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Inhibitory effects of fruit juices on cytochrome P450 3A (CYP3A) activity

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Abstract

There have been very limited reports on the effects of commercial fruit juices on human cytochrome P450 3A (CYP3A) activity. Therefore, the inhibitory effects of readily available commercial fruit juices on midazolam 1`-hydroxylase activity, a marker of CYP3A, were evaluated in pooled human liver microsomes. The fruit juices investigated were black raspberry, black mulberry, plum and wild grape. White grapefruit, pomegranate and orange juice were used as positive and negative controls. The black mulberry juice showed the most potent inhibition of CYP3A except for grapefruit juice. The inhibition depended on the amount of a fruit juice added to the incubation mixture. The inhibitory potential of human CYP3A was in the order: grapefruit > black mulberry > wild grape > pomegranate > black raspberry. The IC_{50} values of all fruit juices tested were reduced after preincubation with microsomes in the presence of NADPH generating system, suggesting that a mechanism based inhibitory component was present in these fruit juices as in the case of grapefruit. The results suggest that like grapefruit juice, commercial fruit juices also have the potential to inhibit CYP3A-catalzyed midazolam 1`-hydroxylation. Therefore, in vivo studies investigating the interactions between fruit juices such as black mulberry and wild grape and CYP3A substrates are necessary to determine whether inhibition of CYP3A activity by fruit juices is clinically relevant.

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Introduction

Several fruits have been reported to cause food-drug interactions, including grapefruit(Bailey et al., 1998) and pomegranate(Hidaka et al., 2005). As a well-known example, consumption of grapefruit juice has been shown to increase the plasma concentration of drugs, such as calcium channel antagonists(Bailey et al., 1993; Bailey et al., 1998), cyclosporine(Yee et al., 1995), midazolam(Kupferschmidt et al., 1995), HIV protease inhibitors(Kupferschmidt et al., 1998), and HMG-CoA reductase inhibitors(Lilja et al., 1998; Lilja et al., 1999). A single glass of grapefruit juice has the potential to augment the oral bioavailability and to enhance the beneficial or adverse effects of a broad range of medications.

Cytochrome P450 (P450) is a representative enzyme involved in hepatic drug metabolism, which is crucial for the elimination of many therapeutic drugs. Among the members of the P450 family, CYP3A is the most important enzyme and is involved in the majority of the P450-catalyzed metabolism(Guengerich, 1996). Studies have shown that grapefruit juices strongly inhibited drug metabolism mediated by CYP3A subfamily in the gut wall (Bailey et al., 1998). On the other hand, it has been documented that orange juice is incapable of inhibiting the catalytic activity of CYP3A, based on the previous results that the systemic exposure of felodipine, a CYP3A substrate, was not affected by orange juice(Bailey et al., 1991). Taking these results into account, the inhibitory effects of a fruit appeared to depend on the fruit species because of the difference of the components contained in each fruit(Guo et al., 2000; Hidaka et al., 2004). These results prompted us to investigate the potency of fruit juices for a possible source of drug interactions. Under these circumstances, not only drug interactions between drugs but also those seen with food have attracted much attention.

So far, there have been few reports on the effects of commercial fruit juices on human CYP3A activity. Therefore, in this study, we investigated whether various commercial fruit juices that people drink inhibit CYP3A activity. We have also compared the inhibitory potential of commercial fruit juices with that of grapefruit juice, a well-known CYP3A inhibitor. The ability of the fruit juices to inhibit the midazolam 1`-hydroxylation mediated by CYP3A was examined by using human liver microsomes.

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Materials and Methods

Chemicals and Reagents. 1`-Hydroxymidazolam and midazolam were purchased from Ultrafine Chemicals (Manchester, UK). Pooled human liver microsomes (H161) were obtained from BD Gentest Co. (Woburn, MA). All chemicals and solvents were of the highest grade that was commercially available.

Samples of Fruit juice. Fruit juices were obtained from local commercial sources and their available information is shown in Table 1. The fruit juice samples were stored at 4 °C until use. All samples were tested soon after the juice package was opened.

