**Ginkgo biloba** Extract Markedly Induces Pentoxyresorufin O-Dealkylase Activity in Rats

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ABSTRACT—We examined the effect of *Ginkgo biloba* extract (GBE) on hepatic drug-metabolizing enzymes, particularly cytochrome P450 (CYP), in rats. Rats were fed a GBE-containing diet or received GBE by intragastric gavage. The concentration of CYP and activity of various CYP enzymes in the liver were increased in a dose- and time-dependent manner. Significant increases in the concentration and activities of CYP enzymes were detected on day 1 of feeding of a 0.5% GBE diet and after administration of 10 mg GBE/kg body weight for 5 days by intragastric gavage. Among the CYP enzymes, the activity of pentoxyresorufin O-dealkylase (PROD), a CYP2B enzyme, was especially markedly increased. The induction of CYP2B enzyme by GBE was confirmed by Western blot analysis. Addition of GBE to a CYP assay system in vitro caused concentration-dependent inhibition of various CYP enzyme activities. The inhibition was more marked for the microsomal enzymes from GBE-treated rats than for those from control rats and more marked against PROD activity among the CYP enzymes tested. When the inhibition of various CYP enzymes activities by GBE in vitro was compared, no marked difference was observed between rat and human hepatic microsomal enzymes. These results indicate that excess intake of GBE induces CYP enzymes, particularly PROD, and may modify the efficacy of drugs taken simultaneously.

Keywords: *Ginkgo biloba*, Cytochrome P450, Rat liver, Pentoxyresorufin O-dealkylase

Herbal medicines have received great attention as alternative medicines in recent years and are sold as a dietary supplement or health food. *Ginkgo biloba* extract (GBE), which is an extract of the leaves of *Ginkgo biloba*, is one of the popular herbal medicines. In Japan and the USA, GBE can be purchased by the general population as a food supplement or health food without any restriction. In European countries, GBE, designated “EGb761”, has been clinically used for cerebral insufficiency, dementia, intermittent claudication, and equilibrium disorders (1 – 4).

With regard to the efficacy of GBE, GBE is thought to be used particularly by elderly people, who tend to take various kinds of medicine. As the effect of GBE would generally be expected after 4 weeks of continuous intakes (5), simultaneous intake of drugs and GBE is very likely to occur. For the effective combined use of drugs and GBE, possible interactions between drugs and GBE should be clarified.

Interactions between drugs and herbal medicines are generally not fully known except in the case of St. John’s Wort, which induces a major hepatic drug-metabolizing enzyme, cytochrome P450 (CYP) 3A4, and reduces the efficacy of drugs that are metabolized by CYP3A4 (6). A component of GBE, Ginkgolide B, is known to be a potent antagonist of platelet activation factor, and thus intake of GBE with anticoagulants may enhance bleeding (7 – 9). However, GBE has generally been recognized as a safe herbal medicine. The reported side effects of GBE are gastrointestinal disturbances and headache (10). Since GBE causes hardly any severe adverse effects such as hepatic dysfunction, interactions between drugs and GBE would probably occur without any overt symptoms. In a human study, GBE was shown not to modify the antipyrene half-life in blood after the intake of 400 mg of GBE for 13 days (11). This finding suggested that GBE has no effect on the hepatic drug-metabolizing enzymes. On the other hand, in rat studies we observed that administration of GBE did not induce hepatic damage, but increased concentrations of hepatic CYP and expression of CYP2B mRNA (12, 13).
However, the details of the induction of hepatic CYP by GBE remain unclear. In this study, we administered GBE to rats and examined time- and dose-dependent changes in hepatic CYP enzyme activities. Using an in vitro assay system, we also examined the influence of GBE on CYP enzyme activities, and we compared the effects of GBE in rats and humans. The type of CYP enzymes examined and the corresponding CYPs were methoxyresorufin O-demethylase, CYP1A2; ethoxyresorufin O-deethylase, CYP1A1; pentoxyresorufin O-dealkylase (PROD), CYP2B; p-nitrophenol O-deethylase, CYP1A1; pentoxyresorufin O-deethylase, CYP2B; p-nitrophenol O-demethylase, CYP1A2; ethoxyresorufin O-demethylase, CYP2E1; testosterone 6β-hydroxylase, CYP3A; and (S)-warfarin 7-hydroxylase, CYP2C9.

