Grapefruit juice (GJ) has been reported recently as showing pharmacokinetic interaction with many drugs after they are taken together (Bailey et al., 1998). Dihydropyridine calcium antagonists, including nifedipine (NFP), nisoldipine, nitrendipine (NTP), and felodipine, constitute a representative group of such drugs (Fuhr, 1998). It is known that when these drugs are taken along with GJ their blood concentrations increase significantly, which can cause serious adverse reactions such as hypotension.

As such, the GJ interaction mechanism has recently drawn much attention. Many of the drugs interacting with GJ serve as substrates for the phase I metabolic enzyme cytochrome P-450 CYP3A and the drug elimination transport protein P-glycoprotein. This interaction occurs only when GJ is taken orally and is not caused by i.v. administration. It is suggested, therefore, that GJ-drug interaction only when GJ is taken orally and is not caused by i.v. administration. This interaction occurs only when GJ is taken orally and is not caused by i.v. administration. It is suggested, therefore, that GJ-drug interaction only when GJ is taken orally and is not caused by i.v. administration.

**ABSTRACT:**

We studied the effects of short- and long-term ingestion of grapefruit juice (GJ) on nifedipine (NFP) pharmacokinetics in rats. Thirty minutes after intraduodenal (id) administration of 2.0 ml of GJ or saline, NFP was i.v. or id administrated at a dose of 3 mg/kg b.wt. No significant differences were observed in pharmacokinetic values between the two groups after i.v. administrations of NFP. By contrast, after id administration, the mean AUC value in the GJ group was approximately 1.62 times that in the control group, and the mean apparent clearance (CL) decreased by approximately 40%. In addition, 2.0 ml of GJ was orally administered twice a day (9:00 AM and 7:00 PM) for 10 consecutive days; on the 11th day the pharmacokinetics of NFP were examined again. Irrespective of route of administration (i.v. or id), NFP CL from plasma in these GJ-treated rats was considerably faster than that in the rats treated with GJ for a short (30-min) period. In microsomes prepared from the intestinal mucosa of animals receiving long-term administration of GJ, NFP oxidation activity (0.21 ± 0.02 nmol/mg/ min) and P-450 content (0.45 ± 0.009 nmol/mg) were significantly lower than those in untreated rats (0.32 ± 0.05 nmol/mg/min and 0.060 ± 0.007 nmol/mg). In hepatic microsomes from the same rats, however, NFP oxidation activity (1.43 ± 0.17 nmol/mg/min) and P-450 content (0.66 ± 0.07 nmol/mg) were distinctly greater than those in untreated rats (1.08 ± 0.06 nmol/mg/min and 0.51 ± 0.04 nmol/mg). In conclusion, short-term id exposure to GJ resulted in increased NFP bioavailability, whereas long-term administration of GJ resulted in reduced bioavailability and increased CL.

**Grapes and Drug Administration.**

Male Wistar-ST rats (Sankyo Labo Service Co., Ltd., Tokyo, Japan) weighing 280 to 300 g were used throughout the study. The rats were housed in stainless steel cages with five animals per cage in a temperature-controlled (24–26°C) room with a 12-h light/dark cycle. The rats were allowed free access to standard rat chow (Sankyo) and water for 1 week before the experiments. The rats were fasted overnight before the experiments. Each animal was anesthetized with 20% (w/v) urethane (1 g/kg b.wt., i.p.). The femoral vein was cannulated with PE-10 tubing (Clay Adams, Parsippany, NJ) for saline infusion and drug administration. The femoral artery was cannulated with PE-50 tubing (Clay Adams, Parsippany, NJ) for arterial blood sampling. Urine samples were collected via a PE-50 tubing bladder cannula into 5-ml glass tubes for 4 consecutive h. To avoid enterohepatic circulation, bile drainage was performed for each rat. The abdominal incision was made, and the common bile duct was cannulated with PE-10 tubing (Clay Adams) to collect bile samples.
and then closed with surgical clips. During the experiment, body temperature was kept at 38 ± 0.5°C to prevent the hypothermic alteration of bile flow. An NFP solution for injection was prepared by dissolving 50 mg of NFP in a mixture of polyethylene glycol 400 (5 ml), ethanol (5 ml), and saline (10 ml).

