DISTINCT ROLE OF BILOBALIDE AND GINKGOLIDE A IN THE MODULATION OF RAT CYP2B1 AND CYP3A23 GENE EXPRESSION BY Ginkgo biloba EXTRACT IN CULTURED HEPATOCYTES

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**ABBREVIATIONS:** BROD, 7-benzyloxyresorufin *O*-dealkylation; DEX; dexamethasone; DMSO, dimethylsulfoxide; P450, cytochrome P450; PB, phenobarbital; PCR; polymerase chain reaction.
In the present study, primary cultures of rat hepatocytes were treated for 48 h with one of several extracts of *Ginkgo biloba* (10, 100, or 1000 µg/ml). Maximal increase in CYP2B1 and CYP3A23 mRNA levels was obtained at 100 µg/ml. This concentration of *Ginkgo biloba* extract also increased CYP3A2 and CYP3A18 mRNA expression in addition to CYP2B-mediated 7-benzyloxyresorufin O-dealkylation (BROD) and CYP3A-mediated testosterone 6β-hydroxylation. In other experiments, cultured hepatocytes were treated for 48 h with bilobalide, ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, kaempferol, quercetin, isorhamnetin, or a flavonol diglycoside at a concentration that represented the level present in an 100 µg/ml concentration of an extract. Only bilobalide (2.8 µg/ml) increased CYP2B1 mRNA expression and the fold-increase (7.9 ± 0.5; mean ± S.E.M.) was similar to that (8.3 ± 1.7) by the extract. By comparison, only ginkgolide A (1.1 µg/ml) increased CYP3A23 mRNA expression, but the extent (2.6 ± 0.5 fold) was less than the 5.3 ± 1.7 fold-increase by the extract. A greater concentration (5 µg/ml) of ginkgolide A was required to elevate CYP3A2 and CYP3A18 mRNA expression. Over the range of 1-5 µg/ml, bilobalide increased CYP2B1 mRNA and BROD, but not CYP3A23 mRNA or testosterone 6β-hydroxylation, whereas ginkgolide A increased CYP3A23 mRNA and testosterone 6β-hydroxylation, but not CYP2B1 mRNA or BROD. Overall, our novel results indicate a distinct role of bilobalide and ginkgolide A in the modulation of CYP2B1 and CYP3A23 gene expression and enzyme activities by *Ginkgo biloba* extract in primary cultures of rat hepatocytes.
The use of herbal medicines has been increasing rapidly, especially in recent years (De Smet, 2002). Many consumers believe that herbal products, unlike conventional drugs, are safe and have no side effects (Barnes, 2003). A survey indicated that among the respondents who consumed herbal medicines, 31% ingested them concomitantly with conventional drugs (Abebe, 2002). Therefore, a concern is the potential risk of adverse effects due to herb-drug interactions (Huang et al., 2004).

According to a 2002 survey conducted in the U.S.A., *Ginkgo biloba* was the third most popular herbal medicine (Barnes et al., 2004). It was used by 21% of adults who consumed herbal medicines. *Ginkgo biloba* extract is used by consumers for the management of a variety of disorders; for example, memory impairment (De Smet, 2002). Many individual chemical constituents are present in *Ginkgo biloba* extract, including terpene trilactones, such as diterpenes (e.g. ginkgolide A, ginkgolide B, ginkgolide C, and ginkgolide J) and sesquiterpenes (e.g. bilobalide), and ginkgo flavonol glycosides (e.g. glycosides of kaempferol, quercetin, and isorhamnetin) (van Beek, 2002).

Previous rodent studies indicated that *Ginkgo biloba* extract, when administered as a single i.p. dose (25 or 50 mg/kg) (Brochet et al., 1999) or fed for 2 weeks via the diet (0.5% or 1% w/w) (Kubota et al., 2004), shortened barbiturate-induced narcosis. *Ginkgo biloba* extract (0.5% w/w in the diet for 2 weeks) has also been shown to reduce the hypotensive action of nicardipine and alter the pharmacokinetics of this drug; specifically, a decrease in the maximum plasma concentration and area under the plasma concentration-time curve (Kubota et al., 2003).

The effect of *Ginkgo biloba* on the pharmacodynamics and pharmacokinetics of these drugs may
be related to induction of cytochrome P450 (P450) enzymes by this herbal medicine. It has been reported that the administration of a Ginkgo biloba extract (contained 24.9% flavonol glycosides and 10.6% terpene trilactones) to rats increased hepatic expression of several P450 enzymes, including CYP2B (Umegaki et al., 2002; Shinozuka et al., 2002). However, it is not known whether the flavonols or the terpene trilactones are responsible for the reported effects of Ginkgo biloba extract. The levels of the terpene trilactones in the extracts used in the previous studies (Umegaki et al., 2002; Shinozuka et al., 2002) were greater than those in many of the commercially available Ginkgo biloba products formulated to contain only 6% terpene trilactones (van Beek, 2002). Therefore, it is not known whether the results from those studies (Umegaki et al., 2002; Shinozuka et al., 2002) can be generalized to other extracts of Ginkgo biloba.

The present study was conducted to: 1) investigate the effect of multiple extracts of Ginkgo biloba containing known amounts of bilobalide, ginkgolides, and flavonol glycosides on CYP2B1, CYP3A23, CYP3A2, and CYP3A18 gene expression in primary cultures of rat hepatocytes, as determined by reverse transcription and real-time polymerase chain reaction (PCR); 2) assess the effect of Ginkgo biloba extract on CYP2B- and CYP3A-mediated enzyme activities in cultured hepatocytes, as analyzed by the 7-benzyloxyresorufin O-dealkylation (BROD) assay and the testosterone 6β-hydroxylation assay, respectively; and 3) determine whether terpene trilactones, such as bilobalide and ginkgolides A, B, C, and J, or flavonol aglycones and glycosides, such as those of kaempferol, quercetin, and isorhamnetin, are responsible for the effect of Ginkgo biloba extract on CYP2B1, CYP3A23, CYP3A2, and CYP3A18 expression. Our results indicate that bilobalide and ginkgolide A are novel inducers of CYP2B1 and CYP3A, respectively, and that these chemicals play a distinct role in the
modulation of CYP2B1 and CYP3A23 mRNA expression and enzyme activities by *Ginkgo biloba* extract.