MATERIALS AND METHODS

Materials

Powdered Ginkgo biloba extract (GBE) was supplied by Tama Seikagaku-Kogyo Co., Tokyo. The 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. Bilobalide, ginkgolide A, ginkgolide B, quercetin, resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, 6β-hydroxytestosterone, corticosterone, testosterone, ketoconazole, p-nitrophenol, 4-nitrocatechol and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO, USA). (S)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). NADPH was obtained from Oriental Yeast (Tokyo). Human and rat microsomes were purchased from Charles River Japan, Inc. (Kanagawa). Antibodies of goat anti-rat CYP2B1/2B2 and horseradish peroxidase labeled anti-goat IgG were obtained from Daiichi Pure Chemical Co., Ltd. (Tokyo) and Santa Cruz Biotechnology, Inc., (Santa Cruz, CA, USA), respectively. Other reagents were obtained from Wako Pure Chemical Ltd., Osaka.

Animals and diets

Male Wistar rats (5-week-old) obtained from SLC (Shizuoka) were housed individually in stainless-steel, wire-bottomed cages at a constant temperature room (23 ± 1°C) under a 12-h light-dark cycle. After being adapted to these conditions for 3 – 5 days, rats were divided into several groups with and without GBE treatment. When GBE was administered via the diet, rats were fed commercial rodent diet (CE2; Japan Clea, Tokyo) containing various concentrations of GBE or the same diet without GBE for predetermined periods. When the dose-response of GBE was examined, GBE was dissolved in water and given to rats at 10 a.m. daily by intragastric gavage at doses of 0, 1, 10, 100 and 1000 mg/kg body weight for 5 days. The day after the last administration, rats were anesthetized with pentobarbital. Their blood was collected from the abdominal aorta, and the liver was removed and weighed. Part of the liver was subjected to histological examination. All procedures were in accordance with National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals.

Analytical methods

Preparation of microsomal and cytosol fractions from the liver: The liver was rinsed with 9 g/L NaCl solution, weighed and homogenized in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose. The homogenate was centrifuged at 10,000 × g at 4°C for 30 min. The supernatant was further centrifuged at 105,000 × g at 4°C for 60 min. The supernatant was used as the cytosol fraction for the assay of glutathione S-transferase, the activity of which was determined by the method of Habig and Jakoby (14) using 1-chloro-2,4-dinitrobenzene as a substrate. The pellet was washed once with 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose by centrifugation at 105,000 × g at 4°C for 60 min and subjected to the analysis of CYP concentration and activities.

Analysis of CYP enzyme activities: CYP concentration was quantified by the method of Omura and Sato (15).

Activities of methoxyresorufin O-demethylase, ethoxyresorufin O-deethylase, and PROD were determined by the method of Hanioka et al. (16) with slight modification. Briefly, 430 μL of substrate (6.6 μmol/L methoxyresorufin, 6.6 μmol/L ethoxyresorufin or 11.6 μmol/L pentoxyresorufin) in 116 mmol/L Tris-HCl (pH 7.7) and 20 μL of microsomes were preincubated for 1 min at 25°C. The reaction was started by the addition of 50 μL of 10 mmol/L NADPH with 60 mmol/L MgCl₂, and incubated for 6 min at 25°C. For the blank, 50 μL of 60 mmol/L MgCl₂ without NADPH was added. The reaction was terminated by adding 500 μL of cold methanol. The mixture was then vigorously mixed, chilled on ice for 15 min, and centrifuged at 10,000 × g for 15 min. The supernatant was filtered through a membrane filter (0.45 μm; Millipore, Bedford, MA, USA) and used as the HPLC sample. Resorufin formed by the reaction was determined by HPLC. The HPLC conditions were as follows: column, Capcell Pack UG120 (3 μm, 150 × 4.6 mm, Shiseido, Tokyo); mobile phase, 20 mmol/L phosphate buffer (pH 6.8)-methanol-acetonitrile (52:45:3, v/v); flow rate, 0.8 ml/min; column temperature, 30°C; fluorescence detection, excitation at 560 nm and emission at 585 nm.

