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Effect of a Dietary Supplement Containing Raspberry Ketone on Cytochrome P450 3A Activity

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Abstract

Objective: Various herbal medicines and dietary supplements are known to alter the effects of drugs and cause severe complications. One of the most famous examples is St. John's wort, which promotes an increase of cytochrome P450 2C9 (CYP2C9), CYP2C19, and CYP3A4 expression, and reduces the effects of a large number of drugs. Raspberry ketone, an aromatic ingredient extracted from raspberries, is sold in Japan as a herbal supplement with a claimed slimming effect; however, its effect on CYP activity is unknown. To clarify the risk of an interaction between raspberry ketone-related supplements and CYP3A substrates, we performed an in vivo pharmacokinetic study using rats.

Methods: We investigated the effect of the oral administration of raspberry ketone on the pharmacokinetics of midazolam, a typical CYP3A substrate, in rats. St. John's wort, as a positive control, and raspberry ketone tablets at a dose of 50 mg/kg were administered every 12 h for 7 days, and at 24 h after the final treatment, 10 mg/kg midazolam was administered orally. The plasma concentration of midazolam was analyzed by high-performance liquid chromatography.

Results: Oral clearance of midazolam in the St. John's wort-treated group increased to 161% of that observed in the control group. Conversely, there was no significant difference between the raspberry ketone-treated and control groups. The mean residence time was essentially the same in all groups.

Conclusion: Because raspberry ketone is considered to suppress the accumulation of body fat, it is mainly taken by young healthy women for weight loss. Considering this population, information about the interaction of raspberry ketone with oral contraceptives, which are substrates of CYP3A, is of clinical importance. In this study, raspberry ketone was found to have little impact on CYP3A activity, unlike St. John's wort. These data indicate the low risk of an interaction between raspberry ketone-related supplements and many drugs metabolized by CYP3A.

Keywords: CYP3A; Raspberry ketone; Herbal supplement; Interaction; Rat; Pharmacokinetic parameters; St. John's wort

Introduction

The cytochrome P450 (CYP) family of enzymes is responsible for the oxidation of various xenobiotic chemicals, including drugs. The regulation of individual CYP enzymes is a complex subject, with examples of induction, direct inhibition, and stimulation. Nutrients and food additives can modify the activity of CYP enzymes and consequently influence the disposition of drugs. Various herbal medicines and dietary supplements are known to change the action of drugs and cause severe complications. One of the most famous examples is St. John's wort (SJW), which promotes an increase in CYP2C9 [1], CYP2C19 [2], and CYP3A4 [3] expression, while reducing the effects of warfarin [1,4], cyclosporine [5], oral contraceptives [6], and other medications.

Raspberry ketone, an aromatic ingredient extracted from raspberries, has been sold in Japan since 2002 as a herbal supplement with claimed slimming effects. The skeletal structure of raspberry ketone is partially similar to capsaicin, the principal component of capsicum fruits (Figure 1), which reportedly increases the secretion of catecholamines [7] and energy expenditure [8] while suppressing body fat accumulation [9]. Capsaicin is also a well-known inhibitor of CYP enzymes [10]. Raspberry ketone also reportedly decreases the body weight of obese rats [11]; however, its effect on CYP activity is unknown.

Since raspberry ketone suppresses the accumulation of body fat, mainly young healthy women may take it to reduce their weight. Considering the population who consume raspberry ketone, information about its interaction with oral contraceptives, which are substrates of CYP3A, is of clinical importance. In this study, we investigated the effect of the oral administration of raspberry ketone

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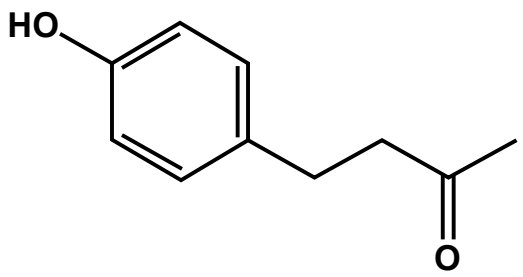

Figure 1: Structure of raspberry ketone.

on the pharmacokinetics of midazolam, a typical CYP3A substrate, in Wistar rats.

