

## UV-Irradiated Grapefruit Juice Loses Pharmacokinetic Interaction with Nifedipine in Rats

Yoshihiro UESAWA\* and Kiminori MOHRI

Clinical Pharmaceutics Laboratory, Department of Pharmaceutics, Meiji Pharmaceutical University; 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan. Received January 31, 2006; accepted March 7, 2006; published online April 21, 2006

In the present study, UV-irradiated grapefruit juice was used to investigate the effects of UV light on nifedipine pharmacokinetics. Grapefruit juice in quartz vessels was UV irradiated (302 nm) with a transilluminator for 0 to 6 h at 4 °C, and furanocoumarins, potent contributors to the pharmacokinetic interaction, in each juice sample were measured using HPLC. The concentrations of all three types of furanocoumarins, bergamottin, 6',7'-dihydroxybergamottin, and bergaptol, decreased in a time-dependent manner. Concentrations of bergamottin, 6',7'-dihydroxybergamottin, and bergaptol were decreased to 1.66, 1.98, and 5.58%, respectively, after UV irradiation for 6 h. Two milliliters of untreated and UV-irradiated grapefruit juice were preadministered into the duodenum in rats to assess the effects of UV irradiation on nifedipine pharmacokinetics *in vivo*. After 30 min, nifedipine was intraduodenally administered at a dose of 3 mg/kg body weight. The nifedipine concentrations in the plasma samples were determined using HPLC. A significant increase in the area under the concentration–time curve of nifedipine was observed in untreated grapefruit juice to 1.6-fold that in the control group, but not in the UV-irradiated grapefruit juice. These findings suggest that UV irradiation is useful to eliminate pharmacokinetic interactions with grapefruit juice.

**Key words** grapefruit juice; furanocoumarin; pharmacokinetic interaction; nifedipine; UV irradiation

It has been reported that grapefruit juice (GJ) causes pharmacokinetic interactions with many drugs such as dihydropyridine calcium antagonists, including nifedipine (NFP).<sup>1,2)</sup> The plasma concentrations of the drugs increase significantly with GJ intake, and serious adverse reactions may possibly result.<sup>3,4)</sup> Most drugs interacting with GJ are substrates for small intestinal cytochrome P450 (CYP) 3A. That is, GJ–drug interactions are caused by the inhibition of CYP3A-mediated drug oxidation during the intestinal absorption process. It has been demonstrated that furanocoumarin (FC) derivatives such as bergamottin (BG) and 6',7'-dihydroxybergamottin (DHB) in GJ induce drug interactions *in vivo* caused by the inhibition of CYP3A activity.<sup>5–12)</sup>

In 1953, Lerner *et al.* reported that if 8-methoxypsoralen, one of the FCs, is exposed to UV irradiation, a decrease in optical density is obtained.<sup>13)</sup> Fowlks confirmed a similar loss of the characteristic peaks of the absorption spectrum of 5-methoxypsoralen, 8-methoxypsoralen, and psoralen after UV exposure.<sup>14)</sup> Furthermore, Knudsen *et al.* examined the reduction of the phototoxic effects of previously UV-irradiated 5-methoxypsoralen, 8-methoxypsoralen, and psoralen.<sup>15)</sup> Photodegradation of BG and bergaptol (BT), major FCs contained in GJ, were also reported.<sup>16,17)</sup> We considered that UV irradiation of GJ might eliminate FCs in the juice and decrease the ability to interact with drugs. However, the effects of UV light on the FCs in GJ and on GJ–drug interactions were not known. Therefore the effects of UV irradiation on the concentration of FCs in GJ were investigated using a comprehensive determination system of FCs in HPLC. Furthermore, NFP pharmacokinetics in rats administered untreated and UV-irradiated GJ were compared to assess the effects of UV irradiation *in vivo*.

### MATERIALS AND METHODS

All handling procedures for NFP were performed under subdued light.

