ORIGINAL RESEARCH

Effect of honokiol on the induction of drug-metabolizing enzymes in human hepatocytes

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Abstract: Honokiol, 2-(4-hydroxy-3-prop-2-enyl-phenyl)-4-prop-2-enyl-phenol, an active component of *Magnolia officinalis* and *Magnolia grandiflora*, exerts various pharmacological activities such as antitumorigenic, antioxidative, anti-inflammatory, neurotrophic, and anti-thrombotic effects. To investigate whether honokiol acts as a perpetrator in drug interactions, messenger ribonucleic acid (mRNA) levels of phase I and II drug-metabolizing enzymes, including cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and sulfotransferase 2A1 (SULT2A1), were analyzed by real-time reverse transcription polymerase chain reaction following 48-hour honokiol exposure in three independent cryopreserved human hepatocyte cultures. Honokiol treatment at the highest concentration tested (50 μM) increased the CYP2B6 mRNA level and CYP2B6-catalyzed bupropion hydroxylase activity more than two-fold in three different hepatocyte cultures, indicating that honokiol induces CYP2B6 at higher concentrations. However, honokiol treatment (0.5–50 μM) did not significantly alter the mRNA levels of phase I enzymes (CYP1A2, CYP3A4, CYP2C8, CYP2C9, and CYP2C19) or phase II enzymes (UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1) in cryopreserved human hepatocyte cultures. CYP1A2-catalyzed phenacetin *O*-deethylase and CYP3A4-catalyzed midazolam 1′-hydroxylase activities were not affected by 48-hour honokiol treatment in cryopreserved human hepatocytes. These results indicate that honokiol is a weak CYP2B6 inducer and is unlikely to increase the metabolism of concomitant CYP2B6 substrates and cause pharmacokinetic-based drug interactions in humans.

Keywords: honokiol, human hepatocytes, drug interactions, cytochrome P450, UDP-glucuronosyltransferases

Introduction

Herb–drug interactions resulting from concurrent use of botanical drugs with prescription and over-the-counter drugs may cause adverse reactions such as toxicity and treatment failure. The mechanisms underlying herb–drug interactions involve inhibition or induction of drug-metabolizing enzymes such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT) enzymes, and drug transporters.[1–7] Herbal drugs such as ginkgo (*Ginkgo biloba*), ginseng (*Panax ginseng*), garlic, danshen (*Salvia miltiorrhiza*), licorice (*Glycyrrhiza glabra*), resveratol, milk thistle (*Silybum marianum*), and St John's wort (*Hypericum perforatum*) reportedly cause drug interactions with anticoagulant, antiretroviral, anticancer, immunosuppressant, and antidepressant drugs.[8–18] Therefore, it is necessary to evaluate herb–drug interactions at an early stage to prevent potentially dangerous clinical outcomes.

Cultured fresh and cryopreserved human hepatocytes enable the integrated evaluation of phase I drug-metabolizing enzymes (eg, CYPs) and phase II drug-metabolizing enzymes (eg, UGTs and SULTs).[19–21] The function of drug-metabolizing enzymes and transporters

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is regulated by their expression at the transcriptional and posttranscriptional levels.[22,23] The primary hepatocyte culture system contains nuclear receptors such as aromatic hydrocarbon receptor, pregnane X-receptor, and constitutive androstane receptor.[20,24] Quantification of CYP messenger ribonucleic acid (mRNA) and protein levels and enzyme activities is recommended to evaluate drug interactions.[25–27] Moreover, drug interaction knowledge might increase efficacy of a drug and reduce its undesirable side effects and toxicity. According to the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines for drug–drug interactions, the effect of a drug on CYP mRNA levels in fresh or cryopreserved human hepatocytes is critical for CYP-induced drug interactions.[28,29] Numerous drug–drug interaction studies using primary or cryopreserved human hepatocyte culture systems have been reported.[30–35]