**Single administration of GJ.** Two milliliters of GJ or saline was injected into the duodenum. After 30 min, NFP was administered at a dose of 3 mg/kg b.w.t. via the femoral vein (i.v. administration) or intraduodenally (i.d. administration). Blood samples (each approximately 0.2 ml) were collected via the femoral artery at 0, 5, 10, 15, 20, 30, and 45 min and 1.0, 1.5, 2.0, 3.0, and 4.0 h after i.v. or i.d administration. The samples were immediately centrifuged at 16,000 g and 4°C for 15 min, and plasma was separated. Bile was collected into 2-ml plastic tubes for 30 consecutive min. Each rat also was given saline via the femoral cannula in a volume equivalent to the volume of blood or bile collected (each approximately 0.2–0.5 ml). The plasma samples collected were stored at −80°C until analysis.

**GJ administration for 10 consecutive days.** Two milliliters of GJ was administered orally twice a day (9:00 AM and 7:00 PM) for 10 consecutive days. On the 11th day, the same experiments described in **Single Administration of GJ** before 30 min were performed.

**HPLC Conditions.** The HPLC system was used that consisted of a PU-980 pump (Jasco Corp., Ltd., Tokyo, Japan) equipped with a UV/Vis detector (JASCO), a 10-ml auto-injector (Hitachi), and a Hitachi 228A spectrophotometer (Hitachi Ltd., Tokyo, Japan). The NFP and its primary metabolite (NFPO) were quantified at wavelengths of 260 and 280 nm, respectively. Plasma samples were analyzed for NFP using a reversed phase analytical Capcell Pak UG-ODS column [4.6 mm (inside diameter) x 25 cm; particle size 5 μm] equipped with a guard column of the same type. A mobile phase consisting of 0.085% phosphoric acid/MeCN (55:45, v/v) was pumped through the column at a speed of 1.0 ml/min. After vigorous shaking, the sample was centrifuged at 16,000 g for 10 min. The cells were homogenized in 5 ml of the supernatant was kept standing on ice for 15 min. The intestinal microsomes were separated by centrifugation at 2000g for 10 min.

**Assay of Cytochrome P-450.** An intestinal (1 mg/ml) or hepatic (2 mg/ml) microsomal suspension in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% (w/v) glycerol and 10 mM EDTA was added at 37°C, and differential spectra were taken using the Hitachi 228A spectrophotometer (Hitachi Ltd., Tokyo, Japan); the cytochrome P-450 concentration in the microsomal fraction was calculated using an extinction coefficient of 91 cm/mol/M for A895 and A630.

**Data Analysis.** All data are expressed as mean ± S.D. Unpaired Student’s t test and one-way ANOVA followed by least-significant difference analysis were used to test for significant differences in mean values. The significance level was set at P < .05.

**Results**

**Pharmacokinetics.** NFP plasma concentration-time curve profiles after single and long-term administrations of GJ to rats are shown in Figs. 1-4.

**Effect of single administration of GJ.** No significant difference was observed between both pharmacokinetic values after i.v. administration of NFP (Fig. 1A, Table 1). In id administration, the mean AUC value of the rats receiving GJ was approximately 1.62 times that of the rats receiving saline, and the mean CL/F value was approximately 0.61 times greater (Fig. 1B, Table 1).

**Effect of single administration of GJ.** Irrespective of administration route (i.v., Fig. 1C; id, Fig. 1D), NFP disappeared from plasma more rapidly in the rats receiving long-term administration of GJ. In i.v. administration, the mean AUC, MRT, CL/F, and Ka values of the rats receiving long-term administration of GJ were approximately 0.74, 0.85, 1.44, 1.51, and 0.64 times those of the rats receiving GJ 30 min previously, respectively. In id administration (Fig. 1D), the mean AUC, MRT, CL/F, and Ka values of the rats receiving long-term administration of GJ were approximately 0.58, 0.65, 1.91, and 1.38 times those of the rats receiving GJ 30 min previously, respectively (Table 1).