**Materials and Methods**

**Chemicals and Reagents.** *Ginkgo biloba* extracts were provided by Indena S.A. (Milan, Italy; batch no. 1306A and batch no. 302831) and Pharmaton S.A. (Bioggio, Switzerland; GK501™, batch no. 63964). Shown in Table 1 is the amount of bilobalide, ginkgolides, and flavonols present in each extract. Bilobalide (batch no. 157/25/1, 97.9% pure) was supplied by Indena S.A. (Milan, Italy). Ginkgolide A, ginkgolide B, ginkgolide C, and ginkgolide J were purchased from LKT Laboratories, Inc. (St. Paul, MN). Kaempferol, kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, isorhamnetin, and isorhamnetin-3-O-rutinoside were bought from INDOFINE Chemical Co., Inc. (Hillsborough, NJ). Quercetin, dexamethasone (DEX), dimethylsulfoxide (DMSO), bovine serum albumin, SYBR™ Green I, Williams’ medium E, fetal bovine serum, collagenase (Type IV), trypsin inhibitor (Type II-S), trypan blue, dicumarol, testosterone, 6β-hydroxytestosterone, and 11β-hydroxytestosterone were purchased from Sigma Chemical Co. (St. Louis, MO). Phenobarbital (PB) was obtained from Wiler PCCA (London, ON, Canada). TriZol®, SuperScript II™ reverse transcriptase, oligo(dT)₁₂₋₁₆ primers, deoxyribonuclease I, dithiothreitol, magnesium chloride, dNTP, 100 base pair-DNA ladder, Platinum® Taq DNA polymerase, penicillin-streptomycin, L-glutamine, human recombinant insulin, 10× Dulbecco’s phosphate-buffered saline, 10× Hanks’ balanced salt solution (without sodium bicarbonate and without phenol red), Liver Perfusion Media®, and Hepatocyte Wash Media® were obtained from Invitrogen, Inc. (Burlington, ON, Canada). β-Glucuronidase and arylsulfatase were bought from Roche Diagnostics (Laval, QC, Canada). Resorufin was obtained...
from Pierce Chemical Co. (Rockford, IL). Matrigel™ basement membrane matrix was purchased from BD Biosciences Canada (Mississauga, ON, Canada). Percoll™ was bought from Amersham Biosciences, Inc. (Baie d’Urfe, QC, Canada). QIAquick™ gel extraction kit was purchased from QIAGEN, Inc. (Mississauga, ON, Canada). RiboGreen™ Total RNA Quantification Kit and PicoGreen™ Double-Stranded DNA Quantification Kit were purchased from Molecular Probes, Inc. (Medford, OR). Forward and reverse primers for CYP2B1, CYP3A23, CYP3A2, and CYP3A18 were synthesized at the University of British Columbia Nucleic Acid and Protein Service Unit (Vancouver, BC, Canada).

**Animals and Isolation of Hepatocytes.** Adult male Sprague-Dawley rats (225-250 g) were purchased from Charles River (Montreal, QC, Canada) and cared for in our animal care facility as described previously (Kuo et al., 2004). Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and hepatocytes were isolated by a two-step collagenase perfusion method (Seglen, 1993). The hepatic portal vein was cannulated with a 21-gauge (1-inch) Teflon catheter (0.9 × 25 mm; Becton Dickinson) and the liver was perfused with calcium-free Liver Perfusion Media® at 37°C at a rate of 25 ml/min using a peristaltic pump. The inferior vena cava was severed immediately to allow for the efflux of the perfusate. The superior vena cava was cannulated with a PE-190 tubing (Becton Dickinson) and the inferior vena cava was then tied to allow for the efflux of the perfusate through the superior vena cava. Subsequently, the liver was perfused at 37°C for 7 min at a rate of 25 ml/min with calcium-free Liver Perfusion Media® followed by perfusion for another 7 min with digest medium (final pH adjusted to 7.5) consisting of Hanks’ balanced salt solution supplemented with 2.38 g/L HEPES, 0.35 g/L NaHCO₃, 0.05 g/L trypsin inhibitor (Type II-S), and 0.5 g/L collagenase (Type IV). Perfused liver was excised and placed in a sterile Petri dish containing Hepatocyte Wash Media®. Cells were mechanically
dispersed using a blunt glass rod. The cell suspension was filtered through sterile Nytex® nylon mesh (64 µm pore size; TETCO, Briarcliff Manor, NY) into a 50 ml Falcon tube. The suspension was centrifuged (50 × g) at 4°C for 5 min and the pellet was suspended in fresh ice-cold Hepatocyte Wash Media®. A portion (20 ml) of the suspension was added to ice-cold Percoll™ solution (26.1 ml Percoll™ and 3.9 ml 10× Dulbecco’s phosphate-buffered saline) and mixed gently by inversion (five times). The mixture was centrifuged (50 × g) at 4°C for 10 min. The supernatant was decanted and the pellet was washed again in Hepatocyte Wash Media® and subsequently suspended in supplemented Williams’ medium E containing heat-inactivated fetal bovine serum (10% v/v). Supplemented Williams’ medium E contained insulin (1 µM), dexamethasone (100 nM), L-glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 µg/ml). Cell viability was 80-90%, as assessed by trypan blue exclusion.

Hepatocyte Cultures. Culture dishes or microplates were coated with Matrigel™ at approximately 1 h prior to use (Schuetz et al., 1988). In the experiments investigating P450 gene expression, 170 µl of Matrigel™ was applied onto a 60 mm-Permanox culture dish. Hepatocyte suspension was diluted to 10⁶ cells/ml in serum-free, supplemented Williams’ medium E and a 3 ml volume was plated onto each culture dish. In experiments investigating P450-mediated enzyme activities, 30 µl of Matrigel™ (diluted 1:1 with culture medium) was applied onto each well in a 24-well microplate. Each well was loaded with 3 × 10⁵ cells in a volume of 500 µl containing serum-free, supplemented Williams’ medium E. Cells were allowed to attach for 4 h in a humidified 37°C incubator with 5% CO₂ and 95% air. Subsequently, the medium was decanted to remove unattached cells and the hepatocytes were cultured in serum-free, supplemented Williams’ medium E. Culture medium was changed daily.
Treatment of Primary Cultures of Rat Hepatocytes. At 48 h after plating, cultured hepatocytes were treated for 48 h with *Ginkgo biloba* extract (10, 100, or 1000 µg/ml) or culture medium (vehicle control). In other experiments, cultured hepatocytes were treated with bilobalide, ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, kaempferol, kaempferol-3-*O*-rutinoside, quercetin, quercetin-3-*O*-rutinoside, isorhamnetin, isorhamnetin-3-*O*-rutinoside, or DMSO (vehicle control) at the concentration indicated in each figure legend. For comparison, cultured hepatocytes were treated with PB (100 µM, positive control for CYP2B1 induction) or DEX (10 µM, positive control for CYP3A23, CYP3A2, and CYP3A18 induction). Culture medium was changed daily.

Isolation of Total RNA and Quantification of Total RNA Concentration. Hepatocytes were harvested at the end of the treatment period. Total cellular RNA was isolated using the TriZol™ reagent and total RNA concentration was determined using the RiboGreen® RNA Quantitation Kit (Jones et al., 1998) as detailed elsewhere (Chang et al., 2003).

