Comparison of Inhibitory Duration of Grapefruit Juice on Organic Anion-Transporting Polypeptide and Cytochrome P450 3A4

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Recently, a new type of interaction has been reported in which fruit juices diminish oral drug bioavailability through inhibition of organic anion-transporting polypeptide (OATP). In this study, we aimed to clarify the duration of OATP inhibition by grapefruit juice (GFJ), and to compare it with the duration of GFJ-induced inhibition of cytochrome P450 (CYP) 3A4 activity. Seven healthy volunteers were enrolled in this open-label, single-sequence study. They were orally administered cefpiroline (100 mg) and midazolam (15 µg/kg) with water on the control day. Three days later, they ingested GFJ (200 mL) 3 times a day for 3 d. On day 1, the same drugs were administered with GFJ. On days 3 and 7, the same drugs were administered with water. Pharmacokinetics of both drugs were evaluated on each trial day. The peak plasma concentration (Cmax) and the area under the plasma concentration–time curve from 0 to 8 h (AUC0–8) of cefpiroline significantly decreased on day 1, and the mean ratios of these values and the corresponding control-day values were 0.18 and 0.25, respectively. The Cmax and AUC0–8 returned to the control levels on days 3 and 7. In contrast, AUC0–8 of midazolam were higher on days 1 and 3 than on the control day (mean ratio, 2.12 and 1.47, respectively). The AUC0–8 returned to the control level on day 7. In conclusion, results of this study indicated that the OATP inhibition caused by GFJ dissipated faster than GFJ-mediated alterations in CYP3A4 activity, which were sustained for at least 48 h.

Key words organic anion-transporting polypeptide; cytochrome P450; grapefruit juice

Food and medication are often taken together. However, certain foods interact with drugs by altering mechanisms that are important determinants of systemic drug availability. In particular, grapefruit juice is known to alter the pharmacokinetics of over 30 prescription drugs by affecting their bioavailability.¹ ³ The mechanism of this interaction is inhibition of intestinal cytochrome P450 (CYP) 3A4 activity and increased systemic exposure of CYP3A4 substrates.⁴ ⁸ In 2012, the United States Food and Drug Administration issued a press release to warn consumers that the intake of grapefruit juice along with medication could cause dangerous side effects.⁹

Recently, a new type of interaction has been reported in which fruit juices diminish oral drug bioavailability through inhibition of the organic anion-transporting polypeptide (OATP) uptake transporter, which is a family of membrane solute carriers (SLC) transporters. OATP influences the intestinal absorption of several drugs in clinical use.¹⁰ ¹⁴ Grapefruit juice decreases the plasma concentrations of some drugs including β-adrenoceptor blocking agents and antihistamines by this mechanism. For example, grapefruit juice was reported to reduce the area under the plasma concentration–time curve (AUC) value for fexofenadine to a mean value of 58% of that when the drug was administered with the corresponding volume of water.¹⁰ Furthermore, cefpiroline, a β-adrenoceptor blocking agent, has been reported to be a substrate of uptake transporters such as OATP1A2 and OATP2B1, and markedly reduced drug absorption when co-administered with grapefruit juice.¹¹ ¹⁵ ¹⁶

In clinical settings, it is important to know how long OATP inhibition will be sustained after the intake of grapefruit juice, so that practitioners can administer drugs without the concern of interactions. Several reports have been published about the duration of CYP3A4 inhibition. These reports show that for some drugs, the magnitude of interaction declines over several days, consistent with enzyme regeneration following inhibition.¹⁷ ¹⁹ However, little attention has been devoted to the duration of the interaction between OATP and grapefruit juice. Thus, our goal was to determine the duration of the inhibitory effect of grapefruit juice on OATP by comparison with the duration of the grapefruit juice-mediated effects on CYP3A4 activity. We administered oral cefpiroline and midazolam, which are substrates of OATP,¹³ and CYP3A4,¹⁴ respectively, on days 1, 3, and 7 (0, 48, and 144 h after the ingestion of grapefruit juice) to healthy subjects.

MATERIALS AND METHODS

Subjects The subjects included 7 healthy Japanese men aged 21–24 years with a body mass index of 18 to 28 kg/ m²; the subjects provided written informed consent before the start of the study. The subjects were non-smokers and medication-free, and were deemed healthy on the basis of their medical history, physical examination results, electrocardiograms, whole blood cell counts, and results of serum chemical analyses. They were asked to refrain from consuming dietary supplements and citrus fruit juices, especially grapefruit juice, starting 2 weeks before the start of the study to the end of the study. They were also asked to refrain from consuming alcoholic beverages 1 d before receiving cefpiroline and midazolam.