p-Nitrophenol hydroxylase activity was determined by HPLC with an electrochemical detector (ECD) (17). Briefly, 430 μL of substrate (p-nitrophenol, 23.4 μmol/L) in 58.4 mmol/L potassium phosphate buffer (pH 6.8) and 20 μL of microsomes were preincubated for 5 min at 37°C. The reaction was started by the addition of 50 μL of 10 mmol/L NADPH with 60 mmol/L MgCl₂ and incubat-
NADPH was added. The reaction was terminated by adding coumarin (internal standard, 5.25 mg/kg) at 37°C by adding 2 mL of ethyl acetate and 100 mg/kg without NADPH was added. The reaction was terminated for 10 min at 37°C. For the blank, 50 µL of 60 mmol/L MgCl₂ without NADPH was added. The reaction was terminated by adding 25 µL of trifluoroacetic acid and the mixture was vigorously mixed, chilled on ice for 15 min, and centrifuged at 10,000 × g for 15 min. The supernatant was used for the analysis of 4-nitrocatechol formed by the reaction. The HPLC conditions were as follows: column, Capcell Pack UG80 (5 µm, 250 × 4.6 mm, Shiseido); mobile phase, trifluoroacetic acid – acetonitrile – water (0.1:25:74.9, v/v); flow rate, 0.8 ml/min; column temperature, 26°C; ECD (+700 mV vs Ag/AgCl).

(5’S)-Warfarin hydroxylase activity was determined by HPLC with a fluorescence detector (18). Briefly, 430 µL of substrate ((5’S)-warfarin, 18.6 µmol/L) in 116 mmol/L potassium phosphate buffer (pH 7.4) and 20 µL of microsomes were preincubated for 3 min at 37°C. The reaction was started by the addition of 50 µL of 10 mmol/L NADPH with 60 mmol/L MgCl₂ and incubated for 60 min at 37°C. For the blank, 50 µL of 60 mmol/L MgCl₂ without NADPH was added. The reaction was terminated by adding 10 µL of 70% perchloric acid and 10 µL of 7-ethoxy-coumarin (internal standard, 5.25 µmol/L), and the mixture was vigorously mixed and chilled on ice. The reaction mixture was centrifuged at 10,000 × g for 15 min, and the resulting supernatant was used for the analysis of 7-hydroxywarfarin formed by the reaction. The HPLC conditions were as follows: column, MightySyl RP-18 (5 µm, 250 × 4.6 mm; Kanto Kagaku Co., Tokyo); mobile phase, 0.5% phosphoric acid – acetonitrile (62:38, v/v); flow rate, 1.5 ml/min; column temperature, 35°C; fluorescence detection, excitation at 320 nm and emission at 415 nm.

