Effects of Furanocoumarin Derivatives in Grapefruit Juice on Nifedipine Pharmacokinetics in Rats

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Purpose. It has been reported that grapefruit juice (GJ) causes a pharmacokinetic interaction with many drugs after co-ingestion. It is postulated that the substances in GJ may inhibit the first-pass metabolism during the intestinal absorption process. In recent years, several furanocoumarin derivatives that inhibit P450 activity in intestinal microsomes were isolated from GJ. In this study, we report the effects of the furanocoumarin derivatives in GJ on the nifedipine (NFP) pharmacokinetics in rats.
Methods. Three furanocoumarin derivatives (bergaptol [BT], bergamottin [BG], and 6′,7′-dihydroxybergamottin [DHB]) found in GJ were used in this study. Each furanocoumarin was reconstituted in orange juice at the same concentration as in the GJ. Two milliliters of each sample was administered into the rat duodenum. After 30 min, NFP was intraduodenally administered at a dose of 3 mg/kg body weight. The NFP concentrations in the plasma samples were determined by HPLC.
Results. A significant increase in the AUC of NFP was observed only in the rats administered BG; 1.5 times that of the control group. The result was quite identical with that of the group that was administered GJ. BT and DHB had no significant effects on the NFP pharmacokinetics.
Conclusions. The results strongly suggested that BG in GJ might be the substance that elevates the NFP plasma concentrations.

KEY WORDS: grapefruit juice; furanocoumarin; nifedipine; pharmacokinetic interaction; bergamottin; first-pass effect.

INTRODUCTION

It has been reported that grapefruit juice (GJ) causes pharmacokinetic interactions with many drugs after co-ingestion (1). Dihydropyridine calcium antagonists, including nifedipine (NFP), nisoldipine, nitrendipine, and felodipine, are such representative drugs (2). When these drugs are taken with GJ, the plasma concentrations of the drugs significantly increase, resulting in serious adverse reactions such as hypotension.

Many of the drugs interacting with GJ include the substrates for the phase I metabolic enzyme cytochrome P450 (CYP) 3A and drug efflux protein P-glycoprotein. Because these interactions occur only after oral co-administration, and not the intravenous (i.v.) administration of the drugs, it is suggested that the GJ-drug interaction may be caused by the inhibition of both the CYP3A and P-glycoprotein-mediated drug transport during the intestinal absorption process (1,3). Recently, it was reported that some furanocoumarin derivatives, bergamottin (BG), 6′,7′-dihydroxybergamottin (DHB), and some furanocoumarin dimers, were isolated from GJ (4–6), which strongly inhibited CYP3A activity in vitro (7–9). However, it has not yet been established whether these furanocoumarins are the substances that cause the GJ-drug interaction in vivo.

To determine the compounds in GJ that cause the GJ-drug interaction in vivo, we studied the effects of three furanocoumarin derivatives (BG, DHB and bergaptol [BT, 5-hydroxypsoralen]), contained in GJ on the NFP pharmacokinetics using rats.

MATERIALS AND METHODS

All handling procedures for nifedipine were performed under subdued light.

Chemicals

NFP and nifedipine oxide (NFPO) were kindly donated by Kanebo Pharmaceuticals, Ltd. (Osaka). Nitrendipine (internal standard 1 [IS 1]) was obtained from Yoshitomi Pharmaceutical Industries, Ltd. (Osaka). BG and DHB were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo). BT and bergapten (BTN, 5-methoxypsoralen) were obtained from Funakoshi Co., Ltd. (Tokyo). Phenylmethylsulfonyl fluoride (PMSF) was purchased from the Sigma Chemical Co. (St. Louis, MO). Anthracene (internal standard 2 [IS 2]) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka). Methanol (MeOH), acetonitrile (MeCN), dimethyl sulfoxide (DMSO), and 85 w/v% phosphoric acid used were of HPLC grade (Wako). All other chemicals were reagent grade (Wako). Water was used after double-distillation in a glass still. GJ (single strength) and orange juice (OJ, single strength) were purchased from Kanda Foods Laboratory Co., Ltd. (Tokyo) and the Kirin Beverage Co., Ltd. (Tokyo), respectively.

