

## Studies on Interactions between Functional Foods or Dietary Supplements and Medicines. III. Effects of *Ginkgo biloba* Leaf Extract on the Pharmacokinetics of Nifedipine in Rats

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The effects of *Ginkgo biloba* leaf extract (GBE), one of the most widely used herbal dietary supplements in Japan and the United States, on the pharmacokinetics of nifedipine (NFP), a typical probe of P450 (CYP) 3A, but not a substrate of the multidrug transporter P-glycoprotein (P-gp), were studied using rats. Simultaneous oral treatment with GBE (20 mg/kg) did not affect the pharmacokinetics after intravenous administration of NFP (2.5 mg/kg). However, the maximal plasma NFP concentration, the area under the concentration–time curve and absolute bioavailability after oral administration of NFP (5 mg/kg) were significantly increased by simultaneous oral treatment with GBE, approximately 1.6-fold, 1.6-fold and 2.1-fold, respectively. These results suggest that the concomitant oral use of GBE appeared to reduce the first-pass metabolism of orally administered NFP, by inhibiting CYP3A, possibly but not P-gp, in rats.

**Key words** *Ginkgo biloba* leaf; nifedipine; pharmacokinetic interaction; P450; P-glycoprotein; rat

Standardized *Ginkgo biloba* leaf extract (GBE) is a widely used dietary supplement for the treatment or prevention of Alzheimer's disease, failing memory, age-related dementias, etc. in Japan as well as the United State, and now a phyto-medicine in many countries in Europe.<sup>1)</sup> GBE consists of many flavonoid glycosides and various unique diterpenes such as ginkgolides, which are potent inhibitors of platelet activating factor.<sup>1)</sup> It is highly likely that GBE is used in combination with various medicines by many patients.

Previously, we reported that the simultaneous addition of GBE to the rat small intestine and liver microsomes inhibited the formation of *N*-demethyl diltiazem (MA), an active metabolite of diltiazem (DTZ) produced by a well-known drug metabolizing enzyme P450 (CYP) 3A, in a concentration-dependent manner.<sup>2)</sup> This inhibition appeared to be caused, at least in part, by a mechanism-based inhibition. Both the rate of formation of MA and total amount of CYP in intestinal or hepatic microsomes after a single oral pre-treatment with GBE (20 mg/kg) decreased transiently. Furthermore, it was found the concomitant use of GBE in rats significantly increased the bioavailability after oral administration of DTZ, a typical substrate of CYP3A, as well as a highly extracted drug, while the elimination after intravenous administration of DTZ was slightly delayed by simultaneous oral treatment with GBE.<sup>2)</sup> These findings suggest that there is a possibility of potential pharmacokinetic interactions between GBE and other drugs that are both substrates for CYP3A and extensively extracted drugs.<sup>3,4)</sup> However, there are still many unclear points regarding the inhibitory mechanism of action because DTZ is a substrate of not only CYP3A, but also esterase in rats and humans,<sup>5)</sup> and of the multidrug transporter P-glycoprotein (P-gp) which is expressed in normal tissues with excretory function, playing an important role in drug pharmacokinetics.<sup>6–8)</sup>

Therefore, in this study, we examined the effects of simultaneous oral treatment with GBE on the pharmacokinetics of nifedipine (NFP), which is a representative substrate of

CYP3A, but not P-gp,<sup>6–8)</sup> and is at first metabolized only to dehydronifedipine (NFPO) in the intestine and liver in both rats and humans,<sup>9–13)</sup> after intravenous and oral administration of NFP to rats.

### MATERIALS AND METHODS

**Chemicals** GBE (Ginkgolon-24; Lot No. 830301034) powders were kindly provided by Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). GBE was produced using a standard method by extraction from milled leaves with ethanol. 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, nifedipine (an internal standard for HPLC analysis) and polyethylene glycol (PEG) 400 were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All other chemicals were reagent- or HPLC-grade commercial products.

**Animals** Nine-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), weighing approximately 260–280 g, were used throughout this study. All animal experimental protocols described below were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

**Pharmacokinetic Experiments** All the procedures of drug preparation, dosing and blood collection were performed under sodium lamps to prevent photo-degradation of NFP. The left carotid artery of each rat was cannulated with polyethylene tubing (PE-50; Clay Adams, Dickinson & Co., Parsippany, NJ, U.S.A.) under pentobarbital anesthesia the day before the pharmacokinetic experiment. The animals were fasted, but allowed free access to water for 18 h before the administration of drugs. Distilled water or GBE suspension (20 mg/2.5 ml/kg) was administered orally *via* gastric intubation to unanesthetized rats, and then NFP dissolved in PEG 400 was immediately administered intravenously or

