Studies on Interactions between Functional Foods or Dietary Supplements and Medicines. IV. Effects of *Ginkgo biloba* Leaf Extract on the Pharmacokinetics and Pharmacodynamics of Nifedipine in Healthy Volunteers

Mutsunobu YOSHIOKA,[a] Noriaki OHNISHI,*[,b] Tomokazu KOISHI,[b] Yukihisa OBATA,[b] Masato NAKAGAWA,[b] Tsuyoshi MATSUMOTO,[b] Kentaro TAGAGI,[b] Koji TAKARA,[b] Tsuyoshi OHKUNI,[c] Teruyoshi YOKOYAMA,[b] and Kazuo KURODA[a]

[a] *Department of Pharmacy, Takarazuka City Hospital; 4–5–1 Kohama, Takarazuka 665–0827, Japan:* [b] *Department of Hospital Pharmacy, Faculty of Pharmaceutical Sciences, Kyoto Pharmaceutical University; 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan: and* [c] *Ohkuni Clinic; 2–5–24 Sonezaki, Kita-ku, Osaka 530–0057, Japan.*
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The effects of *Ginkgo biloba* leaf extract (GBE), a widely used herbal dietary supplement in Japan, on the pharmacokinetics and pharmacodynamics of nifedipine (NFP), a calcium-channel blocker, were studied using 8 healthy volunteers. Simultaneous oral ingestion of GBE (240 mg) did not significantly affect any of the mean pharmacokinetic parameters of either NFP or dehydronifedipine, a major metabolite of NFP, after oral administration of NFP (10 mg). However, the maximal plasma NFP concentrations in 2 subjects were approximately doubled by GBE, and they had severer and longer-lasting headaches with GBE than without GBE, with dizziness or hot flushes in combination with GBE. The mean heart rate after oral administration of NFP with GBE tended to be faster than that without GBE at every time point. Accordingly, it was concluded that GBE and NFP should not be simultaneously ingested as much as possible, and careful monitoring is needed when administering NFP concomitantly with GBE to humans.

Key words *Ginkgo biloba* leaf; nifedipine; healthy volunteer; pharmacokinetic interaction; P450; heart rate

Standardized *Ginkgo biloba* leaf extract (GBE) has been widely used as functional food or dietary supplement for the treatment or prevention of failing memory, age-related dementias, *etc*. in Japan as well as the United States, and as a phytomedicine in Europe for a long time.[1)] Therefore, it is likely that GBE is frequently ingested in combination with various medicines by many patients.

Previously, we reported that the *in vitro* simultaneous addition of GBE and diltiazem (DTZ), a highly extracted typical substrate of P450 (CYP) 3A, one of the most important drug metabolizing enzymes,[2,3)] and of P-glycoprotein (P-gp), a multidrug resistance transporter,[4,5)] to intestinal and hepatic microsomes in rats resulted in decreased activities of DTZ *N*-demethylase.[6)] Furthermore, a single oral treatment with GBE at a dose of 20 mg/kg inhibited the *ex vivo* activity of CYP3A in rat microsomes of the intestine and liver until approximately 12 h after treatment.[6)] In addition, it was found that the elimination after intravenous administration of DTZ was slightly, but significantly, delayed by simultaneous oral treatment with GBE in rats, and that concomitant oral treatment with GBE significantly increased the bioavailability of DTZ after its oral administration, suggesting that GBE may be an inhibitor of CYP3A, like grapefruit juice (GFJ).[6—8)]

Furthermore, using rats, we examined the effects of GBE on the pharmacokinetics of nifedipine (NFP), a representative substrate of CYP3A in both rats and humans, as well as DTZ.[9—13)] However, NFP is thought to be not a substrate for P-gp, or to be a weak inhibitor, unlike DTZ.[4,5,14)] A single oral treatment with GBE at a dose of 20 mg/kg, which is 10-fold the general daily amount ingested in humans, tended to inhibit *ex vivo* activity of NFP dehydrogenase in rat microsomes of the intestine until about 12 h after treatment.[15)] The simultaneous oral treatment with GBE significantly increased the peak plasma NFP concentration (C_{max}), the area under the concentration–time curve (*AUC*) and absolute bioavailability (*F*) after oral administration of NFP to rats. However, the pharmacokinetics after intravenous administration of NFP was unchanged by oral GBE. These results suggest that the concomitant oral use of GBE appears to reduce the first-pass elimination of orally administered NFP in rats.[16)] However, it has not been clarified until now whether or not these interactions occur in humans.