Animals and Drug Administration

Male Wistar-ST rats (Sankyo Labo Service Co., Ltd., Tokyo), weighing 280–300 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 under 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 body weight, intraperitoneally [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) to collect blood samples over time, with an established heparin-lock using 100 units/ml heparin in saline. Urine samples were collected via a PE-50 tubing bladder cannula

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ABBREVIATIONS: BG, bergamottin; DHB, 6′,7′-dihydroxybergamottin; BT, bergaptol; BTN, bergapten; GJ, grapefruit juice; OJ, orange juice; NFP, nifedipine; MeOH, methanol; MeCN, acetonitrile; IS, internal standard; i.d., intraduodenal; NFPO, nifedipine oxide; AUC, area under the concentration-time curve; MRT, mean residence time; Vd_{ss},steady state volume of distribution; CL, apparent plasma clearance; F, bioavailability; $t_{1/2}$, half-life; PMSF, phenylmethylsulfonyl fluoride.

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into 5-ml glass tubes for 4 consecutive h. To avoid enterohepatic circulation, bile drainage was performed for each rat. An 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 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). Furanocoumarin solutions were prepared by dissolving BG (6.3 μg/ml), DHB (3.4 μg/ml), and BT (9.6 μg/ml) in OJ at the same concentrations in GJ.

Two milliliters of GJ, saline, OJ, or each furanocoumarin solution was directly injected into the duodenum by a syringe with a needle. After 30 min, NFP was administered at a dose of 3 mg/kg body weight in the same protheder (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 at 1.0, 1.5, 2.0, 3.0, and 4.0 h. The samples were immediately centrifuged at 16,000 *g* for 15 min at 4°C, and the plasma was separated. Bile was collected into 2-ml plastic tubes for 30 consecutive min. 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°C until analysis.

HPLC Conditions

Measurement of NFP and NFPO

The HPLC system consisted of a PU-980 pump (JASCO Co., Ltd., Tokyo) equipped with a UV-970 UV/VIS detector (JASCO), a SIL-9A auto-injector (Shimadzu Corp., Kyoto), and a C-R4A Chromatopac integrator (Shimadzu). NFP and its 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] × 25 cm; particle size 5 μm; Shiseido Co., Ltd., Tokyo) equipped with a guard column packed with Capcell Pak SG-ODS (4.6 mm [inside diameter] × 1 cm; 5 μm particle size; 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. Another mobile phase consisting of 0.085% phosphoric acid/MeOH/MeCN (55:30:20, v/v) was used for the analysis of NFPO in the microsomal experiments.

Measurement of Furanocoumarin Derivatives in GJ

The concentrations of the four furanocoumarin derivatives (BT, BTN, DHB, and BG) in GJ used in this study were measured by HPLC. The HPLC system consisted of two PU-1580 pumps (JASCO) with a MD-910 multiwavelength UV/VIS detector (JASCO). Data analysis was achieved with a JASCO-Borwin computer program (JASCO). The GJ sample was analyzed using a reversed-phase analytical Capcell Pak SG-Phenyl column (4.6 mm [inside diameter] × 25 cm; particle size 5 μm; Shiseido) equipped with a guard column packed with Capcell Pak SG-ODS (4.6 mm [inside diameter] × 1 cm; particle size 5 μm; Shiseido). A mobile phase consisting of distilled 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; 45–60 min, 100% MeCN.

The three furanocoumarin derivatives (BT, DHB, and BG) contained in the GJ were isolated by the HPLC, and each molecular ion, $[M + H]^+$, of the furanocoumarins was measured by the positive-ion fast atom bombardment (FAB) mass. Positive-ion FAB mass spectra were obtained with a JEOL JMS-DX302 instrument (JEOL, Tokyo), using a direct-inlet system, and an Auto Spec mass spectrometer (Micromass UK, Ltd., Manchester, U.K.) equipped with an FAB source using cesium atoms. The instrument was operated with an accelerating voltage of 3 kV, and Magic Bullet was used as the FAB matrix for the acquisition of positive-ion spectra.

Pretreatment of HPLC Samples

GJ Sample

Four hundred microliters of IS 2 solution (1 μg/ml anthracene in MeCN) was added to 100 μl of GJ in a 2-ml of plastic tube. After vigorous mixing, the sample was centrifuged at 16,000 *g* for 10 min. Fifty microliters of the supernatant was directly injected into the HPLC system.