**Materials** NFP was kindly donated by Kanebo Pharmaceuticals, Ltd. (Osaka, Japan). Nitrendipine [internal standard 1 (IS 1)] was obtained from Yoshitomi Pharmaceutical Industries, Ltd. (Osaka, Japan). BG and DHB were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). BT was obtained from Funakoshi Co., Ltd. (Tokyo, Japan). Anthracene [internal standard 2 (IS 2)] was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methanol (MeOH), acetonitrile (MeCN), and dimethyl sulfoxide (DMSO) were of HPLC grade (Wako). All other chemicals were of reagent grade (Wako). Water was used after double-distillation in a glass still. GJ (*Citrus paradisi*) (single strength) was purchased from Kanda Foods Laboratory Co. (Tokyo, Japan).

**UV Irradiation** GJ in a crystal vessel was irradiated at a distance of 0.3 cm from the filter surface of a TFM-20 UV transilluminator (Ultra-Violet Products, U.S.A.) emitting mainly 302-nm light (9400  $\mu\text{W}/\text{cm}^2$ ) at 5 °C for 0, 1, 2, 3, 4, 5, and 6 h.

**Animals and Drug Administration** Male Wistar-ST rats (Japan SLC Inc., Shizuoka, Japan), 9 weeks old, weighing 280–300 g, were used throughout the study. The rats were housed in stainless steel cages with 3 animals per cage in a temperature-controlled (24–26 °C) room under a 12-h light/dark cycle. The rats were allowed free access to standard rat chow (Sankyo Labo Service Co., Ltd., Tokyo, Japan) 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 body weight, i.p.). The femoral vein was cannulated with PE-10 tubing (Clay Adams, Parsippany, NJ, U.S.A.) for saline infusion and drug administration. The femoral artery was cannulated with PE-50 tubing (Clay Adams) to collect blood samples over time, with an established heparin lock using 100 units/ml heparin in saline. To avoid enterohepatic circulation, bile drainage was performed in each rat. An abdominal incision was made and the common bile duct was cannulated with

\* To whom correspondence should be addressed. e-mail: uesawa@my-pharm.ac.jp

PE-10 tubing (Clay Adams) and then closed with surgical clips. During the experiment, body temperature was kept at  $38 \pm 0.5^\circ\text{C}$  to prevent the hypothermic alteration of the bile flow. An NFP solution for injection was prepared by dissolving 50 mg of NFP in a mixture of polyethyleneglycol 400 (5 ml), ethanol (5 ml), and saline (10 ml). Two milliliters of GJ, 6-h UV-irradiated GJ (UVGJ), and saline was directly injected into the duodenum using a syringe with a needle. After 30 min, NFP was administered at a dose of 3 mg/kg body weight in the same procedure (intraduodenal 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 at 1.0, 1.5, 2.0, 3.0, and 4.0 h. The samples were immediately centrifuged at 16000 *g* for 15 min at  $4^\circ\text{C}$ , and the plasma was separated. Each rat was also 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 collected plasma samples were stored at  $-80^\circ\text{C}$  until analysis.

**Measurement of NFP in Plasma** The HPLC system consisted of a PU-980 pump (JASCO Co., Ltd., Tokyo, Japan) equipped with a UV-970 UV/VIS detector (JASCO), SIL-9A autoinjector (Shimadzu Corp., Kyoto, Japan), and C-R4A Chromatopac integrator (Shimadzu). NFP was quantified at wavelengths of 260 nm. Plasma samples were analyzed for NFP using a reverse-phase analytical Capcell Pak UG-ODS column [4.6 mm (inside diameter)  $\times$  25 cm; particle size 5  $\mu\text{m}$ ; Shiseido Co., Ltd., Tokyo, Japan] equipped with a guard column packed with Capcell Pak SG-ODS [4.6 mm (inside diameter)  $\times$  1 cm; particle size 5  $\mu\text{m}$ ; Shiseido]. A mobile phase consisting of 0.085% phosphoric acid/MeCN (55 : 45, v/v) was pumped through the column at the rate of 1.0 ml/min. One hundred eighty microliters of IS 1 solution (1  $\mu\text{g}/\text{ml}$  of nitrendipine in MeCN) was added to 20 ml of plasma in a 2-ml plastic tube. After vigorous mixing, the sample was centrifuged at 16000 *g* for 10 min at  $4^\circ\text{C}$ . Twenty-five microliters of the supernatant was directly injected into the HPLC system.