Testosterone 6β-hydroxylase activity was determined by HPLC with an ultraviolet detector. Briefly, 430 µL of testosterone (116 µmol/L) in 116 mmol/L phosphate buffer (pH 7.4) containing 116 µmol/L EDTA and 20 µL of microsomes were preincubated for 5 min at 37°C. The reaction was started by the addition of 50 µL of 10 mmol/L NADPH with 60 mmol/L MgCl₂, and incubated for 10 min at 37°C. For the blank, 50 µL of 60 mmol/L MgCl₂ without NADPH was added. The reaction was terminated by adding 2 mL of ethyl acetate and 100 µL of corticosterone (internal standard, 144 µmol/L) and mixing vigorously. The reaction mixture was centrifuged at 2200 × g for 10 min, and 1 mL of the organic phase was evaporated under vacuum, dissolved in 200 µL of mobile phase A and used for the analysis of 6β-hydroxytestosterone formed by the reaction. The HPLC conditions were as follows: column, Capcell Pak UG120 (3 µm, 150 × 4.6 mm, Shiseido); mobile phase, composed of A (water – methanol – acetonitrile, 58:40:2, v/v/v), and B (water – methanol – acetonitrile, 28:40:32, v/v/v), with the B concentration set at 0%, 27%, 100% and 0% at 0, 5, 10 and 15 min, respectively, flow rate, 0.9 ml/min; column temperature, 40°C; ultraviolet detection at 245 nm. In these assay conditions, addition of ketoconazole (1 mol/L), a selective inhibitor of CYP3A, prevented the formation of 6β-hydroxytestosterone.

The influence of GBE or its constituents on CYP enzymes activities in vitro were evaluated by adding these substances to the respective enzyme assay systems.

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

**Western blot analysis of CYP2B enzyme**

Liver microsomes were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel (19) and blotted onto a nitrocellulose membrane (Immobilon transfer membrane, Millapore). The membrane was blocked, incubated with antibodies (first antibody, goat anti-rat CYP2B1/2B2; second antibody, horseradish peroxidase labeled anti-goat IgG), and developed by the enhanced chemiluminescence detection system (ECL Western-Blot kit; Amersham Pharmacia Biotech Inc., Piscatway, NJ, USA) according to the manufacturer’s protocol.

**Statistical analyses**

The data are presented as means with standard error (S.E.M.) for the individual groups. Statistical analysis of the data for the groups was carried out using ANOVA followed by a post hoc test of Fisher’s PLSD. These statistical analyses were performed by a computer program (Stat View 4.5; ASA Institute Inc., Cary, NC, USA).

**RESULTS**

*Time- and dose-dependent changes of hepatic drug metabolizing enzymes*

Feeding rats diets containing either 0%, 0.05%, 0.1% or 0.5% GBE for 2 weeks dose-dependently increased the liver weight and the concentration of CYP in the liver. A significant increase in CYP concentration was detected in the groups given feed containing GBE at the concentrations higher than 0.05%. The liver weight and CYP concentration in the liver of the 0.5% GBE group were about 1.2-fold and 4-fold higher than those in the control group. Histological examination of the liver showed no significant damage even in the 0.5% GBE group (data not shown). Based on these findings, rats were fed either a 0.5% GBE diet or control diet for 1, 3 and 5 days to examine time-dependent changes in various hepatic parameters. Significant increase in liver weight, concentration of CYP, and activity of glutathione S-transferase were detected by the feeding of the 0.5% GBE diet. The increase in CYP concentration was marked at 1.9-fold on day 1, 3.4-fold on day 3 and 3.8-fold on day 5 compared to the control level (0.50 ± 0.03 nmol/mg protein, mean ± S.E.M., n = 5). The
activities of various CYP enzymes also were significantly increased by feeding a GBE diet. The activities of ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, p-nitrophenol hydroxylase and testosterone 6β-hydroxylase on day 5 were increased 2- to 6-fold over the respective control levels. The activity of PROD was most markedly increased, with a 4.1-fold increase on day 1, a 103-fold increase on day 3 and a 106-fold increase on day 5 compared to the control level (15 ± 2 pmol/mg protein/min, mean ± S.E.M., n = 5).

In the next experiment, rats were given various doses of GBE intragastrically for 5 days to examine the dose-dependent changes in various hepatic parameters. Liver weight significantly increased at the dose of 1000 mg/kg (Table 1). The concentration of CYP and the activity of glutathione S-transferase significantly increased at the dose of 10 mg/kg and 1 mg/kg, respectively. Activities of various CYP enzymes were also significantly increased at the dose of 10 mg/kg (Table 2). In this experiment, the activity of (S)-warfarin 7-hydroxylase was measured and showed a 7.7-fold increase at 1000 mg/kg GBE compared to the control levels. At this dose, the increase in PROD activity was 65-fold compared to the control level.

