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White and colored grapefruit juice produce similar pharmacokinetic interactions

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Colored (pink and red) grapefruit pulp contains lower amounts of the furanocoumarin derivatives that cause pharmacokinetic interactions than white grapefruit pulp. However, few studies have examined interactions with colored juice products. Therefore, we examined the potential interactions of both white and colored grapefruit products by measuring the concentrations of furanocoumarin derivatives and inhibition of the metabolizing cytochrome P450 (CYP) 3A enzymes, the target of the furanocoumarins. We measured concentrations of three major furanocoumarin derivatives, bergaptol, bergamottin, and 6′,7′-dihydroxybergamottin, with high-performance liquid chromatography in 21 brands of grapefruit juice sold in Japan, including 14 white and 7 colored brands. The mean difference in bergaptol, bergamottin, and 6′,7′-dihydroxybergamottin concentrations in white grapefruit juice samples was 1.59, 0.902, and 1.03 times, respectively, the amounts in colored samples. White samples inhibited CYP3A-mediated testosterone-6β oxidation in human liver microsomes by 1.04 and 0.922 times (whole juice and furanocoumarin, respectively) the inhibition by colored juice. Thus, colored grapefruit juice may produce drug interactions at the same rate as white grapefruit juice.

1. Introduction

Grapefruit juice (GFJ) increases plasma concentrations of drugs such as calcium channel inhibitors, immunosuppressants, and HMG-CoA reductase inhibitors, which are all metabolized by intestinal cytochrome P450 (CYP) 3A (Fuhr 1998; Kane and Lipsky 2000; García 2003; Dahan and Altman 2004). Furanocoumarin derivatives in GFJ such as bergamottin, 6′,7′-dihydroxybergamottin, and paradisins inhibit intestinal oxidation of these drugs by CYP3A (Bailey 1991; Schmiedlin-Ren 1997; He 1998; Mohri and Uesawa 2001; Row 2006; Uesawa and Mohri 2006a, b). We previously demonstrated that furanocoumarin-dependent CYP3A inhibition (FCI) disappeared after heat treatment of GFJ at 95 °C for 60 min (Uesawa and Mohri 2006b). We also found considerable variation in FCI among GFJ brands (Uesawa and Mohri 2008).
Fukuda et al. showed that bergamottin, paradisin A, and paradisin B levels in pink GFJ from ruby red grapefruits were 66.3%, 57.5% and 70.5% of white GFJ, and that ruby red grapefruit flesh contained one-third the furanocoumarin content of white flesh (Fukuda 2000). De Castro et al. also reported that colored (pink/red) GFJ products contained 41.0% and 35.3% of bergamottin and 6′,7′-dihydrobergamottin compared to white GFJ, and red grapefruit pulp contained little bergamottin (De Castro 2006). Here, we measured the furanocoumarin content and CYP3A inhibition in different GFJ brands.

2. Investigations, results and discussion

2.1. Comparison of furanocoumarin levels between white and colored GFJ

We measured levels of furanocoumarin derivatives in various brands of GFJ. Bergaptol concentrations in white and colored GFJ ranged from 11.0 to 96.0 μM (mean: 48.2, SE: 7.26, n: 14) and from 9.24 to 65.5μM (mean: 30.3, SE: 9.13, n: 7), respectively (Fig. 1a). 6′,7′-Dihydrobergamottin concentrations in white and colored GFJ ranged from 0.351 to 13.3 μM (mean: 6.08, SE: 1.27, n: 14) and from 0.667 to 20.5 μM (mean: 6.74, SE: 2.67, n: 7), respectively (Fig. 1b). Bergamottin concentrations in white and colored GFJ ranged from 1.92 to 29.1 μM (mean: 13.3, SE: 1.77, n: 14) and from 4.21 to 23.7 μM (mean: 12.9, SE: 2.87, n: 7), respectively (Fig. 1c). These differences were not significant.
Bergamottin and 6′,7′-dihydroxybergamottin are critical for the pharmacokinetic interactions of GFJ at intestinal CYP3A (Uesawa and Mohri 2006a, b). These furanocoumarin derivatives were present at the same levels in white and colored GFJ, and therefore might have similar interactions with CYP3A. Other studies showed that levels of furanocoumarin derivatives in fruit meat and juice products made from colored grapefruits were lower than in white grapefruits (Fukuda et al. 2000; De Castro et al. 2006). For example, white grapefruit pulp contained 13 μg/g tissue of bergamottin, and red grapefruits contained only a small percent of this concentration (De Castro et al. 2006). Our