Study Design Our open-label, single-sequence, repeated-measures design is shown in Fig. 1. On the control day, the subjects received a single oral dose of 100 mg cefpiroline (Se-
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lectol®; Nippon Shinyaku Co., Ltd., Kyoto, Japan) and 15 μg/kg midazolam (Dormicum®; Astellas Pharma Inc., Tokyo, Japan) at 0845 h after overnight fasting. The subjects ate a light breakfast at 1100 h. Blood samples were collected via a cannula placed in a forearm vein, which was kept patent with heparinized saline. Fluid remaining within the cannula and a small amount of blood were withdrawn and discarded before each blood sampling. Blood samples (10 mL) were obtained before dosing and at 1, 2, 3, 4, 6, and 8 h after celiprolol and midazolam administration. The samples were collected in heparinized tubes and centrifuged at $3000 \times g$ for 10 min, and plasma was stored at $-20^\circ C$. Before each sampling, systolic and diastolic blood pressure and heart rate were measured twice with the subject in a supine position, and mean values were used in the calculations.

Three days later, all subjects drank 200 mL of grapefruit juice (100% grapefruit juice, Sunkist®; Morinaga Milk Industry Co., Ltd., Tokyo, Japan) 3 times per day with meals for 3 d. On day 1, celiprolol and midazolam were administered orally with grapefruit juice. On days 3 and 7 (48 and 144 h after the last intake of grapefruit juice), the subjects received the same doses of the drugs with water. Blood sampling and measurements of blood pressure and heart rate were performed as on the control day. This study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine and University of Shizuoka, and the study was registered in the UMIN Clinical Trials Registry (UMIN000006072).

Measurement of Plasma Celiprolol and Midazolam Concentrations The plasma concentrations of celiprolol and midazolam were determined using reversed-phase liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) in the positive ion mode by using selected reaction monitoring. HPLC system (Agilent 1100 series; Agilent Technologies, Wilmington, DE, U.S.A.) was connected to a mass spectrometer (API3000; Applied Biosystems, Foster City, U.S.A.) equipped with a Turbo Spray. Pindolol (10 ng) was added to plasma samples as an internal standard, and was then extracted using solid-phase extraction (Oasis® HLB 96-well µElution Plate; Waters Co., Milford, Massachusetts, U.S.A.). HPLC was performed using an analytical column (Cadenza CD-C18, 3 μm, 2.1×150 mm; Intact Co., Kyoto, Japan) with the mobile phase (acetonitrile–0.1% formic acid=35/65) delivered at a flow rate of 0.2 mL/min. The mass transitions were as follows: m/z 380→251 for celiprolol, m/z 326→291 for midazolam, and m/z 249→116 for pindolol. The lower limit of detection was 0.1 ng/mL for both celiprolol and midazolam. The accuracy of...
the analyses for celiprolol and midazolam ranged from 85.5% to 112% and 88.2% to 114%, respectively. The precision was less than 12% for celiprolol and less than 14% for midazolam.

Pharmacokinetic Analyses Pharmacokinetic parameters were determined by a non-compartmental analysis. The linear trapezoidal method was used to calculate the area under the plasma concentration–time curve from 0 to 8 h ($AUC_{0–8}$) after the administration. The elimination half-life ($t_{1/2}$) was calculated using the following formula: $\ln 2/k_e$, where $k_e$ is the terminal slope calculated by linear regression of time vs. concentration. The peak plasma concentration ($C_{max}$) and $t_{max}$ were obtained by inspection.

Statistical Analyses All data are presented as the arithmetic mean and SD. GraphPad Prism (version 5.0; GraphPad Software, San Diego, California, U.S.A.) was used for all statistical analyses. Differences between the 4 periods of the study were analysed using the Wilcoxon signed-rank test with Bonferroni’s correction. Differences between the periods were considered statistically significant when the $p$ value was less than 0.0167 ($=0.05/3$).

RESULTS

All subjects completed the study protocol, and no drug-related adverse events occurred except for mild drowsiness after midazolam dosing.

On the control day, $C_{max}$ of celiprolol (259±83.5 ng/mL) was achieved at 3 h after administration, and the concentration decreased to 63.9 ng/mL at 8 h. On day 1, celiprolol plasma concentrations at all sampling points were lower than the corresponding concentrations observed on the control day (Fig. 2A). The $C_{max}$ and $AUC_{0–8}$ values of celiprolol significantly decreased on day 1, and the mean ratios of these values and the corresponding control-day values were 0.18 and 0.25, respectively (Table 1). The $C_{max}$ and $AUC_{0–8}$ values returned to the control levels on days 3 and 7 (Figs. 2B, 2C, 3A). There were no significant differences in celiprolol $t_{max}$ between the trial days (Table 1).