**NFP-Oxidation Activity and Total Cytochrome P-450 Concentration in the Intestinal and Hepatic Microsomes of Rats Receiving GJ for 10 Consecutive Days.** Intestinal microsomes. Activity for oxidation from NFP to NFPO in a volume equivalent to the volume of blood microsomes of untreated rats were 0.32 ± 0.05 nmol/mg/min and 0.060 ± 0.007 nmol/mg, respectively (Fig. 2), whereas those in the intestinal microsomes of the rats receiving long-term administration...
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**Fig. 1.** Plasma concentration-time curves for NFP after a single i.v. (A) or id (B) administration of NFP 30 min after a single dose of GJ (2 ml) or saline (2 ml) administration into the duodenum, and after a single i.v. (C) or id (D) administration of NFP 30 min after a single dose of GJ (2 ml) into the duodenum or after successive 10-day (p.o.) dosing of GJ.

Dose of NFP: 3 mg/kg. Each point and vertical bar represent the mean and S.D. (n = 5). Symbols: NFP (●) administered 30 min after a single dose of GJ, NFP (○) administered after saline, and NFP (△) administered after successive 10-day (p.o.) dosing of GJ.

**TABLE 1**

Pharmacokinetic parameters of NFP after id and i.v. administrations in rats preadministered with saline or GJ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>id Administration</th>
<th>i.v. Administration</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>GJ (single)</td>
</tr>
<tr>
<td>AUC (µg • h/ml)</td>
<td>4.85 ± 1.75</td>
<td>7.86 ± 2.20*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.03 ± 0.18</td>
<td>1.17 ± 0.10</td>
</tr>
<tr>
<td>Vdss (l)</td>
<td>0.18 ± 0.05</td>
<td>0.11 ± 0.03**</td>
</tr>
<tr>
<td>Ke (h⁻¹)</td>
<td>0.55 ± 0.14</td>
<td>0.60 ± 0.11</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>1.31 ± 0.32</td>
<td>1.19 ± 0.27</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>5.02 ± 1.90</td>
<td>5.98 ± 1.73</td>
</tr>
<tr>
<td>F (%)</td>
<td>54.4</td>
<td>85.9</td>
</tr>
</tbody>
</table>

Saline: the rats were administered saline.

GJ (single): the rats were administered GJ 30 min before NFP.

GJ (10 days): the rats were administered GJ for 10 successive days twice a day.

F: the bioavailability was calculated by the following equations:

\[ F_{\text{saline}} = \frac{AUC_{\text{id, saline}}}{AUC_{\text{i.v. saline}}} \]

\[ F_{\text{GJ-single}} = \frac{AUC_{\text{id, GJ-single}}}{AUC_{\text{i.v. GJ-single}}} \]

\[ F_{\text{GJ-10 days}} = \frac{AUC_{\text{id, GJ-10 days}}}{AUC_{\text{i.v. GJ-10 days}}} \]

* P < .05 and ** P < .01; compared with the values in the group administered saline.

† P < .05 and †† P < .01; compared with the values in the group administered GJ (single).

of GJ were 0.21 ± 0.02 nmol/mg/min and 0.045 ± 0.009 nmol/mg, respectively (Fig. 2).

**Hepatic microsomes.** NFP oxidation activity and P-450 concentration in the hepatic microsomes of untreated rats were 1.00 ± 0.06 nmol/mg/min and 0.51 ± 0.04 nmol/mg, respectively (Fig. 2), whereas those in the hepatic microsomes of the rats receiving long-term administration of GJ were 1.43 ± 0.17 nmol/mg/min and 0.66 ± 0.07 nmol/mg, respectively.
CYP3A4 activity, colic CYP3A5 level, or small intestinal concentration. They administered a 10-mg felodipine extended release tablet and GJ between single intake and 14-day daily intake of GJ. They reported no significant difference in plasma felodipine concentration between the two meal treatments. These two human studies of GJ-felodipine interaction yielded different results. Brunner and coworkers (Brunner et al., 1998) reported that the AUC after oral administration of cyclosporine increases as the number of t.i.d. ingestions of GJ increases. These previous results from human studies did not agree with our results obtained using rats. Although the cause remains unclear, this discrepancy may be due to the use of different drugs and animal species. Felodipine and cyclosporine are known to be likely to undergo first-pass effect during passage through the intestine and liver and to show great variation among patients (Blychert et al., 1991; Kahan et al., 1996). The bioavailability of felodipine and cyclosporine are approximately 10 to 23% (Edgar et al., 1987) and 11 to 30% (Gupta and Benet, 1989), respectively, and that of NFP is approximately 56 to 70% (Raemsch and Sommer, 1983). The proportion of first-pass metabolism during intestinal passage is higher for felodipine and cyclosporine than for NFP. This suggests that the concentration of cyclosporine and felodipine may depend largely on their metabolism in the small intestine, whereas that of NFP may be more dependent on its metabolism in the liver after passage through the small intestine rather than in the small intestine. Therefore, it is assumed that the plasma NFP concentration after long-term administration of GJ is more sensitive to change in hepatic metabolism.

