Inhibition of intestinal and hepatic glucuronidation of mycophenolic acid by

*Ginkgo biloba* extract and flavonoids

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Running title: Inhibition of MPA glucuronidation by ginkgo

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ABBREVIATIONS: HLM, human liver microsomes; HIM, human intestinal microsomes; $K_i$, inhibition constant; $K_m$, Michaelis-Menten constant; LC, liquid chromatography; MPA, mycophenolic acid; MPAG, mycophenolic acid-7-O-glucuronide, PG, phenolphthalein-β-D-glucuronide; MS, mass spectrometry; SRM, single reaction monitoring.
Abstract

Herb-drug interactions have received more attention in recent years because of the widespread popularity of herbal supplements. However, there is limited data on the effect of herbs on glucuronidation in humans. The goal of this work was to examine the effect of *Ginkgo biloba* extract and its main flavonoid and terpene lactone constituents on mycophenolic acid (MPA) 7-O-glucuronidation. Human liver (HLM) and intestinal (HIM) microsomes were incubated with MPA and *G. biloba* extract (unhydrolyzed glycosides or acid-hydrolyzed aglycones), quercetin, kaempferol, ginkgolide A, ginkgolide B, or bilobalide. MPA-7-O-glucuronide (MPAG) formation was inhibited in HLM and HIM incubations by unhydrolyzed (IC$_{50}$ = 84.3 [HLM] and 6.9 [HIM] µg/ml) and hydrolyzed (IC$_{50}$ = 20.9 [HLM] and 4.3 [HIM] µg/ml) *G. biloba* extracts, quercetin (IC$_{50}$ = 19.1 [HLM] and 5.8 [HIM] µM), and kaempferol (IC$_{50}$ = 23.1 [HLM] and 7.7 [HIM] µM). Terpene lactones did not show inhibition of MPA glucuronidation. Quercetin was a mixed-type inhibitor in HLM and HIM incubations ($K_i$ = 11.3 µM [HLM] and 2.8 µM [HIM]), while kaempferol was a non-competitive inhibitor in HLM ($K_i$ = 33.7 µM) and a mixed-type inhibitor in HIM ($K_i$ = 4.5 µM). These results indicate that *G. biloba* extract or quercetin- and kaempferol-rich supplements may inhibit intestinal and hepatic glucuronidation of MPA. Future studies are needed to evaluate the clinical significance of this interaction.
Introduction

Herbal supplement use continues to increase around the globe, especially in populations looking for natural methods to promote health and wellness. In the US, surveys estimate that 20% of the population uses at least one herbal supplement (Bardia et al., 2007). This growing interest in herbals is manifested by annual sales in the US of over $4 billion dollars (NBJ, 2007). Such public interest is met by concerns from health professionals regarding possible deleterious interactions of herbals with conventional drugs. Herbals are considered dietary supplements; hence, they are not routinely screened for interactions with drug metabolizing enzymes (www.fda.gov). However, numerous in vitro, animal, and clinical studies and case reports provide evidence that herbals can interact with conventional drugs and may lead to serious adverse effects (Gardiner et al., 2008).

*Ginkgo biloba* is among the most popular herbals used in the world. Its extract is available over the counter in the US and is commonly prescribed in European countries for cerebral insufficiency (De Smet, 2005). Antioxidant effects as well as beneficial effects on memory and circulation have been attributed to *G. biloba* extract and its components. The primary active constituents of *G. biloba* are terpene lactones (ginkgolides and bilobalide) and flavone glycosides, which are hydrolyzed in vivo to flavone-aglycones (e.g., quercetin and kaempferol) (Figure 1a) (Chan et al., 2007). Several clinical and in vitro studies have investigated the effect of *G. biloba* on drug metabolizing cytochrome P450 enzymes and transporters (Izzo and Ernst, 2009). In contrast, limited research has been conducted to investigate interactions of
G. biloba and its components with conjugation pathways. In vitro studies have shown that quercetin and kaempferol inhibit sulfotransferase 1A1 (Eaton et al., 1996; Ghazali and Waring, 1999); meanwhile, information is lacking regarding effects of G. biloba on drug glucuronidation.