Reverse Transcription and Quantification of Total cDNA Concentration. Reverse transcription was performed and total cDNA concentrations were quantified using the PicoGreen® dsDNA Quantitation Kit (Singer et al., 1997) as detailed elsewhere (Chang et al., 2003).

PCR Primers. The sequences of the forward and reverse primers used to amplify CYP2B1 cDNA (gene accession number J00719) were 5′-CTG-TGG-GTC-ATG-GAG-AGC-TG-3′ and 5′-TCA-CAC-CGG-CTA-CCA-ACC-CT-3′, respectively (Li et al., 1998). The sequences of the forward and reverse primers used to amplify CYP3A2 cDNA (gene accession number NM153312) were 5′-TTG-ATC-CGT-TGT-TCT-TGT-CA-3′ and 5′-GGC-CAG-GAA-ATA-CA.
GAC-AA-3’, respectively (Zhang et al., 1996). The sequences of the forward and reverse primers used to amplify CYP3A18 cDNA (gene accession number NM145782) were 5’-CAA-CTA-CGG-TGA-TGG-CAT-GT-3’ and 5’-CAC-TCG-GTT-CTT-CTG-GTT-TG-3’, respectively (Mahnke et al., 1997). The sequences of the forward and reverse primers used to amplify CYP3A23 cDNA (gene accession number X96721) were 5’-GGA-AAT-TCG-ATG-TGG-AGT-GC-3’ and 5’-AGG-TTT-GCC-TTT-CTC-TTG-CC-3’, respectively (Mahnke et al., 1997). The specificity of the primers was confirmed by sequencing analysis of the purified amplicons as described previously (Yu et al., 2005).

**Real-Time PCR Analysis.** Each 20 µl PCR reaction mixture contained 1 unit Platinum® Taq DNA polymerase in 1× PCR reaction buffer [20 mM Tris-HCl (pH 8.4) and 50 mM KCl], 3 mM magnesium chloride (except that 5 mM was used to amplify CYP3A2 cDNA), 1 ng total cDNA, 200 µM dNTP mix, 0.2 µM each of the forward and reverse primers, 0.25 mg/ml bovine serum albumin, and 2 µl of a 3.3× SYBR® Green I solution. The conditions for the amplification of CYP2B1 cDNA were: 95°C for 1 s (denaturation), 56°C for 6 s (annealing), and 72°C for 23 s (extension). The conditions for the amplification of CYP3A2 cDNA were: 95°C for 1 s, 60°C for 6 s, and 72°C for 17 s. The conditions for the amplification of CYP3A18 cDNA were: 95°C for 1 s, 60°C for 6 s, and 72°C for 30 s. The conditions for the amplification of CYP3A23 cDNA were: 95°C for 1 s, 60°C for 6 s, and 72°C for 14 s. In all cases, the initial denaturation was performed at 95°C for 5 min. Under these conditions, no primer-dimer formation was detected. However, to prevent any potential contribution to the fluorescence signal due to primer-dimer formation, the real-time DNA thermal cycler (LightCycler™, Roche Diagnostics, Mannheim, Germany) was programmed to record fluorescence readings after each
cycle at a temperature several degrees less than the melting temperature of the amplicon. Calibration curves were constructed as described previously (Cheung et al., 2004). The level of gene expression (copies of cDNA) was normalized to the amount of total cDNA used in the amplification, as quantified by the PicoGreen® dsDNA Quantitation Kit (Singer et al., 1997). This approach to normalizing gene expression data was previously shown to be comparable to normalizing it to the amount of a “housekeeping” gene, such as cyclophilin (Yu et al., 2005). Results are expressed as fold-increase over the vehicle-treated control group.

**BROD Assay.** Hepatocytes cultured in 24-well microplates were treated for 48 h with *Ginkgo biloba* extract (100 µg/ml), bilobalide (1-5 µg/ml), ginkgolide A (1.1-5 µg/ml), or PB (100 µM, positive control). Control incubations contained culture medium (vehicle for the extract) or DMSO (0.1% final concentration, vehicle for the individual chemicals). At the end of the treatment period, culture medium was removed and the cells were washed with 1× phosphate-buffered saline. The BROD assay was initiated by the addition of an 150 µl mixture containing 7-benzyloxyresorufin (15 µM) and dicumarol (10 µM) dissolved in DMSO (0.1% final concentration) (Donato et al., 1993). The reaction proceeded for 60 min in a 37°C incubator. Subsequently, a 75 µl aliquot of the reaction mixture was transferred to another well containing a 25 µl mixture of β-glucuronidase (15 Fishman units) and arylsulfatase (120 Roy units) dissolved in 0.1 M sodium acetate (pH 4.5). The microplates were placed in a shaking 37°C incubator for 2 h. Following the addition of ethanol (200 µl), the microplates were centrifuged at 3000 rpm for 10 min. The fluorescence reading of a 200 µl sample of the supernatant was recorded at an excitation wavelength of 530 nm (5 nm bandwidth), an emission wavelength of 580 nm (5 nm bandwidth), and a gain of 50 in a Cytofluor 2350 fluorescence microplate reader (Millipore). A standard curve was constructed with 10-80 pmol resorufin.
Testosterone 6β-Hydroxylation Assay. Hepatocytes cultured in 24-well microplates were treated as described above for the BROD assay, except that the positive control was DEX (10 µM). At the end of the treatment period, culture medium was removed and the cells were washed with 1× phosphate-buffered saline. The testosterone 6β-hydroxylation assay was performed according to a published method (Nicoll-Griffith et al., 2004), but with minor modifications. Testosterone (200 µM) dissolved in DMSO (0.1% final concentration) was added in a volume of 200 µl. Following an incubation period of 60 min in a 37°C incubator, the reaction was stopped with 100 µl of 100% acetonitrile and the mixture was spiked with 20 µl of an internal standard, which was 11β-hydroxytestosterone (82 µg/ml in methanol containing 0.5% acetic acid). The incubation mixture was transferred to a microcentrifuge tube and the content was centrifuged at 10,000 × g for 3 min. A 20 µl sample of the supernatant was analyzed by HPLC. Our HPLC system consisted of a Phenomenex Gemini C_{18} column (150 × 2.0 mm, I.D., 5 µm, Phenomenex Corporation, Torrance, CA) linked to a Phenomenex SecurityGuard™ cartridge (ODS, 4.0 × 2.0 mm, I.D., 5 µm), a Waters Model 1525 Binary HPLC pump, a Waters Model 717 plus autosampler, and a Waters Model 2487 dual λ absorbance detector (Waters Corporation Canada, Mississauga, ON, Canada). Instrument operation and data acquisition were performed using Waters Breeze software (version 3.20). The levels of 6β-hydroxytestosterone and 11β-hydroxytestosterone were determined by reversed-phase HPLC, as modified from a published method (Purdon et al., 1997), and with an ultra-violet detector set at 242 nm. The analytes were eluted at ambient temperature at a flow rate of 0.2 ml/min, with a linear gradient initially with 55% methanol containing 0.5% acetic acid increasing to 90% methanol containing...
0.5% acetic acid over 8 min and staying at 90% for 2 min before re-equilibration at the initial condition. A standard curve was constructed with 30-5000 pmol of 6β-hydroxytestosterone.