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orally at bolus doses of 2.5 mg/ml/kg or 5 mg/2.5 ml/kg, respectively. Considering the difference in metabolic clearance between rats and humans, the oral doses of NFP and GBE in this study were designed to be about 10-fold of those per day to humans. Blood samples (0.25 ml) were collected through the cannula in heparinized plastic microcentrifuge tubes (1.5 ml) before and at 0.083, 0.167, 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 h (i.v.) or 0.05, 0.125, 0.25, 0.5, 1, 1.5, 2, 3 and 4 h (p.o.) after drug administration. The samples were centrifuged at 13000 rpm for 3 min at room temperature in a Centrifuge 5415C (Eppendorf GmbH, Germany), and the plasma fraction was frozen at  $-80^{\circ}\text{C}$  while protected from light until the assay. The assays were performed within one week of collection.

**Assay of NFP** The NFP concentrations in rat plasma were determined by means of the HPLC-UV method reported by Waller *et al.*<sup>13)</sup> and Soons *et al.*<sup>14)</sup> with a slight modification as follows. Plasma samples of 100  $\mu\text{l}$  were placed in shaded glass tubes (10 ml) to prevent photo-degradation, and 5  $\mu\text{g/ml}$  of nicardipine-toluene solution (internal standard; 100  $\mu\text{l}$ ) and 100  $\mu\text{l}$  of 0.1 M sodium hydroxide were added. Then, they were shaken (280 strokes/min) for 10 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  $65^{\circ}\text{C}$  under a stream of nitrogen in a light-proof box. The resulting residue was completely reconstituted with 200  $\mu\text{l}$  of the mobile phase, and then aliquots of 100  $\mu\text{l}$  were injected into the HPLC apparatus (LC-10AS; Shimadzu, Kyoto, Japan) equipped with an ultraviolet detector (SPD-6A; Shimadzu) making use of 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 $\times$ 4.0 mm i.d.; packing, STR ODS-III (Shinwa Chemical Industries, Ltd., Kyoto, Japan); flow rate, 1.0 ml/min; column temperature,  $40^{\circ}\text{C}$ ; wavelength, 350 nm; and sensitivity, 0.00125 a.u.f.s. The retention times for NFP and nicardipine were approximately 24 and 19 min, respectively. The calibration curves for NFP (0.1–10.0  $\mu\text{g/ml}$ ) showed good linearity ( $r^2 > 0.999$ ). The limit of quantitation was about 10 ng/ml.

**Pharmacokinetic Analysis** The peak plasma concentration ( $C_{\text{max}}$ ) and the time to reach  $C_{\text{max}}$  ( $T_{\text{max}}$ ) of NFP were determined from the actual data obtained after intravenous or oral administration. The terminal elimination rate constant ( $\lambda$ ) was calculated by fitting individual data for three terminal points of the plasma concentration profile with a log-linear regression equation using the least-squares method. The corresponding elimination half-life ( $t_{1/2(\lambda)}$ ) was calculated by dividing  $\ln 2$  by  $\lambda$ . The areas under the plasma concentration-time curves from zero to infinity ( $AUC_{0-\infty}$ ) for NFP was calculated using the trapezoidal rule with extrapolation to infinity with  $\lambda$ . The mean residence time from zero to infinity (MRT) for NFP was estimated by moment analysis.<sup>15)</sup> The absolute bioavailability of NFP after oral administration ( $F$ ) was estimated as follows:  $(AUC_{p.o.} \times D_{i.v.}) / (AUC_{i.v.} (\text{NFP alone}) \times D_{p.o.}) \times 100$ .

**Statistical Analysis** Data are expressed as the mean  $\pm$  standard error (S.E.). Comparisons between two groups were performed using the unpaired 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 Treatment with GBE on NFP Pharmacokinetics after Intravenous Administration

The plasma NFP concentration–time curves after its intravenous administration (2.5 mg/kg) with water (control) or oral GBE suspension (20 mg/kg) are shown in Fig. 1, and the two curves were almost equal. There were no significant differences for each pharmacokinetic parameter between control and GBE group (Table 1).