Honokiol, 2-(4-hydroxy-3-prop-2-enyl-phenyl)-4-prop-2-enyl-phenol, is a pharmacologically active component isolated from *Magnolia officinalis*, *Magnolia grandiflora*, and other plants. Honokiol exerts antitumor,[36–39] antioxidative,[40,41] anti-inflammatory,[42–44] antithrombotic,[45] neuroprotective,[46] antiseptic,[47] antinociceptive,[48–50] and antidepressant activities.[51] Honokiol is extensively metabolized to 18 metabolites via hydroxylation, methylation, sulfation, and glucuronidation in rats.[52–54] Honokiol potently inhibits CYP1A2, CYP2C8, CYP2C9, CYP2C19, and UGT1A9 enzyme activities with K_i values of 1.2, 4.9, 0.54, 0.57, and 0.3 μM, respectively, but weakly inhibits those of CYP2B6, CYP2D6, CYP3A4, UGT1A1, and UGT2B7 in human liver microsomes.[55] Thus, honokiol should be examined for potential pharmacokinetic drug interactions in vivo due to its inhibition of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and UGT1A9 enzyme activities.

There are no reports describing the effects of honokiol on the induction of various phase I or II drug-metabolizing enzymes in human hepatocytes after chronic treatment. In this study, we investigated the effect of honokiol on the expression of various drug-metabolizing enzymes using attachable cryopreserved human hepatocytes to evaluate the potential of honokiol as a perpetrator in drug interactions.

Materials and methods
Materials and reagents
Honokiol (>98% by high-performance liquid chromatography), acetaminophen, phenacetin, lansoprazole, phenobarbital, rifampicin, *l*-glutamine, William's medium E, dimethyl sulfoxide (DMSO), and midazolam were purchased from Sigma-Aldrich

Co. (St Louis, MO, USA). $^{13}C_2$,^{15}N-acetaminophen, bupropion, 1′-hydroxymidazolam, hydroxybupropion, cryopreserved human hepatocytes (HMC520, HFC443, and HF382), high-viability cryohepatocyte recovery kit, Biocoat™ Hepatocyte Culture Medium, and Biocoat™ Collagen I 24-Well Plates were obtained from Corning Life Sciences (Woburn, MA, USA). TaqMan® RNA-to-C_T™ 1-Step Kit, TaqMan® Gene Expression Assays, and gene-specific probes and primers (Table 1) for real-time reverse transcription polymerase chain reaction (RT-PCR) were obtained from Applied Biosystems (Foster city, CA, USA). Fetal bovine serum and TRIzol® were obtained from Invitrogen (Carlsbad, CA, USA). Acetonitrile and methanol (liquid chromatography mass spectrometry [LC-MS] grade) were purchased from Burdick & Jackson Inc. (Ulsan, Korea).

Cell culture
Cryopreserved human hepatocyte lines (HF382, HFC443, and HMC520) were thawed in hepatocyte recovery medium, and viability was determined using Trypan Blue according to the manufacturer's suggested protocols (Corning Life Sciences). Viable cells (4×10^5) were seeded in collagen type I precoated 24-well plates in 400 μL of hepatocyte plating medium, maintained at 37°C with 5% CO_2 in an incubator for 4 hours. Plating medium was then removed and the cells were supplied with matrigel medium containing 0.25 mg/mL of Matrigel™ matrix and cultured for 24 hours. Honokiol stock solutions (0.5, 5, or 50 mM in DMSO), 10 mM lansoprazole, 10 mM rifampicin, and 1 M phenobarbital in DMSO were diluted 1,000 times with hepatocyte culture medium. The cells were treated with 0.5, 5, or 50 μM honokiol, vehicle (0.1% DMSO), or prototypical inducers, including lansoprazole (10 μM), phenobarbital (1 mM), or rifampicin (10 μM), in triplicate. The cells were then incubated at 37°C with 5% CO_2 for 48 hours, and the medium was exchanged with fresh medium containing drugs or vehicle (400 μL) every 24 hours.

Table 1 Probe and primer sets used in this study

Target enzymes	Gene symbol	Assay ID
Cytochrome P450	CYP1A2	Hs01070369-m1
	CYP3A4	Hs00430021_m1
	CYP2B6	Hs03044634-m1
	CYP2C8	Hs00426387_m1
	CYP2C9	Hs00426397_m1
	CYP2C19	Hs00559368_m1
UDP-glucuronosyltransferase	UGT1A1	Hs02511055-s1
	UGT1A4	Hs01655285-s1
	UGT1A9	Hs02516855_sH
	UGT2B7	Hs00426592-m1
Sulfotransferase	SULT2A1	Hs00234219-m1

