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Bilobalide in ginkgo biloba extract is a major substance inducing hepatic CYPs

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Abstract

In a search for substances related to the marked induction of hepatic cytochrome P450 (CYP) by ginkgo biloba extract (GBE), mice were given either GBE (1000 mg kg^{-1}) or fractions of GBE for 5 days. The content and activity of CYPs were induced markedly by a bilobalide-rich fraction, but not by flavonoid-rich fractions. The level of induction by the bilobalide-rich fraction was almost the same as that induced by the unfractionated GBE, suggesting that bilobalide is largely responsible for the CYPs induction. To confirm these findings, mice were given various doses of bilobalide (10.5, 21 and 42 mg kg^{-1}), or GBE (1000 mg kg^{-1}, containing bilobalide at 42 mg kg^{-1}). Treatment with bilobalide induced CYPs markedly and in a dose-dependent manner, and the level of induction was quite similar between bilobalide (42 mg kg^{-1}) and GBE. Treatment with GBE and with bilobalide greatly induced pentoxyresorufin O-dealkylase activity. These findings indicate that bilobalide is the major substance in GBE that induces hepatic CYPs.

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Introduction

Ginkgo biloba extract (GBE) is a popular herbal medicine, and is used mostly by the elderly population for the treatment of cerebral insufficiency, dementia and intermittent claudication (Le Bars et al 1997; Blumenthal 1998; Pittler & Ernst 2000). It has been recognized that GBE exerts many pharmacological effects with few adverse reactions. The adverse reactions include headache, gastric symptoms, diarrhoea and allergic skin reactions, but reports of these are rare (De Smet 2002; Ernst 2002).

Recently, adverse reactions to herbal remedies as the result of herb–drug interactions have received a great deal of attention. For example, St John's wort (*Hypericum perforatum*) induces hepatic cytochrome P450 (CYP)3A4 activity and thereby reduces the efficacy of therapeutic drugs such as ciclosporin, indinavir and digoxin (Roby et al 2000). People who take GBE tend to be elderly because of the reported beneficial effects (De Smet 2002; Ernst 2002) but GBE–drug interactions are a matter of concern. In previous studies, we found that feeding GBE to rats increased markedly the concentration of hepatic CYP, the expression of various CYP mRNAs and the activity of some enzymes (Shinozuka et al 2002; Umegaki et al 2002). Moreover, we reported that pretreatment of rats with GBE attenuated the efficacy of co-administered drugs such as tolbutamide (Sugiyama et al 2004a), nicardipine (Kubota et al 2003) and phenobarbital (Kubota et al 2004). Similar findings in rats were reported by other investigators using EGb761, a well-known standardized GBE extract (Chatterjee et al 2005; Zhao et al 2006). In contrast to these rat studies, reports of GBE–drug interactions in clinical studies are inconsistent, and many of them showed no interaction (Markowitz et al 2003; Mauro et al 2003; Jiang et al 2005; Greenblatt et al 2006; Lu et al 2006; Wolf 2006).

GBE is a natural plant product that contains many different chemicals. Most of the commercially available GBE products are standardized according to the content of ginkgo flavonol glycosides (glycosidic derivatives of quercetin, kaempferol, isorhamnetin) and terpenoids (ginkgolides A, B, C, and bilobalide), which comprise 22–27% and approximately 5–7% of GBE, respectively (Blumenthal 1998; DeFeudis 1998). GBE products contain 0.5–1% organic acids, such as vanillic acid and *p*-hydroxybenzoic acid. The exact constituents of GBE may

vary among the products. Without identifying a substance that induces hepatic CYPs, it is difficult to elucidate the GBE–drug interactions. For this purpose, cell-free studies using human and rat hepatic microsomes have been performed; however, there are discrepancies between the results of in-vitro and in-vivo studies (Gaudineau et al 2004; Sugiyama et al 2004c; Mohutsky et al 2006). To our knowledge, there has been little research intended to identify a substance in GBE that relates to the induction of hepatic CYPs in-vivo.