Plasma Samples

One hundred eighty microliters of IS 1 solution (1 μg/ml nitrendipine in MeCN) was added to 20 μl of plasma in a 2-ml of plastic tube. After vigorous mixing, the sample was centrifuged at 16,000 *g* for 10 min at 4°C. Twenty-five microliters of the supernatant was directly injected into the HPLC system.

Analytical Methods

From the NFP/IS peak area ratios vs. various NFP concentrations in the drug-free plasma samples supplemented with an aliquot of standard NFP, calibration curves (0.63–20 μg/ml) were drawn by a linear least-squares regression analysis. The in vitro NFPO concentrations were obtained from the calibration curves for various standard NFPO concentrations (0.5–10 μM) added to the reaction mixture excluding NFP. The concentrations of the furanocoumarin derivatives in GJ were obtained from the calibration curves (5–20 μM) for the three standard furanocoumarins. Each test was performed in duplicate at room temperature (23°C).

Pharmacokinetic Analysis

The plasma concentration-time data (0–4 h) from each subject were analyzed by a model-independent method using the MULTI computer program (10). 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 by moment analysis (11). The apparent steady-state volume of distribution (Vd_{ss}/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 k_{el}, the apparent elimination rate constant, as obtained from the elimination phase gradient.

Preparation of Intestinal Microsomes

Microsomes from rat intestinal epithelia were prepared using the method of Bonkovsky et al. (12). Briefly, a 30-cm portion (from the pars pylorica ventriculi) of the rat small intestine was rinsed with solution A, pH 7.3 containing 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, and 0.23 mM PMSF, then incubated for 15 min at 37°C in solution A. The solution A was then replaced with ice-cooled solution B containing 136.9 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM dithiothreitol, and 0.23 mM PMSF, and the intestine was placed on a glass plate on ice and gently tapped several times. The harvesting procedures of the intestinal epithelia in solution B were repeated three times. The cell suspension was centrifuged at 900 g for 5 min at 4°C. The separated cells were washed two times with solution C, pH 7.0 containing 5.0 mM histidine, 0.25 M sucrose, 0.5 mM EDTA, and 0.23 mM PMSF, and centrifuged at 800 *g* for 10 min at 4°C. The cells were next homogenized in 5 ml of solution C and centrifuged at 15,000 *g* for 10 min at 4°C. After calcium chloride solution was added to the supernatant for a final concentration of 10 mM, the solution was allowed to stand on ice for 15 min. The intestinal microsomes were obtained by centrifugation at 2,000 *g* for 10 min at 4°C. The microsomal pellet was resuspended in 100 mM Tris-HCl (pH 7.4) containing 20 w/v% glycerol, 10 mM EDTA and 0.23 mM PMSF, and stored at −80°C until use. The protein concentration was measured by the method of Lowry et al. (13).

Microsomal Experiments

In our preliminary *in vitro* studies, a Michaelis-Menten plot was drawn. Because the V_{max} value of the NFPO formation was constant from 100 μM of NFP to 500 μM under the incubation conditions, we used 500 μM of the substrate. In the time-course experiments with 500 μM of NFP, the NFPO produced linear up to 20 min.

Each 50 μM of BT, DHB, BTN, or BG (10.1, 18.6, 10.8, or 16.9 μg/ml, respectively) dissolved in 7.5% DMSO was incubated with the microsomes (150 μg) and NADPH (1 mM) in 0.1 M sodium phosphate buffer (pH 7.4) for 10 min (final incubation volume, 30 μl). An aliquot (10 μl) of the reaction mixture was transferred to a 2-ml plastic tube containing NFP (500 μM) and NADPH (1 mM) in 0.1 M sodium phosphate buffer (pH 7.4) (final volume, 120 μl). After 10 min of incubation, MeCN (200 μl) and a saturated aqueous ammonium sulfate solution (120 μl) were added to the reaction mixture. The sample was mixed vigorously for 20 sec and centrifuged at 16,000 *g* for 5 min at 4°C, then the organic layer (25 μl) was injected into the HPLC system. The NFPO produced from NFP by the enzymatic reaction was analyzed by HPLC. Control incubations were carried out by the addition of 7.5% DMSO instead of the furanocoumarins.