**Statistics.** Data analyses were conducted using one-way analysis of variance and the Student Newman-Keuls multiple comparison test (SigmaStat software program, SPSS Inc., Chicago, IL). Statistical significance was set *a priori* at p < 0.05.
Results

Concentration-Dependent Effect of *Ginkgo biloba* extract on CYP2B1 and CYP3A23 Gene Expression. To determine the effect of *Ginkgo biloba* extract on CYP2B1 gene expression, primary cultures of rat hepatocytes were treated for 48 h with various concentrations (10, 100, or 1000 µg/ml) of a *Ginkgo biloba* extract containing known amounts of diterpenes, sesquiterpene, and flavonols (Extract A, Table 1). Control hepatocyte cultures were treated with culture medium (vehicle control). As shown in Fig. 1A, *Ginkgo biloba* extract, at a concentration of 10 µg/ml and 100 µg/ml, increased CYP2B1 mRNA levels by 3.1 ± 0.6 fold (mean ± S.E.M.) and 8.4 ± 2.2 fold, respectively, whereas no increase was obtained with the 1000 µg/ml concentration of the extract. As a positive control, PB (100 µM for 48 h) increased CYP2B1 mRNA expression by 26 ± 2 fold, which was 3.1-fold greater than the effect produced by the 100 µg/ml concentration of *Ginkgo biloba* extract (Fig. 1A). By comparison, treatment of primary cultures of rat hepatocytes for 48 h with *Ginkgo biloba* extract at 10, 100, or 1000 µg/ml also increased CYP3A23 mRNA expression (Fig. 1B). Maximal increase (9.7 ± 5.2 fold) was obtained with the 100 µg/ml concentration of the extract. As a positive control, DEX (10 µM for 48 h) increased CYP3A23 mRNA expression by 27 ± 9 fold, which was 2.8-fold greater than the effect produced by the 100 µg/ml concentration of *Ginkgo biloba* extract (Fig. 1B).

Comparative Effect of Multiple Extracts of *Ginkgo biloba* on CYP2B1 and CYP3A23 Gene Expression. To determine whether *Ginkgo biloba* extract from another lot or from another manufacturer can also modulate CYP2B1 and CYP3A23 gene expression, primary cultures of rat hepatocytes were treated for 48 h with one of two different lots of *Ginkgo biloba* extract from the same manufacturer (i.e. Extract A and Extract B, Table 1) or an extract from a different manufacturer (i.e. Extract C, Table 1) at a concentration of 10, 100, or 1000 µg/ml.
Control hepatocytes were treated with culture medium (vehicle control). As shown in Table 2, all three extracts increased CYP2B1 mRNA levels at a concentration of 10 µg/ml or 100 µg/ml, but not 1000 µg/ml. By comparison, each of the extracts increased CYP3A23 mRNA expression at concentrations of 10, 100, and 1000 µg/ml.

Role of Bilobalide in the Modulation of CYP2B1 and CYP3A23 Gene Expression by *Ginkgo biloba* Extract. *Ginkgo biloba* extract contains various individual chemicals, including bilobalide (Table 1). Therefore, to determine whether this compound was responsible for the induction of CYP2B1 and CYP3A23 by *Ginkgo biloba* extract, primary cultures of rat hepatocytes were treated for 48 h with bilobalide (2.8 µg/ml). This concentration of bilobalide was chosen to represent the level present in a CYP2B1-inducing concentration (100 µg/ml; Fig. 1A) of a *Ginkgo biloba* extract (i.e. Extract A, Table 1). As shown in Figure 2A, bilobalide increased CYP2B1 mRNA expression by 7.9 ± 0.5 fold, which was similar to the increase (8.3 ± 1.7 fold) by the extract. In contrast, it did not increase CYP3A23 mRNA levels (Fig. 2B).

Role of Ginkgolides A, B, C, and J in the Modulation of CYP2B1 and CYP3A23 Gene Expression by *Ginkgo biloba* Extract. *Ginkgo biloba* extracts also contain diterpenes, such as the ginkgolides (Table 1). Therefore, primary cultures of rat hepatocytes were treated for 48 h with ginkgolide A (1.1 µg/ml), ginkgolide B (0.3 µg/ml), ginkgolide C (1.4 µg/ml), ginkgolide J (0.6 µg/ml), or the vehicle (0.1% DMSO). The concentrations of the individual ginkgolides were chosen to reflect the levels present in a CYP3A23-inducing concentration (100 µg/ml; Fig. 1B) of a *Ginkgo biloba* extract (i.e. Extract A, Table 1). None of the ginkgolides influenced CYP2B1 mRNA expression (Fig. 2A), whereas only ginkgolide A increased (2.6 ± 0.5 fold) CYP3A23 mRNA levels (Fig. 2B), although the magnitude of the effect was less than the 5.3 ± 1.7 fold-increase by the extract (Fig. 2B).
Differential Effect of Bilobalide on CYP2B1 and CYP3A23 Gene Expression. In a study that quantified the amount of bilobalide in 27 commercial products of *Ginkgo biloba*, the abundance of this compound ranged from 0.8% w/w to 4.6% w/w (Kressmann et al., 2002). In the present study, a concentration-response experiment was conducted whereby primary cultures of rat hepatocytes were treated for 48 h with bilobalide at 1, 2.8, or 5 µg/ml. As shown in Fig. 3A, bilobalide increased CYP2B1 mRNA expression in a concentration-dependent manner, but it had no effect on CYP3A23 mRNA levels.

Differential Effect of Ginkgolide A on CYP3A23 and CYP2B1 Gene Expression. The abundance of ginkgolide A ranged from 1.1% w/w to 3.8% w/w in a survey of 27 commercial products of *Ginkgo biloba* (Kressmann et al., 2002). Therefore, a concentration-response experiment was performed. Primary cultures of rat hepatocytes were treated for 48 h with ginkgolide A at 1.1, 2.5, or 5 µg/ml. As shown in Fig. 3B, ginkgolide A increased CYP3A23 mRNA levels linearly over this range of concentrations, but it did not affect CYP2B1 mRNA expression.

