \text{h/ml}$ )	394.0 $\pm$ 40.1	572.6 $\pm$ 32.5**	573.9 $\pm$ 44.9*
MRT (h)	8.5 $\pm$ 0.3	8.5 $\pm$ 0.3	8.5 $\pm$ 0.2
$t_{1/2}$ (h)	9.1 $\pm$ 0.6	8.8 $\pm$ 0.5	9.0 $\pm$ 0.4
<b>Carbamazepine 10,11-epoxide</b>			
$C_{\max}$ ( $\mu\text{mol/ml}$ )	17.5 $\pm$ 1.4	21.7 $\pm$ 2.4	22.3 $\pm$ 2.1
$T_{\max}$ (h)	7.3 $\pm$ 1.1	8.0 $\pm$ 1.1	7.6 $\pm$ 1.3
AUC ( $\mu\text{mol} \cdot \text{h/ml}$ )	254.2 $\pm$ 30.5	365.8 $\pm$ 32.3*	367.1 $\pm$ 26.3*
MRT (h)	10.3 $\pm$ 0.8	10.8 $\pm$ 0.3	10.8 $\pm$ 0.4
$t_{1/2}$ (h)	14.9 $\pm$ 1.3	16.7 $\pm$ 0.6	17.2 $\pm$ 1.3
<b>Carbamazepine 10,11-epoxide/carbamazepine</b>			
AUC ratio <sup>a</sup>	0.66 $\pm$ 0.07	0.63 $\pm$ 0.03	0.65 $\pm$ 0.04

\*  $p < 0.05$ , \*\*  $p < 0.01$  vs. control values.

<sup>a</sup> AUC of carbamazepine 10,11-epoxide/AUC of carbamazepine.

hand, the  $t_{1/2}$  values of carbamazepine and carbamazepine 10,11-epoxide and the AUC ratios (carbamazepine 10,11-epoxide/carbamazepine) were not significantly different among the three groups.

We considered that the increased AUC of carbamazepine that was observed in rats treated with pomegranate juice was caused by the inhibition of carbamazepine metabolism as well as by the enhanced absorption of carbamazepine by the component(s) of pomegranate juice. Therefore, to confirm this hypothesis, the effects of pomegranate juice on the absorption of carbamazepine were tested. The absorption of carbamazepine from various parts of the rat intestine was examined in the presence or absence of pomegranate juice by the in situ closed loop technique. Total amounts of carbamazepine eliminated through the duodenum, jejunum, ileum, and cecum during a period of 30 min were evaluated (data not shown). With respect to the absorption of carbamazepine from the four intestinal segments, there was no significant difference between the presence and absence of pomegranate juice. The results indicate that pomegranate juice increases the AUC without affecting the absorption of carbamazepine.

**Recovery of CYP3A Activity.** Our data suggested that pomegranate juice contained the mechanism-based inhibitor(s) of CYP3A (Fig. 2). The mechanism-based inhibitor causes irreversible inhibition of an

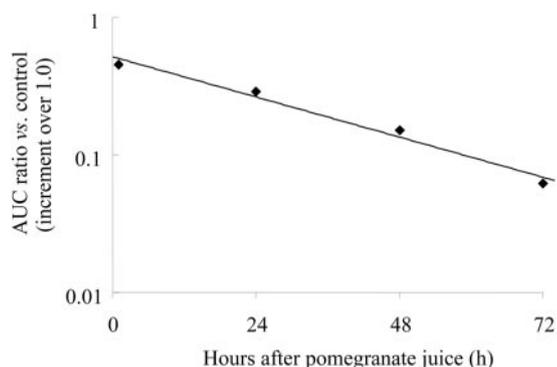


FIG. 4. Relationship between the time after single exposure to pomegranate juice and the fractional increase of area under the plasma concentration-time curve (AUC) of carbamazepine relative to the control value. The recovery half-life was estimated as 25 h ( $r^2 = 0.98$ ). Each point represents the mean value obtained from five or six rats.

enzyme until this enzyme is newly synthesized. Therefore, we evaluated the time course of recovery of CYP3A activity in rats after treatment with pomegranate juice. Carbamazepine was orally administered at 1, 24, 48, and 72 h after exposure to pomegranate juice. The AUCs of carbamazepine obtained after exposure to the juice were normalized by the respective control values in the same manner as that reported by Greenblatt et al. (2003). Figure 4 shows the recovery profiles of the AUC. It was observed that the AUC progressively returned toward the control value as time elapsed. The ratios of mean AUC values at 1, 24, 48, and 72 h after the exposure to pomegranate juice were 1.45, 1.29, 1.15, and 1.06, respectively. A plot of the time after exposure to pomegranate juice versus the ratio of mean AUC values yielded a half-life of recovery estimated as 25 h. These results suggest that CYP3A activity in rats treated with pomegranate juice could recover in approximately 3 days and that mechanism-based inhibitor(s) must be included in the pomegranate juice.