### Effects of Simultaneous Oral Treatment with GBE on NFP Pharmacokinetics after Oral Administration

Figure 2 shows the plasma NFP concentration–time curves after its oral administration (5 mg/kg) with or without simultaneous oral GBE (20 mg/kg) treatment. The plasma NFP concentration 1 h after oral administration of NFP was significantly higher than that in the control group ( $p < 0.05$ ). The pharma-

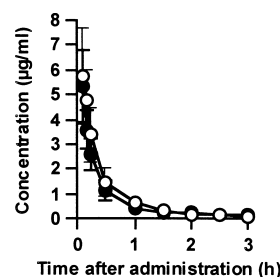


Fig. 1. Effects of Simultaneous Oral Treatment with GBE on the Plasma Concentrations of NFP after Intravenous Administration to Rats

Each point represents the mean  $\pm$  S.E. of 4 or 5 rats. The NFP solution (2.5 mg/kg) was intravenously injected immediately after oral administration with water (control) or a GBE suspension (20 mg/kg) to unanesthetized rats.  $\circ$ , control;  $\bullet$ , GBE.

Table 1. Effects of Simultaneous Oral Treatment with GBE on the Pharmacokinetic Parameters of NFP after Its Intravenous Administration to Rats

Parameter	Control	GBE
$\lambda$ (h)	$0.92 \pm 0.22$	$0.73 \pm 0.15$
$t_{1/2(\lambda)}$ (h)	$0.97 \pm 0.24$	$1.10 \pm 0.19$
$AUC_{0-\infty}$ (mg $\cdot$ h/ml)	$3.27 \pm 0.88$	$2.56 \pm 0.77$
MRT (h)	$0.75 \pm 0.10$	$0.72 \pm 0.14$

Each value represents the mean  $\pm$  S.E. of 4 or 5 rats. The NFP solution (2.5 mg/kg) was intravenously injected immediately after oral administration with water (control) or a GBE suspension (20 mg/kg) to unanesthetized rats.

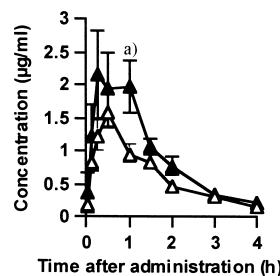


Fig. 2. Effects of Simultaneous Oral Treatment with GBE on the Plasma Concentrations of NFP after Oral Administration to Rats

Each point represents the mean  $\pm$  S.E. of 5 or 7 rats. a)  $p < 0.05$  vs. control at the corresponding time point (unpaired Student's *t*-test). The NFP solution (5 mg/kg) was orally administered immediately after oral administration of water (control) or a GBE suspension (20 mg/kg) to unanesthetized rats.  $\Delta$ , control;  $\blacktriangle$ , GBE.

Table 2. Effects of Simultaneous Oral Treatment with GBE on the Pharmacokinetic Parameters of NFP after Oral Administration to Rats

Parameter	Control	GBE
$C_{\max}$ ( $\mu\text{g/ml}$ )	1.76 $\pm$ 0.20	2.79 $\pm$ 0.40 <sup>a)</sup>
$T_{\max}$ (h)	0.38 $\pm$ 0.06	0.48 $\pm$ 0.15
$\lambda$ (/h)	0.88 $\pm$ 0.15	0.80 $\pm$ 0.25
$t_{1/2(\lambda)}$ (h)	0.95 $\pm$ 0.17	1.31 $\pm$ 0.37
$AUC_{0-\infty}$ ( $\mu\text{g}\cdot\text{h/ml}$ )	2.72 $\pm$ 0.34	4.47 $\pm$ 0.45 <sup>a)</sup>
$MRT$ (h)	1.64 $\pm$ 0.18	1.91 $\pm$ 0.40
$F$ (%)	41.5 $\pm$ 5.2	87.2 $\pm$ 8.9 <sup>b)</sup>

Each value represents the mean $\pm$ S.E. of 5 or 7 rats. a) and b)  $p < 0.05$  and  $p < 0.01$ , respectively vs. each control value (unpaired Student's *t*-test). The NFP solution (5 mg/kg) was orally administered immediately after oral administration with water (control) or a GBE suspension (20 mg/kg) to unanesthetized rats.

cokinetic parameters are summarized in Table 2. The  $C_{\max}$ ,  $AUC_{0-\infty}$  and  $F$  values for NFP were significantly increased by the treatment with GBE, approximately 1.6-fold, 1.6-fold and 2.1-fold, respectively. On the other hand, there were no significant differences in  $T_{\max}$ ,  $\lambda$ ,  $t_{1/2(\lambda)}$  or  $MRT$  values between the two groups. The  $F$  value for NFP in the control group was comparable with that (46.2%) reported by Yoshisue *et al.*<sup>11)</sup>

## DISCUSSION

It is known that NFP is metabolized mainly by CYP3A only to NFPO at the first step of the metabolic pathway in the gastrointestinal tract and liver of rats, as well as humans.<sup>9-13)</sup> Furthermore, NFP has been considered to be not a substrate for P-gp, or to be a weak inhibitor.<sup>6-8)</sup> Therefore, in this study, particularly to clarify the effects of simultaneous oral treatment with GBE on the CYP3A activity alone *in vivo*, we chose NFP as a substrate of CYP3A, but not P-gp, instead of DTZ, a typical probe of CYP3A and P-gp used in our previous similar investigation.<sup>2)</sup>