Role of Flavonol Glycosides and Aglycones in the Modulation of CYP2B1 and CYP3A23 Gene Expression by *Ginkgo biloba* Extract. Flavonol glycosides are present in *Ginkgo biloba* extracts (van Beek, 2002). Therefore, primary cultures of rat hepatocytes were treated for 48 h with kaempferol-3-O-rutinoside (1.9 µg/ml), quercetin-3-O-rutinoside (4 µg/ml), isorhamnetin-3-O-rutinoside (0.6 µg/ml), or the vehicle (0.1% DMSO). The concentration of isorhamnetin-3-O-rutinoside was chosen to reflect the level present in an 100 µg/ml concentration of a *Ginkgo biloba* extract (i.e. Extract A, Table 1). However, the levels of kaempferol-3-O-rutinoside and quercetin-3-O-rutinoside were not known in Extract A. Therefore, we used a concentration that represented the level of all the kaempferol diglycosides...
or quercetin diglycosides present in the extract. As shown in Table 3, isorhamnetin-3-O-
rutinoside, kaempferol-3-O-rutinoside, and quercetin-3-O-rutinoside did not affect CYP2B1 or
CYP3A23 mRNA expression.

Flavonol glycosides are converted to the corresponding aglycones by the action of
intestinal microflora (Walle, 2004). Therefore, primary cultures of rat hepatocytes were treated
for 48 h with kaempferol (6.3 µg/ml), quercetin (10.6 µg/ml), isorhamnetin (4.1 µg/ml), or the
vehicle (0.1% DMSO). These concentrations were selected to reflect the total levels of each of
the flavonols (i.e. sum of the aglycone and the glycosides). As shown in Table 3, kaempferol,
quercetin, and isorhamnetin did not increase CYP2B1 or CYP3A23 mRNA expression.

**Effect of Ginkgo biloba Extract, ginkgolides, bilobalide, and flavonols on CYP3A2 and CYP3A18 Gene Expression.** To determine the effect of *Ginkgo biloba* extract and some of
its individual chemical constituents on CYP3A2 and CYP3A18 gene expression, primary
cultures of rat hepatocytes were treated for 48 h with a *Ginkgo biloba* extract (i.e. Extract A,
Table 1; 100 µg/ml), culture medium (vehicle for the extract), ginkgolide A (1.1 or 5 µg/ml),
ginkgolide B (0.3 µg/ml), ginkgolide C (1.4 µg/ml), ginkgolide J (0.6 µg/ml), bilobalide (2.8
µg/ml), kaempferol-3-O-rutinoside (1.9 µg/ml), quercetin-3-O-rutinoside (4 µg/ml),
isorhamnetin-3-O-rutinoside (0.6 µg/ml), kaempferol (6.3 µg/ml), quercetin (10.6 µg/ml),
isorhamnetin (4.1 µg/ml), DEX (10 µM, positive control), or DMSO (0.1%, vehicle for the
individual chemicals). As shown in Fig. 4A, *Ginkgo biloba* extract increased CYP3A2 mRNA
levels by 4.2 ± 1.2 fold, which was less than the 18 ± 5 fold induction by DEX. The extract also
increased CYP3A18 mRNA expression (4.6 ± 1.7 fold), and the extent was similar to that by
DEX (3.3 ± 0.7 fold) (Fig. 4B). However, ginkgolide A at the level (1.1 µg/ml) in the extract
had no effect on CYP3A2 or CYP3A18 mRNA levels, but an increase was obtained at a
concentration of 5 µg/ml. In contrast, ginkgolide B, ginkgolide C, ginkgolide J, bilobalide, and the flavonols (both the aglycone and the diglycoside) did not influence CYP3A2 or CYP3A18 mRNA expression (data not shown).

Effect of *Ginkgo biloba* Extract, bilobalide, and ginkgolide A on CYP2B and CYP3A Enzyme Activities. To corroborate the findings on gene expression, CYP2B and CYP3A enzyme activities were determined in primary cultures of rat hepatocytes treated with a *Ginkgo biloba* extract (i.e. Extract A, Table 1; 100 µg/ml), culture medium (vehicle for the extract), bilobalide (1, 2.8, or 5 µg/ml), ginkgolide A (1.1, 2.5, or 5 µg/ml), PB (100 µM, positive control for CYP2B activity), DEX (10 µM, positive control for CYP3A activity), or DMSO (0.1%, vehicle for the individual chemicals). *Ginkgo biloba* extract increased CYP2B-mediated BROD by 5 ± 1 fold (Fig. 5A), whereas PB increased it by 23 ± 5 fold (Fig. 5A). The extract also increased CYP3A-mediated testosterone 6β-hydroxylation, although the extent (2.6 ± 0.2 fold) was less than the 9.6 ± 2.3 fold by DEX (Fig. 5B). Concentration-response experiments showed that bilobalide increased BROD, but not testosterone 6β-hydroxylation (Fig. 6A). In contrast, ginkgolide A increased testosterone 6β-hydroxylation, but not BROD (Fig. 6B).
Discussion

The impetus for the present study was the finding that the administration of *Ginkgo biloba* extract to rats increased hepatic P450-mediated enzyme activities (Umegaki et al., 2002; Shinozuka et al., 2002). However, those findings may not be applicable to other extracts of *Ginkgo biloba*. Most of the commercially available *Ginkgo biloba* products contain approximately 6% w/w of terpene trilactones (Kressmann et al., 2002). The extract used in the previous studies (Umegaki et al., 2002; Shinozuka et al., 2002) contained a greater amount (10.6% w/w) of terpene trilactones. The present study was conducted with *Ginkgo biloba* extracts from different lots and different manufacturers. These extracts contained known amounts of terpene trilactones (6.2-6.6% w/w) and flavonol glycosides (21-24.4% w/w) (Table 1). Our results indicated that *Ginkgo biloba* extracts increased rat hepatic CYP2B1, CYP3A23, CYP3A2, and CYP3A18 gene expression.