**Assay of Midazolam 1`-hydroxylase Activity of Human CYP3A.** The assay of midazolam 1`-hydroxylase activity of human CYP3A was performed according to the method of Liu et al. (2004) with minor modifications. Briefly, each incubation was performed with 0.5 mg/ml pooled human liver microsomes (H161, Gentest) in 100 mM phosphate buffer (pH 7.4) at a final volume of 250 μl. Midazolam was dissolved in methanol. The final concentration of methanol did not exceed 0.5%. The incubation mixtures containing microsomes and midazolam (10 μM final concentration) were pre-incubated for 5 min at 37 °C. After preincubation, reactions were initiated by addition of the NADPH-generating system (3.3 mM G6P, 1.3 mM β-$NADP^+$, 3.3 mM $MgCl_2$, and 1.0 unit/ml G6PDH) and stopped after 15 min by placing the incubation tubes on ice and adding 100 μl of ice-cold methanol containing internal standard (lansoprazole, 1μM). Incubation mixtures were centrifuged at 20,000*g* for 10 min at 4 °C, and aliquots of the supernatants were analyzed by HPLC as described previously(Liu et al., 2004a). Reaction rates were linear with incubation time and microsomal protein contents under these conditions.

Inhibitory effects of fruit juices on CYP3A activity. The inhibitory effects of commercial fruit juices on CYP3A activity were investigated according to Guo et al. (2000)

with minor modifications. Briefly, an appropriate amount of commercial fruit juice was dried with a concentrator. The reaction mixture described above (before the addition of NADPH-generating system) was added. The incubation was performed as described above. The inhibitory effects of a fruit juice on midazolam 1`-hydroxylation were expressed as a percentage of the residual activity in comparison with the control in the absence of fruit juice. All incubations were performed in triplicate, and the mean values were used for analysis. To evaluate the effect of preincubation on inhibitory potency, fruit juice was preincubated for 20 min with an NADPH-generating system, buffer, and microsomes. The reaction was started by addition of midazolam (10 μM final concentration).

Data Analysis. The IC_{50} values (concentration of the inhibitor causing 50% inhibition of the original enzyme activity) were determined from the following equation using WinNonlin software (Pharsight, Mountain View, CA).

$$\% \text{ of control activity} = 100A \times (1- (I/(I + IC_{50})))$$

where A is the maximum activity, I is the concentration of inhibitor, and IC_{50} is the inflection point on the curve.

Results and Discussion

The opportunity for a food-drug interaction is an everyday occurrence. The interaction can be particularly important when total drug disposition is altered. In the early 1990s, it was reported that coadministration of grapefruit juice with felodipine or nifedipine resulted in a large increase in the oral bioavailability of these drugs(Bailey et al., 1989; Bailey et al., 1991). Furanocoumarins found in grapefruit juice are now shown to be potent inhibitors of CYP3A in humans(Guo et al., 2000; Guo and Yamazoe, 2004). Furthermore, recent studies revealed that a component(s) of pomegranate inhibits the human CYP3A-mediated metabolism of carbamazepine, and pomegranate juice also alters the carbamazepine pharmacokinetics in rats(Hidaka et al., 2005). So far, there have been few reports on the effects of commercial fruit juices on human CYP3A activity.

In the present study, various commercial fruit juices that people drink have been tested for their effects on human CYP3A-mediated midazolam 1`-hydroxylase activity as a tool for assessing their clinical significances in food-drug interaction. Inhibitory activity of fruit juices is shown in Fig. 1. The addition of commercial fruit juices caused the inhibition of the microsomal CYP3A activity. The inhibitory potential of midazolam 1`-hydroxylase activity of human CYP3A was in the order: grapefruit > black mulberry > wild grape > pomegranate > black raspberry (Table 2). Orange, plum, mandarin orange, carrot, soymilk, and tomato didn’t show inhibition on human liver microsomal CYP3A activity.

The IC_{50} values of black mulberry, black raspberry, and wild grape juice were reduced after preincubation with microsomes in the presence of NADPH generating system, suggesting that a mechanism based inhibitory component was present in these fruit juices as in the case of grapefruit(Guo et al., 2000), pomegranate(Hidaka et al., 2005) and Schizandra fruit(Iwata et al., 2004). Therefore, it may be of interest to determine the identity of the

chemical(s) in these fruit juices that exhibits potent inhibition of CYP3A activity. Understanding the nature of these chemicals would enable health care professionals to avoid food-drug interactions.