First, we examined the effects of simultaneous oral treatment with GBE on the pharmacokinetics of NFP after its intravenous administration to rats. As a result, there were no significant differences in any of the pharmacokinetic parameters between the control and GBE groups (Table 1), unlike the case of DTZ.<sup>2)</sup> These results suggested that GBE orally administered to rats did not affect CYP3A activities in the liver *in vivo*. The elimination of DTZ after intravenous administration of DTZ was significantly delayed by simultaneous oral treatment with GBE.<sup>2)</sup> However, there were no significant changes in the elimination rate for NFP after intravenous administration of NFP by the GBE treatment, as described above. Briefly, the results for NFP and DTZ were different. This reason is now unclear, and further detailed investigations are needed. On the other hand, we have previously reported that a single oral treatment with GBE at the same dose (20 mg/kg) as that used in this study tended to inhibit the *ex vivo* activity of NFP dehydrogenase in rat microsomes of the liver during 1-12 h after treatment.<sup>16)</sup> Thus, the result in the intravenous experiment *in vivo* did not correspond to that *ex vivo*. This difference is thought to be due to some factors *in vivo* except the metabolism of NFP in the liver, but this has not been clarified. Further investigation should be undertaken to clarify this point.

Next, we considered the effects of simultaneous oral treat-

ment with GBE on the pharmacokinetics of NFP after its oral administration to rats. There were no significant differences in the  $T_{\max}$ ,  $t_{1/2(\lambda)}$  and  $MRT$  values for NFP between the two groups, while the  $C_{\max}$ ,  $AUC_{0-\infty}$  and  $F$  values for the GBE group were significantly higher than those for the control group, respectively (Table 2). In addition, this *in vivo* result supports the *ex vivo* data that GBE inhibits the activity of NFP dehydrogenase in the rat intestine.<sup>16)</sup> These results indicate that simultaneous oral treatment with GBE reduced the first-pass metabolism of NFP after its oral administration to rats, resulting in increased NFP bioavailability. Thus, the result in the intravenous experiment is inconsistent with that in the oral experiment. One of the reasons why these results are different might be that the final concentration of some unknown inhibitory substance(s) in the liver is much lower than that in the intestine.

On the other hand, the  $F$  value in the GBE group (87.2%) was 2.1-fold higher than that in the control group (41.5%) and was recovered almost to 100% by simultaneous oral treatment with GBE ( $p < 0.05$ ; Fig. 2 and Table 2). In the case of DTZ, we have already reported that the  $F$  value for DTZ after a single oral administration of DTZ to rats (2.0%) was 2.3-fold increased by simultaneous oral treatment with GBE, reaching only 4.6% ( $p < 0.05$ ),<sup>2)</sup> indicating that the inhibitory ratio of first-pass metabolic extraction of NFP (about 46%: the difference in the two  $F$  values in Table 2) is much higher than that of DTZ (about 2.6%). DTZ has been reported to have two major metabolic pathways at the first step and to be metabolized not only to *N*-demethylidiltiazem by CYP3A, but also in parallel and at a high rate to deacetylidiltiazem by the esterase in the intestine and liver of rats.<sup>5,17)</sup> Also, DTZ is a substrate for P-gp, unlike NFP.<sup>6-8)</sup> Accordingly, it is speculated that the first-pass metabolic extraction ratios of NFP and DTZ by CYP3A are much higher and lower, respectively, when comparing the two drugs, and that GBE given orally to rats seems to inhibit CYP3A, possibly but not P-gp, or the esterase in the intestine and liver.

It is already known that GBE contains many kinds of flavonoids such as quercetin, and terpenes known as ginkgolides A, B and C and bilobalide.<sup>18,19)</sup> Moreover, it is reported that quercetin inhibits the metabolism of NFP by liver microsomes *in vitro*.<sup>20)</sup> However, the substance contained in GBE, which inhibits CYP3A activity *in vivo*, is unclear at present. Further investigation should be undertaken to clarify this point.

In conclusion, these results suggest that the concomitant oral use of GBE might reduce the first-pass metabolism of NFP after oral administration, by inhibiting CYP3A, possibly but not P-gp, in rats. Further detailed studies to verify this conclusion are currently in progress in our laboratory. The findings provide information for future investigations using humans on the potential pharmacokinetic interactions between GBE and a variety of drugs extensively metabolized by CYP3A4.

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