Inhibitory Effects of Commonly Used Herbal Extracts on UDP-Glucuronosyltransferase 1A4, 1A6, and 1A9 Enzyme Activities

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ABSTRACT:

The aim of this study was to investigate the effect of commonly used botanicals on UDP-glucuronosyltransferase (UGT) 1A4, 1A6, and 1A9 activities in human liver microsomes. The extracts screened were black cohosh, cranberry, echinacea, garlic, ginkgo, ginseng, milk thistle, saw palmetto, and valerian in addition to the green tea catechin epigallocatechin gallate (EGCG). Formation of trifluoperazine glucuronide, serotonin glucuronide, and mycophenolic acid phenolic glucuronide was used as an index reaction for UGT1A4, UGT1A6, and UGT1A9 activities, respectively, in human liver microsomes. Inhibition potency was expressed as the concentration of the inhibitor at 50% activity (IC50) and VDI values in the volume in which the dose could be diluted to generate an IC50-equivalent concentration [volume/dose index (VDI)]. Potential inhibitors were EGCG for UGT1A4, milk thistle for both UGT1A6 and UGT1A9, saw palmetto for UGT1A6, and cranberry for UGT1A9. EGCG inhibited UGT1A4 with an IC50 value of (mean ± S.E.) 33.8 ± 3.1 μg/ml. Milk thistle inhibited both UGT1A6 and UGT1A9 with IC50 values of 59.5 ± 3.6 and 33.6 ± 3.1 μg/ml, respectively. Saw palmetto and cranberry weakly inhibited UGT1A6 and UGT1A9, respectively, with IC50 values >100 μg/ml. For each inhibition, VDI was calculated to determine the potential of achieving IC50-equivalent concentrations in vivo. VDI values for inhibitors indicate a potential for inhibition of first-pass glucuronidation of UGT1A4, UGT1A6, and UGT1A9 substrates. These results highlight the possibility of herb-drug interactions through modulation of UGT enzyme activities. Further clinical studies are warranted to investigate the in vivo extent of the observed interactions.

Introduction

Conjugation of compounds with glucuronic acid represents a major disposition pathway for endogenous and exogenous compounds, including drugs and phytochemicals. Human glucuronidation enzymes [UDP-glucuronosyltransferases (UGTs)] are divided into two families, UGT1 and UGT2, which encompass more than 18 enzymes (Tukey and Strassburg, 2000). Substrates for UGT enzymes include many drugs (e.g., mycophenolic acid, trifluoperazine, tamoxifen, lamotrigine, and acetaminophen) and phytochemicals (e.g., quercetin, kaempferol, and epigallocatechin gallate) (Oliveira and Watson, 2000; Lu et al., 2003; Kiang et al., 2005). Because many phytochemicals are glucuronidated primarily by UGT1A enzymes, there is a potential for herb-drug interaction through competition with drug substrates for this pathway (Mohamed and Frye, 2011). We previously reported the inhibitory effects of several commonly used herbal supplements on UGT1A1 and of Ginkgo biloba extract and its polyphenolic compounds quercetin and kaempferol on UGT1A9 (Mohamed and Frye, 2010; Mohamed et al., 2010). The aim of this study was to identify other potential herb-UGT interactions through screening of commonly used herbal extracts for inhibitory effects on the activities of UGT1A4, UGT1A6, and UGT1A9.

Recent surveys estimate that 38% of Americans use complementary and alternative medicine, which includes herbal supplements (Barnes et al., 2008). However, the physiologic and metabolic effects of herbs and phytochemicals are often poorly understood. One of the issues of concern to clinicians is the potential for herb-drug interactions, which may lead to poor clinical outcomes (Gardiner et al., 2008). Several case studies have described deleterious herb-drug interactions that can lead to morbidity or even mortality (Ruschitzka et al., 2000; Kupiec and Raj, 2005). Therefore, much attention has been given to investigating the effects of herbal supplements on cytochrome P450 enzymes, the primary metabolic route for the majority of marketed drugs (Izzo and Ernst, 2009). In contrast, research regarding the potential of herbal products to alter other metabolic routes including glucuronidation is lacking (Mohamed and Frye, 2011).