Among the fruit juices tested, black mulberry juice showed potent inhibition on human CYP3A activities, and the inhibitory effects are somewhat greater than those of pomegranate(Hidaka et al., 2005), known inhibitor of CYP3A. The reproducibility of the effects of mulberry juice to inhibit CYP3A activity was examined (Table 3). Irrespective of the timing of the purchase of the mulberry juice, the same extents of inhibition of CYP3A activity by the respective mulberry juices were observed. Black mulberry is indigenous to western Asia. It is also common to Europe. It has been reported that black mulberry contains certain species of anthocyanins, and it shows potent antioxidant activity(Liu et al., 2004b). Traditionally, mulberry fruit has been used as a medicinal agent to nourish the blood, benefit the kidneys, and treat weakness, fatigue, anemia, and premature graying of hair. Therefore, due to its widespread use, higher mulberry consumption allows for an increased possibility of food-drug interaction. The possibility of the adverse food-drug interaction by the mulberry juice with drugs that are cleared primarily by CYP3A-mediated pathways should be examined in vivo considering pomegranate-carbamazepine interaction potential in rats(Hidaka et al., 2005).

In conclusion, we have shown that a component(s) of commercial juices of fruit such as raspberry, mulberry, and wild grape is an inhibitor or a mechanism-based inhibitor of human CYP3A activity in vitro, and the inhibitory potency of these fruit juices was higher than that observed with pomegranate juice. From these results, it is possible that high amounts of these juices may interact with drug that is metabolised by CYP3A in certain individuals. It is important to note, however, that inhibition of CYP3A activity in vitro does not necessarily translate into drug interactions in vivo. Therefore, in vivo studies investigating the

interactions between fruit juices such as black raspberry and mulberry and CYP3A substrates are necessary to determine whether inhibition of CYP3A activity by fruit juices is clinically relevant.

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Footnotes

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Figure legends

Figure 1. Inhibition of human CYP3A-catalyzed midazolam 1`-hydroxylation activity by commercial fruit juices.

Microsomes were incubated with 10 μM midazolam and various concentrations (1% (black bar) or 5% (white bar))of fruit juice. The corresponding control activity of midazolam 1`-hydroxylation by human liver microsomes was 3.73 nmol min^{-1} mg^{-1}protein. The concentration of fruit juice is equivalent to % of natural strength. Data shown are averages of duplicate experiments.

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TABLE 1.

Available information about the commercial fruit juices used in this study

Fruit juice			Origin	Maker	Processing
Fruit	Trade name	Species			
Grapefruit(white)	Sunup®	*Citrus paradisi*	USA	Maeil, Korea	Squeezed
Orange	Sunkist®	*Citrus sinesis*	USA	Haitai, Korea	Squeezed
Pomegranate	Aysu®	*Punica granatum*	Taiwan	Hanara, Korea	Squeezed
Black raspberry	Aysu®	*Rubus coreanus*	Korea	Hanara, Korea	Squeezed
Black mulberry	Aysu®	*Morus nigra*	China	Hanara, Korea	Squeezed
Plum	Maeone®	*Prunus mume*	Taiwan	Bohae, Korea	Reconstituted
Wild grape	Jirisan®	*Vitis coignetiae*	Korea	Doore, Korea	Squeezed
Mandarin orange	Jeju gamgyul®	*Citrus unshiu Marcovitch*	Korea	Lotte, Korea	Squeezed
Carrot	Nemome®	*Daucus carota*	Korea	Namyang, Korea	Squeezed
Soymilk	Sahmyook®	*Glycine max*	USA	Sahmyook, Korea	Reconstituted
Tomato	Jayeoneun®	*Lycopersicon esculentum*	Chile	Woongjin, Korea	Squeezed

TABLE 2.

Effects of components contained in commercial fruit juices on midazolam 1`-hydroxylase activity of human liver microsomes.

Values are presented as mean ± S.D. of triplicate assays. The amount of fruit juice added to the incubation mixture was 0, 0.2, 0.5, 1, 2.5, 5, 10, and 20% (v/v). The control activity of midazolam 1`-hydroxylation by human liver microsomes determined in the absence of fruit juice was 3.73 nmol min^{-1} mg^{-1} protein.

Fruit juice	IC_{50} (%)	
	With preincubation	No preincubation
White grapefruit	0.09 ± 0.03	0.32 ± 0.11
Pomegranate	11.3 ± 1.0	34.2 ± 7.2[a]
Black raspberry	15.0 ± 2.3	25.3 ± 9.1[a]
Black mulberry	2.96 ± 0.33	6.22 ± 0.47
Wild grape	4.40 ± 0.77	5.92 ± 0.30

[a] Values were extrapolated.

TABLE 3.