CYP1A2, CYP2B6, and CYP3A4 activity measurement

A cocktail (200 µL) containing 20 µM phenacetin, 10 µM bupropion, and 5 µM midazolam in William's E buffer solution was added to each well treated with vehicle (0.1% DMSO), rifampicin, lansoprazole, phenobarbital, or honokiol, and the plate was returned to the CO_2 incubator. After incubation for 1 hour, 200 µL of the medium was removed from each well and stored in a −70°C deep freezer until sample analysis. $^{13}C_2,^{15}N$-acetaminophen (50 µL; 0.15 µg/mL, internal standard for acetaminophen) and d_9-hydroxybufuralol (0.01 µg/mL, internal standard for hydroxybupropion and 1′-hydroxymidazolam) in methanol were added to 50 µL of the medium obtained from each well. The mixtures were vortex-mixed for 2 minutes and then centrifuged at 13,000 rpm for 4 minutes at 4°C. The supernatant (40 µL) was diluted with 60 µL of deionized water and then mixed for 2 minutes by vortexing. An aliquot (5 µL) was analyzed by LC-MS/MS.[56] The LC-MS/MS system consisted of a tandem mass spectrometer (TSQ Quantum Access, Thermo Scientific, San Jose, CA, USA) coupled with a Nanospace SI-2 LC system (Shiseido, Tokyo, Japan). Separation was performed on an Atlantis dC18 (5 µm, 2.1 mm internal diameter ×100 mm; Waters Co., MA, USA) using a gradient elution of 5% methanol in 0.1% formic acid (mobile phase A) and 95% methanol in 0.1% formic acid (mobile phase B) at a flow rate of 0.4 mL/min: 15% mobile phase B for 1.5 minutes, 15%–95% mobile phase B for 0.5 minute, and 95% mobile phase B for 5 minutes. The column and autosampler temperatures were 50°C and 6°C, respectively. After 1.5 minutes, the LC eluent was diverted from waste to a mass spectrometer fitted with an electrospray ionization source and operated in the positive ion mode. Electrospray ionization source settings for ionization of the metabolites were as follows: electrospray voltage, 4,000 V; vaporizer temperature, 350°C; capillary temperature, 330°C; sheath gas pressure, 35 psi; and auxiliary gas pressure, 15 psi. Quantification of each metabolite was performed by selected reaction monitoring mode: acetaminophen, m/z 152.0>110.0; $^{13}C_2,^{15}N$-acetaminophen (internal standard for acetaminophen), m/z 155.0>111.0; hydroxybupropion, m/z 256.2>238.2; 1′-hydroxymidazolam, m/z 342.1>203.0; and d_9-1-hydroxybufuralol, 287.1>187.0 (internal standard). CYP1A2, CYP2B6, and CYP3A4 enzyme activities are expressed as formation rates (pmol/million cells/h).

Total RNA isolation

At the end point of the experiment, William's E buffer solutions containing substrate cocktail were recovered and total RNA was immediately isolated using TRIzol. Briefly, TRIzol (500 µL) was added to each well of 24-well culture plates and transferred into 1.5 mL microcentrifuge tubes. Cell lysates were combined with 100 µL of chloroform, mixed by vortexing, and the RNA aqueous layer was separated by centrifugation. Total RNA was obtained by ethanol precipitation and dissolved in diethylpyrocarbonate-treated water. The RNA concentration and purity were determined by absorbance test at OD260/280 nm using a NanoVue Plus spectrophotometer (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) and stored at −70°C until RT-PCR analysis.

RT-PCR

RT-PCR was conducted using an RT-PCR detection system (Bio-Rad, Foster City, CA, USA) with a TaqMan® RNA-to-C_T™ 1-Step Kit and TaqMan® Gene Expression Assays Kit (Table 1), as follows. Total RNA (25 ng) in each reaction sample was used for RT-PCR: 48°C for 25 minutes for reverse transcription, 95°C for 15 minutes for enzyme activation, 44 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute. The relative (ΔCt) values of all samples were normalized to the glyceraldehyde 3-phosphate dehydrogenase Ct value, and then the relative Ct values (ΔΔCt) of each sample, including CYP3A4, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1, were obtained by comparison (ΔCt) of vehicle and rifampicin, lansoprazole, phenobarbital, or honokiol (0.5, 5, and 50 µM). The relative gene expression rate was calculated using the (ΔΔCt) increase/decrease compared with the vehicle control.