Identification of selective substrates for UGT enzymes allows screening of herb-UGT interactions using human liver microsomes. Trifluoperazine, serotonin, and mycophenolic acid were reported to be...
selective in vitro probe substrates for UGT1A4, UGT1A6, and UGT1A9, respectively (Court, 2005). In this study, formation of trifluoperazine glucuronide, serotonin glucuronide, and mycophenolic acid phenolic glucuronide was used as an index reaction for UGT1A4, UGT1A6, and UGT1A9 enzymatic activities, respectively.

**Materials and Methods**

**Chemicals and Reagents.** Trifluoperazine (TFP) (≥99%), serotonin (≥98%), potassium phosphate dibasic, Tris-HCl, UDP-glucuronic acid (UDPGA), β-glucuronidase, magnesium chloride, bovine serum albumin (BSA), alamethicin, niflumic acid, and epigallocatechin gallate (EGCG) (≥97%) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, ethanol, methanol, acetic acid, hecogenin acetate (93%), and 1-naphthol (≥99%) were purchased from Thermo Fisher Scientific (Waltham, MA). Serotonin-β-β-glucuronide was provided by RTI International (Research Triangle Park, NC) through the National Institute of Mental Health Chemical Synthesis Program. Mycophenolic acid (MPA) (98%), mycophenolic acid β-β-glucuronide (MPAG) (98%), and mycophenolic acid-δ-δ-glucuronide (MPA-δ3-G) (98%) were purchased from Toronto Research Chemicals (North York, ON, Canada). Herbal extracts (black cohosh, Cimicifuga racemosa; cranberry, Vaccinium marocarpon, echinacea, Echinacea purpurea; garlic, Allium sativum; ginkgo, Ginkgo biloba; ginseng, Panax ginseng; milk thistle, Silybum marianum; saw palmetto, Serenoa repens; and valerian, Valeriana officinalis)) were generously provided by Finzelberg and Co. KG (Andernach, Germany) as dry powder. Table 1 summarizes the properties of the extracts screened. UltraPool human liver microsomes (HLM), which are microsomes pooled from 150 donors to minimize lot-to-lot variability, were purchased from BD Biosciences Discovery Labware (Bedford, MA).

**Preparation of Herbal Working Solutions.** Herbal extracts were reconstituted with the solvents originally used for extraction and standardization by the vendor (Table 1). To remove any insoluble contents, the mixture was centrifuged at 20,000g for 5 min, and the liquid supernatant was removed. Working solutions were freshly prepared so that final herbal concentrations in screening incubations would represent the recommended daily intake of each extract in 53, 53, and 0.53 liters. These volumes roughly represent total body fluids, and two extremes of a range of concentrations that could appear in the small intestine, assuming 100% bioavailability as described previously by Hellum et al. (2007). For confirmation experiments, a range of concentrations around the rough IC50 of herbal extracts was used in incubations. The concentration of organic solvents in incubations was the same in all incubations including controls and was limited to 1%. For EGCG, working solutions were freshly prepared in 10% methanol and 1.5 mM ascorbic acid, which was added to ensure EGCG stability during the experiment (Lu et al., 2003). Ginseng and ginkgo were tested as both unhydrolyzed and acid-hydrolyzed extracts due to