**Measurement of FCs in GJ** The concentrations of the three FC derivatives (BT, DHB, and BG) in GJ used in this study were measured using the HPLC system reported previously.<sup>12</sup> Briefly, the HPLC system consisted of two PU-1580 pumps (JASCO) with an MD-910 multiwavelength UV/VIS detector (JASCO). Data analysis was performed with a JASCO-Borwin computer program (JASCO). The GJ sample was analyzed using a reverse-phase analytical Capcell Pak SG-Phenyl column [4.6 mm (inside diameter)  $\times$  25 cm; particle size 5  $\mu\text{m}$ ; Shiseido] equipped with a guard column packed with Capcell Pak SG-ODS [4.6 mm (inside diameter)  $\times$  1 cm; particle size 5  $\mu\text{m}$ ; Shiseido]. A mobile phase consisting of water and MeCN was pumped through the column at a speed of 1.0 ml/min with the following gradient: 0–15 min, 40% MeCN; subsequently from 40 to 100% MeCN in 30 min. The three FC derivatives (BT, DHB, and BG) contained in the GJ were isolated using HPLC with 5.2-, 7.1-, and 31.9-min retention times, respectively. The FC derivatives had a maximum UV absorption at 311 nm commonly certified by the multiwavelength UV/VIS detector.

**Pretreatment of GJ Samples** Four hundred microliters of IS 2 solution (1  $\mu\text{g}/\text{ml}$  of anthracene in MeCN) was added to 100  $\mu\text{l}$  of GJ in a 2-ml plastic tube. After vigorous mixing,

the sample was centrifuged at 16000 *g* for 10 min at  $4^\circ\text{C}$ . Fifty microliters of the supernatant was directly injected into the HPLC system.

**Analytical Methods** From the NFP/IS peak area ratios *versus* various NFP concentrations in the drug-free plasma samples supplemented with an aliquot of standard NFP, calibration curves (0.63–20  $\mu\text{g}/\text{ml}$ ) were drawn using linear least-squares regression analysis. The concentrations of the FC derivatives in GJ were obtained from the calibration curves (1–50  $\mu\text{g}/\text{ml}$ ) for the three standard FCs. Each test was performed in duplicate at room temperature ( $23^\circ\text{C}$ ).

**Pharmacokinetic Analysis** The plasma concentration–time data (0–4 h) from each rat were analyzed with a model-independent method using the MULTI computer program.<sup>18</sup> The area under the concentration–time curve (*AUC*) was calculated from the values obtained using the trapezoidal rule (0–4 h). The mean residence time (*MRT*) was calculated using moment analysis.<sup>19</sup> The apparent steady-state volume of distribution ( $V_{\text{dss}}/F$ ) was calculated by multiplying the dose by *MRT/AUC*, where *F* is the bioavailability. The apparent plasma clearance (*CL/F*) was calculated by dividing the dose by the *AUC*. The half-life ( $t_{1/2}$ ) was obtained by dividing  $\ln 2$  by *kel*, the apparent elimination rate constant, as obtained from the elimination-phase gradient.

**Data Analysis** All data are expressed as mean  $\pm$  S.D. The unpaired Student's *t*-test and one-way ANOVA, followed by least-significant difference analysis, were used to test for significant differences in the mean values. The significance level was set at  $p < 0.05$ .