Data analysis

The data are presented throughout as means ± standard deviation for three organ donors. On the basis of EMA and FDA guidelines, the induction results were evaluated separately for each donor. The treatment mRNA level is compared with those of the vehicle incubations. Enzyme induction in an in vitro study was considered to be demonstrated if drug treatment resulted in a more than two-fold increase in mRNA level in a concentration-dependent manner. A 50% decrease in mRNA that was not attributable to cytotoxicity was taken to indicate downregulation of the enzyme in question.

Results

Effect of honokiol on CYP mRNA levels in human hepatocytes

The effect of honokiol on the mRNA levels of CYP1A2, CYP3A4, CYP2B6, CYP2C8, CYP2C9, and CYP2C19

enzymes was evaluated by RT-PCR. Honokiol and prototypical inducers did not exert toxicity at the indicated concentrations (0.5–50 µM) using 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium calorimetric assay. Rifampicin, a pregnane X-receptor inducer, and phenobarbital, a constitutive androstane receptor inducer, increased the mRNA levels of CYP3A4, CYP2B6, CYP2C8, CYP2C9, and CYP2C19 in three human hepatocyte lines (Table 2). Lansoprazole, an aromatic hydrocarbon receptor inducer, significantly increased the CYP1A2 mRNA level by 6.93- to 70.0-fold (Table 2). The fold increase was 6.61–21.2 for CYP2B6 mRNA in phenobarbital-treated hepatocytes and 36.6–113 for CYP3A4 mRNA in rifampicin-treated hepatocytes (Table 2). These results demonstrated that the cryopreserved human hepatocytes significantly responded to prototypical inducers and that these test systems were adequate to address FDA and EMA guidelines.

Honokiol did not affect the mRNA levels of CYP1A2, CYP3A4, CYP2C8, CYP2C9, or CYP2C19 at the three concentrations tested (0.5, 5, and 50 µM) in three human hepatocyte lines (Table 2, Figure 1). Honokiol (50 µM) induced a more than two-fold increase in the CYP2B6 mRNA level in three human hepatocyte lines (HF382, HFC443, and HMC520; Table 2), indicating that honokiol

at higher concentrations may cause drug interactions in vivo due to CYP2B6 induction, based on FDA and EMA guidelines.

Effects of honokiol on CYP1A2, CYP2B6, and CYP3A4 enzyme activities in three human hepatocyte lines

The effects of honokiol and prototypical inducers on CYP1A2, CYP2B6, and CYP3A4 enzyme activities were evaluated in three human hepatocyte lines (Table 3). CYP1A2-catalyzed phenacetin O-deethylase activity was significantly increased by 12.1- to 47.8-fold by lansoprazole treatment in three human hepatocyte lines (Table 3). Rifampicin and phenobarbital treatment significantly increased CYP2B6-catalyzed bupropion hydroxylase activities by 4.14- to 12.7-fold, and CYP3A4-catalyzed midazolam 1′-hydroxylase activities by 2.04- to 4.40-fold, compared with the vehicle control (Table 3). CYP1A2-catalyzed phenacetin O-deethylase and CYP3A4-catalyzed midazolam 1′-hydroxylase activities were not significantly altered by treatment with 0.5–50 µM honokiol in three human hepatocyte lines, indicating that honokiol did not affect CYP1A2 and CYP3A4 enzyme activities (Table 3). However, chronic 50 µM honokiol treatment resulted in 1.94- to 4.66-fold increases in CYP2B6-catalyzed bupropion hydroxylase activity in three human hepatocyte lines (Table 3).

Table 2 Effect on CYP1A2, CYP2B6, and CYP3A4 mRNA levels of 48-hour treatment with honokiol or the positive control (rifampicin, lansoprazole, or phenobarbital) in three human hepatocyte lines (HF382, HFC443, and HMC520)

