Inhibitory effect of honokiol and magnolol on cytochrome P450 enzyme activities in human liver microsomes

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Abstract: Honokiol and magnolol, the major bioactive neolignans of magnolia officinalis, are the most important constituents of the crude drug prescriptions that are used in the therapy of neuroses and various nervous disorders. There have been limited reports on the effects of neolignoid compounds on human cytochrome P450 activity. Therefore, the inhibitory effects of honokiol and magnolol on seven human cytochrome P450s were evaluated in human liver microsomes. Honokiol and magnolol showed the most potent inhibition of CYP1A2-mediated phenacetin O-deethylase activity (IC<sub>50</sub> values of 3.5 and 5.4 mM, respectively) among the seven P450s tested. These in vitro data indicate that neolignan compounds can inhibit the activity of CYP1A2 and suggest that these compounds should be examined for potential pharmacokinetic drug interactions in vivo.

Key words: Cytochrome P450, Drug interaction, Honokiol, Magnolol, Microsomes

Introduction

Magnolia officinalis has been used in a number of traditional medicine preparations in China and Japan. The genus Magnolia is a rich source of several biologically active compounds. Several neolignan ingredients, including magnolol, honokiol, 4-O-methylhonokiol, and obovatol, have been the focus of studies on the diverse pharmacological effects of Magnolia. Among these, magnolol and honokiol are the major bioactive components of Magnolia officinalis. They can relieve smooth muscle spasms and stop vomiting. They also are the most important constituents of the crude drug prescriptions that are used in the therapy of neuroses and various nervous disorders, including Parkinsonism, and gastrointestinal abnormalities. Magnolol has anti-allergic, anti-asthma and anti-inflammatory effects, and honokiol has an anxiolytic effect and a cardiac muscle protective effect. So far, there have been few reports on the effects of neolignan compounds on human cytochrome P450 isoform activities. Therefore, in this study, we used human liver microsomes and tandem mass spectrometry to investigate the inhibitory effects of honokiol and magnolol on the metabolism of seven major P450 isoform-specific substrates to assess the probability of drug interactions.

Experimental

Materials

D-glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH), honokiol, magnolol, β-nicotinamide adenine dinucleotide phosphate (β-NADP<sup>+</sup>), magnesium chloride (MgCl<sub>2</sub>), potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), and terfenadine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were LC-MS grade (Fisher Scientific Co., Pittsburgh, PA, USA) and the other chemicals were of the highest quality available. Pooled human liver microsomes (coded HLM 150) were obtained from BD Biosciences (Woburn, MA, USA). The manufacturer supplied information regarding protein content, P450 content, and enzyme activity (available: www.bdgiosciences.com).

Inhibitory effects of honokiol and magnolol on P450 activity

The inhibitory potency of honokiol and magnolol were determined with cytochrome P450 assays in the absence and presence of these compounds (final concentration of 0.5~50 µM with methanol concentration less than 0.5%) using pooled human liver microsomes. Phenacetin O-deethylase, bupropion 4-hydroxylase, amodiaquine N-deethylase, tolbutamide 4-hydroxylase, omeprazole 5-hydroxylase, dextromethorphan O-demethylase, and midazolam 1'-hydroxylase activities were measured as probe activities for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A, respectively, using cocktail incubation and tandem mass spectrometry, as described previously with some modification. In brief, the incubation mixtures containing pooled human liver microsomes (0.25 mg/mL), P450-selective sub-strates, and honokiol or magnolol (0.5~50 µM) were pre-incubated for 5 min at 37°C. The reaction was initiated by adding a NADPH-generating system containing 3.3 mM glucose-6-phosphate, 1.0 unit/mL glucose-6-phosphate dehydrogenase, 3.3 mM MgCl<sub>2</sub>,

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and 1.3 mM NADP\(^+\). Incubations were performed for 15 min at 37°C in a thermo-shaker. The reaction was terminated by the addition of ice-cold acetonitrile containing terfenadine (internal standard, 5 ng/mL final concentration). All incubations were performed in triplicate, and mean values were used for analysis.

**LC-MS/MS analysis**

All metabolites of the P450 isoform-specific substrates were measured by tandem mass spectrometry as described previously. Briefly, the system consisted of a Thermo Vantage Triple quadrupole Mass Spectrometer (Thermo Fischer Scientific, San Jose, CA, USA), coupled with a Thermo Accela HPLC system (Thermo Fischer Scientific). The separation was performed on a Luna C18 column (2 mm i.d. × 30 mm, 5 µm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B). Gradient elution was conducted as follows: 8% A was linearly increased to 95% over 7 min, and then immediately stepped back down to 8% for re-equilibration until 11 min, at a flow rate of 0.2 mL/min. The electrospray interface was operated in positive ion mode at 4000 V. The operating conditions were as follows: capillary temperature, 350°C; vaporizer temperature, 300°C; sheath gas pressure, 35 Arb; Auxiliary gas, 10 Arb; nitrogen gas flow rate, 8 L/min. Quantitation was performed by selected reaction monitoring (SRM) of the [M+H]\(^+\) ion and the related product ion for each metabolite. The SRM transitions and collision energies determined for each metabolite and internal standard are listed in Table 1. The analytical data were processed by Xcalibur (version 2.1).