Reproducibility of CYP3A inhibition by mulberry juice

Values are presented as mean ± S.D. of triplicate assays. The amount of fruit juice used in assays was 12.5 μl (5.0%, *v/v*). The control activity of midazolam 1`-hydroxylation by human liver microsomes determined in the absence of fruit juice was 3.65 nmol min^{-1} mg^{-1} protein.

Purchase Date of Mulberry Juice	Residual Activity (%)
April 9, 2005	40.0 ± 1.9
June 18, 2005	39.0 ± 3.8
August 13, 2005	40.9 ± 3.1
October 15, 2005	36.6 ± 5.2

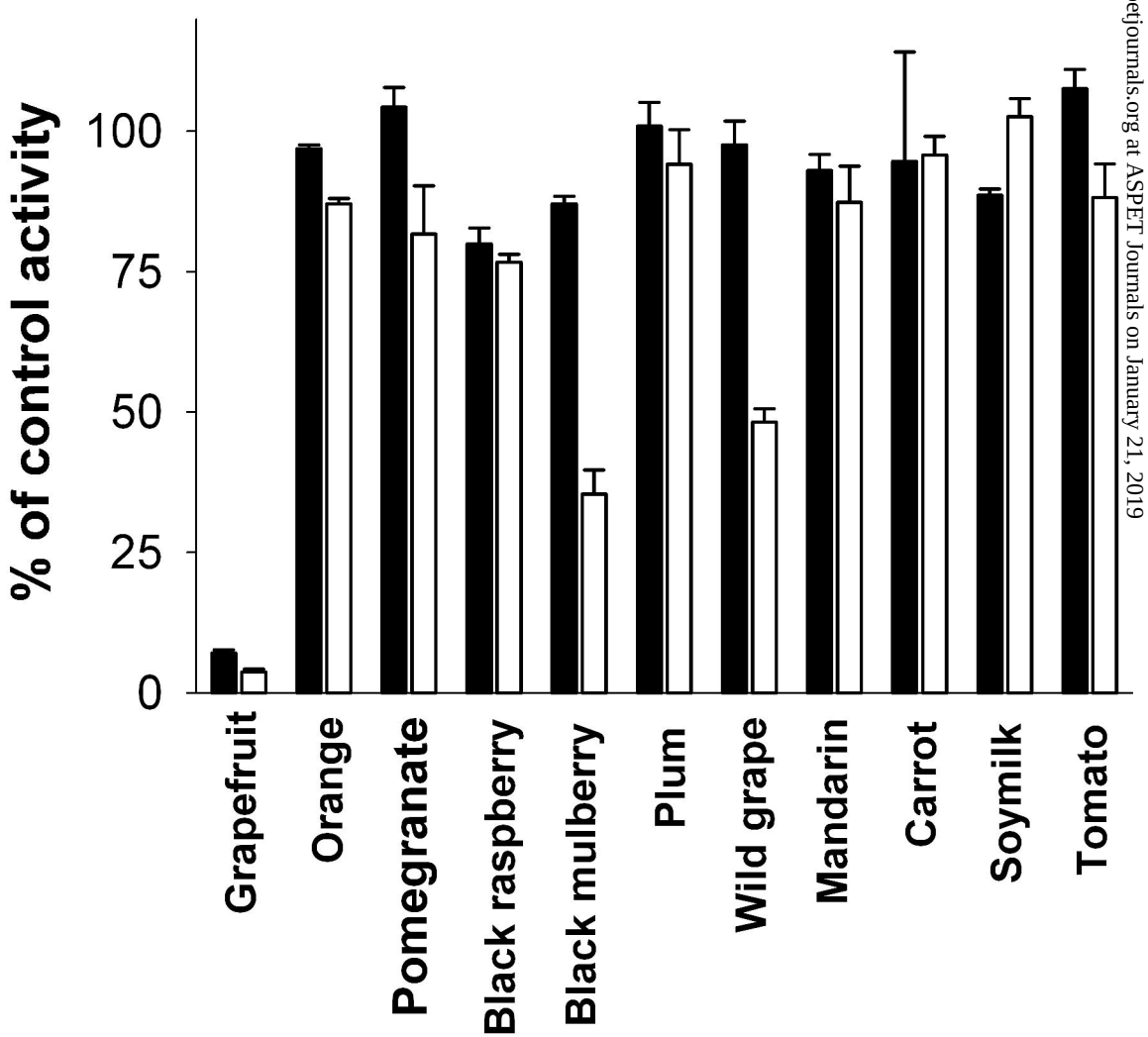

Fig. 1