Therefore, in this study, we examined the effects of simultaneous oral ingestion of GBE on the pharmacokinetics and pharmacodynamics of NFP after oral administration of NFP in 8 healthy male volunteers.

MATERIALS AND METHODS

Chemicals GBE (Ginkgolon-24; Lot No. 830301034) powders capsuled were kindly provided by Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). GBE was produced using a standard method by extraction from milled leaves with ethanol, and the yield was about 2.0%. The final quality of this extract was assured by maintaining the prescribed range of index components (over 24% flavonoid glycosides and 6% terpene lactones and less than 1 ppm ginkgolic acids). NFP and nicardipine (an internal standard for HPLC analysis) were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Dehydronifedipine (NFPO) was obtained from Sumitomo Chemical Ind., Ltd. (Osaka, Japan). All other chemicals were reagent- or HPLC-grade commercial products.

Healthy Volunteers Eight healthy, male volunteers, aged 22 to 45 (mean±S.E., 29±3 years old), from whom informed consent was obtained after a full explanation of the procedures, participated in this study. Their body weights

* To whom correspondence should be addressed. e-mail: ohnishi@mb.kyoto-phu.ac.jp

ranged from 50 to 76 kg (mean±S.E., 60±3 kg). The subjects abstained from any other medication, grapefruit juice and St. John's wort for 2 weeks prior to, and throughout, each study, and from food from 22:00 on the previous night to 12:00 on the test day. Lunch, which was a light meal, was unified among all volunteers and between the two test days. All experimental protocols described below were approved by the Institutional Review Board at Kyoto Pharmaceutical University.

Pharmacokinetic and Pharmacodynamic Experiments This study was performed in an open, random crossover manner with each subject serving as his own control in the two different studies with an interval of 2 weeks. The control studies were carried out by administering a single oral dose of an NFP-containing soft capsule (Adalat®: 10 mg) with 200 ml of water at around 9:00. In the GBE co-administration studies, the volunteers were given an NFP capsule (10 mg) and two capsules containing GBE (a total GBE dose of 240 mg: general daily dose in human[1]) with 200 ml of water. Venous blood samples (5 ml) were collected directly from the left jugular vein into heparinized disposable plastic syringes at zero (just before the start of administration), and 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h after each dose. The procedures of blood collection and centrifugation were performed under sodium lamps to prevent photo-degradation of NFP. The blood obtained from the subjects was centrifuged at 3000 rpm for 10 min at room temperature, and the plasma fraction was frozen at −80 °C and protected from light until the assay. The assays were performed within 2 weeks of collection.

The blood pressure and heart rate were measured about 30 min earlier under a stable condition and immediately after the blood sampling at 1, 2, 3, 4, 6 and 8 h. Furthermore, headaches, dizziness and hot flushes, which are characteristic side-effects appearing transiently and frequently after oral administration of NFP, were monitored by the subjects until 12 h after administration of the drug.