PROD is a CYP2B enzyme, and our previous study showed that GBE markedly induced expression of CYP2B mRNA in rats (13). To confirm the induction of CYP2B enzyme, Western immunoblot analysis was performed using anti-rat CYP 2B1/2B2 antibody. Marked signals of CYP2B1 and 2B2 were detected in microsomes of rats treated with 1000 mg/kg GBE compared to untreated rats (Fig. 1).

### Table 1. Dose-dependent changes in weight of body, liver and hepatic drug metabolizing enzymes in rats given various doses of GBE for 5 days

<table>
<thead>
<tr>
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<th>Untreated control</th>
<th>GBE-treated (GBE/kg body weight)</th>
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<tr>
<td></td>
<td>(1 mg/kg)</td>
<td>(10 mg/kg)</td>
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<td></td>
<td>(100 mg/kg)</td>
<td>(1000 mg/kg)</td>
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<tr>
<td>Body weight (g)</td>
<td>195 ± 3.3</td>
<td>193 ± 3.2</td>
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<td>Liver weight (g)</td>
<td>6.24 ± 0.22</td>
<td>6.26 ± 0.18</td>
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<td>Hepatic drug metabolizing enzymes</td>
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<tr>
<td>Cytochrome P450 content</td>
<td>0.57 ± 0.02</td>
<td>0.59 ± 0.04</td>
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<tr>
<td>(nmol·mg protein⁻¹)</td>
<td></td>
<td>0.78 ± 0.05*</td>
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<tr>
<td>GSH-transferase activity</td>
<td>578 ± 58</td>
<td>720 ± 34*</td>
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<tr>
<td>(nmol·mg protein⁻¹·min⁻¹)</td>
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<td>760 ± 47*</td>
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Rats were given various doses of GBE by intragastric gavage for 5 days. Data are the mean ± S.E.M. for 5 rats.

*Significance versus untreated control, *P*<0.05

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<td>(1 mg/kg)</td>
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<td></td>
<td>(100 mg/kg)</td>
<td>(1000 mg/kg)</td>
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<tr>
<td>Ethoxyresorufin O-deethylase (CYP1A1)</td>
<td>57 ± 3</td>
<td>67 ± 6</td>
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<tr>
<td>Methoxyresorufin O-demethylase (CYP1A2)</td>
<td>33 ± 2</td>
<td>43 ± 3*</td>
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<td>Pentoxylesorufin O-dealkylase (CYP2B)</td>
<td>19 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>p-Nitrophenol hydroxylase (CYP2E1)</td>
<td>2918 ± 166</td>
<td>3238 ± 198</td>
</tr>
<tr>
<td>Testosterone 6β-hydroxylase (CYP3A)</td>
<td>261 ± 24</td>
<td>309 ± 40</td>
</tr>
<tr>
<td>(S)-Warfarin 7-hydroxylase (CYP2C9)</td>
<td>3.2 ± 0.2</td>
<td>3.9 ± 0.5</td>
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Rats were given various doses of GBE by intragastric gavage for 5 days. Data are the mean ± S.E.M. for 5 rats.

*Significance versus untreated control, *P*<0.05
Induction of CYP by Ginkgo biloba Extract

Inhibition of CYP enzyme activities by GBE and its constituents in vitro

The effects of GBE on the activities of CYP enzymes were examined in vitro. As the enzyme source, hepatic microsomes prepared from rats given 0.5% GBE for 5 days or from the control rats were used. The activity of PROD, which was markedly induced by GBE treatment, was significantly inhibited as the concentration of GBE added to the assay medium was increased. The inhibition of PROD activity by GBE was more marked in the microsomal enzyme preparation from the GBE-treated rats than in that from the control rats. The IC₅₀ was about 32 µg/mL for the control rats and 6 µg/mL for the GBE-treated rats. Using the microsomal enzyme preparation from the GBE-treated rats, the degrees of inhibition of various CYP enzyme activities by GBE in vitro were compared. The inhibition curve of PROD activity was shifted to the left compared to those of other CYP enzymes, indicating that PROD activity was most strongly influenced by GBE (Fig. 2).