Glucuronidation constitutes the main pathway of conjugative metabolism for a wide variety of compounds (Ouzzine et al., 2003); substrates for UDP-glucuronosyltransferase enzymes (UGTs) include endogenous compounds, drugs and many phytochemicals. Many flavonoids (e.g., quercetin and kaempferol) are substrates for UGT enzymes. Moreover, inhibitory effects of flavonoids on UGT1A enzymes have been reported in the literature (Williams et al., 2002; D’Andrea et al., 2005). For substrates metabolized mainly through glucuronidation, modulation of UGT activities can lead to significant effects on pharmacokinetics (Kiang et al., 2005).

Mycophenolic acid (MPA) is an immunosuppressive drug that acts by inhibiting the production of guanosine nucleotides in lymphocytes, ceasing their proliferation (Allison and Eugui, 2005). Therefore, it is used to prevent graft rejection in transplant recipients and to delay progression of the autoimmune disorders (Heatwole and Ciafaloni, 2008). MPA is available as either a prodrug mofetil ester (CellCept®) or as an enteric-coated sodium salt (Myfortic®). Although both formulations have similar pharmacokinetic and efficacy profiles, absolute oral bioavailability of mycophenolate sodium is 72% compared to 94% for mycophenolate mofetil (Staatz and Tett, 2007). This difference is attributed to higher presystemic glucuronidation of MPA from the mycophenolate sodium formulation. Following oral absorption, MPA is metabolized
by UGTs to the major phenolic conjugate 7-O-MPA-glucuronide (MPAG) (Figure 1b). In the liver, UGT1A9 is the main isoform catalyzing the formation of MPAG, while UGT1A7, UGT1A8, and UGT1A10 contribute to MPAG formation extra-hepatically – mainly in the kidneys and intestine (Picard et al., 2005). MPA is a narrow therapeutic index drug with wide inter- and intra-individual variability and complex pharmacokinetics in transplant recipients (Staatz and Tett, 2007). Therefore, an alteration in MPA glucuronidation may cause changes in exposure to the immunosuppressive drug, and consequently, undesired clinical outcomes. The aim of this study was to investigate the effect of G. biloba extract and its main components on MPAG formation in human intestinal and liver microsomes. The results demonstrate that G. biloba and its primary constituents have the ability to inhibit MPA glucuronidation in the intestine and liver.
Materials and methods

Chemicals and Reagents. Mycophenolic acid (MPA; 98%) and mycophenolic acid-7-O-glucuronide (MPAG; 98%) were purchased from Toronto Research Chemicals (North York, ON, Canada). Potassium phosphate dibasic, uridine diphosphate glucuronic acid, magnesium chloride, alamethicin, phenolphthalein-β-D-glucuronide (PG; internal standard), niflumic acid, and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol Quercetin dihydrate (99% purity) and kaempferol (90%) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ginkgolide A (95.1%), ginkgolide B (82.8%), and bilobalide (99.7%) were purchased from ChromaDex (Irvine, CA, USA). Pooled human liver and intestinal microsomes were purchased from BD Biosciences Discovery Labware (Woburn, MA, USA).

Herbal Extracts. Ginkgo biloba extract was provided by Finzelberg & Co. KG (Andernach, Germany) as dry powder. The extract was standardized by the supplier to contain 24% flavonglycosides, 6% terpene lactones, and < 5 ppm ginkgolic acids using 60% acetone as the extraction solvent. Unhydrolyzed and acid-hydrolyzed G. biloba working solutions were freshly prepared by dissolving 30 mg of the powder extract in 1 ml of either 60% acetone or 60% acetone/40% 5N HCl to prepare the unhydrolyzed and acid-hydrolyzed working extracts, respectively. The acid treated extract was heated at 90°C for one hour and neutralized with 2N KOH. The acetone-rich extracts were serially diluted to prepare working solutions of G. biloba with concentrations of 0.05 to 5 mg/ml and acetone content of 10%.
Inhibition of MPA Glucuronidation Assay. The incubation conditions were optimized with respect to time of incubation and microsomal protein concentration. A typical 100 µl incubation mixture contained HLM or HIM (protein concentration, 0.16 mg/ml), alamethicin (100 µg/mg microsomal protein), MgCl₂ (5 mM), MPA, and different concentrations of each test extract or test compound in 100 mM phosphate buffer, pH 7.4. Microsomes were pre-incubated on ice with alamethicin for 15 minutes to activate UGT enzymes. The reaction was started by adding UDPGA (1 mM) and placing incubation tubes in a water bath at 37°C for 30 minutes. The reaction was stopped by adding 300 µl of ice-cold acetonitrile and 20 µl of internal standard (0.5 mg/ml phenolphthalein glucuronide). Tubes were vortex-mixed for two minutes and centrifuged for 10 min at 20,000 x g. The supernatant was diluted 12-fold with purified water and 5 µl was injected into the HPLC system. Incubations with herbal extracts and the corresponding controls contained 1% acetone. The HLM and HIM used in all experiments were from the same lot.