**Data analysis**

The enzyme inhibition parameter IC\(_{50}\) values (concentration of the inhibitor causing 50% inhibition of the original enzyme activity) were calculated using nonlinear least-squares regression analysis of the plot of percent control activity versus concentration of honokiol or magnolol, using Win-Nonlin version 4.0 (Pharsight, Mountain View, CA, USA).

**Results and Discussion**

Inhibition of P450 activity was evaluated at concentrations of up to 50 µM honokiol and magnolol to investigate their effects on P450-mediated drug interactions in human liver microsomes. The activities of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A were determined in human liver microsomes using a cocktail assay containing phenacetin for CYP1A2, bupropion for CYP2B, amodiaquine for CYP2C8, tolbutamide for CYP2C9, omeprazole for CYP2C19, dextromethorphan for CYP2D6, and midazolam for CYP3A. The LC-MS/MS system in SRM mode was optimized for detection of each metabolite (Table 1 and Figure 1). Honokiol and magnolol inhibited the metabolism of CYP1A2-mediated phenacetin deethylase in vitro with IC\(_{50}\) values of 3.5 and 5.4 µM, respectively, which is similar to the IC\(_{50}\) (4.4 µM) of obovatol, another neolignan compound. Co-administration of honokiol or magnolol with CYP1A2 substrates may increase the concentrations of CYP1A2 substrates in blood. They also inhibited CYP2C9-catalyzed tolbutamide hydroxylase activity with IC\(_{50}\) values of 9.6 and

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**Table 1.** SRM parameters for the major metabolites of the seven P450 probe substrates used in all assays

<table>
<thead>
<tr>
<th>P450 enzyme</th>
<th>Substrate</th>
<th>Concentration (µM)</th>
<th>Metabolite</th>
<th>Transition (m/z)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Phenacetin</td>
<td>100</td>
<td>Acetaminophen</td>
<td>152&gt;110</td>
<td>25</td>
</tr>
<tr>
<td>2B6</td>
<td>Bupropion</td>
<td>50</td>
<td>Hydroxybupropion</td>
<td>256&gt;238</td>
<td>20</td>
</tr>
<tr>
<td>2C8</td>
<td>Amodiaquine</td>
<td>1</td>
<td>Desethylamodiaquine</td>
<td>328&gt;283</td>
<td>17</td>
</tr>
<tr>
<td>2C9</td>
<td>Tolbutamide</td>
<td>100</td>
<td>Hydroxytolbutamide</td>
<td>287&gt;89</td>
<td>60</td>
</tr>
<tr>
<td>2C19</td>
<td>Omeprazole</td>
<td>20</td>
<td>Hydoxyomeprazole</td>
<td>362&gt;214</td>
<td>10</td>
</tr>
<tr>
<td>2D6</td>
<td>Dextromethorphan</td>
<td>5</td>
<td>Dextrorphan</td>
<td>285&gt;157</td>
<td>35</td>
</tr>
<tr>
<td>3A</td>
<td>Midazolam</td>
<td>5</td>
<td>1'-Hydroxymidazolam</td>
<td>342&gt;203</td>
<td>25</td>
</tr>
<tr>
<td>IS</td>
<td>Terfenadine</td>
<td>5 ng/mL</td>
<td>-</td>
<td>472&gt;436</td>
<td>25</td>
</tr>
</tbody>
</table>
Honokiol and magnolol at a concentration of 40 µM did not affect the activities of CYP2C8, CYP2D6, and CYP3A isoforms. These findings suggest that clinical interactions between these compounds and P450s such as CYP2C8, CYP2D6, and CYP3A would not occur.

**Conclusion**

In the present study, we investigated the inhibitory effect of honokiol and magnolol using human liver microsomes. Honokiol and magnolol moderately inhibited the metabolism of CYP1A2-mediated phenacetin O-deethylase *in vitro* with IC₅₀ values of 3.5 and 5.4 µM, respectively. It is important to note, however, that inhibition of CYP1A2 activity *in vitro* does not translate into drug-drug interaction *in vivo*. Therefore, *in vivo* studies of honokiol and magnolol must be further evaluated to rule out the inhibitory potential of these compounds with CYP1A2 substrates such as caffeine and theophylline and to determine whether inhibition of CYP1A2 activity by neolignan compounds is clinically relevant.

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**References**

7. Kim, M. J.; Kim, H.; Cha, I. J.; Park, J. S.; Shon, J. H.;
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