On the control day, $C_{max}$ of midazolam (4.47±2.56 ng/mL) was achieved at 1 h after administration, and the plasma concentration decreased to 0.59 ng/mL at 8 h. On days 1 and 3, plasma concentrations of midazolam at all sampling points

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Control</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
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<tbody>
<tr>
<td><strong>Celiprolol</strong></td>
<td></td>
<td></td>
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<tr>
<td>$AUC_{0–8}$ (ng·h/mL)</td>
<td>814±214</td>
<td>200±125</td>
<td>711±324</td>
<td>700±277</td>
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<td>ratio vs. control</td>
<td>—</td>
<td>0.251±0.142*</td>
<td>0.881±0.358</td>
<td>0.872±0.390</td>
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<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>259±83.5</td>
<td>46.0±32.8*</td>
<td>219±126</td>
<td>225±104</td>
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<tr>
<td>ratio vs. control</td>
<td>—</td>
<td>0.181±0.106*</td>
<td>0.899±0.579</td>
<td>0.920±0.522</td>
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<tr>
<td>$t_{max}$ (h)</td>
<td>2.57±0.572</td>
<td>2.86±1.07</td>
<td>3.43±0.787</td>
<td>2.86±0.690</td>
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<tr>
<td>ratio vs. control</td>
<td>—</td>
<td>1.14±0.513</td>
<td>1.36±0.339</td>
<td>1.17±0.385</td>
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<tr>
<td><strong>Midazolam</strong></td>
<td></td>
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</tr>
<tr>
<td>$AUC_{0–8}$ (ng·h/mL)</td>
<td>11.3±6.18</td>
<td>22.9±13.8</td>
<td>16.9±9.63</td>
<td>15.1±10.5</td>
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<td>ratio vs. control</td>
<td>—</td>
<td>2.12±0.758</td>
<td>1.47±0.440*</td>
<td>1.33±0.450</td>
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<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>4.47±2.56</td>
<td>7.56±3.80</td>
<td>6.82±3.36*</td>
<td>5.15±2.87</td>
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<tr>
<td>ratio vs. control</td>
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<td>2.12±1.21</td>
<td>1.64±0.694*</td>
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<tr>
<td>$t_{max}$ (h)</td>
<td>1.14±0.378</td>
<td>1.14±0.378</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
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<tr>
<td>ratio vs. control</td>
<td>—</td>
<td>1.07±0.450</td>
<td>0.929±0.189</td>
<td>0.929±0.189</td>
</tr>
</tbody>
</table>

* The parameters are expressed as the means and S.D. $AUC_{0–8}$ area under the plasma concentration–time curve from 0 to 8 h after drug administration; $C_{max}$ peak plasma concentration; $t_{max}$ time to peak plasma concentration.* $p<0.0167$ compared to control.
were higher than the corresponding concentrations observed on the control day (Figs. 2D, E). Midazolam $AUC_{0-8}$ values on days 1 and 3 were higher than that on the control day, and the mean ratios of the corresponding values and the control-day value were 2.1 and 1.5, respectively (Table 1). The $AUC_{0-8}$ values returned to the control level on day 7 (Table 1, Fig. 3B).

Similarly, the $C_{max}$ of midazolam on days 1 and 3 increased by 1.7-fold and 1.5-fold of that on the control day, and returned to the control level only on day 7.

Hemodynamic parameters were measured at every sampling point after administration of celiprolol and midazolam. Systolic and diastolic blood pressure and heart rate slightly decreased 3 to 5 h after drug administration on the control day and on days 1, 3, and 7. At 1 and 3 h after drug administration on the control day and day 1, decreases in blood pressure and heart rate were calculated as percentages (%) of the values observed before drug administration. Decreases in systolic blood pressure and heart rate at 1 h after drug administration on day 1 were lower than the corresponding decreases at the same time point on the control day (systolic blood pressure: 10.5% vs. 4.8%, heart rate: 22.3% vs. 10.6%, Figs. 4A, C). However, these differences were not significant. Similarly, there were no significant differences in the decreases in hemodynamic parameters at 3 h after drug administration between the control day and day 1 (Fig. 4).

Fig. 4. Percent Changes in Systolic Blood Pressure (A), Diastolic Blood Pressure (B), and Heart Rate (C) 1 and 3 h after Drug Administration on Day 1 and Control Day Compared to the Values Prior to Administration of Celiprolol and Midazolam (Baseline)

The horizontal line in each column indicates the mean.
DISCUSSION

In this study, we aimed to clarify the duration of OATP inhibition by grapefruit juice by comparison with the duration of the grapefruit juice-mediated effect on CYP3A4. We orally administered celiprolol and midazolam, which are substrates of OATP and CYP3A4, respectively, on days 1, 3, and 7. Grapefruit juice greatly decreased the \( C_{\text{max}} \) and \( AUC_{0-8} \) values of celiprolol, suggesting that grapefruit juice induced OATP inhibition. The decreased celiprolol \( C_{\text{max}} \) and \( AUC_{0-8} \) values returned to the corresponding control levels 48 h after intake of grapefruit juice. In contrast to administration of celiprolol with grapefruit juice, administration of midazolam with grapefruit juice produced 2-fold and 1.5-fold increase in \( AUC_{0-8} \) of midazolam at 0 and 48 h, respectively, after the last grapefruit juice ingestion. This increased \( AUC_{0-8} \) of midazolam had returned to the control level on day 7.