Assays of NFP and NFPO Plasma NFP and NFPO (a major metabolite of NFP) concentrations were simultaneously determined by means of the HPLC-UV method reported by Soons *et al.*[17] with slight modifications as follows. Plasma samples of 1 ml were placed in shaded glass tubes (10 ml) to prevent photo-degradation, and 500 ng/ml of nicardipine-toluene solution (internal standard; 0.1 ml) and 1 ml of 0.1 M sodium hydroxide were added. Then, they were shaken (280 strokes/min) for 20 min after the addition of 5 ml of toluene. The mixture was centrifuged at 3000 rpm for 15 min, and then the organic layer (4 ml) was evaporated to dryness at 65 °C under a stream of nitrogen in a light proof box. The resulting residue was completely reconstituted with 200 μl of the mobile phase, and then aliquots of 100 μl were injected into an HPLC apparatus (LC-10AS; Shimadzu, Kyoto, Japan) equipped with an ultraviolet detector (SPD-6A; Shimadzu) with an automatic injector (SIL-9; Shimadzu). The mobile phase consisted of methanol/0.1 M sodium acetate/acetic acid (48 : 51 : 1, pH 4.6). The conditions for analysis were as follows: column size, 25 cm×4.0 mm i.d.; packing, STR ODS-III (Shinwa Chemical Industries, Ltd., Kyoto, Japan); flow rate, 1.0 ml/min; column temperature, 40 °C; wavelength, 250 nm; and sensitivity, 0.00125 a.u.f.s. The retention times for NFP, NFPO and nicardipine were approximately 24, 15 and 19 min, respectively. The calibration curves for NFP (10—200 ng/ml) or NFPO (5—100 ng/ml) showed good linearity (r^2>0.999). The limit of quantitation for NFP or NFPO was about 5 ng/ml.

Pharmacokinetic Analysis The C_{max} and the time to reach C_{max} (T_{max}) for NFP or NFPO were determined from the actual data obtained after oral administration of NFP with or without GBE. The areas under the plasma concentration–time curves from zero to 8 h ($AUC_{0—8}$) for NFP and NFPO were calculated with the trapezoidal rule.

Statistical Analysis Data are expressed as the mean± standard error (S.E.). Comparisons between two groups were performed using the paired Student's *t*-test with StatView J5.0 for Macintosh (Abacus Concepts Inc., Berkeley, CA, U.S.A.), and differences were considered statistically significant when p<0.05.

RESULTS

Effects of Simultaneous Oral Ingestion of GBE on NFP Pharmacokinetics The mean plasma concentration–time curves of NFP and NFPO, an inactive and major metabolite of NFP in the blood, after oral administration of NFP (10 mg) with or without simultaneous oral GBE ingestion (240 mg) are shown in Figs. 1A and B. The mean plasma NFP and NFPO concentration profiles in the two groups were almost the same. Tables 1 and 2 summarize the pharmacokinetic parameters for NFP and NFPO. None of the mean pharmacokinetic parameters for either NFP or NFPO were significantly changed by concomitant treatment with GBE. However, the simultaneous ingestion of GBE tended to increase the mean C_{max} value for NFP by approximately 30%. Particularly, in the No. 1 and 3 subjects, the values in combination with GBE were about 2-fold greater than that in the control, though the increased rates were below 1.3 in other individuals.

Effects of Simultaneous Oral Ingestion of GBE on NFP Pharmacodynamics Figure 2A shows the systolic and diastolic pressures before and after oral administration of NFP (10 mg) with and without GBE (240 mg). The stable systolic pressures before the NFP administration were 124±4 and 124±4 mmHg, and the diastolic pressures were 79±3 and 74±3 mmHg in the control and GBE groups, respectively. In the control group, the mean systolic pressure decreased maximally by about 9% at 2 h and the mean diastolic pressure de-

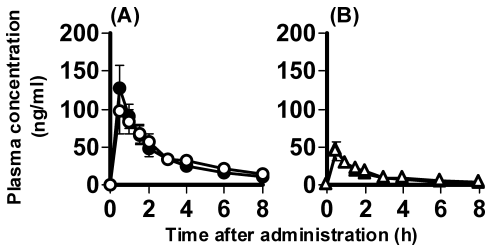

Fig. 1. Effects of Simultaneous Oral Ingestion with GBE on the Mean Plasma Concentrations of NFP and NFPO after Oral Administration of NFP to Healthy Volunteers

Each point represents the mean±S.E. of 8 adult male volunteers. An NFP capsule (10 mg) was administored orally with water (200 ml) in combination with or without GBE (240 mg) to volunteers. (A) NFP concentration: ○, control; ●, GBE. (B) NFPO concentration: △, control; ▲, GBE.

creased by about 16% at 1 h after drug administration; they were almost normalized after 8 h in both groups. However, no significant differences in their values at any time point between the two groups were observed.