**TABLE 1**

List of herbal extracts screened for UGT1A4, UGT1A6, and UGT1A9 inhibition

<table>
<thead>
<tr>
<th>Extract</th>
<th>Scientific Name of Origin</th>
<th>% Key Components (w/w)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black cohosh rhizome extract</td>
<td>Cimicifuga racemosa</td>
<td>≥5% Total triterpenylglycosides</td>
<td>50% Ethanol</td>
</tr>
<tr>
<td>Cranberry press juice</td>
<td>Vaccinium marocarpon</td>
<td>&gt;40% Total proanthocyanidins</td>
<td>96% Ethanol</td>
</tr>
<tr>
<td>Echinacea root extract</td>
<td>Echinacea purpurea</td>
<td>3% Chlorogenic acids</td>
<td>60% Ethanol</td>
</tr>
<tr>
<td>Garlic bulb extract</td>
<td>Allium sativum</td>
<td>≥1.25% Allin</td>
<td>80% Methanol</td>
</tr>
<tr>
<td>Ginkgo biloba leaf extract</td>
<td>Ginkgo biloba</td>
<td>≥24% Ginkgo flavoglycosides</td>
<td>60% Ace tone</td>
</tr>
<tr>
<td>Ginseng root extract</td>
<td>Panax ginseng</td>
<td>≥5% Total ginsenosides</td>
<td>60% Ethanol</td>
</tr>
<tr>
<td>Milk thistle herb extract</td>
<td>Silybum marianum</td>
<td>37.9% Total silymarin flavonolignans</td>
<td>80% Ace tone</td>
</tr>
<tr>
<td>Saw palmetto fruit extract</td>
<td>Serenoa repens</td>
<td>&gt;85% Total fatty acids</td>
<td>96% Ethanol</td>
</tr>
<tr>
<td>Valerian root extract</td>
<td>Valeriana officinalis</td>
<td>≥0.1% Valericen acids</td>
<td>70% Ethanol</td>
</tr>
<tr>
<td>EGCG</td>
<td>Camellia sinensis</td>
<td>&gt;97% EGCG</td>
<td>100% Methanol</td>
</tr>
</tbody>
</table>

* Values provided by manufacturer.

**TABLE 2**

Effect of commonly used herbal extracts on UGT1A4, UGT1A6, and UGT1A9 activity

<table>
<thead>
<tr>
<th>Extract</th>
<th>RDI</th>
<th>UGT1A4</th>
<th>UGT1A6</th>
<th>UGT1A9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rough IC50</td>
<td>VDI</td>
<td>Rough IC50</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>µg/ml</td>
<td>l/dose</td>
<td>µg/ml</td>
</tr>
<tr>
<td>Black cohosh</td>
<td>40</td>
<td>69.7 ± 4.8</td>
<td>0.6</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cranberry</td>
<td>100</td>
<td>742.1 ± 118.7</td>
<td>1.3</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Echinacea</td>
<td>400</td>
<td>116.1 ± 25.1</td>
<td>3.4</td>
<td>241.0 ± 23.4</td>
</tr>
<tr>
<td>Garlic</td>
<td>100</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>220</td>
<td>268.2 ± 48.9</td>
<td>0.9</td>
<td>N.A.</td>
</tr>
<tr>
<td>Acid-hydrolyzed Ginkgo biloba</td>
<td>240</td>
<td>Interf</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Ginseng</td>
<td>550</td>
<td>368.4 ± 66.6</td>
<td>1.5</td>
<td>N.A.</td>
</tr>
<tr>
<td>Acid-hydrolyzed ginseng</td>
<td>550</td>
<td>Echinacea</td>
<td>388.0 ± 42.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Milk thistle</td>
<td>600</td>
<td>Interf</td>
<td>66.9 ± 3.5</td>
<td>9.0*</td>
</tr>
<tr>
<td>Saw palmetto</td>
<td>320</td>
<td>70.6 ± 9.3</td>
<td>4.5</td>
<td>131.8 ± 21.5</td>
</tr>
<tr>
<td>Valerian</td>
<td>1000</td>
<td>406.5 ± 35.3</td>
<td>2.5</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>EGCG</td>
<td>250</td>
<td>34.3 ± 4.1</td>
<td>7.3*</td>
<td>183.6 ± 29.8</td>
</tr>
</tbody>
</table>

* N.A., data points did not fit the IC50 curve.

a Volume/dose index values that exceed the cutoff for further investigation.

b Interf: addition of herb interfered with florescence detection of glucuronide.
the high content of glycosides, which are extensively hydrolyzed in vivo by stomach acids and intestinal bacteria. Acid-hydrolyzed ginseng extract was prepared by dissolving 60 mg of the powder extract in 1 ml of 60% ethanol-40% 0.5 M HCl (Sloley et al., 2006). After 90 min at 37°C, the extract was neutralized with 0.1 M KOH and was serially diluted to prepare working solutions containing 10% ethanol. Acid-hydrolyzed ginkgo extract was prepared by dissolving 30 mg of the powder extract in 1 ml of 60% acetone-40% 5 M HCl. The acid-treated extract was heated at 90°C for 1 h and neutralized with 2 M KOH. Working solutions were prepared so that their concentrations were 10-fold higher than the final concentrations in incubations.