Sample	mRNA (fold change, mean ± SD, n=3)		
	HF382	**HFC443**	**HMC520**
CYP1A2 mRNA			
Vehicle	1.00	1.00	1.00
Lansoprazole (10 µM)	15.0±1.19	70.0±1.61	6.93±1.55
Honokiol (0.5 µM)	1.06±0.39	0.88±0.33	0.90±0.05
Honokiol (5 µM)	1.10±0.09	1.48±0.53	1.10±0.06
Honokiol (50 µM)	1.43±0.16	1.85±0.42	0.81±0.09
CYP2B6 mRNA			
Vehicle	1.00	1.00	1.00
Rifampicin (10 µM)	8.65±0.49	19.3±1.54	4.86±0.58
Phenobarbital (1 mM)	11.3±3.38	21.2±2.22	6.61±0.7
Honokiol (0.5 µM)	1.12±0.34	0.93±0.31	0.90±0.23
Honokiol (5 µM)	0.89±0.12	1.15±0.24	1.05±0.16
Honokiol (50 µM)	2.12±0.09	5.12±0.65	2.21±0.12
CYP3A4 mRNA			
Vehicle	1.00	1.00	1.00
Rifampicin (10 µM)	93.4±3.75	112.6±15.6	36.6±1.00
Phenobarbital (1 mM)	134.6±3.47	74.6±4.78	34.7±2.51
Honokiol (0.5 µM)	1.15±0.54	0.91±0.33	0.87±0.26
Honokiol (5 µM)	0.87±0.02	1.13±0.19	0.90±0.13
Honokiol (50 µM)	1.12±0.21	1.63±0.13	0.68±0.14

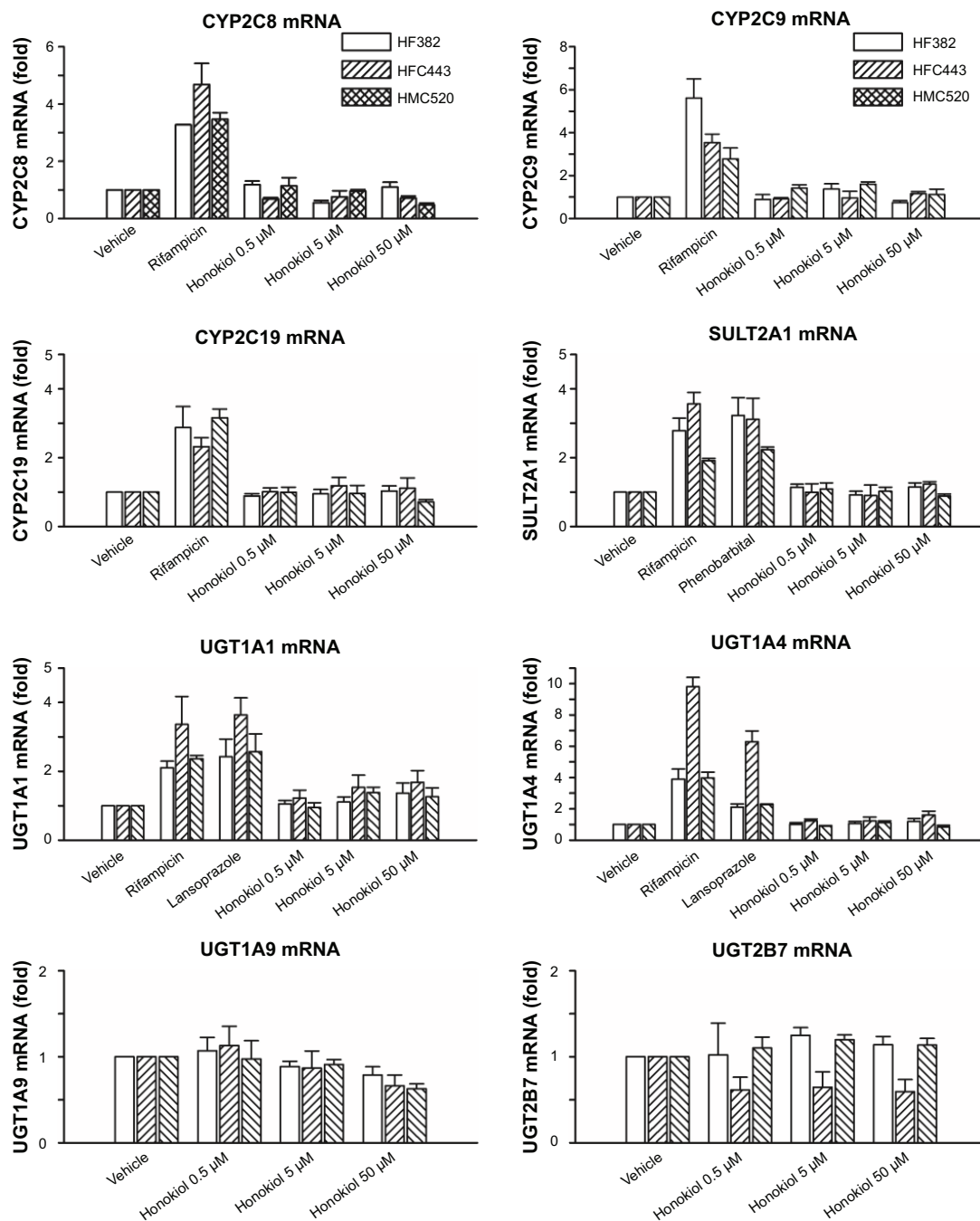**Abbreviations:** CYP, cytochrome P450; mRNA, messenger ribonucleic acid; SD, standard deviation.

