Food-drug Interaction of Tacrolimus with Pomelo, Ginger, and Turmeric Juice in Rats

Kanoko EGASHIRA¹,², Hitoshi SASAKI¹, Shun HIGUCHI³ and Ichiro IEIRI²,*

¹Department of Hospital Pharmacy, Nagasaki University Hospital, Nagasaki, Japan
²Department of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Science, Kyushu University, Fukuoka, Japan
³Academic, Industrial and Governmental Liaison Center, Fukuoka University, Fukuoka, Japan

Summary: Tacrolimus is a well-known potent immunosuppressant agent, which has various drug-drug or food-drug interactions. Previously, we found a renal transplant recipient who increased tacrolimus blood concentrations after ingestion of pomelo as a rare case. So, we investigated the effect of pomelo after its administration for one day or 3 consecutive days on the pharmacokinetics of tacrolimus in rats. We also confirmed the effects of grapefruit, turmeric, and ginger. The tacrolimus blood concentrations of the rats pre-treated with 100% pomelo juice were significantly higher than those pre-treated with water. On the other hand, the tacrolimus blood concentrations of the rats pre-treated with 50% pomelo juice were not significantly different from those pre-treated with water. The pomelo-tacrolimus interaction showed concentration dependency. Even low concentration of pomelo juice could enhance the blood concentrations of tacrolimus by repeated administration. The inhibitory effect of 100% pomelo juice disappeared 3 days after intake. The AUC values of tacrolimus in the rats pre-treated with grapefruit juice, ginger juice, and turmeric juice were significantly larger than those pre-treated with water. We could confirm the pomelo-tacrolimus interaction, which we discovered in a case study, quantitatively. We newly found the influence of turmeric and ginger on tacrolimus pharmacokinetics, comparable to pomelo.

Keywords: food-drug interaction; tacrolimus; pomelo; pharmacokinetics; ginger; turmeric; CYP3A

Introduction

Tacrolimus is a well-known potent immunosuppressant agent, and has been widely used for prophylaxis and/or treatment of rejection after organ transplantation and graft-versus-host disease after bone marrow transplantation.¹ The peroral bioavailability of tacrolimus is poor and variable, ranging from 4 to 89%.² Tacrolimus has a narrow therapeutic range (from 5 to 20 ng/mL), and often causes critical adverse events such as nephro- and neurotoxicity at high blood concentration. Therefore it is essential to control carefully the blood concentration. Tacrolimus is also a substrate of cytochrome P450 (CYP) 3A.³⁻⁵ CYP3A enzymes metabolize tacrolimus in the intestine as well as in the liver.⁶ Its absorption is further limited due to the involvement of P-glycoprotein (P-gp).⁷ Therefore, drugs or foods that inhibit the CYP3A/P-gp can increase the blood concentration of tacrolimus. In fact, it is reported that concomitant administration of tacrolimus and clotrimazole, which known as a potent inhibitor of CYP3A, increased the blood concentrations of tacrolimus.⁸,⁹

In 2001, we found a renal transplant recipient who increased tacrolimus blood concentrations after ingestion of pomelo as a rare case.¹⁰ Pomelo is botanically close to grapefruit and contains furanocoumarins, which inhibit activity of CYP3A. In our previous report, it was revealed that the extract of pomelo could inhibit activity of CYP3A and metabolism of tacrolimus.¹¹ The increase of tacrolimus blood concentrations in our case must have been caused by ingestion of pomelo. However the effects of pomelo on the pharmaco-
kinetics of tacrolimus in vivo have never been evaluated. Therefore, we investigated the effect of pomelo after its administration for one day or 3 consecutive days on the pharmacokinetics of tacrolimus in rats. On the other hand, grapefruit, ginger, and turmeric were reported to influence activity of CYP3A.\textsuperscript{11,12} So, we confirmed their effects on the pharmacokinetics of tacrolimus under the same conditions.

**Materials and Methods**

**Chemicals:** Tacrolimus (Prograf®, 5 mg/mL) was purchased from Astellas Pharma Inc. (Tokyo, Japan). A tacrolimus reagent kit was purchased from Abbott Laboratories (Abbott Park, IL, USA). Grapefruit juice (GFJ) and orange juice (OJ) were the products of the Dole Food Company, Inc. (Westlake Village, CA, USA). Pomelo (Citrus grandis from Kumamoto, Japan; Banpeiyu), ginger (Zingiber officinale from Nagasaki, Japan), and turmeric dry powder (Curcuma longa from Nagasaki, Japan) were purchased from markets.

**Preparation of pomelo juice, ginger juice, and turmeric juice:** Pomelo was squeezed by hand to obtain juice. Ginger juice was obtained by grating and filtering. Turmeric juice was prepared by suspending 17 mg of turmeric dry powder in 1 mL distilled water.

**Animals:** Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University with approval from the Institutional Animal Care and Use Committee. Male SD rats (7 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The rats were fasted overnight before the experiments.

**Pharmacokinetic experiments:** Each rat was anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal), and the left femoral artery was cannulated with polyethylene tubes to collect blood samples. The body temperature of the rats was maintained at 37°C with a heat lamp during the experiment.