Data Analysis

All data are expressed as mean ± SD. 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 Furanocoumarin Derivatives in GJ

Four furanocoumarin derivatives, BT (5), BTN (6), DHB, and BG (Fig. 1), in GJ were identified by the retention times on the HPLC chromatogram and by the comparison with the UV spectra of each furanocoumarin standard. The furanocoumarin derivatives had a maximum UV absorption at 311 nm commonly (λ_{max} 311 nm) certified by the multiwavelength UV/VIS detector. The HPLC chromatograms at 311 nm are shown in Fig. 2. The retention times of BT, DHB, BTN, and BG were 5.2, 7.1, 7.9, and 31.9 min, respectively, under the HPLC conditions described above. The concentrations of BT, DHB, and BG in the GJ used in the study were 9.6, 3.4, and 6.3 μg/ml, respectively (Fig. 2A). BTN could not be detected in the GJ used (it was below the detection limit), which was further certified by HPLC using another mobile phase (30% MeCN, retention time 11.3 min). The four furanocoumarin derivatives could not be detected in the evaluated OJ (Fig. 2B). The furanocoumarins isolated from GJ were identical with each authentic standard. FAB MS ($[M + H]^+$) *m/z:* 203 forBT, 373 forDHB, and 339 BG, respectively.

Animal Experiments

Figure 3 shows the plasma NFP concentration-time profiles in rats preadministered with GJ, OJ, saline, and three furanocoumarin (BT, DHB, and BG) solutions into the duodenum. The pharmacokinetic parameters are shown in Table 1. The mean AUC value of the rats administered GJ was about 1.62 times that of the rats receiving saline, and the mean CL/F value was about 0.61 times greater (Fig. 3A, Table 1). No statistically significant difference was observed among the pharmacokinetic data of the rats administered saline, OJ, BT, and DHB (Fig. 3A, B, and C), whereas the NFP plasma concentrations of the rats preadministered BG were significantly increased. BG caused a significant increase in the AUC (1.5 times) and C_{max} (1.4 times), and a decrease in the appar-

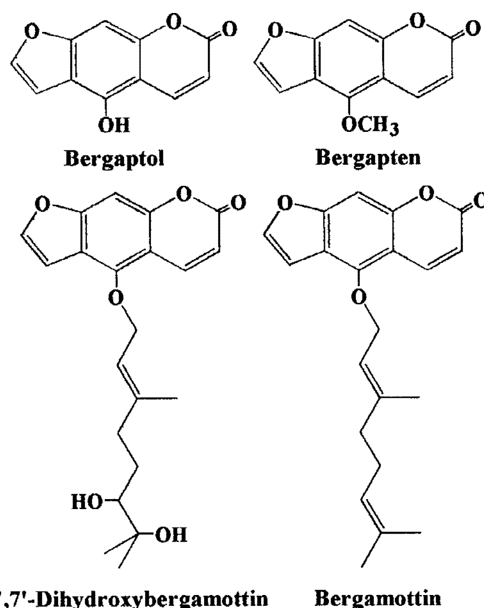

Fig. 1. Chemical structures of furanocoumarin derivatives in GJ.

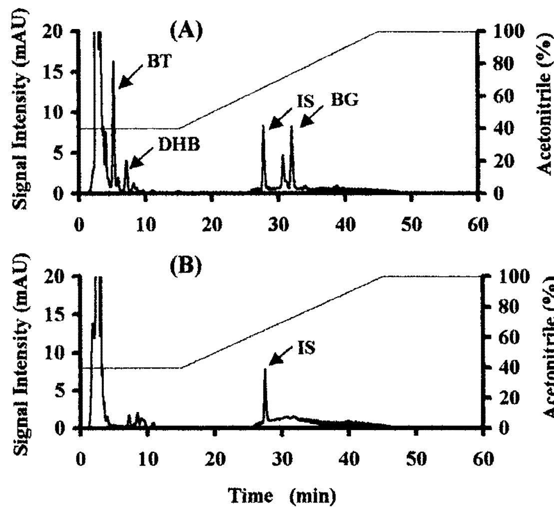

Fig. 2. HPLC chromatograms of GJ (A) and OJ (B) at 311nm.

ent CL/F (0.63 times) compared with OJ (Fig. 3D). No NFPO, a primary metabolite of NFP, could be detected in the plasma, bile, or urine samples of the rats.

Microsomal Experiments

Figure 4 shows the effects of the furanocoumarins on the NFP-oxidation activity in the intestinal microsomes. The NFP-oxidation activities with the control, BT, BTN, DHB, and BG were 0.18 ± 0.017, 0.19 ± 0.007, 0.15 ± 0.019, 0.12 ± 0.003, and 0.070 ± 0.003 nmol/min/mg protein, respectively.

