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# Drug interaction potentials among different brands of grapefruit juice

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The discrepancy of drug-interaction potential among different brands of grapefruit juice was estimated based on inhibition of CYP3A activity caused by furanocoumarin derivatives in the grapefruit juice. Heat treatment of the grapefruit juice at 95 °C for 1 h was utilized to degrade the furanocoumarins. Initial velocity of testosterone 6β-oxidation using human liver microsomes was determined as an indicator of the CYP3A activities. Changes in the velocities of the reaction mixture were observed when 10% of each brand of untreated grapefruit juice or heat-treated grapefruit juice was added. The differences in the velocities between untreated and heat-treated grapefruit juice were defined as the potentials of furanocoumarin-caused CYP3A-inhibitions.

## 1. Introduction

It has been suggested that cytochrome P450 (CYP) 3A in intestinal mucosal cells has the potential to assemble one of the most important barrier systems on intravasation of xenobiotics (Watkins 1997; Benet et al. 1999; Wacher et al. 2001). Many types of medications such as calcium channel blocking agents, immunosuppressive agents, and HMG-CoA reductase inhibitors are metabolized and CYP3A controls these intestinal first-pass effects (Fuhr 1998; Kane and Lipsky 2000; García et al. 2003; Dahan and Altman 2004). Grapefruit juice (GJ) intake causes breakdown of the system due to a mechanism-based inhibition of the intestinal CYP3A. A number of studies have shown that furanocoumarin derivatives in GJ such as bergamottin, 6′,7′-dihydroxybergamottin, and furanocoumarin dimers are potent inhibitors of CYP3A activity (Bailey et al. 1991; Schmiedlin-Ren et al. 1997; He et al. 1998; Mohri and Uesawa 2001; Row et al. 2006; Uesawa and Mohri 2006a, b). As a result, drug interaction with GJ may lead to adverse effects due to the elevation of the bioavailability and concentration of the drug in circulating blood (Kane and Lipsky 2000; Sagir et al. 2003). For example, headache may occur in subjects who simultaneously take GJ and antihypertensive dihydropyridine drugs such as felodipine (Lundahl et al. 1995). On the other hand, GJ also includes flavonoid concomitants such as naringin and naringenin (Hagen et al. 1965). Although these flavonoids indicate CYP3A inhibition as demonstrated by *in vitro* experiments (Guengerich and Kim 1990), there are no reports demonstrating that they cause the pharmacokinetic interactions with GJ (Bailey et al. 1993). Recently, we discovered that heat treatment of GJ decreased concentrations of furanocoumarin derivatives, bergamottin and 6′,7′-dihydroxybergamottin, depending on the temperature and the treatment period, thereby causing the inhibitory effect on CYP3A to decrease and the pharmacokinetic interaction potential to disappear (Uesawa and Mohri 2006b). These findings suggest that heat treatment of GJ may be applicable in the evaluation of GJ-drug interactions from furanocoumarins, suggesting that the decrease in the CYP3A inhibitory potential of GJ by the heat treatment was related to the concentrations of furanocoumarins present in GJ. In the present study, variations in the drug-interactions among 21 different brands of GJ were estimated using heat treatment to analyze the potentials of furanocoumarin-caused CYP3A-inhibitions (FCIs).

## 2. Investigations and results

In this study, the initial rates of CYP3A dependent testosterone 6β-oxidation in human liver microsomes were measured with various brands of GJ and HGJ. As a result, when compared with the corresponding brand of untreated GJ, all brands of HGJs indicated significantly lower efficacy in the inhibition of the CYP3A oxidation, except for one brand that showed no significant change (Fig. 1). The inhibitory effects of untreated GJ and HGJ ranged from 54.2 to 85.9 % ($72.4 \pm 11.2$) and from 25.0 to 71.1% ($53.4 \pm 12.2$), respectively. The differences between the two, caused by the loss of furanocoumarins in heating, were defined as net potentials of furanocoumarin-induced CYP3A inhibitions (FCIs) and expressed as percentages compared with the control velocity in a 6β-hydroxytestosterone – production reaction without GJ (Fig. 2). The results show that FCIs ranged from 4.0 to 35.9 % ($19.0 \pm 8.5$).