On the other hand, as shown in Fig. 2B, the mean value for heart rate after oral administration of NFP in the two groups increased by about 5—11% at 1 h after administration of the drug, compared with those before NFP administration (control, 78±4 beats/min; GBE, 77±3 beats/min). The heart rate in the GBE group was faster by 2—9% than that in the control group at every observed time point (maximal increased rate, approximately 9% at 4 h; Fig. 2B), and there were no significant differences in the values at any time point between the two groups because the inter-individual variations in these experiments were large.

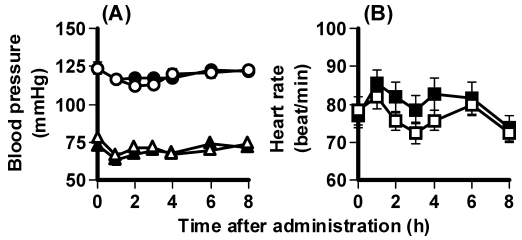

Fig. 2. Effects of Simultaneous Oral Ingestion with GBE on the Blood Pressure and Heart Rate after Oral Administration of NFP to Healthy Volunteers

Each point represents the mean±S.E. of 8 adult male volunteers. An NFP capsule (10 mg) was administored orally with water (200 ml) in combination with or without GBE (240 mg) to volunteers. (A) blood pressure: ○, control (systolic); ●, GBE (systolic); △, control (diastolic); ▲, GBE (diastolic). (B) heart rate: □, control; ■, GBE.

As described above, subjects No. 1 and 3 showed similar results with 2-fold increased C_{max} values; subject No. 1 experienced dizziness, and subjects 2, 3 and 4 had hot flushes only when NFP was co-administered with GBE. Furthermore, subjects No. 1, 3, 5 and 8 had headaches in administration of NFP alone, and subjects 1, 3 and 8 experienced severer and longer-lasting headaches in combination with GBE than without GBE. In cases No. 1 and 3, a light headache continued for 2—4 h after the last time point (8 h) in this study (data not shown).

DISCUSSION

First, we examined the effects of simultaneous oral ingestion of GBE on the pharmacokinetics of NFP and NFPO after its oral administration to humans (Fig. 1). As a result, simultaneous GBE ingestion did not significantly affect any of the mean pharmacokinetic parameters for either NFP or NFPO (Tables 1 and 2). The mean C_{max} value for NFP in the GBE group was about 1.3-fold that in the control group, and particularly the values for subjects No. 1 and 3 were about 2-fold increased by the combination with GBE. It is known that

Table 1. Effects of Simultaneous Oral Ingestion with GBE on the Pharmacokinetic Parameters of NFP after Oral Administration of NFP to Healthy Volunteers

Subject No.	C_{max} (ng/ml)		T_{max} (h)		$AUC_{0—8}$ (ng·h/ml)	
	Control	GBE	Control	GBE	Control	GBE
1	109	199	1.5	0.5	433	387
2	141	61	0.5	0.5	242	199
3	142	276	1.0	0.5	595	648
4	57	52	0.5	1.0	172	130
5	101	121	1.0	1.0	275	264
6	94	97	0.5	0.5	148	138
7	119	149	0.5	0.5	309	322
8	120	161	0.5	0.5	264	277
Mean	110	140	0.8	0.6	305	296
S.E.	10	26	0.1	0.1	52	59

Each point represents the mean±S.E. of 8 adult male volunteers. An NFP capsule (10 mg) was administored orally with water (200 ml) in combination with or without GBE (240 mg) to volunteers.

Table 2. Effects of Simultaneous Oral Ingestion with GBE on the Pharmacokinetic Parameters of NFPO after Oral Administration of NFP to Healthy Volunteers

Subject No.	C_{max} (ng/ml)		T_{max} (h)		$AUC_{0—8}$ (ng·h/ml)	
	Control	GBE	Control	GBE	Control	GBE
1	19	32	1.0	0.5	77	55
2	45	25	0.5	0.5	70	80
3	27	45	0.5	0.5	102	85
4	23	37	0.5	1.0	67	70
5	47	36	0.5	1.0	126	94
6	115	73	0.5	0.5	141	101
7	22	55	0.5	0.5	74	94
8	72	102	0.5	0.5	168	130
Mean	47	51	0.6	0.6	103	89
S.E.	12	45	0.3	0.1	13	8

Each point represents the mean±S.E. of 8 adult male volunteers. An NFP capsule (10 mg) was administored orally with water (200 ml) in combination with or without GBE (240 mg) to volunteers.