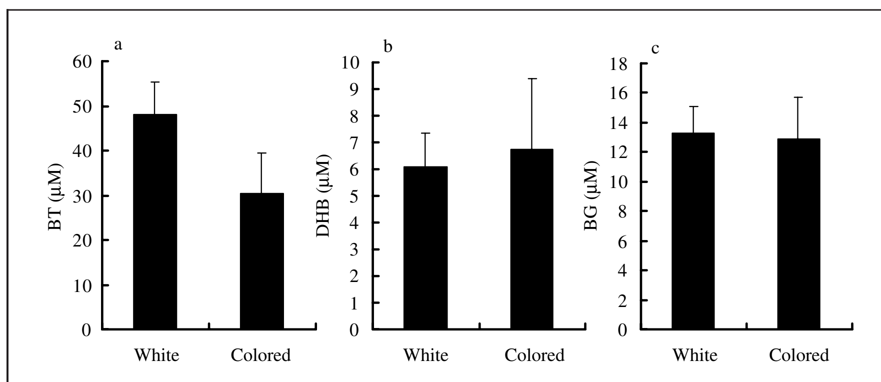

Fig. 1:
Concentrations of bergaptol (BT, a), 6′,7′-dihydrobergamottin (DHB, b), and bergamottin (BG, c) in white and colored GFJ samples. Each point and vertical bar represents the mean and SE. (n = 14 and 7 in white and colored GFJ, respectively).

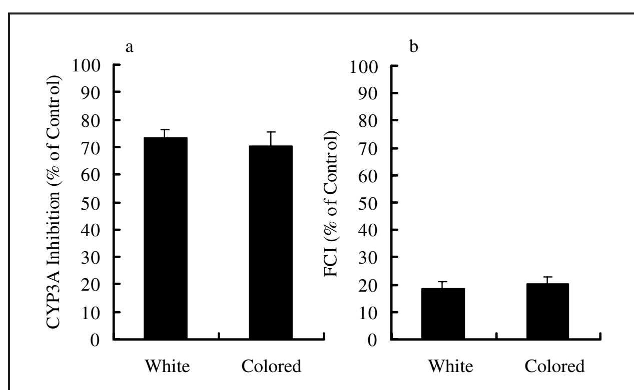

Fig. 2: Inhibitory effects (a) and FCI values (b) on the 6β-oxidation rates of testosterone with human liver microsomes with white and colored GFJ samples. The control mixture included no GFJ. Each point and vertical bar represents the mean and SE. (n = 14 and 7 in white and colored GFJ, respectively).

findings of similar levels in both white and colored GFJ may be due to variability in GFJ brands, which result from differences in the production process. GFJ brands made from colored cultivars may also include furanocoumarin derivatives from the non-pulp tissues.

2.2. Inhibition of CYP3A oxidation and FCI in white and colored GFJ

We measured CYP3A-dependent testosterone 6β oxidation in human liver microsomes after addition of GFJ. Oxidation levels with white GFJ were 58.8% to 85.9% (mean: 73.4%, SE: 2.83%, n: 14) and with colored GFJ, from 54.2% to 83.9% (mean: 70.4%, SE: 4.95%, n: 7) of control (Fig. 2a), and were not significantly different. Oxidation levels with white juice FCI were 4.04% to 35.9% (mean: 18.5%, SE: 2.53%, n: 14) and with colored juice FCI, from 13.4% to 29.1% (mean: 20.1%, SE: 2.59%, n: 7) (Fig. 2b), again were not significantly different.