### Discussion

In recent times, pomegranate has been widely consumed in Japan as well as in the United States and the Middle Eastern countries. Pomegranate is a rich source of several chemicals such as pectin, tannins, flavonoids, and anthocyanins. However, limited data are available on whether the component(s) of pomegranate inhibits CYP3A activity. It has been well documented that the components of grapefruit, such as bergamottin and (*R*)-6',7'-dihydroxybergamottin, demonstrate potent inhibition of CYP3A activity, depending on the preincubation period (Guo et al., 2000; Paine et al., 2004). According to the data presented by us, the manner in which inhibition is caused by the component(s) of pomegranate is similar to that caused by grapefruit. Hence, pomegranate might contain the same component(s) mentioned above. Therefore, it is of interest to determine the identity of the chemical(s) in pomegranate juice that exhibits potent inhibition of CYP3A activity. Understanding the nature of these chemicals would enable health care professionals to avoid food-drug interactions. Furthermore, such information will be useful for identifying situations in which the inhibition of CYP3A may be of therapeutic benefit.

In this study, we demonstrated that pomegranate juice influenced the pharmacokinetics of carbamazepine in rats (Fig. 3). In particular, in comparison with the effect of water, the AUC of carbamazepine increased approximately 1.5-fold in rats upon exposure to pomegranate juice 1 h before the administration of the drug. Another interesting observation was that the elimination half-life of carbamazepine and the AUC ratio of carbamazepine 10,11-epoxide to carbamazepine were not altered by pomegranate juice (Table 1). If the component(s)

that inhibits CYP3A is absorbed into the systemic circulation, it might decrease the elimination of carbamazepine and the formation of carbamazepine 10,11-epoxide by inhibiting hepatic CYP3A. These findings suggest that the uptake of the component(s) of pomegranate juice into the systemic circulation is not sufficient to inhibit hepatic CYP3A. Therefore, we considered that pomegranate juice resulted in food-drug interactions via the gastrointestinal tract. Generally, the mechanism of food-drug interactions that occur in the intestine consists of several systems (Lilja et al., 2003). It is mainly divided into metabolism and absorption. The P-glycoprotein is believed to play an important role in the efflux of numerous drugs, which results in poor absorption of these drugs. It is well known that there is an overlap between the inhibitors of CYP3A and P-glycoprotein (Kim et al., 1999). Therefore, pomegranate juice might be an inhibitor of P-glycoprotein in the intestine and enhance the absorption of drugs. Since a previous report suggested that carbamazepine was not a substrate for P-glycoprotein, considering P-glycoprotein in this study may be unnecessary (Owen et al., 2001). However, it has recently been reported that grapefruit juice affects intestinal uptake transporters as well as P-glycoprotein (Dresser and Bailey, 2003; Lilja et al., 2003). Therefore, we determined the effect of pomegranate juice on carbamazepine absorption. The report by Owen et al. (2001) supports our results that pomegranate juice had no influence on the absorption of carbamazepine in the rat intestine (data not shown). Thus, we conclude that the increase in the AUC of carbamazepine by pomegranate juice could be due to the inhibition of enteric CYP3A activity.

In rats, inhibition of the enteric CYP3A activity by a single exposure to pomegranate juice appears to continue for approximately 3 days (Fig. 4). The recovery pattern in humans is roughly consistent with the time course of enzyme regeneration after irreversible (mechanism-based) inhibition (Venkatakrishnan et al., 2001; Greenblatt et al., 2003). According to these studies, the activity of human enteric CYP3A recovered within 3 days, and this was similar to our results. There are few reports on rat CYP3A regeneration; however, enzyme regeneration after irreversible inhibition is considered to also occur in the rat intestine. Therefore, we considered that the recovery pattern obtained from our results depends on enzyme regeneration after irreversible inhibition. However, this hypothesis is not confirmed in this study and needs to be explored in future studies.

The overall rate of biotransformation of carbamazepine in rats is markedly different from that in humans; the metabolic clearance is more than 10-fold faster in rats (Faigle and Feldman, 1995). However, the major metabolic pathways of the drug are almost similar in both species (Lertratanangkoon and Horning, 1982). In both rats and humans, CYP3A is known to be involved in the metabolism of carbamazepine to carbamazepine 10,11-epoxide (Kerr et al., 1994; Panesar et al., 1996). According to our results (Fig. 1), pomegranate juice might influence the pharmacokinetics of CYP3A-mediated drugs in humans. However, it is difficult to extrapolate our results, which were obtained in rats, to humans. Quantitative evaluation of pomegranate-drug interaction in humans needs to be verified by studies in humans. Therefore, further investigations in humans are necessary to develop our findings.

In conclusion, we have shown that a component(s) of pomegranate inhibits the CYP3A-mediated metabolism of carbamazepine. Furthermore, pomegranate juice has an influence on the pharmacokinetics in rats.

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