## RESULTS

**Measurement of FCs in GJ** BT, DHB, and BG were identified as major FCs in GJ based on the retention times on the HPLC chromatogram and by the comparison with the UV spectra of each FC standard. The concentrations of BT, DHB, and BG in the GJ used in this study were 9.58, 3.42, and 6.29  $\mu\text{g}/\text{ml}$ , respectively. All FCs showed a consistent decrease during UV irradiation for 6 h (Fig. 1). After 6 h, the concentrations of BT, DHB, and BG in GJ treated with UV

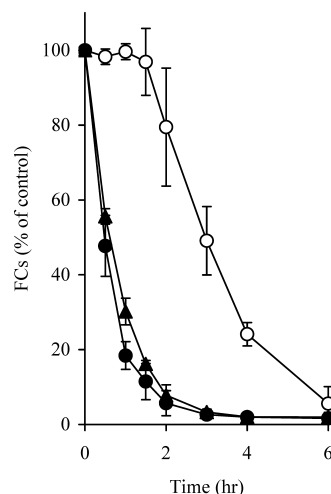


Fig. 1. Time Course of Concentrations of BT(○), DHB(●), and BG(▲) in GJ after UV Irradiation

Each point and vertical bar represent the mean and S.D. ( $n = 3$ ). The experimental conditions are described in Materials and Methods.

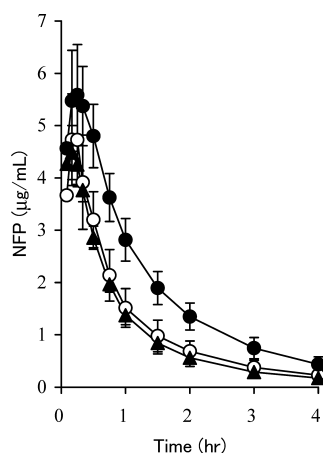


Fig. 2. Plasma NFP Concentration–Time Curves after Intraduodenal Administration of NFP (3 mg/kg Body Weight) 30 min after Preadministered Saline (○), GJ (●), and UVGJ (▲)

Each point and vertical bar represent the mean and S.D. ( $n=5$ ). The experimental conditions are described in Materials and Methods.

Table 1. Pharmacokinetic Parameters of NFP after Intraduodenal Administration to Rats Preadministered Saline, GJ, or UVGJ

Parameter	Saline	GJ	UV-GJ
AUC (mg·h/ml)	4.85±1.75	7.86±2.29*	4.38±1.25
MRT (h)	1.03±0.18	1.17±0.10	0.96±0.13
CL/F (l/h)	0.18±0.06	0.11±0.03*	0.18±0.05
$V_{dss}$ (l)	0.21±0.07	0.14±0.03	0.19±0.06
$t_{1/2}$ (h)	1.31±0.32	1.19±0.27	1.23±0.36
$T_{max}$ (h)	0.20±0.05	0.27±0.14	0.20±0.05
$C_{max}$ (mg/ml)	5.02±1.90	5.98±1.73	4.89±1.45

Saline, the rats were administered saline 30 min before NFP; GJ, the rats were administered GJ 30 min before NFP; UVGJ, the rats were administered UVGJ before NFP. \* $p<0.05$  compared with the values in the group administered saline.

were 0.54, 0.07, and 0.10  $\mu\text{g/ml}$ , respectively.

**Animal Experiments** Figure 2 shows the plasma NFP concentration–time profiles in rats preadministered saline, GJ, and UVGJ into the duodenum. The pharmacokinetic parameters were shown in Table 1. The mean AUC value of the rats administered GJ was about 1.62-fold that of the rats receiving saline, and the mean apparent clearance (CL/F) value was about 0.61-fold greater (Fig. 1, Table 1). No statistically significant difference was observed between the pharmacokinetic data of the rats administered saline and UVGJ (Fig. 2).

## DISCUSSION

GJ has been known to require attention in clinical settings because of its interactions with many types of drugs including dihydropyridine antihypertensive drugs like NFP.<sup>1–4</sup> In the interactions, the concentrations of drugs in the blood are increased by GJ. Many patients receiving pharmaceuticals for the treatment of chronic diseases such as essential hypertension are forbidden to drink GJ lifelong. GJ can be considered a luxury drink because of its unique flavor and tartness. Therefore prohibiting GJ consumption may be a factor that decreases the quality of life (QOL). However, positive approaches to remove the GJ components related to drug interactions have not been investigated. In this paper, we report a method to eliminate the interactive elements from GJ.