Similarly, the inhibition of the activities of various CYP enzymes by GBE in vitro was examined using the hepatic microsomal enzymes from rats and humans, which were obtained commercially. The addition of GBE to the incubation medium caused a concentration-dependent inhibition of various CYP enzymes activities. Comparable inhibition curves were obtained with the human and rat microsomal enzymes in response to the addition of GBE. The calculated IC₅₀ (µg/mL) of rats vs human were 81 vs 82 for the methoxyresorufin O-demethylase, 85 vs 94 for the ethoxyresorufin O-deethylase, 83 vs 120 for the PROD, 53 vs 68 for the p-nitrophenol hydroxylase, 77 vs 114 for the testo-

Fig. 1. Western blot analysis of hepatic microsomes from GBE-treated rats and control rats by rat CYP2B1/2B2 antibody. Hepatic microsomes prepared from either GBE-treated (1000 mg/kg for 5 days) or the control rats were subjected to Western blotting analysis using anti-rat CYP2B1/2B2 antibody.

Fig. 2. Inhibition of various CYP enzyme activities by GBE in vitro in microsomes from GBE-treated rats. Various CYP enzyme activities were measured in the presence of various concentrations of GBE using hepatic microsomes prepared from GBE-treated rats. Each point represents the mean of 2 determinations. Left panel: Methoxyresorufin O-demethylase (closed square, dotted line), ethoxyresorufin O-deethylase (open triangle), pentoxyresorufin O-dealkylase (closed circle). Right panel: Testosterone 6β-hydroxylase (closed triangle), (S)-warfarin 7-hydroxylase (diamond, dotted line), p-nitrophenol hydroxylase (open square, dashed line).
The inhibitory activity of constituents of GBE toward PROD was examined using the microsomal enzymes from GBE-treated rats. Bilobalide, ginkgolide A and ginkgolide B did not inhibit PROD activities up to the concentration of 6 µg/mL. Quercetin and kaempferol only slightly inhibited PROD activity, with IC_{50} values of about 10 – 14 µg/mL. Mixtures of bilobalide, ginkgolide A, ginkgolide B, quercetin and kaempferol did not inhibit PROD activity up to the dose of 4 µg/mL. Under these conditions, GBE inhibited PROD activity with an IC_{50} of about 6 µg/mL.

**DISCUSSION**

We previously reported that GBE administration to rats markedly increased the concentration of CYP (12) and the level of CYP2B mRNA in the liver (13). In this study, we showed that GBE administration increased CYP concentrations and the activities of various CYP enzymes, especially PROD, in the liver in a time- and dose-dependent manner. As PROD is a CYP2B enzyme, the increases in both the activity and mRNA corresponded well. Furthermore, marked induction of CYP2B1/2B2 by GBE administration was confirmed by Western blot analysis (Fig. 1). Taken together with the findings of previous studies, these results suggest that intake of GBE induced various hepatic CYP enzymes, especially CYP2B-type enzymes.