In this study, we prepared fractionated GBE samples and identified a substance that induces hepatic CYPs in an animal study. To minimize the amount of test samples, we used mice instead of rats, which were used in our previous studies (Shinozuka et al 2002; Umegaki et al 2002; Sugiyama et al 2004a). The results of this study provide important indications for the elimination of GBE–drug interactions and for the standardization of GBE products in the market.

Materials and Methods

Materials

Powdered ginkgo biloba extract (GBE) was supplied by Tama Seikagaku-Kogyo Co. (Tokyo, Japan). GBE contained 24.9% flavonoids and 10.6% total terpene, which consisted of 2.9% ginkgolide A, 1.4% ginkgolide B, 2.1% ginkgolide C and 4.2% bilobalide. Resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, testosterone, 6β-hydroxytestosterone, corticosterone, *p*-nitrophenol, 4-nitrocatechol, 7-ethoxycoumarin, quercetin, ginkgolide A, ginkgolide B, ginkgolide C and bilobalide were purchased from Sigma-Aldrich (St Louis, MO). (*S*)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, UK). NADPH was obtained from Oriental Yeast (Tokyo, Japan). All other reagents were obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan).

Preparation of fractionated GBE samples

Powdered GBE (100 g) was dissolved in water (2000 mL). Diethyl ether (2000 mL) was added and the mixture was shaken in a separating funnel to prepare a diethyl ether extract, which was evaporated under vacuum to dryness (fraction 1, yield 12.11 g). Then, the aqueous layer was extracted with ethyl acetate (2000 mL) to prepare fraction 2 (yield 15.81 g). The resulting aqueous layer was dried, acetone (1000 mL) was added and the mixture was stirred for 4 h, then filtered to obtain the acetone-soluble matter, which was evaporated to dryness (fraction 3, yield 21.79 g). Methanol was added to the acetone-insoluble residue (1000 mL), and the methanol-soluble matter was prepared as described for fraction 3 (fraction 4, yield 38.15 g). Deionized water (500 mL) was added to the methanol-insoluble residue; the mixture was stirred, filtered and dried (fraction 5, yield 8.02 g). Finally, 0.05% NaOH (500 mL) was added to the insoluble residue; the mixture was stirred, filtered and dried (fraction 6, yield 1.84 g). Terpenoids (ginkgolide A, ginkgolide B, ginkgolide C and bilobalide) in the samples were determined by HPLC with an evaporative light-scattering detector (Shimadzu, Kyoto) according to the method of Tang et al (2003). For the analysis of flavonoids (quercetin, isorhamnetin, kaempferol), samples were treated with 6% HCl in 60% aqueous methanol at 90 °C for 60 min to hydrolyse flavonoid glycosides, and the resulting flavonoid aglycones were determined by HPLC with a UV detector measuring absorbance at 360 nm (Shimadzu, Kyoto, Japan). The HPLC conditions were as follows: column material, L-column ODS (4.6 mm × 250 mm; Chemical; Inspection & Testing Institute, Tokyo, Japan); temperature, 40°C; flow-rate, 1 mL min^{-1}; mobile phase, 0.5% citric acid–acetonitrile–isopropanol (100:35:4, by vol.). Typical chromatograms of unfractionated GBE sample are shown in Figure 1.