**Incubations of Herbal Extracts with TFP.** TFP was used as a probe substrate for UGT1A4 in HLM. Incubations with TFP were performed as described previously by Uchaipichat et al. (2006). In brief, the incubation mixture (final volume, 250 μl) consisted of TFP, 5 mM MgCl₂, 50 mM Tris-HCl buffer (pH 7.4), 0.1 mg/ml microsomal proteins, and alamethicin (100 μg/mg protein). The concentration of TFP in incubations was 60 μM.

**Fig. 1.** Effect of commonly used herbal extracts on UGT1A4, UGT1A6, and UGT1A9 enzyme activities. HLM were coincubated with herbal extracts and TFP for UGT1A4 activity (A), serotonin for UGT1A6 activity (B), and mycophenolic acid for UGT1A9 activity (C). Three concentrations were tested for each herbal extract, which represent extract daily intake in 53 liters (dotted bars), 5.3 liters (checkered bars), and 0.53 liters (striped bars). Formation of TFPG, serotonin glucuronide, and MPAG was detected in the respective herbal incubations. Percentage of activity was calculated as the percentage of the glucuronide peak area in herbal incubations compared with that in negative controls. Each value represents mean of duplicate incubations. Error bars represent positive S.E.
which corresponds to the $K_m$ in HLM (Uchaipichat et al., 2006). The mixture was preincubated on ice for 15 min. The reaction was started by adding UDPGA (final concentration, 5 mM). After the mixture was incubated for 20 min at 37°C, the reaction was stopped by adding 250 µl (4% acetic acid-96% methanol), vortex-mixing, and placing tubes on ice. Tubes were centrifuged for 10 min at 20,000g, and the supernatant was transferred to HPLC tubes. Screening experiments were performed by adding herbal extracts at three different concentrations to the incubation mixture. Incubations with and without hecogenin (50 µM) were performed to serve as positive and negative controls, respectively.

Chromatographic Analysis of TFPG. HPLC analysis was performed with a Shimadzu LC-10AD VP pump (Shimadzu Scientific Instruments, Columbia, MD) connected to a Waters 717 autosampler and Waters 2475 fluorescence detector (Waters, Milford, MA). Fifty microliters of the incubation supernatant were injected on ice with alamethicin for 15 min. The reaction was started by adding UDPGA (final concentration, 5 mM). After the mixture was incubated for 60 min at 37°C, the reaction was stopped by adding 10 µl of 70% HClO$_4$, vortex-mixing, and centrifugation at 20,000g for 10 min. The total run time was 15 min. TFPG was detected at an excitation wavelength of 310 nm and emission wavelength of 475 nm (Rele et al., 2004).

The identity of the TFPG peak was verified through enzymatic hydrolysis using β-glucuronidase. TFP (60 µM) was incubated with HLM as described above for 1 h at 37°C. Then, 25 µl of 100 mM potassium phosphate buffer (pH 4.0) and 2500 units of β-glucuronidase were added. Tubes were incubated for 16 h at 37°C. The reaction was stopped by adding 10 µl of 70% HClO$_4$, vortex-mixing, and centrifugation at 20,000g for 10 min. The supernatant was transferred to HPLC tubes for injection. Control incubations were performed in the same way but did not contain β-glucuronidase enzyme. The TFP peak was detected in the control incubation but not in the hydrolyzed incubation.

Incubations of Serotonin with Herbal Extracts. To investigate the effect of herbal products on UGT1A6 activity, incubations of herbal extracts with HLM were performed using serotonin as a probe substrate as described by Krishnaswamy et al. (2003) with modifications. In brief, the incubation mixture (final volume, 100 µl) consisted of serotonin at a concentration of approximately the $K_m$ value in HLM (8 mM), 5 mM MgCl$_2$, 50 mM Tris-HCl buffer (pH 7.4), 0.5 mg/ml microsomal proteins, and alamethicin (100 µg/mg protein). The mixture was preincubated on ice for 15 min. The reaction was started by adding UDPGA (final concentration, 5 mM). After the mixture was incubated for 60 min at 37°C, the reaction was stopped by adding 10 µl of 24% perchloric acid-acetonitrile (1:1, v/v), vortex-mixing, and placing tubes on ice. Tubes were centrifuged for 10 min at 20,000g, and the supernatant was transferred to HPLC tubes. 1-Naphthol (50 µl) was used as a positive control in the screening assays (Fujiiwara et al., 2008).