Screening experiments were conducted to generate IC₅₀ values by incubating MPA at the estimated Kₘ value in the presence of five concentrations of *G. biloba* unhydrolyzed and hydrolyzed extracts (final concentrations ranging from 5 - 500 µg/ml) or *G. biloba* individual components (final concentrations ranging from 1 - 100µM). In addition to IC₅₀ values, inhibitory potency was also expressed as the volume per dose index, which is defined as the volume in which one dose would be dissolved in to obtain the corresponding IC₅₀ concentration as described by Strandell et. al. (2004). Comparison of this unit to physiological volumes facilitates an
assessment of inhibitory potential.

A $K_i$ value was determined if the IC₅₀ value was lower than 100 µM. In such cases, MPA (60 - 600 µM with HLM or 30 - 600 µM with HIM) and a range of concentrations of individual ginkgo components (10 - 100 µM with HLM or 3 - 20 µM with HIM) were used for the construction of Dixon plots and estimation of $K_i$ values.

Detection of MPA-7-O-glucuronide. MPAG was determined by LC/MS/MS on a ThermoFinnigan Surveyor series HPLC system connected to a TSQ Quantum triple quadrupole mass spectrometer (Thermo Corp., San Jose, CA, USA) using electrospray ionization (ESI), as described previously (Mohamed et al., 2008). Average assay within-day and between-day relative standard deviations were 5.2% and 6.9%, respectively and accuracy expressed as relative error was within 8%.

Briefly, 5 µl of each sample was injected on a reversed-phase Phenomenex (Torrance, CA, USA) Synergi Fusion-RP18 column (100 × 2 mm, 4 µm). The two mobile phases consisted of (A) 1 mM acetic acid in deionized water and (B) 1 mM acetic acid in acetonitrile. Gradient elution at a flow-rate of 0.22 mL/min was employed with the following steps: at start of the run, 30% B for one min, then increased to 90% B in 0.75 min, held at 90% B between 1.75 and 3.1 min, and from 3.6 to 6.5 min, the column was re-equilibrated at 30% B. Analysis was carried out in the single reaction monitoring (SRM), negative ion mode using the mass transitions of $m/z$ 495 → 319 and $m/z$ 493 → 175 for MPAG and PG, respectively. MPAG standard solutions were freshly prepared for each experiment with concentration ranges of 100 - 4 µM for HIM or 1 - 20 µM for HLM incubations.
Enzyme Kinetics Analysis. $K_m$ and $V_{max}$ were determined by nonlinear regression analysis of the MPAG formation data using eight different MPA concentrations (0.02 to 1 mM). Data points were fitted to the Michaelis-Menten model using Prism 4.0 (GraphPad software, San Diego, CA, USA).

IC$_{50}$ values were similarly determined by nonlinear regression fitting of the inhibition data to the IC$_{50}$ equation (Copeland, 2005) using Prism 4.0. The $K_i$ values were determined by fitting competitive, noncompetitive, uncompetitive, and mixed-type inhibition models to the MPAG formation data (Copeland, 2005). The mode of inhibition was determined on the basis of visual inspection of the Dixon plot and the Akaike information criterion (Akaike, 1974) using SigmaPlot v.11 Enzyme Kinetics Module 1.3 (Systat Software, Inc., Chicago, IL, USA).
Results

MPA-7-\textit{O}-glucuronide formation was best explained by Michaelis-Menten kinetics. The $K_m$ and $V_{\text{max}}$ were $103.9 \pm 19.5$ µM and $2.6 \pm 0.2$ nmol/min/mg protein (mean ± SEM), respectively, with pooled HLM, whereas with pooled HIM, these values were $67.2 \pm 10.1$ µM and $408.7 \pm 17.1$ pmol/min/mg protein (mean ± SE), respectively. These values are similar to values previously reported (Shipkova et al., 2001; Miles et al., 2005; Chang et al., 2009).