Materials and Methods

Chemicals and materials

Raspberry ketone was kindly supplied by Kanebo (Tokyo, Japan) as Vitarosso® tablets containing 16.7 mg of raspberry ketone per tablet. The tablets also contained *Gymnema sylvestris* and adlay seed extracts, inositol, and ascorbic acid as minor constituents. SJW extract was kindly supplied by Fancl (Yokohama, Japan). Midazolam was purchased from Yamanouchi Pharmaceuticals (Tokyo, Japan) as an injection (Dormicum®). Clonazepam, an internal standard for high-performance liquid chromatography (HPLC) analysis, was generously supplied by Sumitomo Pharmaceuticals (Osaka, Japan). All other chemicals and reagents were obtained from commercial sources and used without further purification.

Animal experiments

The animal experimental protocol was approved by the Animal Research Ethics Committee of Gunma University (Permit Number: 10-014), and its procedures were performed in accordance with the ethics guidelines of the committee. All surgery was performed under anesthesia, and all efforts were made to minimize suffering. Male Wistar rats (7 weeks of age, weighing 180-250 g) were purchased from Charles River (Tokyo, Japan). The rats were kept under a 12-h light/dark cycle at 24 ± 1°C, and allowed free access to standard laboratory rodent chow and water.

Five rats were used in each group. In the raspberry ketone group, raspberry ketone tablets were crushed and suspended in saline and administered to the rats at a dose of 50 mg/kg every 12 h for 7 days [12]. SJW extract was suspended in saline and administered to the rats in the SJW-treated group at a dose of 1,000 mg/kg every 24 h for 7 days [13]. The doses of raspberry ketone and SJW were chosen according to previous reports [12,13]. The same volume of saline was administered every 12 h to the rats in the control group for the raspberry ketone-treated group and every 24 h to the rats in the control group for the SJW-treated group. On the 7th day of treatment, the rats were fasted overnight. At 24 h after the final treatment with raspberry ketone or SJW, 10 mg/kg midazolam in a buffered solution was administered orally and blood samples were collected at 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min after administration. The blood samples were centrifuged immediately at 1,000×*g* for 10 min to obtain plasma. The plasma samples were stored at -20°C until HPLC analysis.

HPLC

The plasma concentration of midazolam was measured using HPLC. Briefly, 0.1 mL plasma was mixed with 0.1 mL internal standard solution (1 μg/mL clonazepam), 1 mL of 0.1 mol/L borate buffer (pH 10.0), and 2.5 mL n-hexane:dichloromethane (1:1, v/v). The mixture was shaken for 10 min and then centrifuged at 3,000×*g* for 5 min. The upper organic layer was transferred to a clean glass tube and evaporated to dryness at 40°C. The dried residue was dissolved in 0.1 mL of 65% methanol, and a 20-μL aliquot was injected into the HPLC system. A Waters 2960 separation module was used for the HPLC pump, and the absorbance at 245 nm was detected using a Waters 996 photodiode-array detector (Waters, Milford, MA, USA). The column was a YMC Pack Pro C18 ODS column (YMC, Tokyo, Japan) with a length of 250 mm and a diameter of 4.6 mm, and maintained at 40°C. The mobile phase was 65% methanol and pumped at a flow rate of 1.0 mL/min. The detection limit of midazolam was 50 ng/mL.

Data analysis

The pharmacokinetics of midazolam after the oral administration of raspberry ketone or SJW were analyzed using the model-independent moment analysis method. The area under the plasma concentration curve (AUC) and area under the moment curve (AUMC) were calculated using the trapezoidal method from 0 to 180 min. The oral clearance rate (CL_{oral}) and mean residence time (MRT) of midazolam were calculated using the following equations:

$$CL_{oral}=dose/AUC$$

$$MRT=AUMC/AUC$$

The apparent volume of distribution (Vd) divided by bioavailability (F) was calculated using the following equation:

$$Vd/F=MRT \bullet CL_{oral}$$

Comparisons of these values between the treated and control groups were performed using Welch's t-test.