Figure 1 Effects of honokiol (0.5, 5, and 50 μM) and positive controls (ie, rifampicin [10 μM], lansoprazole [10 μM], and phenobarbital [1 mM]), on CYP2C8, CYP2C9, CYP2C19, UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1 mRNA levels after 48-hour treatment in three human hepatocyte lines (HF382, HFC443, and HMC520). Data represent the mean ± standard deviation (n=3).
Abbreviations: CYP, cytochrome P450; mRNA, messenger ribonucleic acid; UGT, UDP-glucuronosyltransferase; SULT, sulfotransferase.

Effect of honokiol on SULT and UGT mRNA levels in human hepatocytes

Phase II drug metabolic enzymes such as UGT and SULT play key roles in drug metabolism to increase the hydrophilicity of xenobiotics by conjugation of chemical moieties such as glucuronide or sulfate. The mRNA levels of human UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1

were determined and compared against the fold induction levels of vehicle-treated hepatocytes. The UGT1A1 mRNA level was increased 2.10- to 3.36-fold by rifampicin and 2.42- to 3.63-fold by lansoprazole compared with vehicle in three human hepatocyte lines (Figure 1). UGT1A4 mRNA levels were increased 3.89- to 9.81-fold by rifampicin and 2.10- to 6.29-fold by lansoprazole compared with vehicle in

Table 3 Effect on CYP1A2, CYP2B6, and CYP3A4 activities following 48-hour treatment with honokiol or the positive control (rifampicin, lansoprazole, or phenobarbital) in three human hepatocyte lines (HF382, HFC443, and HMC520)

Sample	Activity (pmol/10^6 cells/h, mean $\pm$ SD, n=3)		
	HF382	HFC443	HMC520
CYP1A2-catalyzed phenacetin O-deethylase			
Vehicle	311.2±62.9	74.4±17.3	466.2±114.5
Lansoprazole (10 μM)	4,940.6±313.3	3,559.4±58.2	5,636.3±56.2
Honokiol (0.5 μM)	300.4±42.3	95.1±26.3	626.1±197.1
Honokiol (5 μM)	340.8±46.1	87.5±27.5	820.3±21.0
Honokiol (50 μM)	552.1±17.4	102.3±7.8	899.5±43.9
CYP2B6-catalyzed bupropion hydroxylase			
Vehicle	20.0±3.0	16.9±3.3	146.9±34.5
Rifampicin (10 μM)	207.4±24.1	99.0±4.5	607.5±17.2
Phenobarbital (1 mM)	254.5±9.7	82.0±13.3	727.7±6.5
Honokiol (0.5 μM)	25.4±4.4	17.6±3.6	193.4±62.6
Honokiol (5 μM)	29.3±5.1	18.7±5.6	235.0±7.3
Honokiol (50 μM)	93.3±5.3	44.8±3.7	284.8±26.5
CYP3A4-catalyzed midazolam 1′-hydroxylase			
Vehicle	113.8±2.7	182.9±16.1	269.3±23.0
Rifampicin (10 μM)	501.0±50.6	489.4±33.7	301.8±12.0
Phenobarbital (1 mM)	409.3±16.9	449.4±4.6	550.4±7.5
Honokiol (0.5 μM)	101.3±2.0	176.9±11.8	293.6±40.6
Honokiol (5 μM)	127.5±11.7	175.4±18.8	333.7±13.1
Honokiol (50 μM)	107.0±11.8	175.7±6.9	296.4±8.9

Abbreviations: CYP, cytochrome P450; SD, standard deviation.

three human hepatocyte lines (Figure 1). SULT2A1 mRNA levels were induced 1.92- to 3.56-fold by rifampicin and 2.23- to 3.22-fold by phenobarbital in three human hepatocyte lines (Figure 1). We found that honokiol did not affect the UGT1A1, UGT1A4, UGT1A9, UGT2B7, or SULT2A1 mRNA levels in three human hepatocyte lines (Figure 1). Therefore, honokiol likely does not induce drug–drug interactions by up- or downregulating the expression of phase II drug-metabolizing enzymes such as UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1.