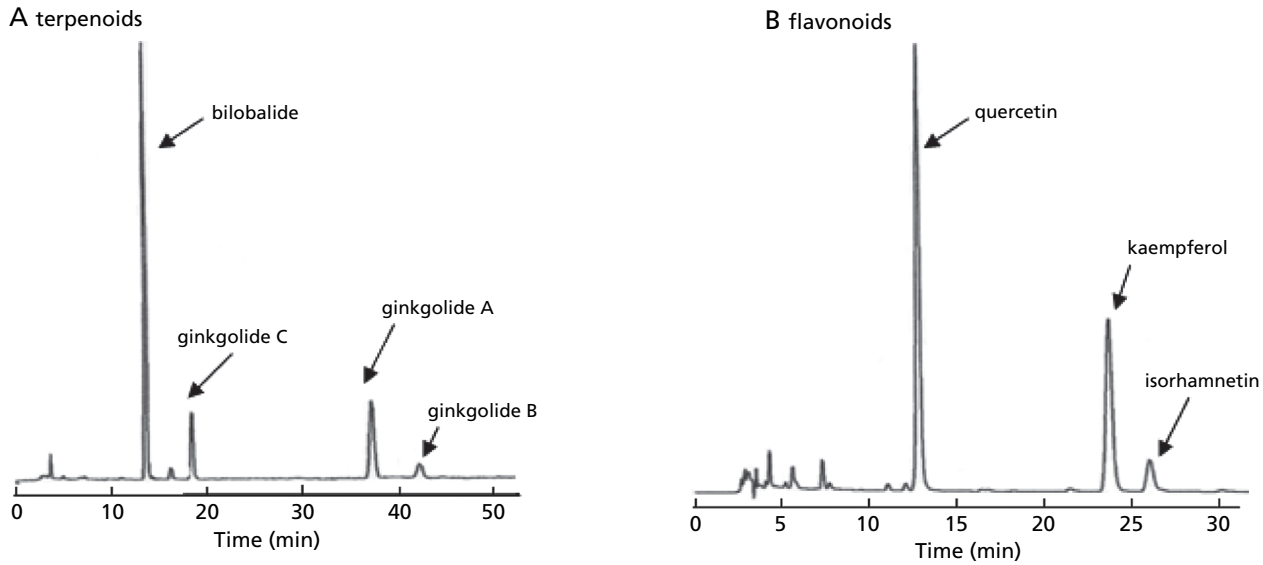

Figure 1 HPLC chromatogram of terpenoids (A) and flavonoids (B) in unfractionated GBE sample. Terpenoids (ginkgolide A, ginkgolide B, ginkgolide C, bilobalide) were detected by HPLC with evaporate light-scattering detector. Flavonoids aglycones (quercetin, isorhamnetin, kaempferol) were detected by HPLC with a UV detector (360 nm). Detailed conditions are shown in the text.

Animal experiments

Male ICR mice, 5 weeks old (Japan Clea, Tokyo, Japan), were kept at a constant temperature (23 ± 1°C) with a 12-h light–dark cycle in polypropylene cages with free access to laboratory feed (CE2; Japan Clea) and tap water. Mice were given the test samples orally (GBE, various fractions of GBE, or bilobalide) suspended in 0.5% carboxymethylcellulose or vehicle for 5 days. One day after the last administration, mice were anaesthetized with pentobarbital and sacrificed. The liver was removed immediately and weighed. All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals, and approved by the ethical committee.

Preparation of microsomes from the liver and analysis of CYP enzyme activity

The liver was rinsed with 0.9% (w/v) NaCl, and homogenized in 50 mmol L^{-1} Tris-HCl buffer (pH 7.4) containing 0.25 mol L^{-1} sucrose. The homogenate was centrifuged at 10 000 *g* at 4°C for 30 min. The supernatant was centrifuged at 105 000 g at 4°C for 60 min. The pellet was washed once with 50 mmol L^{-1} Tris-HCl buffer (pH 7.4) containing 0.25 mol L^{-1} sucrose by centrifugation at 105000 *g* at 4°C for 60 min, and the concentration and activity of CYP were analysed. The CYP content was quantified by the method of Omura & Sato (1964), and the activity of various CYP enzymes were determined by HPLC methods as described (Umegaki et al 2002). The subtypes of CYP enzymes examined and the corresponding CYPs were: ethoxyresorufin O-deethylase, CYP1A1; methoxyresorufin O-demethylase, CYP1A2; pentoxyresorufin O-dealkylase, CYP2B; (*S*)-warfarin 7-hydroxylase, CYP2C9; p-nitrophenol hydroxylase, CYP2E1; and testosterone 6β-hydroxylase, CYP3A.

Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL).

Statistical analysis

The data are presented as the mean and standard deviation (s.d.) for the individual groups. Statistical analysis of the data was carried out by analysis of variance with Fisher's PLSD post-hoc test. $P < 0.05$ was considered to be significant. These statistical analyses were performed with Stat View 5.0 (ASA Institute Inc., Cary, NC).