Inhibition of MPA Glucuronidation by \textit{Ginkgo biloba}. Both unhydrolyzed and acid-hydrolyzed \textit{G. biloba} extracts inhibited MPA glucuronidation in pooled HIM and HLM (Figure 2). MPA concentration was 100 µM for HLM incubations and 70 µM for HIM incubations. Results showed that unhydrolyzed and acid-hydrolyzed \textit{G. biloba} extracts inhibited MPA glucuronidation in HLM with best fit IC$_{50}$ values of $84.3 \pm 11.6$ and $20.9 \pm 3.6$ µg/ml, respectively. More potent inhibition of MPA glucuronidation was observed in HIM with IC$_{50}$ values of $6.8 \pm 0.8$ and $4.3 \pm 1.2$ µg/ml for the unhydrolyzed and acid-hydrolyzed extracts, respectively (Table 1). The volume/dose index values, calculated to estimate the clinical significance of the inhibition as described previously (Strandell et al., 2004), are shown in Table 1.

Effect of Ginkgo Compounds on MPA Glucuronidation. Ginkgo flavonoids (quercetin and kaempferol) and terpene lactones (ginkgolides A and B, and bilobalide) were incubated with MPA to determine whether or not these compounds inhibit MPA glucuronidation. Ginkgo flavonoids but not terpene lactones showed inhibition with IC$_{50}$ values < 100 µM (Table 1). Quercetin and kaempferol inhibited
MPA glucuronidation in HLM with IC$_{50}$ values of 19.1 ± 1.3 and 23.1 ± 5.5 µM, respectively. In agreement with results from incubations with *G. biloba* extracts, inhibition of MPA glucuronidation was more potent in HIM, with IC$_{50}$ values of 5.8 ± 0.3 and 7.6 ± 0.6 µM for quercetin and kaempferol, respectively.

**Inhibition Kinetics Analysis.** To further characterize the inhibition of MPA glucuronidation by ginkgo flavonoids, enzyme inhibition kinetic experiments were carried out. Based on the analysis of nonlinear regression of inhibition data and Dixon plots presented in Figure 2, quercetin exhibited mixed-type inhibition against MPA glucuronidation in both HLM and HIM. Kaempferol exhibited non-competitive inhibition in HLM and mixed-type inhibition in HIM. In HLM, $K_i$ values were 11.3 ± 1.7 and 33.6 ± 2.5 µM for quercetin and kaempferol, respectively (Table 2; Fig 2-A ).

Again, inhibitory potency of quercetin and kaempferol to MPA glucuronidation in HIM was three to four-fold higher than that in HLM with $K_i$ values of 2.8 ± 0.4 and 4.5 ± 1.2 µM, respectively (Table 2; Figure 2-B ).
Discussion

Scientific and public interest in *G. biloba* has grown enormously in recent years because of its purported beneficial effects on memory and circulation (Bardia et al., 2007). *G. biloba* supplements have been widely used with little awareness of the potential for drug interactions with conventional drugs. Although *G. biloba* is considered generally safe, clinical studies and case reports have demonstrated that it can interact with conventional drugs and may lead to severe adverse effects (Hu et al., 2005; Kupiec and Raj, 2005). In the current study, *G. biloba* extract and flavone aglycones inhibited the UGT-mediated metabolism of mycophenolic acid in human intestinal and liver microsomes.

In intestinal microsomes, *G. biloba* extracts inhibited MPAG formation with IC₅₀ values of 4.3 and 6.8 µg/ml for acid-hydrolyzed and unhydrolyzed extracts, respectively. The clinical significance of this interaction can be postulated based on the recommended dose of *G. biloba* supplements and the fraction of MPA metabolized by intestinal enzymes. *G. biloba* extracts are usually taken at a dose of 120 mg to 240 mg per day. Therefore, IC₅₀-equivalent concentrations can be achieved in the intestine if a 120 mg *G. biloba* dose is mixed with 18 to 28 L of fluid (i.e., 6.7 to 4.3 mg/L) or if a 240 mg dose is mixed with 35 to 56 L of fluid. Thus, based on estimates of intestinal volume that range from about 0.5 to 5 L (Hellum et al., 2007), concentrations in the intestine after ingestion of a *G. biloba* supplement are expected to be much higher than IC₅₀ values; accordingly, inhibition of intestinal UGT enzymes in vivo is likely. The potential for interaction is greater with enteric-
coated mycophenolate sodium, since about 28% of the dose is eliminated through first pass metabolism (Myfortic prescribing information: http://www.pharma.us.novartis.com/product/pi/pdf/myfortic.pdf). Inhibiting first pass metabolism of MPA could result in higher systemic concentrations, enhanced immunosuppressive effect and increased potential for side effects.