NFP is metabolized, mainly by CYP3A, only to NFPO at the first step of the metabolic pathway in the gastrointestinal tract and liver of humans, as well as rats.[9—13] Also, NFP is thought to be not a substrate for P-gp, or to be a weak inhibitor.[4,5,14] In addition, we have not found any report that NFP may be a substrate for multidrug resistance-associated protein (MRP), organic cation transporter (OCT), organic cation/carnitine transporter (OCTN), organic anion transporting polypeptide-B (OATP), *etc*., except P-gp, in human intestine or liver. Further detailed research should be carried out to clarify the pharmacokinetic mechanism of action for 2-fold elevation in C_{max}s in two subjects described above.

Next, the effects of simultaneous oral ingestion of GBE on the pharmacodynamics of NFP after oral administration of NFP were examined. There were no significant differences in the systolic and diastolic pressures at any time point between the two groups (Fig. 2A), but subjects No. 1 and 3, in which the C_{max}s of NFP were approximately doubled by GBE, had severer and longer-lasting headaches with GBE than without GBE, and they experienced dizziness and hot flushes, respectively, only with the combination. Betocchi *et al*. reported that peripheral vascular resistance decreased as a function of NFP concentration ($p<0.001$), however evidence for a pure vasodilator effect of NFP was inconsistent in patients with nonobstructive hypertrophic cardiomyopathy.[18] Furthermore, it has been reported that marked interindivisual variation is found for the correlation between plasma NFP levels and the change in blood pressure although minimum effective concentration of NFP in plasma for patients with hypertension is believed to be 12 ng/ml.[19,20] Also, it is well-known that NFP induces headaches, dizziness and hot flushes, which are characteristic side-effects of the peripheral vasodilator appearing rapidly, transiently or frequently after oral administration of NFP, but there is no information on the relationship between plasma NFP concentrations and their adverse reactions. On the other hand, Rowin and Lewis reported severe side-effects such as spontaneous bilateral subdural hematomas or intracerebral hemorrhage associated with chronic GBE ingestion alone.[21,22] In addition, headache, mild gastrointestinal complaints and allergic skin reactions have been reported with the single use of GBE in rare cases.[1,23] Furthermore, GBE has been reported to improve the blood flow in peripheral blood vessels in the brain.[1,24] Accordingly, the occurrence of severer and longer-lasting headaches in subjects 1 and 3 might be attributed to 2-fold elevation in C_{max}s of NFP or synergic action due to the simultaneous ingestion of GBE.

On the other hand, the mean value for heart rate after oral administration of NFP in combination with GBE tended to be faster maximally, by approximately 9%, than that without GBE (Fig. 2B). This result suggests that there might be a pharmacodynamic interaction by which the heart rate is accelerated, between NFP and GBE when they are used simultaneously. It has been reported that NFP increases heart rate as one of many side-effects of NFP in healthy volunteers,[25] and that NFP increased heart rate immediately and remained elevated for the duration of the infusion at the mean steady-state concentration of about 30 ng/ml when NFP was given intravenously to healthy volunteers with the rapid regimen (within 3 min).[26] In contrast, we were unable to find any report on significant heart rate increases as a side-effect due to GBE alone. Therefore, palpitations due to NFP administration alone might be accelerated in combination with GBE. However, this interactive mechanism of action between NFP and GBE has not been clarified, and further investigation should be undertaken to clarify this point.

In conclusion, it is recommended that GBE and NFP, or other similar calcium-channel blockers, should not be simultaneously ingested as much as possible, and careful monitoring is needed when administering such drugs concomitantly with GBE to humans.

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