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 P450 s were evaluated in human liver microsomes. Honokiol and magnolol showed the most potent inhibition of CYP1A2-mediated phenacetin *O*-deethylase activity (IC_{50} 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.[1] The genus *Magnolia* is a rich source of several biologically active compounds. Several neolignan ingredients, including magn-olol, honokiol, 4-*O*-methylhonokiol, and obovatol, have been the focus of studies on the diverse pharmacological effects of *Magnolia*.[1] Among these, magnolol and honokiol are the major bioactive components of *Magnolia officinalis*.[2] They can relieve smooth muscle spasms and stop vomiting.[3] 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. Mag-nolol has anti-allergic, anti-asthma and anti- inflammatory effects,[4] and honokiol has an anxiolytic effect and a cardiac muscle protective effect.[5,6] 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-$NADP^+$), magnesium chloride ($MgCl_2$), potassium phosphate (KH_2PO_4), 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, dextrometh-orphan *O*-demethylase, and midazolam 1'-hydro–xylase activ-ities were measured as probe activities for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A, respectively, using cocktail incubation and tandem mass spectro-metry, as described previously with some modifi-cation.[7] 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 dehyd-rogenase, 3.3 mM $MgCl_2$,

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and 1.3 mM $NADP^+$. Incu-bations 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 tripli-cate, 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.[7,8] Briefly, the system consisted of a Thermo Vantage Triple quadrupole Mass Spectrometer (Thermo Fischer Scientific, San Jose, CA, USA), coupled with an Thermo Accela HPLC system (Thermo Fischer Scientific). The separation was performed on a Luna C18 column (2 mm i.d. × 30 mm, 5 μm, 100 Å, 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-squa-res 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 CYP2B6, 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.[8] 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

Table 2. Inhibitory potency of honokiol and magnolol on seven P450 activities in human liver microsomes

Enzyme activity	P450	IC_{50} (μM)		
		Honokiol	Magnolol	Obovatol[8]
Phenacetin *O*-deethylation	1A2	3.5	5.4	4.4
Bupropion hydroxylation	2B6	18.8	44.9	13.9
Amodiaquine *N*-deethylation	2C8	40.8	>50	11.1
Tolbutamide 4-methylhydroxylation	2C9	9.6	10.2	3.3
Omeprazole 5-hydroxylation	2C19	32.9	>50	-*
S-Mephenytoin 4-hydroxylation	2C19	-*	-*	0.8
Dextromethorphan *O*-demethylation	2D6	>50	>50	>50
Midazolam 1'-hydroxylation	3A	>50	>50	>50

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

P450 enzyme	Substrate	Concentration (μM)	Metabolite	Transition (*m/z*)	Collision energy (eV)
1A2	Phenacetin	100	Acetaminophen	152>110	25
2B6	Bupropion	50	Hydroxybupropion	256>238	20
2C8	Amodiaquine	1	Desetyhylamodiaquine	328>283	17
2C9	Tolbutamide	100	Hydorxytolbutamide	287>89	60
2C19	Omeprazole	20	Hydoxyomeprazole	362>214	10
2D6	Dextromethorphan	5	Dextrorphan	258>157	35
3A	Midazolam	5	1'-Hydroxymidazolam	342>203	25
IS	Terfenadine	5 ng/mL	-	472>436	25

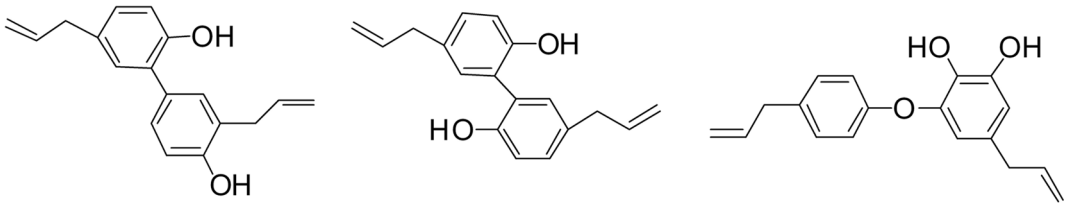

Figure1. Chemical structures of honokiol, magnolol and obovatol.