CYP2B1 is a major PB-inducible P450 enzyme in rat liver (Waxman, 1999). In the present study, treatment of primary cultures of rat hepatocytes with *Ginkgo biloba* extract increased CYP2B1 gene expression level to approximately one-third of that by 100 µM PB, which is a maximal CYP2B1-inducing concentration (LeCluyse et al., 1999). It also increased CYP2B-mediated BROD to approximately one-quarter of the level by PB. At a concentration of 1000 µg/ml, *Ginkgo biloba* extract did not increase CYP2B1 gene expression. A potential explanation could be that a chemical(s) present in the extract might have suppressed the CYP2B induction response. Another explanation might be general cellular toxicity, although this was not assessed in our hepatocyte cultures. However, the same concentration of the extract resulted in a maximal increase in CYP3A23 gene expression.
Several hepatic CYP3A enzymes, such as CYP3A23, CYP3A2, and CYP3A18, are expressed in male rats (Mahnke et al., 1997; Matsubara et al., 2004). The constitutive expression of hepatic CYP3A23 is low, but it is a major DEX-inducible P450 enzyme (Mahnke et al., 1997). Our results obtained with a *Ginkgo biloba* extract (i.e. Extract A, Table 1) showed that it induced CYP3A23 gene expression to a level that was approximately one-third of that by 10 µM DEX, which is a maximal CYP3A23-inducing concentration (Lu et al., 2001). However, minor differences existed in the magnitude of the increase among the extracts tested. Similar to CYP3A23, CYP3A2 and CYP3A18 are also inducible by DEX (Mahnke et al., 1997). As shown in the present study, when compared to a maximal CYP3A-inducing concentration (10 µM) of DEX (Lu et al., 2001), *Ginkgo biloba* extract was less effective in elevating CYP3A23 and CYP3A2 mRNA levels, but both were similarly effective in inducing CYP3A18. Consistent with the CYP3A gene expression data, treatment of cultured hepatocytes with *Ginkgo biloba* extract increased testosterone 6β-hydroxylation, which is mediated by CYP3A23, CYP3A2, CYP3A18, and other CYP3A enzymes (Matsubara et al., 2004). Our cell culture results are in agreement with a previous study indicating that treatment of 5-week old male Wistar rats with an extract of *Ginkgo biloba* (10, 100, or 1000 mg/kg via intragastric gavage once daily for 5 days) increased hepatic microsomal testosterone 6β-hydroxylation activity (Umegaki et al., 2002).

Bilobalide is the most abundant terpene trilactone in commercial preparations of *Ginkgo biloba* (Kressmann et al., 2002). According to the German Commission E monograph, the amount of bilobalide in a *Ginkgo biloba* product should be within the range of 2.6-3.2% w/w (Blumenthal, 1998). A novel finding from the present study is that bilobalide is responsible for the modulation of CYP2B1 gene expression and CYP2B-mediated BROD by *Ginkgo biloba* extract. Our results also indicated a concentration-dependent relationship on the effect of...
bilobalide (1-5 µg/ml) on CYP2B1 mRNA and enzyme activity. We tested this range of concentrations because according to a survey of commercial *Ginkgo biloba* products, the bilobalide concentrations were between 0.8-4.6 µg/ml (Kressmann et al., 2002). Interestingly, an earlier study reported that the oral administration of bilobalide (30 mg/kg once daily for four days) to mice increased hepatic microsomal 7-methoxycoumarin *O*-demethylation activity (Sasaki et al., 1997). However, it is not known which P450 enzyme(s) is responsible for this PB-inducible activity in mouse liver microsomes.

Another group of terpene trilactones present in *Ginkgo biloba* extracts are the diterpenes, which include ginkgolides A, B, C, and J (van Beek, 2002). According to the German Commission E monograph, the total amount of ginkgolides in a *Ginkgo biloba* product should be within the range of 2.8-3.4% w/w (Blumenthal, 1998), although there is no specified amount for the individual ginkgolides. As shown for the first time in the present study, ginkgolide A is partially responsible for the modulation of CYP3A23 gene expression by *Ginkgo biloba* extract. This compound, when determined at a concentration (1.1 µg/ml) that reflected the level present in a CYP3A-inducing concentration (100 µg/ml) of a *Ginkgo biloba* extract (i.e. Extract A, Table 1), increased CYP3A23 mRNA expression to approximately one-half of that by the extract. Ginkgolide A increased CYP3A23 mRNA expression and CYP3A-catalyzed testosterone 6β-hydroxylation over the range of 1.1-5 µg/ml, which reflected the concentrations (1.1-3.8%) of ginkgolide A present in a survey of 27 commercial products of *Ginkgo biloba* (Kressmann et al., 2002). Interestingly, ginkgolide A, at the concentration (1.1 µg/ml) present in Extract A (Table 1) did not increase CYP3A2 or CYP3A18 mRNA expression. A greater concentration (5 µg/ml) was required for the modulation of these two CYP3A genes by ginkgolide A.
A novel finding from the present study is the differential induction of CYP2B1 and CYP3A23 gene expression by bilobalide and ginkgolide A. The molecular basis for these effects is not known. The current evidence indicates that the constitutive androstane receptor mediates the induction of CYP2B1 (Muangmoonchai et al., 2001), whereas the pregnane X receptor is a regulator of CYP3A23 induction (Zhang et al., 1999). Therefore, a potential explanation for the differential effect of the two compounds on CYP2B1 and CYP3A23 expression is the preferential activation of constitutive androstane receptor and pregnane X receptor by bilobalide and ginkgolide A, respectively. Studies are planned to elucidate the molecular mechanism of CYP2B and CYP3A induction by these naturally occurring chemicals.

Another major group of constituents in *Ginkgo biloba* extracts are the flavonol glycosides, including those of quercetin, kaempferol, and isorhamnetin (van Beek, 2002). However, as demonstrated in the present study, the aglycone and the 3-O-rutinoside of each of these flavonols did not increase CYP2B1, CYP3A23, CYP3A2, or CYP3A18 gene expression. This finding is consistent with the result from a previous study showing a lack of an increase in hepatic microsomal CYP2B-mediated pentoxyresorufin O-dealkylation activity in rats administered via the diet for two weeks with quercetin at a dosage of 1% w/w (Brouard et al., 1988).

It is not known whether *Ginkgo biloba* extract alters CYP2B-mediated drug clearance in humans, but conflicting data appear to exist for the effect of this herbal medicine on the clearance of drugs metabolized by human CYP3A enzymes. In one study, the oral ingestion of *Ginkgo biloba* extract (60 mg four times daily for 28 days) by healthy human volunteers did not alter the 1-h serum ratio of 1-hydroxymidazolam/midazolam (Gurley et al., 2002), which is used as an *in vivo* metabolic index for CYP3A activity (Thummel et al., 1994). However, in another
study, the oral ingestion of *Ginkgo biloba* extract (120 mg twice daily) for 14 days resulted in a modest but statistically significant reduction in the area under the plasma alprazolam concentration-time curve (Markowitz et al., 2003). This result suggests a CYP3A-inductive effect by *Ginkgo biloba* because alprazolam is metabolized predominantly by CYP3A enzymes (Gorski et al., 1999). In the studies that investigated the effect of *Ginkgo biloba* extract on drug elimination (Gurley et al., 2002; Markowitz et al., 2003), the levels of bilobalide and ginkgolide A in the extracts were not reported. Studies are in progress to determine directly whether *Ginkgo biloba* extract and some of its individual chemical constituents are capable of modulating CYP2B6 and CYP3A4 expression and function in primary cultures of human hepatocytes.

