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Short Communication

POTENT INHIBITION BY STAR FRUIT OF HUMAN CYTOCHROME P450 3A (CYP3A) ACTIVITY

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ABSTRACT:

There has been very limited information on the capacities of tropical fruits to inhibit human cytochrome P450 3A (CYP3A) activity. Thus, the inhibitory effects of tropical fruits on midazolam 1′-hydroxylase activity of CYP3A in human liver microsomes were evaluated. Eight tropical fruits such as common papaw, dragon fruit, kiwi fruit, mango, passion fruit, pomegranate, rambutan, and star fruit were tested. We also examined the inhibition of CYP3A activity by grapefruit (white) and Valencia orange as controls. The juice of star fruit showed the most potent inhibition of CYP3A. The addition of a star fruit juice (5.0%, v/v) resulted in the almost complete inhibition of midazolam 1′-hydroxylase activity (residual activity of 0.1%). In the case of grapefruit, the residual activity was 14.7%. The inhibition depended on the amount of fruit juice added to the incubation mixture (0.2–6.0%, v/v). The elongation of the preincubation period of a juice from star fruit (1.25 or 2.5%, v/v) with the microsomal fraction did not alter the CYP3A inhibition, suggesting that the star fruit did not contain a mechanism-based inhibitor. Thus, we discovered filtered extracts of star fruit juice to be inhibitors of human CYP3A activity in vitro.

In early 1990s, grapefruit juice was documented to increase the oral bioavailability of felodipine to higher than 250% compared with that seen with water (Bailey et al., 1989, 1991). Adverse experiences, mainly accounted for by headaches, facial flushing, and lightheadedness, were reported to be more frequent after intake of grapefruit juice than of water. Subsequently, various drugs that were orally administered have been proven to interact with grapefruit juice (Bailey et al., 1993; Dcharme et al., 1995; Kupferschmidt et al., 1995). These drugs differ in their chemical structure and pharmacological property but are commonly metabolized by a form of cytochrome P450, namely cytochrome P450 3A4 (CYP3A4). Studies have shown that the furanocoumarin derivatives identified from grapefruit juice strongly inhibited the catalytic activity of CYP3A4 and caused the decrease of the first-pass metabolism of orally administered therapeutic drugs catalyzed by CYP3A4 (Fukuda et al., 1997; Guo et al., 2000; Tassaneeyakul et al., 2000). On the other hand, it has been documented that common orange juice is incapable of inhibiting the catalytic activity of CYP3A4 (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 (Bailey et al., 1991; Guo et al., 2000).

In areas with a warm climate, a wide variety of fruits have been produced. These fruits, namely tropical fruits, are taken by people living all over the world. So far, there have been few reports on the effects of these tropical fruits on human CYP3A activity.

Therefore, in this study, we investigated whether the components present in the tropical fruits inhibited CYP3A activity. The ability of the fruits to inhibit the midazolam 1′-hydroxylase activity of CYP3A was examined by using human liver microsomes.

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Materials and Methods

Chemicals. 1′-Hydroxymidazolam and midazolam were purchased from Wako Pure Chemicals (Tokyo, Japan). Pooled human liver microsomes were obtained from Daiichi Pure Chemicals (Tokyo, Japan). All chemicals and solvents were of the highest grade commercially available.

Fruit Samples. Tropical fruits, common papaw (*Carica papaya*) (Hawaii), dragon fruit (*Hylocereus undatus Brit. et Rose*) (Okinawa, Japan), kiwi fruit (*Actinidia chinensis*) (Bay of Plenty, New Zealand), mango (*Mangifera indica* L.) (Queensland, Australia), passion fruit (*Punica granatum*) (Fukushima, Japan), pomegranate (*Nephelium lappaceum*) (California), rambutan (*Passiflora edulis SIMS*) (Queensland, Australia), star fruit (*Averrhoa carambola*) (Miyazaki, Japan), grapefruit (white) (*Citrus paradise*) (Florida), and Valencia orange (*Citrus sinensis cv. Valencia*) (Australia) were obtained from local commercial sources. Fruit samples were stored at 4°C until use. All the fruit samples were tested soon after they were squeezed and filtered.

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 Fujita et al. (2003) with minor modifications. Briefly, a typical incubation mixture consisted of 100 mM sodium potassium phosphate buffer (pH 7.4), 50 μM EDTA disodium salt, an NADPH-generating system (0.5 mM $NADP^+$, 5 mM $MgCl_2$, 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 concentration of midazolam was 10 μM. The protein content and reaction time were predetermined based on linearity between microsomal protein concentration (up to 0.2 mg/ml) and reaction time (up to 4 min) versus metabolite formation rate. According to the results, the protein content and the reaction time were determined to 0.2 mg/ml and 4 min, respectively. Reactions were initiated by the addition of midazolam and terminated by the addition of 5 ml of ethyl acetate. Analysis of the 1′-hydroxymidazolam metabolite was performed by high-performance liquid chromatography as described by Fujita et al. (2003).