1) **Single administration**

Tacrolimus (0.6 mg/kg) was intraduodenally administered in rats 1, 38, or 62 h after oral administration of 10 mL/kg 100% pomelo juice or 1 h after that of 10 mL/kg 50% pomelo juice, GFJ, OJ, ginger juice, turmeric juice, or water.

2) **Repeated administration for 3 consecutive days**

The 10 mL/kg of pomelo juice (100% or 50%) was orally administered once daily for 2 days. On the 3rd day, tacrolimus (0.6 mg/kg) was intraduodenally administered in rats 1 h after oral administration of 10 mL/kg pomelo juice (100% or 50%).

All blood samples (approximately 0.5 ml) were collected through the left femoral artery at 5, 10, 15, 30, 45, 60, 90, and 120 min after intraduodenal administration of tacrolimus. Tacrolimus blood concentrations were assayed by a chemiluminescent immunoassay (CLIA) using an ARCHITECT® analyzer i1000 (Abbott Laboratories, Abbott Park, IL, USA). All procedures, calibration curve, and validation followed the working protocols provided by the supplier. The assay was calibrated from 2.4 to 30.0 ng/mL, and the lower detection limit was 0.3 ng/mL.

**Pharmacokinetic analysis:** The blood concentration-time profile data (0–120 min) from each rat was analyzed by a model-independent method using the MULTI computer program.\textsuperscript{13} The area under the blood concentration-time curve (AUC) was calculated from the values obtained (0–120 min) using the trapezoidal rule. The maximum tacrolimus blood concentration (C\textsubscript{max}) was obtained directly from the blood concentration time profile. The mean residence time (MRT) was calculated by moment analysis.\textsuperscript{14} The half-life (t\textsubscript{1/2}) was obtained by dividing the natural logarithm of 2 by K\textsubscript{e}, the apparent elimination rate constant, as obtained from the elimination phase gradient.

**Data analysis:** All data are expressed as the mean ± S.D. Statistical significance was determined with one-way analysis of variance (ANOVA) followed by Dunnett’s test or Student’s t test. A p value of less than 0.05 was considered statistically significant.

**Results**

**Effects of single administration of pomelo juice:**

Figure 1 shows the blood concentration-time profiles of tacrolimus after its intraduodenal administration to rats following oral administration of pomelo juice (100%, 50%) or water. The tacrolimus blood concentrations of the rats pre-treated with 100% pomelo juice were significantly higher than those pre-treated with water. The tacrolimus blood concentrations of the rats pre-treated with 50% pomelo juice, however, were not significantly different from those pre-treated with water. Pharmacokinetic parameters for tacrolimus profiles were calculated and are shown in Table 1. The AUC value of tacrolimus in the rats pre-treated with 100% pomelo juice was 1.9 times larger than for those pre-treated with water.

![Fig. 1. Blood concentration-time profiles of tacrolimus after its intraduodenal administration to rats following oral administration of 10 mL/kg 100% pomelo juice (○), 50% pomelo juice (△), or water (■)](image)
Effects of repeated administration of pomelo juice for 3 consecutive days: Figure 2 show the blood concentration-time profiles of tacrolimus after its intraduodenal administration to rats following oral administration of 10 mL/kg 100% pomelo juice ( ), 50% pomelo juice (△), 100% pomelo juice ( ), 38 h following oral administration of 100% pomelo juice ( ). Each point represents the mean ± S.D. (n = 3–5).

Recovery from the effect of pomelo juice pretreatment: Figure 3A shows the blood concentration-time profiles of tacrolimus after its intraduodenal administration to rats at 1, 38, and 62 h following oral administration of 100% pomelo juice. Figure 3B shows the recovery profiles of the AUC values. The AUC values of tacrolimus obtained after pre-treatment of 100% pomelo juice were normalized by the control value (water) in the same manner as that reported by Greenblatt et al.15) The ratios of mean AUC values (increment over 1.0) at 1, 38, and 62 h following oral administration of 100% pomelo juice were 0.9, 0.6, and 0.3, respectively. It was observed that the AUC values progressively returned toward the control value as time elapsed. A plot of time after pre-treatment of 100% pomelo juice versus ratio of mean AUC values yielded a half-life of recovery estimated as 36 h.

Effects of GFJ, OJ, ginger juice, and turmeric juice: Figure 4 shows the AUC values calculated from the blood concentration-time profiles of tacrolimus after its intraduodenal administration to rats following oral administration of 10 mL/kg 100% pomelo juice and water ( ) in rats. Each point represents the mean ± S.D. (n = 5, 6). *, p < 0.05 (vs. water).
istration of GFJ, OJ, ginger juice, and turmeric juice. The AUC values of tacrolimus in the rats pre-treated with GFJ, ginger juice, and turmeric juice were significantly larger than those pre-treated with water. These AUC values were almost equal to the values for those pre-treated with 100% pomelo juice. There was no significant difference in the AUC values between OJ and water.