## 3. Discussion

We reported that furanocoumarin derivatives in GJ were eliminated by heat treatment. The concentrations of bergamottin and 6′,7′-dihydroxybergamottin were decreased in a time- and temperature-dependent manner, by 82.5 and 97.9% respectively, after incubation for 1 h at 95 °C (Uesawa and Mohri, 2006b). In addition, the effect of each GJ sample on the microsomal testosterone 6β-oxidation

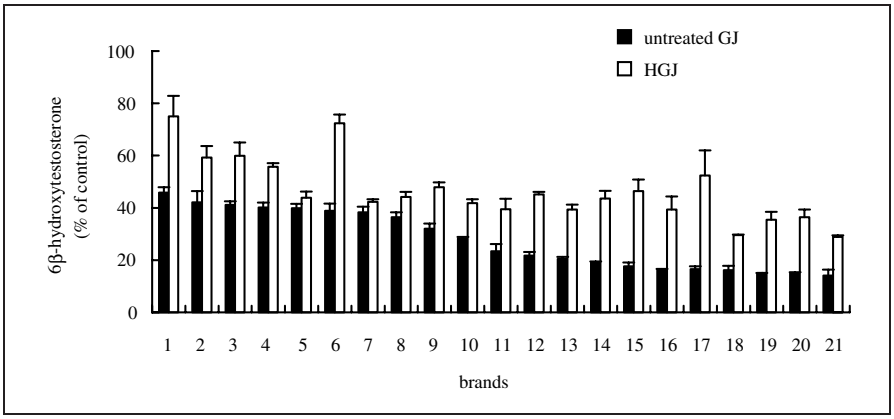


Fig. 1:
6β-Oxidation rates of testosterone with human liver microsomes and untreated GJ or HGJ. The control mixture included no GJ. Each point and vertical bar represents the mean and S.D. (n = 3)