Induction of specific types of CYP enzymes by GBE in vivo suggests that GBE may inhibit the specific type of CYP enzyme activity in in vitro assays by competition with assay substrates such as ethoxy-, methoxy- and pentoxy-resorufin, resulting in decrease in calculated activities. As expected, addition of GBE to the enzyme assay medium markedly attenuated the activity of PROD in a GBE dose-dependent manner. The inhibition of PROD activity by GBE was more marked in the microsomal enzymes obtained from GBE-treated rats than in those from control rats, and PROD was the most sensitive of the CYP enzymes tested (Fig. 2). The calculated IC_{50}s of GBE toward the PROD activity were 6 µg/mL in GBE-treated rats and 32 µg/mL in the control rats. Similar high values of IC_{50} were observed in human and rat microsomes, which were commercially available. These facts indicated that only the microsome from GBE-treated rats contained a high amount of PROD, which was specifically induced by GBE-treatment in vivo; thus, the addition of GBE to the enzyme assay system in vitro markedly attenuated the activity of PROD due to the competition of pentoxy-resorufin and GBE toward the PROD. In the case of St. John’s Wort, intake has been shown to increase the ratio of 6β-hydroxy-cortisol/cortisol in human urine samples (6), indicating the induction of CYP3A4 enzyme in vivo. However, it has also been shown that constituents of St. John’s Wort inhibited human CYP enzyme activity in vitro through competitive inhibition (20). These findings are similar to those in the present study and indicate that the induction of some kinds of CYP enzymes in vivo can be detected as an inhibition of the respective CYP enzyme activity in vitro assays.

It is possible to identify constituents of GBE responsible for the induction of PROD in vivo by assaying the inhibition of PROD activity in vitro using liver microsomal enzymes prepared from GBE-treated rats. Flavonoids have been shown to cause structure-specific induction of CYP enzymes in vitro (21 – 23). Rahden-Staron et al. (23) showed that the S9 fraction obtained from the livers of quercetin-treated rats enhanced the metabolism of cyclophosphamide which is catalyzed by CYP2B enzyme. In this study, quercetin and kaempferol slightly inhibited the PROD activity in microsomal enzymes prepared from GBE-treated rats. The IC_{50} toward the PROD activity was 6 µg/mL for GBE and 10 – 14 µg/mL for quercetin and kaempferol. The contents of quercetin and kaempferol in GBE were less than 25%, even as their glycosides. If quercetin and/or kaempferol were the constituents responsible for the PROD inhibition, their IC_{50} should be less than 1.5 µg/mL. Therefore, it is highly unlikely that quercetin and kaempferol are the major constituents of GBE responsible for the inhibition of PROD activity. Similarly, ginkgolides A and B and bilobalide did not inhibit PROD activity at their estimated concentrations in GBE. These results suggest the presence of other substances in GBE that inhibit the PROD activity and induce PROD in vivo. Further studies will be needed to identify the substances in GBE that induce PROD in vivo. The PROD inhibition assay system using liver microsomal enzymes obtained from GBE-treated rats would be useful in such studies.

Duche et al. (11) have reported that daily intake of 400 mg of EGB761, a standardized GBE, for 13 days did not modify the antipyrine half-life in human subjects. As the metabolism of antipyrine is catalyzed by various CYP enzymes in the liver, that study suggested that GBE did not influence hepatic drug-metabolizing enzymes. The composition of EGB761 and the GBE used in the present study are similar in terms of flavonoids and terpenoids. In addition, the inhibition of various CYP enzymes by GBE was comparable in human and rat microsomes as shown in this study. Therefore, we speculate that neither a difference in the constituents of GBE nor a species difference between humans and rats is related to the different results obtained in Duche’s human study and rat studies. In this study, significant induction of CYP enzyme was detected at the dose of 10 mg/kg GBE, which corresponded to more than 600 mg in humans. Generally, the daily intake level of
GBE in humans is 80 – 120 mg. Thus, different intake levels of GBE may be responsible for the different results obtained in Duché’s and our studies. It is thought that the beneficial effect of GBE may be obtained after 4 weeks of continuous intakes (5). If people consume an excess of GBE compared to ordinary intake levels, significant induction of CYP enzymes may occur, as shown in this study.

GBE induced CYP enzymes but did not cause hepatic damage. This fact may indicate that interaction between GBE and drugs could occur without any overt symptoms, resulting in modification of the efficacy of drugs taken at the same time as GBE. People who take GBE tend to be elderly, and thus may be likely to take various drugs simultaneously. As GBE is safe and effective for various diseases, it is important to know how GBE intake influences the efficacy of drugs that are metabolized by CYP enzymes, particularly by PROD. Future studies focusing on these matters will be needed to ensure effective use of GBE.

Acknowledgment

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