These results are consistent with the similar levels of furanocoumarin derivatives seen in white and colored GFJ. The FCI values at CYP3A should relate to the pharmacokinetic inhibition of each juice brand *in vivo* (Uesawa and Mohri 2006b). Thus, both GFJs seem to possess equal risk of causing drug interactions.

3. Experimental

3.1. Materials

Anthracene [internal standard (IS)] was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Bergamottin and 6′,7′-dihydrobergamottin were purchased from Daiichi Pure Chemicals Co. Ltd. (Tokyo, Japan). Bergaptol was obtained from Funakoshi Co. Ltd. (Tokyo, Japan). Testosterone was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 6β-Hydroxytestosterone and corticosterone [internal standard (IS2)] were purchased from Daiichi Pure Chemicals and Wako Pure Chemical Industries, 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). Different GFJ brands, including 14 white and 7 colored GFJ, were purchased from 15 local markets in Japan.

3.2. Detection of furanocoumarin derivatives in GFJ

The detection of furanocoumarin derivatives in GFJ was performed as previously described (Mohri and Uesawa 2001). Briefly, juice samples were analyzed using HPLC equipped with a reverse-phase analytical Capcell Pak SG-Phenyl column [4.6 mm (inside diameter) × 25 cm; particle size 5 μm; Shiseido Co. Ltd., Tokyo, Japan]. A photodiode array detector (MD-910, JASCO Corp. Ltd., Tokyo, Japan) was used because it reveals the characteristic UV-absorption spectrum of furanocoumarin derivatives, commonly with 311 nm as the maximum wavelength (Uesawa and Mohri 2005). A mobile phase consisting of 0.1% phosphoric acid and acetonitrile was pumped through the column at 1.0 mL/min with a gradient of 0 to 5 min, 40% acetonitrile, and subsequently from 40% to 100% acetonitrile in 30 min. Then, 400 μL of IS solution (10 μg/mL anthracene in acetonitrile) were added to 100 μL of the GFJ in a 2 mL plastic tube. After being shaken vigorously, the sample was centrifuged at 16,000 × g and 4 °C for 10 min; 50 μL of the supernatant were injected directly into the HPLC system. Calibration curves (1 to 50 μg/mL of bergamottin, 6′,7′-dihydroxybergamottin, and bergaptol) were drawn by linear least-squares regression analysis. The detection limits for bergamottin, 6′,7′-dihydroxybergamottin, and bergaptol were 0.1, 0.1, and 0.05 μg/mL, respectively.

3.3. Heat treatment of GFJ

One mL samples of each GFJ brand in 1.5-mL microtubes were treated at 95 °C for 60 min to remove furanocoumarin derivatives, such as bergamottin and 6′,7′-dihydroxybergamottin, related to CYP3A inhibition. A TAITEC Dry-Thermo Unit TAH-1G (Taitec Co. Ltd., Saitama, Japan) was used in setting the temperatures. The heated GFJ samples (HGJs) were used to measure the inhibition of microsomal oxidation *in vitro* after neutralizing with sodium hydroxide (Uesawa and Mohri 2006b, 2008).

3.4. Assay of testosterone 6β-oxidation activities and furanocoumarin-induced CYP3A inhibition (FCIs)

An assay of testosterone 6β-oxidation activity with human liver microsomes was performed according to the manual of the NADPH Regenerating System (BD Biosciences, Inc., CA, USA). Briefly, microsomes (150 μg) were incubated in 10% of neutralized GFJ or HGJ samples in 50 mM sodium phosphate buffer (pH 7.4) with 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/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 IS2 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 a 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, and 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 °C 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 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. The differences between testosterone 6β-oxidation activities with GFJ and HGJ were defined as net potentials of furanocoumarin-induced CYP3A inhibition (FCIs) and expressed as percentages of the control velocity in a 6β-hydroxytestosterone-production reaction without GFJ.

3.5. Data analysis

All data are expressed as mean ± SE. Unpaired Student's t-test were used to test for significant differences in mean values. The significance level was set at $P < 0.05$.

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