Chromatographic Analysis of Serotonin Glucuronide. Isocratic chromatography was carried out at ambient temperature on a reverse-phase C18 column (3.9 x 150 mm, 5 µm) at 100 µl/min. The mobile phase consisted of 5% acetonitrile-95% 2 mM ammonium acetate (pH 2.7). Isocratic elution at a flow rate of 1.0 ml/min was used. The total run time was 10 min, and the injection volume was 30 µl. The HPLC system consisted of a Shimadzu LC-10AD VP pump connected to a Waters 717 autosampler and Waters 2475 fluorescence detector. Serotonin glucuronide was detected at an excitation wavelength of 225 nm and emission wavelength of 320 nm. To confirm the identity of the serotonin glucuronide peak, the retention time was compared with the serotonin glucuronide standard. In addition, the serotonin glucuronide peak was collected from the HPLC eluate and analyzed using MS/MS. The isolated fraction showed abundant ions with m/z 353, which matches the m/z 353 of serotonin glucuronide ions in the positive mode. Upon fragmentation of the parent ion, a product ion with m/z 177 was produced, which matches the m/z 177/153, which matches the m/z 177/153 peak area of the glucuronide metabolites formed in herbal extract incubations expressed as a percentage of the negative control. Remaining enzyme activity and herbal extract concentration data were fitted to eq. 1 using Prism 5.02 (GraphPad Software Inc., San Diego, CA) to estimate IC$_{50}$ values.

Volume per dose index (VDI) was calculated using eq. 1 and was used as a measure of the potential of IC$_{50}$ concentrations to be reached in vivo as described by Strandlell et al. (2004). The VDI is defined as the volume in which one dose should be dissolved to obtain the corresponding IC$_{50}$ concentration:

\[
\text{VDI (liters)} = \frac{\text{RDI}}{\text{IC}_{50}}
\]  

where RDI is recommended daily intake.

Results

A total of 35 herb-UGT enzyme pairs were evaluated, each at three different concentrations. Results from the screening experiments are summarized in Table 2. Rough IC$_{50}$ and VDI values were estimated on the basis of the remaining enzyme activity data at the three concentrations of each herbal extract. VDI was used to select the herb-UGT interactions to investigate further. A VDI cutoff value was considered to be 5 liters for UGT1A4 interactions and 2 liters for UGT1A6 and UGT1A9 interactions. This value was based on an expression study that showed that UGT1A6 and UGT1A9 are expressed in the intestine and the liver, whereas UGT1A4 is mainly expressed in the liver (Ohno and Nakajin, 2009). Herbal extracts that met this criterion were selected for further analysis.

| UGT Enzyme | Extract | RDI | IC$_{50}$ | VDI | R
|-------------|---------|-----|----------|-----|---
| UGT1A4     | EGCG    | 250 | 33.8 ± 3.1 | 7.4 | 1.0
| UGT1A6     | Milk thistle | 600 | 59.5 ± 3.6 | 6.3 | 1.1
| UGT1A9     | Cranberry | 100 | 203.4 ± 22.9 | 3.1 | 1.0
|             | Milk thistle | 600 | 33.6 ± 3.1 | 17.9 | 0.8

RDI, recommended daily intake.
Effect of Herbal Extracts on TFPG Formation. The effect of 10 herbal extracts on UGT1A4 activity was achieved through incubations of pooled HLM with TFP and monitoring formation of TFP glucuronide (TFPG) as an index for UGT1A4 activity in HLM incubations. Each data point represents the mean of duplicate incubations. Error bars represent two-sided S.E.M. Data points were fitted to the IC$_{50}$ equation as described under Materials and Methods. The goodness-of-fit $r^2$ value was 0.98.