Discussion

Drug-metabolizing enzymes play major roles in the metabolism of xenobiotics or endogenous compounds, and are up- or downregulated following xenobiotic exposure.[57] Hyperforin, an active component of St John's wort, potently inhibited CYP2C9 (K_i, 1.8 μM) and CYP3A4 (K_i, 0.49 μM) activity in human complementary deoxyribonucleic acid-expressing CYP microsomes,[58] but chronic 48-hour hyperforin treatment (1 μM) in human hepatocytes resulted in significant increases in the mRNA, activity, and protein levels of CYP3A4 and CYP2C9.[31] These results suggest that cotreatment with hyperforin in the clinic significantly decreases the plasma concentrations of CYP3A4 substrates, including cyclosporine, digoxin, simvastatin, and oral contraceptives.[59]

In our previous study,[55] 30-minute honokiol treatment resulted in marked inhibition of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and UGT1A9 activities in pooled human liver microsomes, with K_i values of 1.2, 4.9, 0.54, 0.57, and 0.3 μM, respectively. However, chronic treatment with a higher concentration (50 μM) of honokiol increased CYP2B6 mRNA and activity levels without affecting the expression of CYP1A2, CYP3A4, CYP2C8, CYP2C9, CYP2C19, UGT1A1, UGT1A4, UGT1A9, UGT2B7, or SULT2A1 in three human hepatocyte lines (Tables 2 and 3, Figure 1). CYP1A1-catalyzed phenacetin O-deethylase and CYP3A4-catalyzed midazolam 1′-hydroxylase activities were unaffected in honokiol-treated hepatocytes compared with vehicle-treated hepatocytes (Table 3), suggesting that honokiol had no effect on CYP1A2 and CYP3A4 mRNA levels in three human hepatocyte lines. These results indicate that honokiol coadministration may not result in any clinically significant drug interactions with CYP1A2, CYP3A4, CYP2C8, CYP2C9, CYP2C19, UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1 substrates in vivo.

Treatment with a higher concentration (50 µM) of honokiol increased the CYP2B6 mRNA level and CYP2B6-catalyzed bupropion hydroxylase activity by more than two-fold in three human hepatocyte lines, indicating that a higher concentration of honokiol induces CYP2B6 expression in vivo according to FDA and EMA drug interaction guidelines. High doses of honokiol should be used cautiously with CYP2B6-metabolized drugs such as cyclophosphamide, ifosfamide, tamoxifen, bupropion, valproic acid, and efavirenz to avoid drug interactions.[60]

Honokiol has poor oral absorption and is extensively metabolized in liver with an elimination half-life of 49.05 minutes and 56.24 minutes for 5 mg/kg and 10 mg/kg intravenous doses, respectively, in rats.[36] After intravenous administration of honokiol in rats, 18 metabolites via hydroxylation, sulfation, glucuronidation, and methylation were identified in plasma.[53] Incubation of human hepatocytes with honokiol (5 µM) for 1 hour resulted in the detection of metabolites such as honokiol glucuronide, honokiol sulfates, hydroxyhonokiol, hydroxyhonokiol glucuronide, and hydroxyhonokiol sulfate, but not honokiol, by LC high-resolution orbitrap mass spectrometry. At present, no data on honokiol pharmacokinetics in humans are available; such data are indispensable for prediction of honokiol–drug interactions.

Conclusion

In conclusion, the present study demonstrated the potential of honokiol to increase CYP2B6 mRNA and activity levels following chronic exposure to a high concentration (50 µM). However, honokiol had negligible effects on the CYP1A2, CYP3A4, CYP2C8, CYP2C9, CYP2C19, UGT1A1, UGT1A4, UGT1A9, UGT2B7, and SULT2A1 mRNA levels in human hepatocytes. Our results suggest that honokiol at the usual dosage may not cause pharmacokinetic-based drug interactions with the substrates of these drug-metabolizing enzymes in humans via enzyme induction.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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