Incubations with HLM also showed inhibition of MPA glucuronidation by *G. biloba* extracts. In the liver, UGT1A9 selectively metabolizes MPA to MPAG (Picard et al., 2005); therefore, MPAG formation can be used as an in vitro UGT1A9 index reaction. An effect observed on MPAG formation is expected to reproduce with other UGT1A9 substrates like propofol. In vitro screening of *G. biloba* components for inhibition indicates that the observed inhibition can be attributed to *G. biloba* flavonoid components, but not to terpene lactones. $K_i$ values for inhibition of hepatic MPA glucuronidation by quercetin and kaempferol were 11.3 and 33.6 µM, respectively.

To understand the clinical significance of this observation, adequate knowledge of the bioavailability and hepatic concentrations of the inhibitors is necessary. Quercetin and kaempferol are classified as flavonols, which is a class of flavonoids ubiquitously found in plants, beverages, and dietary supplements, e.g., tea, onions, apples, red wine, St. John’s wort, and *G. biloba* (Nijveldt et al., 2001). A typical diet contains about 14-16 mg/day quercetin and 4-6 mg/day kaempferol according to dietary surveys in the Netherlands and US (Hertog et al., 1993; Sampson et al., 2002); however, the intake can reach several hundred mg in dietary supplements and herbal products and several grams in anticancer therapy (Lamson and Brignall,
In contrast to kaempferol, a relatively large number of studies concerning the absorption of quercetin have been published. However, the extent to which quercetin reaches the liver remains largely unknown. Most studies were not able to detect free quercetin concentrations in plasma and absorption was estimated from the quantities of quercetin and quercetin conjugates detected in the urine (0.3-1.4% of quercetin dose) (Scalbert and Williamson, 2000); thus it was assumed that quercetin was poorly absorbed. However, an early study in healthy ileostomy subjects estimated quercetin absorption to be 17-52% of orally ingested amount (Hollman et al., 1995). The authors reported that only 0.3% of the oral quercetin dose was recovered in urine and concluded that it might be possible that some quercetin accumulated in tissues and was released slowly over time. A recent study investigating tissue distribution of quercetin in pigs following long-term dietary supplementation reported that total quercetin concentration in liver was 5 to 6 fold higher than that in plasma (Bieger et al., 2008). Interestingly, 93% of quercetin found in the liver was in the aglycone form. Taken together, further studies are needed to investigate whether long-term G. biloba or flavonoid-rich supplements may lead to accumulation of quercetin in human liver to levels that could inhibit mycophenolic acid glucuronidation.

Incubations with intestinal microsomes exhibited 3- to 12-fold more potent inhibition of MPAG formation than in liver microsomes by G. biloba extracts, quercetin and kaempferol (Tables 1 and 2). This difference in inhibition potency can
be explained by differentially expressed UGT enzymes in liver and intestine (Ohno and Nakajin, 2009) and the difference in catalytic activities towards MPA glucuronidation between liver and intestine microsomes. In this study, microsomal intrinsic clearance ($V_{max}/K_m$) for MPAG formation was 4-fold higher by HLM as compared to HIM (25.12 vs. 6.08 µl/min/mg protein). This is in accordance with previously reported values (Bowalgaha and Miners, 2001; Shipkova et al., 2001; Picard et al., 2005). In the intestine, UGT1A7, 1A8, 1A9 and 1A10 conjugate MPA to MPAG with different affinities, while in the liver, MPAG is selectively formed by UGT1A9 (Picard et al., 2005). In addition, UGT1A10 exhibits a much lower catalytic activity towards MPA glucuronidation than UGT1A8 and UGT1A9, while its expression in the intestine is 13- and 25-fold greater than UGT1A8 and UGT1A9, respectively (Picard et al., 2005; Ohno and Nakajin, 2009). Due to these differences, interactions may not always translate from liver to intestinal microsomes with the same magnitude. Therefore, using intestinal microsomes to screen for interactions may be necessary for drugs metabolized by intestinal glucuronidation.