GJ decreases the small intestinal metabolism of drugs dependent on CYP3A because of the mechanism-based inhibition of the enzyme.<sup>20</sup> As a result, the bioavailability of drugs increases. The FC derivatives BG and DHB in GJ were shown to be inhibitors of CYP3A in *in vivo* and *in vitro* studies.<sup>5–12</sup> On the other hand, the FC 8-methoxypsoralen is widely used as in the treatment of dermatologic conditions because of photosensitivity.<sup>21</sup> This FC is unstable when UV irradiated, as are 5-methoxypsoralen, BT, and BG.<sup>13,16,17</sup> The degradation of 8-methoxypsoralen by UV irradiation might prevent the DNA-binding activities, a major mechanism in this agent. Another important physiologic function of FCs is the mechanism-based inhibition of P450 s. It was reported that 8-methoxypsoralen and 5-methoxypsoralen suppress CYP2A6 and 2B1 as suicide inhibitors.<sup>22–24</sup> GJ FCs such as BG and DHB, which are CYP3A inhibitors, cause adverse reactions to drugs in the clinical setting as mentioned above. If UV irradiation of GJ eliminates the risk of GJ, this process will be useful to create “harmless” GJ for patients receiving long-term pharmacotherapy. The effects of UV irradiation on GJ and drug interactions have not studied previously. Furthermore, the stability of DHB, one of the most important FCs in GJ in drug interactions in humans,<sup>7</sup> after UV irradiation has not been reported. Therefore changes in the concentrations of the major FCs BG, DHB, and BT in GJ during UV irradiation were investigated.

Rapid decreases were observed in the concentrations of BG and DHB with UV irradiation from the transilluminator at 5°C. Under these experimental conditions, the time to reduce the concentration of BG and DHB by one-half was about 0.5 h. The concentration of BT, which has not been reported to cause drug interactions, also decreased time dependently with UV irradiation, but more slowly than that of BG and DHB. About 3.0 h was needed to reduce the concentration by one-half. BG, DHB, and BT were reduced to 1.6, 2.0, and 5.6% of the baseline concentration in GJ after UV irradiation for 6 h, respectively.

It was speculated that drug interactions of FCs are eliminated due to degradation after UV irradiation. Therefore the drug interaction of GJ irradiated with UV for 6 h was investigated in *in vivo* pharmacokinetic experiments in rats. We reported that the plasma concentration–time profile (AUC) of NFP, a typical substrate of CYP3A, administered intraduodenally increased at 30 min after preadministration of GJ into the duodenum.<sup>25</sup> After intravenous administration of NFP under similar conditions, the pharmacokinetic parameters of NFP were not altered by GJ preadministration. Furthermore, NFP oxidation by rat intestinal microsomes was inhibited by GJ.<sup>12</sup> These results indicate that the NFP-GJ interaction in rats was caused by an increase in bioavailability due to the inhibition of the intestinal CYP3A-oxidation of NFP in rats, as also occurs in humans.<sup>26</sup> On the other hand, preadministration of UVGJ did not result in any significant difference in the pharmacokinetic parameters including  $C_{max}$ , AUC, and MRT compared with the controls. This confirms that UV irradiation of GJ inhibits the drug interaction of GJ due to a decrease in the concentration of FCs. These observations in rats may be reproduced in humans because the mechanism in the inhibition of interaction must result from the elimination of FCs in GJ by UV irradiation. It was reported, however, that 8-methoxypsoralen and 5-methoxypsoralen generate cy-

totoxic singlet oxygen during UV-irradiation.<sup>27)</sup> The physiologic safety of the photodegradation products of FCs in GJ has not been confirmed. Further investigations are therefore necessary to apply our findings to the clinical setting from the viewpoint of safety.

In conclusion, UV irradiation of GJ eliminates FCs in GJ. Furthermore, UVGJ lost the ability to interact with intraduodenal NFP in rats. These observations demonstrate that UV irradiation is a useful method to prevent interactions between GJ and pharmacotherapeutic agents.

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