In summary, the present study in primary cultures of rat hepatocytes shows: 1) induction of CYP2B1, CYP3A23, CYP3A2, and CYP3A18 gene expression by *Ginkgo biloba*, as shown with multiple extracts containing known amounts of terpene trilactones and flavonol glycosides; 2) bilobalide and ginkgolide A are novel inducers of CYP2B1 and CYP3A, respectively, as demonstrated by real-time PCR and enzyme activity assays; and 3) bilobalide is responsible for the modulation of CYP2B1 expression by *Ginkgo biloba* extract, whereas ginkgolide A contributes partially to the modulation of CYP3A23 expression by this herbal medicine.
Acknowledgements. The authors thank Pharmaton S.A. (Bioggio, Switzerland) for providing the GK501™ Ginkgo biloba extract and Indena S.A. (Milan, Italy) for the bilobalide and the two lots of Ginkgo biloba extract.
References


Mahnke A, Strotkamp D, Roos PH, Hanstein WG, Chabot GG, and Nef P (1997) Expression and inducibility of cytochrome P450 3A9 (CYP3A9) and other members of the CYP3A subfamily in rat liver. _Arch Biochem Biophys_ **337**:62-68.


Footnotes

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Address correspondence to: Dr. Thomas K. H. Chang, Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, BC, V6T 1Z3, Canada. E-mail: tchang@interchange.ubc.ca
FIG. 1. Concentration-dependent effect of *Ginkgo biloba* extract on CYP2B1 and CYP3A23 mRNA expression. Primary cultures of rat hepatocyte cultures were treated for 48 h with various concentrations (10, 100, or 1000 µg/ml) of a *Ginkgo biloba* extract (i.e. Extract A, Table 1) or culture medium (vehicle control). As a positive control, hepatocytes were treated for the same length of time with PB (100 µM) or DEX (10 µM) dissolved in DMSO (0.1% final concentration). CYP2B1 (panel A) and CYP3A23 (panel B) mRNA levels were determined by reverse transcription and real-time PCR. Data are expressed as mean ± S.E.M. for hepatocytes from three individual rats per treatment group. *Significantly different from the corresponding vehicle-treated control group (p < 0.05).

FIG. 2. Role of ginkgolides A, B, C, and J and bilobalide in the modulation of CYP2B1 and CYP3A23 mRNA expression by *Ginkgo biloba* extract. Primary cultures of rat hepatocyte cultures were treated for 48 h with culture medium, DMSO (0.1%, vehicle control), ginkgolide A (1.1 µg/ml), ginkgolide B (0.3 µg/ml), ginkgolide C (1.4 µg/ml), ginkgolide J (0.6 µg/ml), bilobalide (2.8 µg/ml), or *Ginkgo biloba* extract (Extract A, Table 1; 100 µg/ml). The concentrations of the individual chemicals were chosen to reflect the levels present in an 100 µg/ml concentration of Extract A. CYP2B1 (panel A) and CYP3A23 (panel B) mRNA expression were determined by reverse transcription and real-time PCR. Data are expressed as mean ± S.E.M. for hepatocytes from three individual rats per treatment group. *Significantly different from the corresponding vehicle-treated control group (p < 0.05).
FIG. 3. Differential effect of bilobalide and ginkgolide A on CYP2B1 and CYP3A23 mRNA expression. Primary cultures of rat hepatocytes were treated with bilobalide (1, 2.8, or 5 µg/ml; panel A), ginkgolide A (1.1, 2.5, or 5 µg/ml; panel B), or DMSO (0.1%, vehicle control). CYP2B1 and CYP3A23 mRNA levels were determined by reverse transcription and real-time PCR. Data are expressed as mean ± S.E.M. for hepatocytes from three individual rats.

FIG. 4. Effect of *Ginkgo biloba* extract and ginkgolide A on CYP3A2 and CYP3A18 mRNA expression. Primary cultures of rat hepatocyte cultures were treated for 48 h with *Ginkgo biloba* extract (i.e. Extract A, Table 1; 100 µg/ml), culture medium (vehicle for the extract), ginkgolide A (1.1 or 5 µg/ml), DEX (10 µM, positive control), or DMSO (0.1%, vehicle for ginkgolide A and DEX). CYP3A2 (panel A) and CYP3A18 (panel B) mRNA levels were determined by reverse transcription and real-time PCR. Data are expressed as mean ± S.E.M. for hepatocytes from three individual rats per treatment group. *Significantly different from the corresponding vehicle-treated control group (p < 0.05).

FIG. 5. Effect of *Ginkgo biloba* extract on CYP2B and CYP3A enzyme activities. Rat hepatocytes cultured for 48 h in 24-well microplates were treated with *Ginkgo biloba* extract (100 µg/ml) or an equivalent volume (800 µl) of culture medium (vehicle control) for 48 h. In control experiments, cultured hepatocytes were treated with PB (100 µM, positive control for CYP2B activity) or DEX (10 µM, positive control for CYP3A activity) dissolved in DMSO (0.1%). CYP2B-mediated BROD (panel A) and CYP3A-mediated testosterone 6β-hydroxylation (T-6β-OH; panel B) were determined as described in Materials and Methods.
Data are expressed as mean ± S.E.M. for hepatocytes from three or five individual rats per treatment group.

FIG. 6. Effect of bilobalide and ginkgolide A on CYP2B and CYP3A enzyme activities. Primary cultures of rat hepatocytes were treated with bilobalide (1, 2.8, or 5 µg/ml; panel A), ginkgolide A (1.1, 2.5, or 5 µg/ml; panel B), or DMSO (0.1%, vehicle control). CYP2B-mediated BROD and CYP3A-mediated testosterone 6β-hydroxylation (T-6β-OH) activities were determined as described in the Materials and Methods. Data are expressed as mean ± S.E.M. for hepatocytes from three or five individual rats.
The levels of terpene trilactones in *Ginkgo biloba* extracts A and B were quantified by gas chromatography (Indena S.A., Milan, Italy) and those of flavonols in these extracts were quantified by liquid chromatography – mass spectrometry (ChromaDex, Inc., Santa Ana, CA). The levels of terpene trilactones and flavonols in *Ginkgo biloba* extract C (GK501) were determined by high performance liquid chromatography (Pharmaton S.A., Bioggio, Switzerland).