Results

A preliminary study showed that oral administration of GBE to mice at doses of 100 mg kg^{-1} and 1000 mg kg^{-1} induced an increase of the hepatic content and activity of CYPs in a dose-dependent manner, as observed in the earlier study with rats (Umegaki et al 2002). Thus, in this mouse study, screening for a substance that induced hepatic CYPs in GBE was performed at a dose of 1000 mg kg^{-1}. As shown in Table 1, GBE was separated into fractions 1–6. Typical substances were terpenoids in fractions 1 and 2 and flavonoids in fractions 3 and 4. In particular, fraction 1 contained the greatest amount of bilobalide (80% of the GBE). For 5 days, mice were given either GBE (1000 mg kg^{-1}) or a sample of one of the fractions, the dose of which was equivalent to the amount contained in GBE (1000 mg kg^{-1}). No significant influence of body weight or liver weight was observed due to the administration. Induction of an increased CYP content was greatest for fraction 1 and lower for fractions 3–6 (Figure 2). The level of induction was comparable between fraction 1 and GBE. Also, the activity of various CYPs was highest with fraction 1, and the level was almost the same as that induced by GBE (Table 2). These findings suggested that bilobalide is the major component of GBE that induces hepatic CYPs. To confirm this hypothesis, mice were given various doses of bilobalide (10.5, 21 and 42 mg kg^{-1}), or GBE (1000 mg kg^{-1}) that contained bilobalide at a dose of 42 mg kg^{-1}. The administration of bilobalide induced an increase in the content and activity of CYPs in a dose-dependent manner (Figure 3, Table 3). The induction levels of the CYPs at the 42 mg kg^{-1} dose of bilobalide and GBE were quite similar. GBE markedly induced pentoxyresorufin O-dealkylase activity, as reported previously (Umegaki et al 2002), and marked induction of pentoxyresolfin O-dealkylase by the administration of bilobalide was detected in this study. The enzyme induction by bilobalide at the dose of 10.5 mg kg^{-1} was 13.8 times higher than in the untreated control (Table 3).

Table 1 Major constituents in fractionated samples of ginkgo biloba extract (GBE)

Fraction No.	Amount in GBE (%)	Content in fractionated samples from GBE						
		Flavonoids			Terpenoids			
		Quercetin	Kaempferol	Isorhamnetin	Ginkgolide A	Ginkgolide B	Ginkgolide C	Bilobalide
1	12.11	2.93	0.42	0.34	7.64	5.36	3.33	15.33
2	15.81	12.46	12.65	0.31	5.18	1.77	6.94	3.56
3	21.79	15.42	10.92	2.60	ND	ND	0.1	ND
4	38.15	14.05	9.32	1.74	ND	ND	ND	ND
5	8.02	6.53	4.24	0.78	ND	ND	ND	ND
6	1.84	2.43	2.52	0.06	ND	ND	ND	ND

ND, not detected.