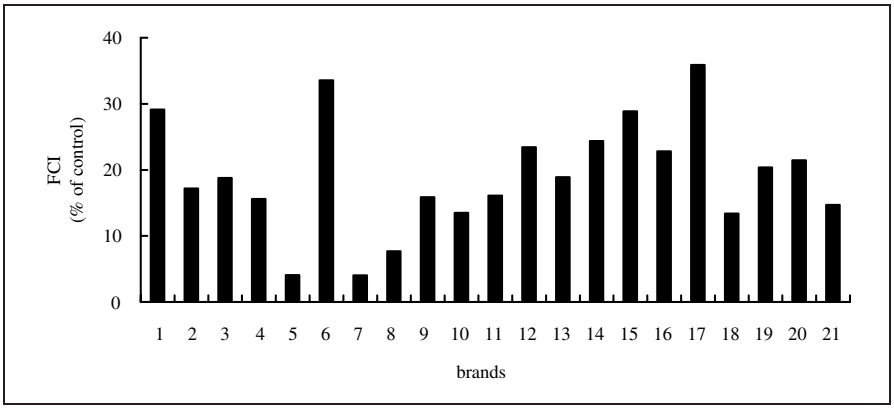


Fig. 2:
FCI values in each GJ sample

was observed in the previous study. The inhibitory effects of heated GJ were decreased in a time- and temperature-dependent manner, as in the case of bergamottin and 6′,7′-dihydroxybergamottin concentrations. Furthermore, 2 mL of HGJ treated for 60 min at 95 °C was administered into the rat duodenum. After 30 min, nifedipine was administered intraduodenally. No significant increase in the area under the plasma concentration-time curve (AUC) of nifedipine was observed in the rats given HGJ. These results suggest that heat treatment of GJ reduces the concentrations of furanocoumarins, thus eliminating the potential for drug interactions thereby indicating that the retained inhibitory effect of HGJ *in vitro* is not reflected in the pharmacokinetic interaction potential because of the loss of furanocoumarins. Meanwhile, the component inhibitory potentials eliminated by the heat treatment of GJ may be able to reflect the action *in vivo*. The results indicate that heat treatment could be useful in evaluating the potencies of GJs in the drug interactions caused by furanocoumarins. It is believed that the *in vitro* evaluation systems using only untreated GJ do not properly reflect the GJ-drug interactions *in vivo* because these interactions are induced by furanocoumarin derivatives such as bergamottin and 6′,7′-dihydroxybergamottin in GJ. Figures 1 and 2 show that order of each brand on the interaction potential estimated by FCI is not necessarily correspond to the case estimated by only untreated GJ. Therefore, we suggest that the inhibition potential of GJ may be estimated by subtracting the microsomal CYP3A activity with HGJ from those activities obtained with the corresponding untreated GJ. It is anticipated that the technical measurement of the GJ-drug interaction potentials using FCI established in the present study, may be an effective method to identify the intensity of GJ in the interactions.

## 4. Experimental

### *4.1. Materials*

Testosterone was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 6β-Hydroxytestosterone and corticosterone [internal standard (IS)] were purchased from Daiichi Pure Chemicals (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Pooled human hepatic microsomes were obtained from BD Biosciences (San Jose, CA, USA). Methanol, acetonitrile, and phosphoric acid of HPLC grade were used (Wako). All other chemicals were of reagent grade (Wako). The different brands of GJ were purchased from 15 local markets.

### *4.2. Heat treatment of GJ*

One mL samples of each of the GJ brands in 1.5-mL microtubes were treated at 95 °C for 60 min. TAITEC Dry-Thermo Unit TAH-1G (Taitec Co. Ltd., Saitama, Japan) was used in setting the temperatures. The heated GJ samples (HGJs) were used to measure the inhibition of microsomal oxidation *in vitro* after neutralizing with sodium hydroxide.

### *4.3. Assay of testosterone 6β-oxidation activities*

An assay of testosterone 6β-oxidation activity with human liver microsomes was performed according to a manual of NADPH Regenerating System (BD Biosciences, Inc., CA, USA). Briefly, the microsomes (150 μg) were incubated in 10% GJ or HGJ samples in 50 mM sodium phosphate buffer (pH 7.4) with 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride, and 0.2 mM testosterone (final incubation volume, 100 μL) at 37 °C. After 30 min of incubation, 1 mL of IS solution (2 μg/mL corticosterone in acetonitrile) was added to the reaction mixture. The sample was mixed vigorously for 20 s and centrifuged at 16,000 g for 5 min at 4 °C; then the supernatant (5 μL) was injected into LC/ESI/MS. ESI mass spectra were obtained using Shimadzu LCMS-2010EV LCMS system with an ESI probe (Shimadzu Co. Ltd.) equipped with a reversed-phase analytical Capcell Pak MGII-ODS column [2.0 mm (inside diameter) × 15 cm; particle size 5 μm (Shiseido Co. Ltd., Kyoto, Japan)]. The flow rate was set at 0.2 mL/min. $[M - H]^-$ ions at m/z 303.2 and 345.2 for 6β-hydroxytestosterone and corticosterone, respectively, were monitored for negative ions; the interface voltage was 4.5 kV, and the detector voltage was 1.5 kV. The heat block and CDL temperatures were 200 and 250 °C, respectively. Nitrogen was used as the nebulization gas at flow rates of 1.5 L/min. A mobile phase consisting of water and acetonitrile was

pumped through the column at a flow rate of 0.2 mL/min using a gradient ranging from 10 to 100% acetonitrile in 8 min and subsequently 100% for 12 min. Calibration curves (1 to 60 μM of 6β-hydroxytestosterone) were constructed using linear regression analysis.

### *4.4. Data analysis*

All data were expressed as mean ± SD. Pearson's correlation analysis and unpaired Student's t-test were used to test for a significant correlativity and significant differences in mean values, respectively. The significance level was set at $P < 0.05$.

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