Two limitations are acknowledged for this study. First, the study does not rule out the possibility of induction of MPA metabolism by *G. biloba*. A recent study showed that *G. biloba* and its components induce cytochrome P450 enzymes, transporters, and UGT1A1 (Li et al., 2009). The effect of *G. biloba* on MPA-metabolizing enzymes in hepatocytes warrants further research. Second, the study did not control for the possible inhibition of UGT activities by fatty acids released from the microsomal membrane, which may inhibit UGT1A9 and result in underestimation of inhibition.
potency (Rowland et al., 2008). Although the effect of released fatty acids on MPA glucuronidation has not been documented, it is possible that the actual potency of inhibition is greater than what we observed.

Based on our findings, *G. biloba* supplements taken concomitantly with mycophenolate sodium could lead to increased MPA exposure secondary to inhibition of presystemic glucuronidation. Therefore, patients should be advised to avoid *G. biloba* supplements while taking enteric-coated mycophenolate sodium—the form of MPA that is more subject to presystemic metabolism. Effect of *G. biloba* on MPA systemic metabolism cannot be predicted, due to lack of information on hepatic concentrations of quercetin and kaempferol, but will likely be weaker than the presystemic inhibition. MPA is used in HLM as a probe of UGT1A9 activity because of selective formation of MPAG by UGT1A9. Therefore, the observed hepatic inhibition would be expected to extrapolate to other UGT1A9 substrates like propofol and flavopiridol. The actual in vivo effect of this interaction should be verified in clinical studies.
Acknowledgement

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DMD 30080

References:


(UGT) 1A9 substrates but not UGT1A1 and UGT1A6 activities. *Drug Metab Dispos* **36**:1056-1062.


Legends for Figures:

**Figure 1.** Chemical structures of (A) main bioactive ginkgo components and (B) mycophenolic acid (MPA) and MPA-7-O-glucuronide.

**Figure 2.** Effect of *Ginkgo biloba* extracts on mycophenolic acid 7-O-glucuronidation in vitro.

Alamethicin-activated pooled human liver (panel A) or intestinal (panel B) microsomes (0.16 mg/ml) were incubated with UDPGA (1 mM) and various concentrations of unhydrolyzed (square with solid line) and acid-hydrolyzed (triangle with dotted line) *G. biloba* extracts (5, 10, 50, 100, and 500 µg/ml). Incubations were performed using 100 or 70 µM MPA for HLM and HIM, respectively. Reactions were stopped after 30 minutes by adding 300 µl ice-cold acetonitrile. MPAG was detected by LC-MS/MS as described under *Materials and Methods*. Each point represents the mean of duplicate measurements.

**Figure 3.** Inhibition of mycophenolic acid 7-O-glucuronidation by quercetin and kaempferol.

Alamethicin-activated pooled human liver (panels A and B) or intestinal (panels C and D) microsomes (0.16 mg/ml) were incubated with UDPGA (1 mM), various concentrations of MPA, and various concentrations of quercetin (panels A and C) or kaempferol (panels B and D). Data shown are representative Dixon plots. Each point represents the mean of duplicate measurements.
Table 1. Inhibition of MPA-7-O-glucuronidation by *Ginkgo biloba* extracts.

Pooled human liver or intestine microsomes (0.16 mg/ml) were incubated with UDPGA (1 mM) and various concentrations of ginkgo extracts, and ginkgo compounds. IC$_{50}$ values and volume/dose index were determined as described under Materials and Methods. All incubations were performed in duplicate. Data are expressed as the best-fit IC$_{50}$ values ± standard error. Goodness of fit $r^2$ values for the nonlinear regression model were > 0.9 for unhydrolyzed and acid-hydrolyzed extracts, quercetin, and kaempferol.