**Discussion**

Food and drugs are often taken together. Unintended outcomes often arise when they are concomitantly consumed. Grapefruit is well known as a food that causes interaction with drugs. Ingestion of GFJ has been shown to increase the blood concentrations of several drugs including dihydropyridine calcium antagonists, HMG-CoA reductase inhibitors, antibiotics, anti-HIV agents, and immunosuppressants. GFJ inhibited the activities of both P-gp and CYP3A. These interactions occurred only after oral co-administration, and not intravenous administration, of the drugs. The GFJ-drug interaction was caused by inhibition of presystemic drug metabolism.

Previously, we found a pomelo-tacrolimus interaction in a clinical case. Grapefruit as well as pomelo contains both monomer and dimer forms of furanocoumarins such as bergamottin (BG), 6',7'-dihydroxybergamottin (DHB), GF-I-1, and GF-I-4, which inhibited CYP3A activity in vitro. In an in vitro experiment, we reported that the extract of pomelo inhibited both 6β-hydroxylation of testosterone (a typical CYP3A probe substrate) and metabolism of tacrolimus. We also showed that the extract of pomelo had little effect on the transport of [3H]digoxin (a typical P-gp probe substrate) and tacrolimus in LLC-GA5-COL150 cells over expressing human P-gp. However, the effect of pomelo on the pharmacokinetics of tacrolimus in vivo was unknown.

We studied the effects of single administration of 100% and 50% pomelo juice on tacrolimus pharmacokinetics in rats (Fig. 1). The dose of tacrolimus was decided as 0.6 mg/kg because linearity of AUC was confirmed in a preliminary experiment. The dose of juice was decided as 10 mL/kg according to the method previously reported. This dose was equivalent to about half of a pomelo. The tacrolimus blood concentrations of the rats pre-treated with 100% pomelo juice were significantly higher than those pre-treated with water (Fig. 1). The AUC values of tacrolimus in rats pre-treated with 100% pomelo juice were about 2 times larger than those pre-treated with water (Table 1). This result matched our previous in vitro data that metabolism of tacrolimus was almost completely inhibited by the extract of pomelo. The half-life of tacrolimus was not altered by pre-treatment of 100% pomelo juice. This was consistent with the currently understood mechanism of CYP3A inhibition by GFJ.

The tacrolimus pharmacokinetic values of the rats pre-treated with 50% pomelo juice, however, were almost equal to those of rats pre-treated with water (Table 1). Therefore, the concentration dependency of pomelo juice was shown in pomelo-tacrolimus interaction.

We also examined the effects of repeated administration for 3 consecutive days of 100% and 50% pomelo juice on tacrolimus pharmacokinetics in rats. There was no difference between single and repeated administration of 100% pomelo juice. On the other hand, the repeated administration of 50% pomelo juice increased the tacrolimus blood concentrations compared with single administration (Figs. 1 and 2). This result indicated that even a low concentration of pomelo juice could enhance the tacrolimus blood concentrations by repeated administration.

In this study, at 38 and 62 h after the pre-treatment of 100% pomelo juice, the blood concentration-time profiles of tacrolimus gradually reverted to the profile in the pre-treatment of water. This result was consistent with our previous report that the inhibitory effect of pomelo on the metabolism of tacrolimus was exerted in a mechanism-based manner and that the inhibitory effect lasted for several days.

Thus, we were able to confirm the pomelo-tacrolimus interaction in rats. Under the same experimental conditions, we compared the effects of GFJ, ginger juice, and turmeric juice with that of pomelo juice. OJ was used as a negative control, because it is incapable of inhibiting the catalytic activity of CYP3A. The AUC values of tacrolimus in the rats pre-treated with GFJ were almost equal to those for pomelo juice. There was no significant difference in the AUC values between OJ and water.

Turmeric (*Curcuma longa*) is a yellow-coloured spice which has been used as a folk remedy in Japan. It is particularly effective in hangover prevention. Ginger, the roots of *Zingiber officinale*, is popularly used as a spice and herbal medicine. Ginger is clinically effective for the treatment of nausea resulting from pregnancy, and motion sickness. Given this background, ginger and turmeric are consumed widely in Japan. The hidden risk of herb-drug interaction is often overlooked.

In fact, pre-treatment of both ginger and turmeric juice significantly increased the tacrolimus blood concentrations. The effects of ginger juice and turmeric juice were almost equal to those of GFJ and pomelo juice (Fig. 4). Curcumin, which is a component of turmeric, has been reported to change both the function and expression of the P-gp and the CYP3A enzymes. Ginger was reported to change the activity of CYP3A4 and P-gp. We clearly found the turmeric-tacrolimus interaction and ginger-tacrolimus interaction in our in vitro experiment.

The major metabolic pathways of tacrolimus were almost identical in rats and humans although the rate of tacrolimus metabolism was a little different. Further investigations in humans are necessary to apply our results to clinical use. We also have to consider the seasonal difference of the inhibitory component.
In conclusion, the pomelo-tacrolimus interaction, which we discovered in a case study, was quantitatively confirmed in the in vivo experiment. We newly found the influence of turmeric and ginger on tacrolimus pharmacokinetics, comparable to pomelo.

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References