The mechanism underlying CYP3A4 inhibition by grapefruit juice can be both reversible and irreversible (mechanism-based).38 This mechanism-based inhibition results in a prolonged effect of grapefruit juice on CYP3A4 activity. The magnitude of this interaction has been reported to decline over 3 to 7 d for CYP3A4 substrates such as simvastatin, midazolam, and triazolam.19,20 The results of our pharmacokinetic analysis of midazolam are consistent with those of previous analyses. Grapefruit juice interactions with OATPs expressed in the intestine may be mediated through inhibition of transporter activity rather than through a reduction in protein expression.46 Regarding the duration of the inhibitory effect of grapefruit juice on OATPs, only one previous study has reported no effect of grapefruit juice ingestion 4 h before fexofenadine administration.20 Our results indicate that the inhibitory effect of grapefruit juice on OATP dissipates faster than its effect on CYP3A4 activity, which is sustained for at least 48 h. Very recently, Shirasaka et al. reported the effects of pre-incubation with fruit juices on OATP2B1-mediated transport of specific substrates in vitro.22 Their results indicated that OATP2B1 is functionally impaired through both competitive and long-lasting inhibition mechanisms by apple juice and orange juice, but not by grapefruit juice. Our results from a clinical investigation are in agreement with these in vitro findings. It will be interesting to clarify the clinical significance of the duration of inhibition induced by other fruit juices such as apple and orange juice.

In previous studies, the \( AUC_{0-8} \) of celiprolol decreased to 13% and 17% of the control level following grapefruit juice administration to healthy volunteers.11,16 The decrease \( AUC_{0-8} \) of celiprolol in the previous two studies was higher than that observed in our study. These differences between studies may be attributable to the volume of grapefruit juice ingested. In the previous studies, the subjects drank 200 mL grapefruit juice 3 times a day for 2 d. On the next day, each subject ingested 100 mg celiprolol with 200 mL of water or grapefruit juice 1 h after ingestion of grapefruit juice. Furthermore, water or 200 to 400 mL of grapefruit juice was ingested after celiprolol administration. In our study, subjects did not drink grapefruit juice after celiprolol administration. Therefore, the grapefruit juice-induced inhibition of OATP may be dose-dependent. Although OATP-mediated transport is a major mechanism involved in this drug interaction, several alternative mechanisms could also account for these findings. It has been suggested that P-glycoprotein may be involved in the gastrointestinal absorption of celiprolol in mice.19 In addition, a decreased intraduodenal pH induced by grapefruit juice could lead to reduced absorption of celiprolol because the nonionized form is more lipid-soluble (and thus better absorbed) than the ionized form. Therefore, possible involvement of other factors such as P-glycoprotein-mediated efflux transport and a physicochemical factor, in the drug interaction shown in this study cannot be ruled out.

We also evaluated pharmacodynamic parameters of celiprolol and midazolam following their oral administration in healthy subjects. Systolic and diastolic blood pressure and heart rate decreased slightly after the administration of these drugs. At 1 h after drug administration on day 1, lowering effect on systolic blood pressure and heart rate from baselines was attenuated by 50% than the corresponding values on the control day. However, these inter-day differences were not significant. These results suggest that grapefruit juice might suppress the celiprolol-induced reduction in blood pressure and heart rate by decreasing the systemic exposure of celiprolol. However, it is unclear whether these effects were caused by grapefruit juice because of the limited number of subjects enrolled and because the effect of celiprolol on heart rate was relatively weak. Further studies are needed to elucidate the effects of grapefruit juice on the pharmacodynamics of OATP substrates.

In conclusion, we aimed to clarify the duration of OATP inhibition by grapefruit juice by comparison with the duration of the grapefruit juice-mediated effects on CYP3A4 activity. Our results indicated that the OATP inhibition caused by grapefruit juice dissipated faster than the grapefruit juice-mediated alterations in CYP3A4 activity, which were sustained for at least 48 h. Our results are clinically relevant because many patients take OATP substrates daily. Further studies are needed to clarify the effect of grapefruit juice both in more short-term and in pharmacodynamics of OATP substrates.

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