DISCUSSION

It has been reported (1,2,14) that when the drugs are taken with GJ, the bioavailability of the drugs increases. Bailey et al. (15) reported that the bioavailability of oral NFP (10 mg) taken with GJ was 1.35 times greater than that with water in humans. We represented the GJ-drug interaction observed in humans with NFP and GJ (single strength) using rats.

In the rats that received GJ 30 min prior to the NFP i.d. administration, the plasma NFP concentrations significantly increased, compared to that with saline or OJ (controls) (Fig. 3, Table 1), which was very similar to the drug-GJ interaction in humans.

The AUC of NFP was approximately 1.6 times greater than that of the controls (Table 1). Grundy et al. (16) reported that although the concentrated GJ concentrate (twice concentrations) significantly increased the NFP bioavailability (relative bioavailability 2.02 compared with tap water) in the rat, the regular-strength GJ had no significant effect on the NFP bioavailability. However, the single-strength GJ we used significantly increased the plasma NFP concentrations. This might be due to different kinds and concentrations of the components in GJ.

In recent years, it was reported that some furanocoumarin derivatives isolated from GJ significantly inhibited the P450 activities in the hepatic and intestinal microsomes, re-

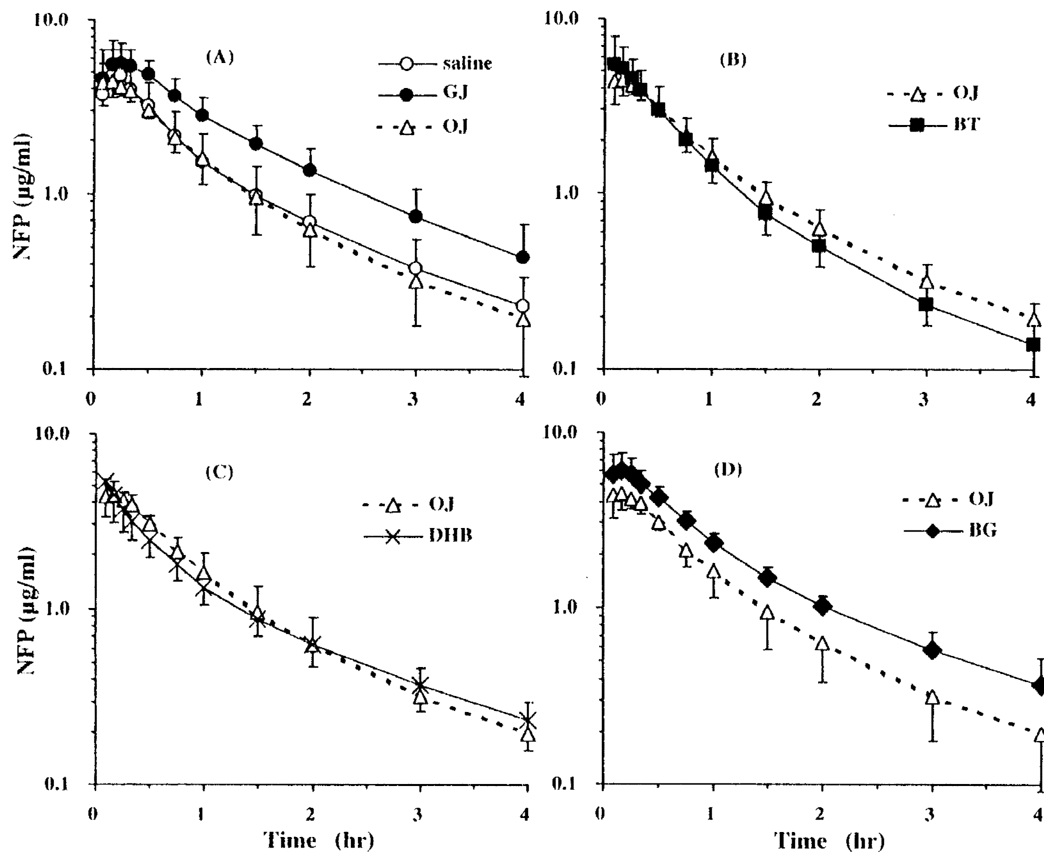

Fig. 3. Plasma NFP concentration-time curves after i.d. administration of NFP (3 mg/kg body weight) 30 min preadministered saline (A), GJ (A), OJ (A–D), BT (B), DHB (C), and BG (D), respectively.