Effect of Herbal Extracts on MPAG Formation. Black cohosh, echinacea, ginseng, acid-hydrolyzed ginseng, and milk thistle inhibited MPAG formation (Fig. 1). However, only milk thistle (IC$_{50}$ = 35.9 ± 4.3 μg/ml; VDI = 16.7 liters) and cranberry (IC$_{50}$ = 260.5 ± 33.0 μg/ml; VDI = 3.8 liters) exceeded the VDI cutoff of 2 liters and were selected for further study (Table 2). Precise best-fit IC$_{50}$ values for milk thistle and cranberry were 33.6 ± 3.1 μg/ml and 17.9 liters and 230.4 ± 32.9 μg/ml and 3.1 liters, respectively (Table 3; Fig. 4).

**Discussion**

In this study, 10 commonly used herbal extracts were screened for their effects on the glucuronidation activity of UGT1A4, UGT1A6, and UGT1A9 in pooled HLM. UGT enzyme activities were measured in vitro using selective substrates: TFP for UGT1A4, serotonin for UGT1A6, and MPA for UGT1A9 (Court, 2005). On the basis of VDI values, the most potent inhibitors were EGCG for UGT1A4, milk thistle for both UGT1A6 and UGT1A9, saw palmetto for UGT1A6, and cranberry for UGT1A9. These findings highlight the possibility of herb-drug interactions through modulation of UGT enzyme activity. The likelihood of the observed in vitro interactions to occur in vivo depends on characteristics of the herb, the drug substrate, the specific enzyme, and the potency of the inhibition.

UGT1A4 is known to be the primary enzyme that catalyzes N-glucuronidation of primary, secondary, and aromatic amines, which include TFP, lamotrigine, tamoxifen, tacrolimus, and azole antifungals (Kiang et al., 2005; Rowland et al., 2006; Bourcier et al., 2010; Zhou et al., 2010; Lavédière et al., 2011). In addition, UGT1A4 shows O-glucuronidation activity toward steroidal compounds (Green and Tephly, 1996). Hecogenin is a known inhibitor of UGT1A4-mediated TFP glucuronidation with IC$_{50}$ values of 1.5 μM (Uchaipichat et al., 2006). Compared with hecogenin, EGCG is a nonselective UGT1A4 inhibitor with moderate potency. EGCG has previously been shown to inhibit estradiol-3-glucuronidation, an index for UGT1A1 activity, with a lower IC$_{50}$ value (7.8 μg/ml) (Mohamed et al., 2010). In addition, in this study EGCG showed some weak inhibitory activities toward UGT1A6 and UGT1A9 (Fig. 1). Pharmacokinetic studies show that maximum plasma concentrations of EGCG are more than 10-fold less than the observed IC$_{50}$ values after hepatic first-pass metabolism of UGT1A4 substrates is possible and will be augmented with higher EGCG doses. EGCG has been studied...
at doses that reach 800 mg daily for its antioxidant and anticancer effects (Chow et al., 2005). With higher doses of EGCG (800 mg), the VDI will be 23.6 liter, indicating that the 800-mg dose can be diluted in up to 23.6 liters and still inhibit UGT1A4 activity by up to 50%. The effect of EGCG on glucuronidation of the UGT1A4 substrates TFP, lamotrigine, tamoxifen, and imipramine warrants further investigation.

UGT1A6 is typically a low-affinity enzyme that catalyzes glucuronidation of drug substrates including acetaminophen, naproxen, and deferiprone (Bovalgaha et al., 2005; Kiang et al., 2005; Limenta et al., 2008). Milk thistle and saw palmetto inhibited serotonin glucuronidation with IC₅₀ concentrations attainable if the daily doses of milk thistle (600 mg) or saw palmetto (320 mg) are diluted with 6.3 and 3.1 liters, respectively. The observed milk thistle IC₅₀ for UGT1A6 is equivalent to a total flavonolignan concentration of 22.6 g/ml, which is approximately 1000-fold higher than the observed plasma concentration after intake of 600 mg of milk thistle extract (Schrieber et al., 2008). Taken together, these results indicate that milk thistle extract is more likely to inhibit UGT1A6-mediated first-pass rather than systemic metabolism. On the other hand, no pharmacokinetic data on saw palmetto are available. On the basis of an IC₅₀ value exceeding 100 g/ml, milk thistle is expected to have mild, if any, inhibition of UGT1A6-mediated metabolism in vivo (Table 2).