<table>
<thead>
<tr>
<th>Extract/ Ginkgo compound</th>
<th>IC$_{50}$ values Mean ± SE</th>
<th>Volume/Dose Index* (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unhydrolyzed <em>G. biloba</em></td>
<td>84.3 ± 11.6 µg/ml 6.8 ± 0.8 µg/ml</td>
<td>1.4 17.6</td>
</tr>
<tr>
<td>Acid-hydrolyzed <em>G. biloba</em></td>
<td>20.9 ± 3.6 µg/ml 4.3 ± 1.2 µg/ml</td>
<td>5.8 27.8</td>
</tr>
<tr>
<td>Quercetin</td>
<td>19.1 ± 1.3 µM 5.8 ± 0.3 µM</td>
<td>0.7 2.2</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>23.1 ± 5.5 µM 7.6 ± 0.6 µM</td>
<td>0.5 1.4</td>
</tr>
<tr>
<td>Ginkgolide A</td>
<td>&gt; 100 µM &gt; 100 µM</td>
<td>&lt; 0.01 &lt; 0.01</td>
</tr>
<tr>
<td>Ginkgolide B</td>
<td>&gt; 100 µM &gt; 100 µM</td>
<td>&lt; 0.06 &lt; 0.06</td>
</tr>
<tr>
<td>Bilobalide</td>
<td>&gt; 100 µM &gt; 100 µM</td>
<td>&lt; 0.03 &lt; 0.03</td>
</tr>
</tbody>
</table>

*Volume/Dose index was calculated by dividing daily dose by the IC$_{50}$ value (Strandell et al., 2004).
Daily dose was considered to be 120 mg G. biloba extract containing (%w/w) 10.75% quercetin, 8.75% kaempferol, 1.2% ginkgolide A, 0.48% ginkgolide B, and 2.94% bilobalide.
Table 2. Inhibition of MPA-7-O-glucuronidation by ginkgo flavonoids.

Alamethicin-activated pooled human liver or intestine microsomes (0.16 mg/ml) were incubated with UDPGA (1 mM), various concentrations of MPA and various concentrations of quercetin or kaempferol. $K_i$ values were determined as described under Materials and Methods. All incubations were performed in duplicate. Data are expressed as the best-fit $K_i \pm$ standard error.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ (µM)</th>
<th>Mode of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
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<tr>
<td>HLM</td>
<td>11.3 ± 1.7</td>
<td>Mixed</td>
</tr>
<tr>
<td>HIM</td>
<td>2.8 ± 0.4</td>
<td>Mixed</td>
</tr>
<tr>
<td>Kaempferol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>33.6 ± 2.5</td>
<td>Non-competitive</td>
</tr>
<tr>
<td>HIM</td>
<td>4.5 ± 1.2</td>
<td>Mixed</td>
</tr>
</tbody>
</table>
Figure 1

A.

Ginkgolide A: $X = H$
Ginkgolide B: $X = OH$

Bilobalide

Kaempferol: $X' = H$
Quercetin: $X' = OH$

Kaempferol

B.

MPA

UGT1A7, 1A8, 1A9, 1A10

MPAG
Figure 2

A. 

- Unhydrolyzed ginkgo extract
- Acid-hydrolyzed ginkgo extract

HLMs

B. 

- Unhydrolyzed ginkgo extract
- Acid-hydrolyzed ginkgo extract

HIMs

MPAG Formation Percent of Control

Herbal Extract Concentration μg/ml
Figure 3

A.  
- [MPA] = 60 μM
- [MPA] = 150 μM
- [MPA] = 300 μM
- [MPA] = 600 μM

HLM

1/Velocity (nmol/min/mg protein)

[Quercetin] (μM)

r² = 0.99

B.  
- [MPA] = 60 μM
- [MPA] = 150 μM
- [MPA] = 300 μM
- [MPA] = 600 μM

HLM

1/Velocity (nmol/min/mg protein)

[Kaempferol] (μM)

r² = 0.99

C.  
- [MPA] = 30 μM
- [MPA] = 60 μM
- [MPA] = 150 μM
- [MPA] = 300 μM
- [MPA] = 600 μM

HIM

1/Velocity (nmol/min/mg protein)

[Quercetin] (μM)

r² = 0.99

D.  
- [MPA] = 30 μM
- [MPA] = 60 μM
- [MPA] = 150 μM
- [MPA] = 300 μM
- [MPA] = 600 μM

HIM

1/Velocity (nmol/min/mg protein)

[Kaempferol] (μM)

r² = 0.97