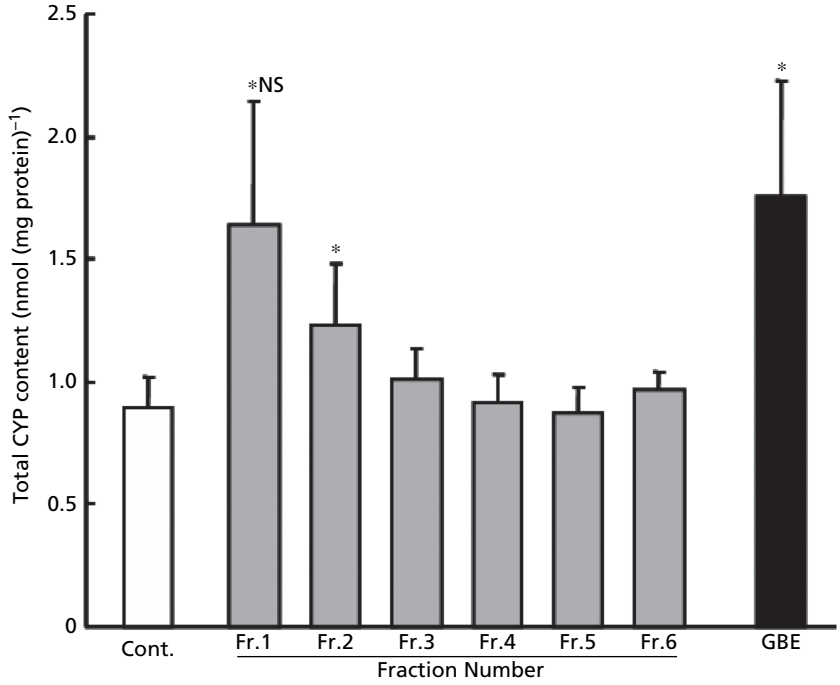

Figure 2 Hepatic CYP content in mice given either ginkgo biloba extract (GBE) or its fractionated samples. Mice were given either GBE (1000 mg kg^{-1}) or fractionated GBE samples (at the same amount of each constituent in GBE) for 5 days. The dose of the fractionated samples was 121 mg kg^{-1} in fraction 1, 158 mg kg^{-1} in fraction 2, 218 mg kg^{-1} in fraction 3, 382 mg kg^{-1} in fraction 4, 80 mg kg^{-1} in fraction 5 and 18 mg kg^{-1} in fraction 6. Each value is expressed as the mean ± s.d. for 5 mice. *$P < 0.05$ vs control; ^{NS}not significant vs GBE (1000 mg kg^{-1}).

Table 2 Activity of hepatic CYPs in mice given either ginkgo biloba extract (GBE) or its fractionated samples

	Control	**Fractionated GBE**						**GBE 1000 mg kg^{-1}**
Dose (mg kg^{-1})	0	Fraction 1 121	Fraction 2 158	Fraction 3 218	Fraction 4 382	Fraction 5 80	Fraction 6 18	
	Activity (pmol (mg protein)$^{-1}$ min^{-1})							
Ethoxyresorufin O-deethylase (CYP1A1)	42.9 ± 8.6	123.6 ± 37.3 [2.9]*^{NS}	64.8 ± 21.6 [1.5]	56.9 ± 11.8 [1.3]	35.6 ± 15.4 [0.8]	31.4 ± 7.7 [0.7]	33.0 ± 8.9 [0.8]	101.1 ± 39.3 [2.4]*
Methoxyresorufin O-demethylase (CYP1A2)	35.4 ± 8.7	75.5 ± 12.8 [2.1]*^{NS}	46.6 ± 14.1 [1.3]	37.2 ± 5.7 [1.1]	30.4 ± 6.7 [0.9]	25.8 ± 3.8 [0.7]	23.2 ± 4.4 [0.7]	58.8 ± 17.8 [1.7]*
Pentoxyresorufin O-dealkylase (CYP2B)	6.8 ± 2.3	113.5 ± 41.2 [16.7]*^{NS}	34.8 ± 13.4 [5.1]*^{NS}	8.7 ± 3.3 [1.3]	6.4 ± 1.3 [0.9]	5.5 ± 1.4 [0.8]	8.1 ± 1.7 [1.2]	116.1 ± 32.0 [17.1]*
(s)-Warfarin 7-hydroxylase (CYP2C9)	1.12 ± 0.22	3.25 ± 1.32 [2.9]*^{NS}	1.79 ± 0.54 [1.6]	1.52 ± 0.48 [1.4]	1.11 ± 0.31 [1.0]	1.04 ± 0.13 [0.9]	1.41 ± 0.31 [1.3]	3.24 ± 1.42 [2.9]*
p-Nitrophenol hydroxylase (CYP2E1)	4710 ± 549	6146 ± 1069 [1.3]*^{NS}	4304 ± 946 [0.9]	5316 ± 740 [1.1]	5004 ± 778 [1.1]	4967 ± 888 [1.1]	5082 ± 570 [1.1]	6619 ± 1096 [1.4]*
Testosterone 6β-hydroxylase (CYP3A)	2560 ± 433	5249 ± 1724 [2.1]*^{NS}	3464 ± 550 [1.4]	2428 ± 586 [0.9]	2434 ± 528 [1.0]	2092 ± 171 [0.8]	3147 ± 257 [1.2]	5509 ± 2110 [2.2]*

Mice were given either GBE (1000 mg kg^{-1}) or fractionated GBE samples (Fractions 1–6) at the same amount of each constituent in GBE for 5 days. Each value is expressed as the mean ± s.d. for 5 mice. Number in brackets indicates the increase ratio to control. *$P < 0.05$, vs control; ^{NS}not significant vs GBE (1000 mg kg^{-1}).