UGT1A9 catalyzes glucuronidation of a wide range of substrates including MPA, propofol, raloxifene, and flavopiridol (Kiang et al., 2005). In the current study, milk thistle and cranberry inhibited MPAG formation, which was used as an index reaction for UGT1A9 activity in HLM (Court, 2005). For milk thistle extract, the IC₅₀ value was 33.6 µg/ml, which is equivalent to 12.7 µg/ml flavonolignans. This concentration is much higher than the expected plasma concentration of flavonolignans after milk thistle intake (Schrieber et al., 2008). Therefore, inhibition of systemic metabolism of UGT1A9 substrates by milk thistle extract is not likely. Conversely, on the basis of the range of intestinal fluid volume of 0.5 to 5 liters, a single 600-mg dose of milk thistle may result in putative concentrations of 120 to 1200 µg/ml. Thus, inhibition of first-pass metabolism of UGT1A9 substrates by milk thistle extract is possible.

In this study, we screened specific UGT enzyme activities using HLM rather than human intestine microsomes (HIM) or expressed enzymes. The difference between expressed enzymes and HLM is that the first contain single UGT enzymes, whereas the latter contain all the hepatic isoforms. Therefore, HLM are closer to the in vivo environment because of the availability of other UGT enzymes that may form heterodimers, which has been reported for some UGT enzymes and may affect enzyme activity (Ouzzine et al., 2003). Because our goal was to screen for interactions that may have clinical significance, the use of HLM was more appropriate. This was made feasible by the availability of selective substrates for different UGT enzymes in HLM (Court, 2005). Likewise, HIM contain all the intestinal UGT enzymes. However, no selective substrates for individual UGT enzymes have been described in HIM.

Calculation of VDI provides a helpful tool to predict the likelihood of achieving IC₅₀-equivalent concentrations in the intestine or plasma in the absence of clinical data (Strandell et al., 2004). Although this approach is sufficient for the purpose of screening and hypothesis generation, it is limited by not considering the extent of absorption of phytochemicals through tissue and cellular barriers. Use of VDI assumes that the concentration in the gastrointestinal lumen is equivalent to that in the endoplasmic reticulum of intestinal epithelial cells where UGT enzymes are located. This assumption may lead to overestimate the extent of the inhibition, because many phytochemicals are poorly absorbed through the intestinal wall. Therefore, the results need to be confirmed in clinical studies and, where available, IC₅₀ values need to be compared with unbound in vivo concentrations. It is worth noting that using VDI to describe inhibition potency changes the order of significance of inhibitors compared with IC₅₀ values. For example, based on rough IC₅₀ values, black cohosh and saw palmetto are equipotent inhibitors of UGT1A4 activity (rough IC₅₀ = 69.7 and 70.6 µg/ml) (Table 2). However, the daily dose of saw palmetto is 8-fold higher than that of black cohosh (320 versus 40 mg). Thus, ingestion of 320 mg of saw palmetto is expected to result in a higher extent of UGT1A4 inhibition than ingestion of 60 mg of black cohosh.

In summary, in this study, 10 herbal extracts were screened for inhibition of three UGT1A enzymes: UGT1A4, UGT1A6, and UGT1A9. We report inhibition of UGT1A4 by EGCG, UGT1A6 by milk thistle and saw palmetto, and UGT1A9 by cranberry and milk thistle extracts. Among these, EGCG inhibition of UGT1A4 and milk thistle inhibition of UGT1A6 and UGT1A9 are likely to affect first-pass glucuronidation of substrates, which will be important for drugs with high first-pass extraction ratios. The in vivo effects of these interactions on the pharmacokinetics of UGT1A4, UGT1A6, and UGT1A9 substrates remain to be determined in clinical studies.

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Authorship Contributions

Participated in research design: Mohamed and Frye.
Conducted experiments: Mohamed.
Performed data analysis: Mohamed.
Wrote or contributed to the writing of the manuscript: Mohamed and Frye.
References