Results

The plasma concentration profiles and pharmacokinetic parameters of midazolam in rats treated with raspberry ketone or SJW for 7 days are shown in Figure 2 and Table 1, respectively. The CL_{oral} of midazolam in the SJW-treated group increased to 161% of the

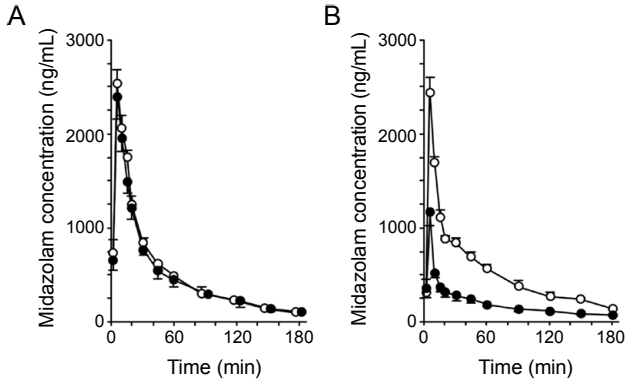

Figure 2: Concentration profile of midazolam in plasma after oral administration.
Closed circles: treated with raspberry ketone (100 mg/day) (A) or SJW (1000 mg·kg^{-1}·day^{-1}) (B) for 7 days.
The open circles in panels A and B show the control group for each treated group.
Each symbol and vertical bar represents the mean ± standard deviation of 5 rats.

	Raspberry ketone		St John's wort	
	Treated (n=5)	Control (n=5)	SJW (n=5)	Control (n=5)
CL_{oral} (mL/min)	115 ± 12	107 ± 5	184 ± 6.2*	114 ± 40
MRT (min)	50 ± 3	49 ± 1	52 ± 1	53 ± 9
Vd / F (L/kg)	5.7 ± 0.3	5.3 ± 0.2	9.6 ± 0.2	6.2 ± 3.0

All of data were shown as mean ± S.D.
CL_{oral}: oral clearance rate; MRT: mean residence time; Vd: volume of distribution; F: bioavailability; SJW: St John's wort
*Statistically significant difference from control (Welch's t-test, P<0.05)

Table 1: Pharmacokinetic parameters of midazolam.

control group at 7 days; conversely, there was no significant difference between the raspberry ketone-treated and control groups. The MRT was essentially the same in all groups.

Discussion

Drug-drug and food-drug interactions are two of the most important causes of inter-individual differences in drug efficacy. Tobacco, alcohol, coffee, and other supplements sometimes alter the efficacy of drug therapy. Recently, serious interactions between SJW and clinically important drugs drew our attention to the potential drug interactions of food supplements and herbal medicines. The effects of various herbal medicines on drug-metabolizing enzymes and drug transporters have been reported and are under investigation [1,5,6].

In the present study, the effects of raspberry ketone on the pharmacokinetics of midazolam, a substrate of CYP3A, were investigated. Because raspberry ketone is considered to suppress the accumulation of body fat, it is mainly taken by young healthy women for weight loss. Considering this population, information about the interaction of raspberry ketone with oral contraceptives, which are substrates of CYP3A, is of clinical importance. In the present study, we could not detect any effects of raspberry ketone treatment on the pharmacokinetics of midazolam, even with an extremely high dose (50-fold larger than the usual human dose) for 7 days. Therefore, raspberry ketone seems to have a minimal effect on CYP3A activity, unlike SJW. There have been reported cases of unwanted pregnancies during the concomitant use of SJW and oral contraceptives [14,15]; however, raspberry ketone does not seem to reduce the efficacy of oral contraceptives. Capsaicin, which has a partially similar structural formula to raspberry ketone, reportedly has an inhibitory effect on CYP3A *in vitro* [16,17]. In a recent study, Zhai et al. [18] reported that the blood concentration of cyclosporine A was significantly increased by capsaicin treatment and, conversely, the mRNA and protein levels of CYP3A and p-glycoprotein (p-gp) in the liver and intestine were decreased by capsaicin treatment [18]. In our study, although we did not evaluate the expression of CYP3A or p-gp, raspberry ketone did not affect the blood concentration of midazolam. This result indicates that raspberry ketone is expected to have no effect on the expression levels or activities of proteins affecting the pharmacokinetics of midazolam. Conversely, because the effect of raspberry ketone on the activities of many CYPs other than CYP3A was not evaluated in this study, we should clarify its impact on those enzymes to characterize further the interaction of raspberry ketone with other drugs. When a drug is administered orally, we must also consider first-pass metabolism. The intestinal and/or hepatic elimination of midazolam may be induced by SJW; however, we could not separate these effects in the present study.

Despite the limitations described above and the small sample size used in the present study, which is considered to lead to a large coefficient of variation for the CL_{oral} of midazolam in the SJW control group, we showed that raspberry ketone has little effect on CYP3A activity. We believe that our data provide useful and important information for physicians when prescribing drugs for patients using raspberry ketone.

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