Discussion

As GBE is a natural product that gives a variety of products with different compositions, identification of a substance that induces hepatic CYPs is critical for standardizing GBE products and to avoid GBE–drug interactions, which are suggested by studies in animals (Kubota et al 2003, 2004; Sugiyama et al 2004a; Zhao et al 2006) and man (Yin et al 2004; Uchida et al 2006). Various studies have been performed to identify the active substance in GBE, but no clear data have been obtained so far. In this study, we tried to

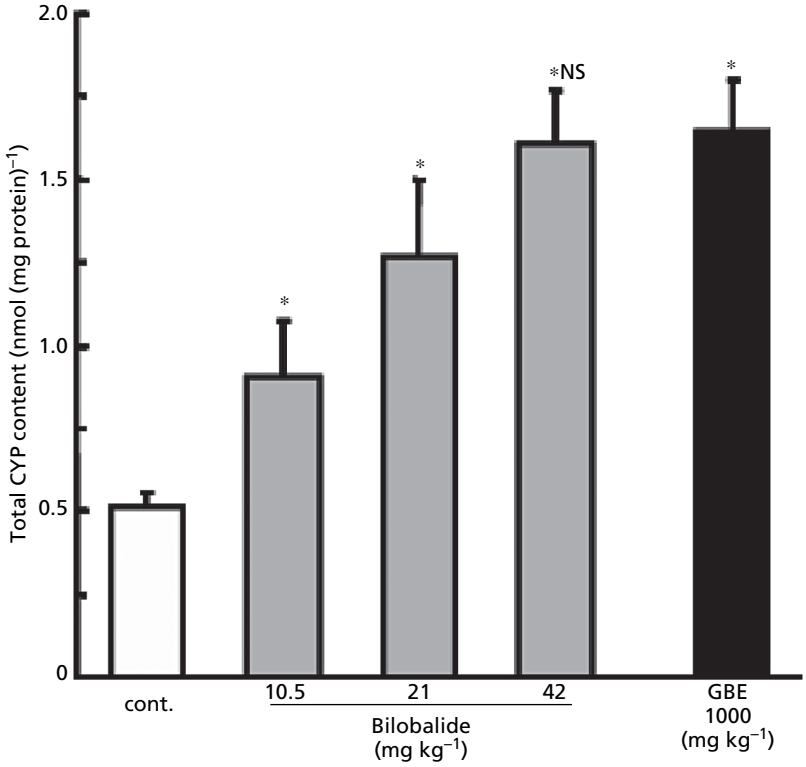

Figure 3 Hepatic CYP content in mice given bilobalide or ginkgo biloba extract (GBE). Mice were administered either bilobalide (10.5, 21, 42 mg kg^{-1}) or GBE (1000 mg kg^{-1}; 42 mg kg^{-1} as bilobalide) for 5 days. Each value is expressed as the mean ± s.d. for 5 mice. *$P < 0.05$ vs control; ^{NS}not significant vs GBE (1000 mg kg^{-1}).