The mutually reverse results of long-term GJ administration on the CL of oral felodipine and cyclosporine may be explained by selective contribution of P-glycoprotein to cyclosporine first-pass (Lown et al., 1997), which is not observed for felodipine. The short- and long-term effects of GJ on small intestinal P-glycoprotein activity are not well known (Soldner et al., 1999), which may complicate the mechanistic interpretation of the in vivo blood concentration-time profiles for drugs serving as substrates for both metabolism and efflux transporter.

Bearing in mind that the rapid NFP CL by long-term administration of GJ is due to the induction of the NFP-metabolizing enzymes, we examined the induction of the NFP-metabolizing enzymes in the liver and small intestine. NFP oxidation activity in the gastrointestinal microsomes was significantly lower in rats receiving GJ for 10 consecutive days than that in untreated rats (Fig. 2). P-450 content in the gastrointestinal microsomes also decreased significantly. Previously, Schmiedlin-Ren et al. (1997) showed that in humans GJ reduced not only gastrointestinal P-450 activity but also P-450 content in the gastrointestinal microsomes. Our results agree well with their results in that GJ components inhibit the enzymes and reduce P-450 content in the intestinal epithelia.

Unexpectedly, in the hepatic microsomes prepared from the same rats, the NFP oxidation activity and P-450 content were approximately 1.43 and 1.30 times greater than those in the microsomes of untreated rats, respectively (Fig. 2). These findings strongly suggest the induction of rat hepatic P-450 by repeated administration of GJ.

Recently, some furanocoumarin derivatives showing strong inhibitory action on P-450 activity (e.g., bergamottin, 6’7’-dihydroxybergamottin) were isolated from GJ (Fukuda et al., 1997; Schmiedlin-Ren et al., 1997; He et al., 1998). It is likely that these GJ components,
which directly inhibit the enzyme activity of P-450 in small intestinal epithelia, increased NFP bioavailability after id administration of GJ.

The furanocoumarin derivative 8-methoxypsoralen has been reported to induce various metabolic enzymes in the liver (Mandula et al., 1978; Bickers and Patbak, 1984; Letteron et al., 1986; Gwang, 1996; Diawara et al., 1999). Based on the fact that 8-methoxypsoralen is structurally very similar to bergamottin derivatives, it is likely that the hepatic metabolic enzymes are induced by such derivatives or other molecules contained in GJ during its long-term ingestion. This report, for the first time, suggests the possibility of induction of the hepatic drug metabolic enzyme P-450 by long-term repeated ingestion of GJ. It is known, however, that there are interspecific differences in enzyme induction between rats and humans. For example, rifampicin causes enzyme induction in humans but not in rats. Further investigations in humans are therefore necessary to apply our rat findings to humans.

In conclusion, no significant difference was observed between both pharmacokinetic values after i.v. administrations of NFP to the rats administered GJ and saline 30 min previously, respectively. However, the NFP bioavailability in rats receiving id administration of GJ 30 min previously was approximately 1.6 times that of control rats. However, irrespective of administration route (i.v. or id), rapid NFP CL from plasma was observed in the rats receiving long-term administration of GJ. In these rats, although the oxidation activity and P-450 content in the intestinal mucosa decreased, those in the liver increased significantly. We demonstrated for the first time that repeated administration of GJ increased the metabolic enzyme P-450 level in the liver.

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References