Inhibitory Effects of Tropical Fruits on CYP3A Activity. The inhibitory effects of tropical fruits on CYP3A activity were investigated by the method of

TABLE 1

Effects of components contained in tropical fruits on midazolam 1′-hydroxylase activity of human liver microsomes

Values are presented as mean ± S.D. of triplicate assays. The amount of fruit juice used in assays was 25 μl (5.0%, v/v). The control activity of midazolam 1′-hydroxylation by human liver microsomes determined in the absence of fruit juice was 1.68 nmol/min/mg protein.

Tropical Fruits	Residual Activity
	(%)
Common papaw	11.4 ± 1.9
Dragon fruit	94.3 ± 2.1
Kiwi fruit	82.2 ± 3.4
Mango	66.4 ± 6.3
Passion fruit	97.5 ± 1.9
Pomegranate	3.2 ± 0.1
Rambutan	75.2 ± 4.7
Star fruit	0.1 ± 0.0
Grapefruit (white)	14.7 ± 0.5
Valencia orange	60.8 ± 4.9

Guo et al. (2000) with minor modifications. Briefly, an appropriate amount of a tropical fruit juice was dried with a concentrator. The reaction mixture described above (before the addition of midazolam) was added and the residue of the tropical fruit juice was resuspended with a vortex mixer at full power for 2 s. After preincubation of the mixture at 37°C for 5 min, the substrate midazolam was added. The reaction was performed as mentioned above. The inhibitory effects of a tropical fruit on midazolam 1′-hydroxylation were expressed as a percentage of the residual activity compared with the control in the absence of a tropical juice. Each assay was performed in duplicate.

Effects of Preincubation of a Fruit Juice on Human CYP3A Activity. As an index of mechanism-based inhibition, a fruit juice was preincubated at 37°C for 5, 10, 15, or 20 min in the reaction mixture according to the method mentioned above.

Results and Discussion

Inhibition of midazolam 1′-hydroxylase activity of human CYP3A by filtered extracts of various tropical fruits was examined. The results are summarized in Table 1. Among the tropical fruit juices tested, star fruit juice showed the strongest inhibition. The addition of star fruit juice (5.0%, v/v) caused the almost complete inhibition of midazolam 1′-hydroxylase activity of human CYP3A (0.1%) (Table 1). The inhibition of star fruit was stronger than that of grapefruit. The reproducibility of the effects of star fruit juice to inhibit CYP3A activity was examined (Table 2). Irrespective of the timing of the purchase of the star fruits, the period from purchase of the fruit to preparation of the juice, and the shops from which the star fruits were obtained, the same extents of inhibition of CYP3A activity by the respective star fruit juices were observed. As shown in Fig. 1, the inhibition depended on the amount of fruit juice added to the reaction mixture (0.2 to 6.0%, v/v).

TABLE 2

Reproducibility of CYP3A inhibition by star fruit

Values are presented as mean ± S.D. of triplicate assays. The amount of fruit juice used in assays was 25 μl (5.0%, v/v). The control activity of midazolam 1′-hydroxylation by human liver microsomes determined in the absence of fruit juice was 1.63 nmol/min/mg protein.

Purchase Date of Star Fruit	Shop	Preparation Date of Juice	Residual Activity
			(%)
November 19, 2003	A	November 20, 2003	0.1 ± 0.0
December 18, 2003	A	December 18, 2003	0.3 ± 0.1
January 27, 2004	A	February 3, 2004	0.4 ± 0.1
January 27, 2004	B	February 3, 2004	0.3 ± 0.1

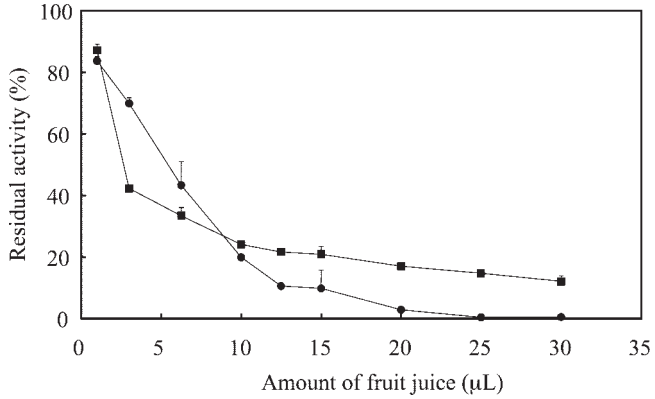

FIG. 1. *Inhibition of human CYP3A activity by star fruit juice or grapefruit juice.*

The amount of fruit juice added to the incubation mixture was 1.0, 3.0, 6.25, 12.5, 15, 20, 25, and 30 μl (0.2, 0.6, 1.25, 2.5, 3.0, 4.0, 5.0, and 6.0%, v/v), respectively. The control activity of midazolam 1′-hydroxylation by human liver microsomes determined in the absence of fruit juice was 1.64 nmol/min/mg protein. ●, star fruit; ■, grapefruit. Each point represents the means of three independent assays.