Table 3 Activity of hepatic CYPs in mice given bilobalide or ginkgo biloba extract (GBE)

	Control	**Bilobalide**			**GBE 1000 mg kg^{-1}**
Dose (mg kg^{-1})	0	10.5	21	42	
	Activity (pmol (mg protein)$^{-1}$ min^{-1})				
Ethoxyresorufin O-deethylase (CYP1A1)	41.4 ± 5.6	96.5 ± 24.9 [2.3]*	133.2 ± 20.9 [3.2]*^{NS}	147.0 ± 24.3 [3.6]*^{NS}	147.0 ± 40.7 [3.6]*
Methoxyresorufin O-demethylase (CYP1A2)	31.4 ± 3.0	76.9 ± 14.9 [2.4] *	108.6 ± 23.3 [3.5]*^{NS}	136.7 ± 15.2 [4.4]*^{NS}	124.7 ± 25.7 [4.0]*
Pentoxyresorufin O-dealkylase (CYP2B)	6.1 ± 1.2	84.3 ± 12.6 [13.8]*	98.6 ± 6.7 [16.2]*^{NS}	113.8 ± 2.6 [18.7]*^{NS}	105.6 ± 13.5 [17.3]*
(s)-Warfarin7-hydroxylase (CYP2C9)	1.07 ± 0.22	2.31 ± 0.76 [2.2]*	3.18 ± 0.49 [3.0]*^{NS}	4.41 ± 0.87 [4.1]*^{NS}	3.83 ± 1.41 [3.6]*
p-Nitrophenol hydroxylase (CYP2E1)	5253 ± 111	6594 ± 558 [1.3]*	7093 ± 504 [1.4]*^{NS}	8107 ± 509 [1.5]*^{NS}	7919 ± 1329 [1.5]*
Testosterone6β-hydroxylase (CYP3A)	2242 ± 247	3596 ± 629 [1.6]*	4422 ± 616 [2.0]*	6603 ± 1144 [2.9]*	7875 ± 120 [3.5]*

Mice were administered either bilobalide (10.5, 21, 42 mg kg^{-1}) or GBE (1000; 42 mg kg^{-1} as bilobalide) for 5 days. Each value is expressed as the mean ± s.d. for 5 mice. Number in brackets indicates the increase ratio to control. *$P < 0.05$ vs control; ^{NS}not significant vs GBE (1000 mg kg^{-1}).

identify the substance that induces hepatic CYPs in an animal study.

As shown by our results, it is suggested strongly that bilobalide is the major substance in GBE that induces hepatic CYPs. The GBE used in our study contained 42 mg of bilobalide in 1000 mg GBE, and induction levels of CYPs by 42 mg kg^{-1} bilobalide and those by 1000 mg GBE were quite similar. Induction of CYPs by bilobalide has been reported by

other investigators. Sasaki et al (1997) has reported that administration of bilobalide to mice at 30 mg kg^{-1} (per os) for 4 days increased the amount of hepatic drug-metabolizing enzymes. Chang et al (2006a) has observed the induction of CYP2B1 mRNA, and the respective enzyme activity, by bilobalide in primary cultured rat hepatocytes. In our previous studies, we showed that administration of GBE to rats increased markedly the expression of CYP2B mRNA, and the content and activity of enzyme (i.e. pentoxyresorufin O-dealkylase (CYP2B)) (Shinozuka et al 2002; Umegaki et al 2002). As indicated in Tables 2 and 3, the activity of pentoxyresorufin O-dealkylase was highest in bilobalide-rich fraction, and was markedly induced by bilobalide. The GBE used in our studies contained higher amount of bilobalide as shown in Figure 1. Taken together, from these findings it is reasonable to conclude that bilobalide is a major substance in GBE inducing hepatic CYPs. Marked induction of CYPs was not observed for GBE samples that were rich in flavonoids (i.e. fractions 3 and 4). As reported previously (Sugiyama et al 2004c), feeding quercetin, a major flavonoid in GBE, for 1 week at a dose of 0.125% in the diet did not induce hepatic CYPs in rats. These findings indicate that the involvement of flavonoids in GBE in induction of hepatic CYPs is highly unlikely or very low. On the other hand, ginkgolide A and B in GBE may be also responsible for the induction of CYPs, because both compounds are also rich in the fraction 1, which showed the highest induction of CYPs (Figure 2, Table 2). In a cell culture study, it has been reported that ginkgolide A in GBE increased CYP3A mRNA and CYP3A-mediated enzyme activity (Rajaraman et al 2006), although the effect of GBE can not be explained only by ginkgolide A. The content of ginkoglide A and B in GBE is lower than that of biloblide (Figure 1), therefore the contribution of ginkgolide A and B to the induction of CYPs by GBE may be very low. Further detailed study will be needed to clarify the exact contribution of ginkgolide A and B to the induction of CYPs by GBE.