Table I. Pharmacokinetic Parameters of NFP After i.d. Administration in Rats Preadministered with Saline, GJ, OJ, or Furanocoumarin Derivatives[a]

Parameteres	Saline	GJ	OJ	BT[b]	DHB	BG
AUC (μg · hr/ml)	4.85 ± 1.75	7.86 ± 2.29**††	4.71 ± 1.03	4.59 ± 1.52	4.58 ± 1.10	6.91 ± 1.13††
MRT (hr)	1.03 ± 0.18	1.17 ± 0.10	0.97 ± 0.13	1.07 ± 0.08	1.01 ± 0.16	0.77 ± 0.07
CL/F (l/hr)	0.18 ± 0.05	0.11 ± 0.03*†	0.18 ± 0.04	0.18 ± 0.07	0.17 ± 0.04	0.10 ± 0.02*†
Vdss/F (l)	0.21 ± 0.07	0.14 ± 0.03	0.18 ± 0.03	0.16 ± 0.08	0.20 ± 0.08	0.12 ± 0.02*
Kel (hr^{-1})	0.55 ± 0.14	0.60 ± 0.11	0.62 ± 0.24	0.77 ± 0.34	0.50 ± 0.11	0.54 ± 0.16
$t_{1/2}$ (hr)	1.31 ± 0.32	1.19 ± 0.27	1.30 ± 0.57	1.06 ± 0.49	1.43 ± 0.33	1.41 ± 0.53
Tmax (hr)	0.20 ± 0.05	0.27 ± 0.14†	0.15 ± 0.07	0.13 ± 0.05	0.10 ± 0.04*	0.18 ± 0.07
Cmax (μg/ml)	5.02 ± 1.90	5.98 ± 1.73	4.54 ± 0.98	5.61 ± 2.26	5.29 ± 1.97	6.13 ± 1.63*†

[a]Saline: the rats were administered saline. GJ and OJ: the rats were administered each citrus juice 30 min before NFP administration. BT, DHB and BG: the rats were administered bergaptol, 6′,7′-dihydroxybergamottin and bergamottin 30 min before NFP administration, respectively. * $p < 0.05$ and ** $p < 0.01$; compared with the values in the group administered saline. † $p < 0.05$ and †† $p < 0.01$; compared with the values in the group administered OJ.

[b]The concentrations of BT, DHB, and BG in OJ used were prepared to the same concentrations in GJ, respectively.

sulting in the down-regulation of intestinal CYP3A (4,7–9). The GJ used in this study contained three furanocoumarin derivatives, BG, DHB, and BT. Because BG and DHB are insoluble in water, we reconstituted each furanocoumarin in OJ at the same concentration in the GJ. The OJ increased the solubility of these furanocoumarin derivatives. We administered GJ, OJ, each furanocoumarin in OJ, or saline into the rat duodenum before the 30 min NFP i.d. administration.

Because the NFP pharmacokinetic profile in the rats administered OJ was very similar to that of the rats administered saline (Fig. 3A), it is obvious that OJ does not affect the NFP pharmacokinetics. This finding was identical with the previous reports in humans (15). As shown in Fig. 3 and Table I, only GJ and BG i.d. administrations increased the bioavailability of NFP. The saline, OJ, BT, and DHB had no significant effects on the NFP plasma concentrations (Fig. 3, Table I). The mean AUC value of the rats receiving BG was about 1.5 times greater than that with OJ, and the mean CL/F value was about 0.56 times. The mean AUC and CL/F values of the rats administered GJ were 1.6 and 0.61 times, respectively, compared to those with saline (Fig. 3, Table I). The pharmacokinetic parameters in the rats pretreated with GJ were very similar to those with BG.