<table>
<thead>
<tr>
<th>Ginkgo biloba Extract</th>
<th>Extract A</th>
<th>Extract B</th>
<th>Extract C</th>
</tr>
</thead>
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<tr>
<td><strong>Ginkgolide A</strong></td>
<td>1.1</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Ginkgolide B</strong></td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Ginkgolide C</strong></td>
<td>1.4</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Ginkgolide J</strong></td>
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<td>0.6</td>
<td>0.4</td>
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<td><strong>Total</strong></td>
<td>3.4</td>
<td>3.3</td>
<td>3.4</td>
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</table>

**Sesquiterpene**

<table>
<thead>
<tr>
<th></th>
<th>Extract A</th>
<th>Extract B</th>
<th>Extract C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bilobalide</strong></td>
<td>2.8</td>
<td>2.9</td>
<td>3.2</td>
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**Total Terpene Trilactones**

<p>| | | | |</p>
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<tr>
<th></th>
<th></th>
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<td><strong>6.2</strong></td>
<td>6.2</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Flavonol and its Glycosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>Kaempferol (aglycone)</td>
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<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kaempferol (diglycosides)</td>
<td>1.9</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Kaempferol (other glycosides)</td>
<td>4.4</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kaempferol (sum of aglycone and glycosides)</td>
<td>6.3</td>
<td>11.2</td>
<td>9.5</td>
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<tr>
<td>Quercetin (aglycone)</td>
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<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Quercetin (diglycosides)</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Quercetin (other glycosides)</td>
<td>6.6</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Quercetin (sum of aglycone and glycosides)</td>
<td>10.6</td>
<td>10.9</td>
<td>11.6</td>
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<tr>
<td>Isorhamnetin (aglycone)</td>
<td>none</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Isorhamnetin (3-O-rutinoside)</td>
<td>0.6</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Isorhamnetin (other glycosides)</td>
<td>3.5</td>
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<td>n.d.</td>
</tr>
<tr>
<td>Isorhamnetin (sum of aglycone and glycosides)</td>
<td>4.1</td>
<td>2.3</td>
<td>2.9</td>
</tr>
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</table>

Total Flavonol Glycosides
21 24.4 24
TABLE 2

*CYP2B1 and CYP3A23 mRNA expression in primary cultures of rat hepatocytes treated with different brands and different lots of Ginkgo biloba extract*

Primary cultures of rat hepatocytes were treated for 48 h with an extract (100 µg/ml) of *Ginkgo biloba* (i.e. Extract A, Extract B, or Extract C; Table 1) or culture medium (vehicle). Data are expressed as the mean (± S.E.M.) fold-increase in mRNA expression (relative to the vehicle-treated control group) for hepatocyte cultures from three individual rats.

<table>
<thead>
<tr>
<th>Ginkgo biloba Extract</th>
<th>CYP2B1 mRNA</th>
<th>CYP3A23 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/ml)</td>
<td>Fold-Increase Relative to Control</td>
</tr>
<tr>
<td>Extract A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.1 ± 0.6*</td>
<td>2.3 ± 0.3*</td>
</tr>
<tr>
<td>100</td>
<td>8.4 ± 2.2*</td>
<td>9.7 ± 5.2*</td>
</tr>
<tr>
<td>1000</td>
<td>1.4 ± 0.6</td>
<td>7.6 ± 4.1*</td>
</tr>
<tr>
<td>Extract B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.2 ± 0.3*</td>
<td>3.8 ± 2.3*</td>
</tr>
<tr>
<td>100</td>
<td>4.0 ± 0.6*</td>
<td>4.5 ± 2.3*</td>
</tr>
<tr>
<td>1000</td>
<td>2.1 ± 1.1</td>
<td>17.6 ± 12.5*</td>
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<tr>
<td>Extract C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.2 ± 0.3*</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>100</td>
<td>4.2 ± 0.2*</td>
<td>6.5 ± 4.3*</td>
</tr>
</tbody>
</table>
1000 1.9 ± 0.6 11.2 ± 6.6*

*Significantly different from the control group ($p < 0.05$).
TABLE 3

*Effect of flavonol glycosides and aglycones on CYP2B1 and CYP3A23 mRNA expression in primary cultures of rat hepatocytes*

Primary cultures of rat hepatocytes were treated with a *Ginkgo biloba* extract (i.e. Extract A, Table 1; 100 µg/ml), kaempferol-3-*O*-rutinoside (1.9 µg/ml), quercetin-3-*O*-rutinoside (4 µg/ml), isorhamnetin-3-*O*-rutinoside (0.6 µg/ml), kaempferol (6.3 µg/ml), quercetin (10.6 µg/ml), or isorhamnetin (4.1 µg/ml). Control hepatocytes were treated with culture medium (vehicle for the extract) or DMSO (0.1%, vehicle for the individual chemicals). Data are expressed as the mean ± S.E.M. fold-increase in mRNA expression (relative to the vehicle-treated control group) for hepatocyte cultures from three individual rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP2B1 mRNA</th>
<th>CYP3A23 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fold-Increase Relative to Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-<em>O</em>-rutinoside</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Quercetin-3-<em>O</em>-rutinoside</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Isorhamnetin-3-<em>O</em>-rutinoside</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
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<tr>
<td>Kaempferol</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>Isorhamnetin</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td><em>Ginkgo biloba</em> extract</td>
<td>8.3 ± 1.7*</td>
<td>5.3 ± 0.3*</td>
</tr>
</tbody>
</table>
*Significantly different from the corresponding vehicle-treated control group ($p < 0.05$).
Fig. 1

(A) CYP2B1 mRNA

(B) CYP3A23 mRNA
Fig. 2

(A) CYP2B1 mRNA

(B) CYP3A23 mRNA
Fig. 3

(A) Bilobalide

Relative mRNA Expression (fold-increase over control)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>CYP2B1</th>
<th>CYP3A23</th>
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<tr>
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</tr>
<tr>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
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</table>

(B) Ginkgolide A

Relative mRNA Expression (fold-increase over control)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>CYP2B1</th>
<th>CYP3A23</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>5</td>
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</table>
(A) CYP3A2 mRNA

Relative mRNA Expression (fold-increase over control)

- Culture Medium
- Ginkgo biloba Extract
- DMSO
- Ginkgolide A (1.1 μg/ml)
- Ginkgolide A (5 μg/ml)
- DEX

* indicates significant increase.

(B) CYP3A18 mRNA

Relative mRNA Expression (fold-increase over control)

- Culture Medium
- Ginkgo biloba Extract
- DMSO
- Ginkgolide A (1.1 μg/ml)
- Ginkgolide A (5 μg/ml)
- DEX

* indicates significant increase.
Fig. 5

(A) BROD

Product Formation (fold-increase over control)

- Culture Medium
- Ginkgo biloba Extract
- DMSO
- PB

(B) T-6β-OH

Product Formation (fold-increase over control)

- Culture Medium
- Ginkgo biloba Extract
- DMSO
- DEX

* indicates a significant increase.
Fig. 6

(A) Bilobalide

Product Formation (fold-increase over control)

Concentration (μg/ml)

(B) Ginkgolide A

Product Formation (fold-increase over control)

Concentration (μg/ml)