The lack of an effect of bilobalide on hepatic CYPs has been reported in cell-free enzyme studies using microsomes from rats or man (Kuo et al 2004; Sugiyama et al 2004c; von Moltke et al 2004; Chang et al 2006b). Studies performed in a cell-free enzyme system are very effective for screening for a substance in GBE that influences hepatic CYPs, but the results are not always applicable to the in-vivo situation. This discrepancy in the effect of GBE in-vivo and in-vitro has been pointed out by several studies (Gaudineau et al 2004; Sugiyama et al 2004c; Mohutsky et al 2006). In the cell-free study using microsomes, an unphysiological situation, such as concentration or form of the substances, might contribute to the difference in results obtained in the in-vivo situation. Direct interaction between enzymes and substances examined in the cell-free study may give rise to misunderstanding of the real phenomena that occur the in-vivo situation. GBE–drug interactions have been reported in clinical studies, and the results are inconsistent. The interaction of GBE (280–360 mg daily for 12–28 days) with omeprazole (Yin et al 2004) and tolbutamide (Uchida et al 2006) has been demonstrated. It should be mentioned that these findings are similar to those obtained in animal studies (Kubota et al 2003; Sugiyama et al 2004a; Zhao et al 2006) but the GBE–drug interaction, particularly the pharmacodynamic interaction, is very slight. On the other hand, the lack of interaction of GBE (120–240 mg, for less than 14 days) with flurbiprofen and tolbutamide (Greenblatt et al 2006; Mohutsky et al 2006), dextromethorphan and alprazolam (Markowitz et al 2003), S-warfarin (Jiang et al 2005), digoxin (Mauro et al 2003) and acetylsalicylic acid (Wolf 2006) has been reported. It is reasonable to speculate that the dose (in particular bilobalide in GBE) and periods of treatment with GBE are related to the inconsistent results of GBE–drug interaction in most of the clinical studies and the animal studies. Generally, the recommended dose of standardized GBE is less then 240 mg per day, and positive interaction data are found at doses higher than this. In animal studies, marked induction of hepatic CYPs was detected with a high dose (>10 mg kg^{-1}) of GBE, equivalent to more than 600 mg per day in man. A difference of hepatic drug-metabolizing enzymes between species is well known. Thus, species difference of CYPs may be involved, in part, in the different findings of GBE–drug interactions between man and rats.

There are many chemicals in GBE but it is not clear which is (or are) responsible for the beneficial effect of GBE in cases such as cerebral insufficiency and peripheral vascular disease (Le Bars et al 1997; Blumenthal 1998; Pittler & Ernst 2000). The marked effect of bilobalide on hepatic CYPs shown in this study indicates that bilobalide is a biologically active substance within GBE, and may be related to the beneficial effect of GBE. In fact, several studies have shown a neuroprotective effect of bilobalide in-vivo (Chandrasekaran et al 2003; Mdzinarishvili et al 2007). The reported half-life of bilobalide in blood is approximately 2 h in rats and man (Biber & Koch 1999; Mauri et al 2001), indicating it is eliminated easily from blood. These data agree well with the results of our previous study (Sugiyama et al 2004b), in which continuous and excess GBE administration to rats induced hepatic CYPs, and discontinuation of the treatment led to the recovery of the normal level of CYPs within 1 week. Therefore, GBE–drug interactions are not of particular concern when healthy subjects are using the recommended dose (< 240 mg daily) of standardized products. Even in patients who have taken GBE and worry about an interaction with a drug, the interaction could be rapidly preventable by discontinuation of GBE administration.

In conclusion, we identified that bilobalide in GBE is a major substance that induces hepatic CYPs in this animal study, in which absorption, metabolism and excretion of the substances in GBE can be evaluated. The results will be helpful when considering the detail of interactions between GBE and co-administered drugs in the clinical field.

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