We examined whether the component(s) of star fruit inhibited human CYP3A in a mechanism-based manner. In contrast to the case with grapefruit, the inhibition potency of a juice from star fruit was not altered by the elongation of the preincubation period, suggesting that star fruit did not contain a mechanism-based inhibitor (Fig. 2).

Judging from these results, we concluded that filtered extracts of star fruit were inhibitors of human CYP3A activity in vitro, and the inhibition potency of them was almost the same as that of grapefruit components.

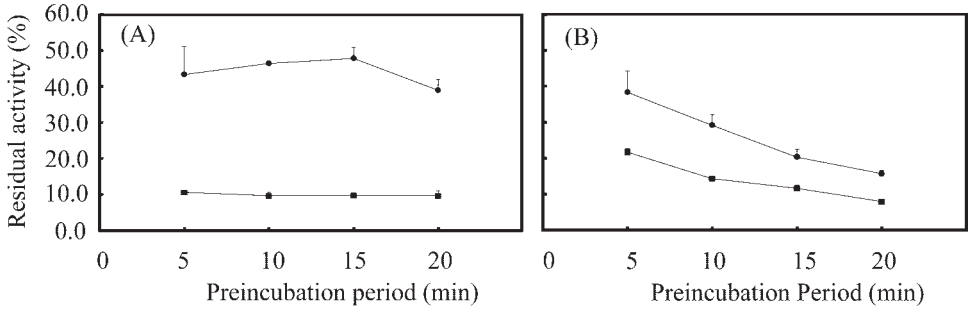

FIG. 2. *The effect of preincubation period on the inhibition of midazolam 1′-hydroxylase activity by star fruit or grapefruit juice.*

The amount of star fruit or grapefruit juice added to the incubation mixture was 6.25 or 12.5 μl (1.25 or 2.5%, v/v). Midazolam concentration was 10 μM. A fruit juice was added to the reaction mixture and preincubated for the indicated period before the reaction was started by the addition of a substrate. The control activity of midazolam 1′-hydroxylation by human liver microsomes determined in the absence of a fruit juice was 1.47 nmol/min/mg protein. A, star fruit; B, grapefruit; ●, 6.25 μl; ■, 12.5 μl. Each point represents the means of three independent assays.

Star fruit is believed to have originated in Ceylon and the Moluccas, but it has been cultivated in southeastern Asia and Malaysia for many centuries. It is commonly grown in south China, Taiwan, and India, and also in southern parts of Japan such as Kyushu and Okinawa islands. The star fruit was introduced in Southern Florida before 1887 (Morton 1987; Neto et al., 2003). It has been rather popular in the Philippines and Queensland (Australia), and moderately so in some of the South Pacific islands, in Central America, and in tropical western Africa. It is also common in Brazil.

Star fruit can be eaten out of hand or sliced and used in salads. The star fruit juice could be used in tropical drinks and smoothies. In Brazil, the star fruit is served as a fresh beverage, in natura, or as an industrialized juice, as it is also served throughout the world. People living in Brazil sometimes drink up to 500 ml of star fruit juice in a day (Neto et al., 1998, 2003). The fruit is also applied in chutney, curries, and tarts.

So far, there has been no clinical report suggesting the adverse food-drug interaction caused by the intake of star fruit. It has been demonstrated that the inhibition of a drug-metabolizing enzyme by a certain chemical observed in vitro was not necessarily consistent with that observed in vivo, since the chemical might be digested, might not be absorbed, or might interact with various components present in vivo (Gorski et al., 2004). Accordingly, the inhibition of CYP3A by star fruit juice may not be observed in vivo. However, the results obtained by us raised the hypothesis that the filtered extracts of the star fruit were capable of altering pharmacokinetics of therapeutic drugs coadministered via CYP3A inhibition, similar to the case with grapefruit. Thus, the possibility of the adverse food-drug interaction by the star fruit or star fruit juice with medicine through CYP3A metabolism should be examined in vivo.

It has been well documented that the components of grapefruit, such as (*R*)-6′,7′-dihydroxybergamottin, GF-I-1, GF-I-4, and GF-I-6 show the preincubation period-dependent inhibition of CYP3A activity (Guo et al., 2000). However, according to the data demonstrated by us (Fig. 2), the component(s) of star fruit appears not to be the mechanism-based inhibitor(s). It is of interest to know the chemical(s) in star fruit showing the potent inhibition of human CYP3A activity.

In the present study, we used a filter for the preparation of fruit juice to remove the residue of the fruit. It has been reported that filtering of fruit juice has influenced the inhibitory effects of the juice on CYP3A activity (Bailey et al., 1998). Therefore, the comparison of the inhibitory effects on CYP3A activity between star fruit juice and grapefruit juice observed in this study may not necessarily reflect that between juices that people drink.

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