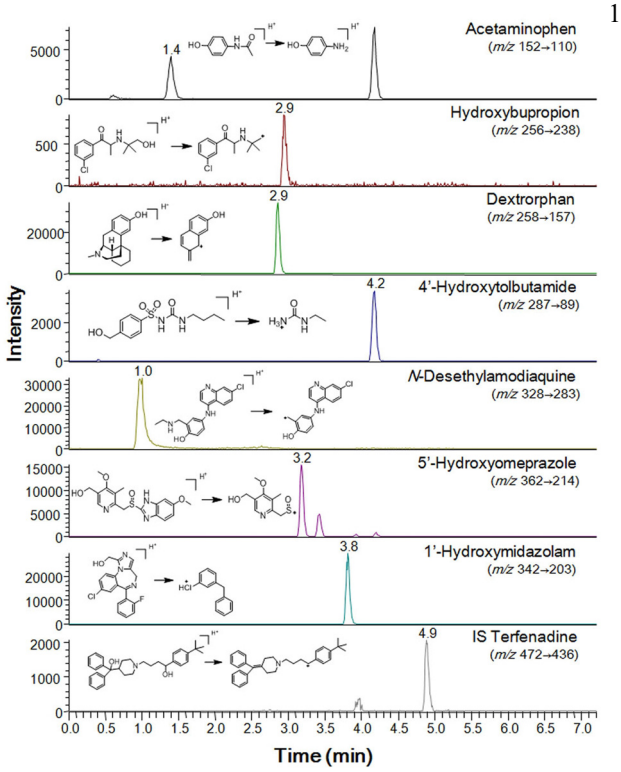

Figure2. SRM chromatograms of the analyzed metabolites in human liver microsomes.

0.2 μM. These *in vitro* data suggest that *in vivo* interaction studies of honokiol or magnolol should be further evaluated to rule out the possible inhibitory potential of honokiol or magnolol against CYP1A2 and CYP2C9 isoform activities.

Honokiol and magnolol had negligible inhibitory effect (IC_{50} > 30 μM) on CYP2C19-mediated omeprazole hydroxylase activity whereas obovatol showed strong inhibitory potential (IC_{50} = 0.8 μM) against CYP2C19-mediated *S*-mephenytoin hydroxylase activity. This result might be explained by the difference in substrates used. This difference is not an unusual finding, given that the inhibitory effects of compounds on CYP-isoform activities also vary according to the substrate used.[9, 10] For example, ketoprofen was a moderate inhibitor of the metabolism of *S*-mephenytion (IC_{50} = 4.15 μM), but it was a very poor inhibitor of the metab-olism of omeprazole (IC_{50} > 100 μM).[9]

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 IC_{50} 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.[11,12]

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References

1. Lee, Y. J.; Lee, Y. M.; Lee, C. K.; Jung, J. K.; Han, S. B.; Hong, J. T. *Pharmacol Ther.* **2011**, 130, 157.
2. Teng, C. M.; Chen, C. C.; Ko, F. N.; Lee, L. G.; Huang, T. F.; Chen, Y. P.; Hsu, H. Y. *Thromb Res.* **1988**, 50, 757.
3. Zhang, W. W.; Li, Y.; Wang, X. Q.; Tian, F.; Cao, H.; Wang, M. W.; Sun, Q. S. *World J. Gastroenterol.* **2005**, 11, 4414.
4. Wang, J. P.; Ho, T. F.; Chang, L. C.; Chen, C. C. *J. Pharm. Pharmacol.* **1995**, 47, 857.
5. Kuribara, H.; Kishi, E.; Hattori, N.; Yuzurihara, M.; Maruyama, Y. *Phytother Res.* **1999**, 13, 593.
6. Tsai, S. K.; Huang, S. S.; Hong, C. Y. *Planta Med.* **1996**, 62, 503.
7. Kim, M. J.; Kim, H.; Cha, I. J.; Park, J. S.; Shon, J. H.;

Liu, K. H.; Shin, J. G. *Rapid Commun. Mass Spectrom.* **2005**, 19, 2651.
8. Joo, J.; Lee, D.; Wu, Z.; Shin, J. H.; Lee, H. S.; Kwon, B. M.; Huh, T. L.; Kim, Y. W.; Lee, S. J.; Kim, T. W.; Lee, T.; Liu, K. H. *Biopharm. Drug Dispos.* **2013**. [*Epub ahead of print*]
9. Foti, R. S.; Wahlstrom, J. L. *Drug Metab. Dispos.* **2008**, 36, 523.
10. Kumar, V.; Wahlstrom, J. L.; Rock, D. A.; Warren, C. J.; Gorman, L. A.; Tracy, T. S. *Drug Metab. Dispos.* **2006**, 34, 1966.
11. Berthou, F.; Flinois, J. P.; Ratanasavanh, D.; Beaune, P.; Riche, C.; Guillouzo, A. *Drug Metab. Dispos.* **1991**, 19, 561.
12. Sarkar, M. A.; Jackson, B. J. *Drug Metab. Dispos.* **1994**, 22, 827.