It is reported that NFP is not a substrate for P-glycoprotein (17). Therefore, it has been considered that the PK-interaction of NFP-GJ occurred by only inhibiting the CYP3A mediated metabolism of the drug. To further ensure the effects of furanocoumarins on the intestinal P450 activity, we studied the inhibitory effects of the four furanocoumarin derivatives on the NFP oxidation activity using rat intestinal microsomes. Preincubation of the microsomes with BG, among the four furanocoumarins tested, had the greatest effect on the NFP oxidation activity, by the mechanism-based inhibition (Fig. 4), which is well described by the in vivo results. In contrast, although DHB had a weaker inhibitory effect than BG on the NFP oxidation activity, BT and BTN showed no inhibition. DHB had no pharmacokinetic effect in the rats despite the inhibition of NFP-oxidation during the microsomal experiments. Although the reason is currently unclear, it may be due to intracellular circumstances (e.g., concentrations of NFP, DHB, and metabolic enzyme CYP3A in the intestinal enterocyte) where the in vivo results are different from those in vitro. It may be possible that DHB could not pass though the intestinal enterocyte as does BG, because DHB is more hydrophilic than BG.

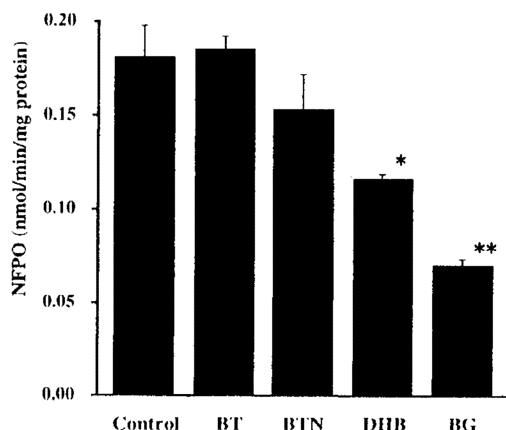

Fig. 4. Effects of 50 μM BT, BTN, DHB, and BG on the NFP oxidation activity in rat intestinal microsomes. * $p < 0.05$ and ** $p < 0.01$; compared with the control value.

Schmiedlin-Ren et al. (7) and He et al. (9) reported the inhibition of BG and DHB against the testosterone 6-beta hydroxylase activity using recombinant CYP3A4. He et al. showed that BG inhibited the CYP3A4 activity more strongly than DHB. Furthermore, Bailey and co-workers (18) also supported that DHB was not responsible for the GJ-felodipine interaction in humans. Edwards et al. (19) also reported that Seville OJ including DHB did not give any pharmacokinetic interaction with cyclosporine after oral coadministration. Considering the results of the inhibitory effects on the NFP oxidation activity and the NFP pharmacokinetic profile in the rats pretreated with DHB, it appears that DHB may not be a key compound in GJ causing the drug-GJ pharmacokinetic interaction.

On the other hand, Tassaneeyakul et al. (20) and Ohnishi et al. (21) reported that DHB was a stronger inhibitor against CYP3A than BG using human hepatic microsomes. We conclude that we cannot compare our data to the previous groups' data, because there is a species difference in both data. We used the intestinal microsomes of the rats, and they used human hepatic microsomes. It is expected that NFP is metabolized by CYP3A4 in the human liver and by CYP3A1

in the rat intestine. Furthermore, although they used less than 1% methanol to solubilize the furanocoumarins, we could not make BG soluble in less than 1% methanol. Therefore, we used 7.5% DMSO to make 50 μM of BG soluble in the enzymatic reaction. It was likely that the previous two groups might underestimate the inhibitory effect by BG against CYP 3A in order to make BG insoluble in the enzymatic reaction. The GJ used included 3.4 μg/ml of DHB, 6.3 μg/ml of BG, and 9.6 μg/ml of BT. To exert an influence on the NFP pharmacokinetics in vivo, concentrations higher than 3.4 μg/ml DHB may be required.

Recently, it was reported that some furanocoumarin dimers were more potent P450 inhibitors when compared with BG and DHB (4,20). Although we measured the furanocoumarin dimers in the GJ used in this study, the dimers could not be detected because they were under the detection limit (20 ng/ml).

In conclusion, BG among the three furanocoumarins (BT, DHB, and BG) contained in GJ, increased the AUC of NFP approximately 1.5 times over that of the control group, which was almost equal to the AUC of the GJ group. From the results using intestinal microsomes and the NFP pharmacokinetics in rats pretreated with furanocoumarins, we propose here that BG in GJ may be the substance that causes elevation of the NFP plasma concentrations in rats, which may be similar to the drug-GJ interaction in humans. Therefore, further investigations in humans are necessary to apply our rat findings to humans.

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