0090-9556/08/3606-1010–1015$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 36, No. 6
19612/3338648
Printed in U.S.A.

DMD 36:1010–1015, 2008

Identification and Characterization of Potent CYP2B6 Inhibitors in Woohwangcheongsimwon Suspension, an Herbal Preparation Used in the Treatment and Prevention of Apoplexy in Korea and China

Hyunmi Kim, Kwon-Bok Kim, Hei-Young Ku, Soo-Jin Park, Hoon Choi, Joon-Kwan Moon, Byeoung-Soo Park, Jeong-Han Kim, Sung Su Yea, Choong-Hwan Lee, Hye Suk Lee, Jae-Gook Shin, and Kwang-Hyeon Liu

Department of Pharmacology and PharmacoGenomics Research Center (H.K., K.-B.K., H.-Y.K., S.-J.P., J.-G.S., K.-H.L.) and Department of Biochemistry (S.S.Y.), Inje University College of Medicine, Busan, Korea; Frontier Inje Research for Science and Technology, Inje University, Busan, Korea (S.S.Y., K.-H.L.); School of Agricultural Biotechnology, Seoul National University, Seoul, Korea (H.C., J.-K.M., B.-S.P., J.-H.K.); Department of Biosciences and Biotechnology, IBST, Konkuk University, Seoul, Korea (C.-H.L.); and College of Pharmacy, Wonkwang University, Iksan, Korea (H.S.L.)

Received November 1, 2007; accepted March 5, 2008

ABSTRACT:

Woohwangcheongsimwon is a traditional medicine for treating hypertension, arteriosclerosis, coma, and stroke in China and Korea. To assess potential interactions of herb and drug metabolism, commercially available Woohwangcheongsimwon suspensions were examined for their potential to inhibit the activity of nine human cytochrome P450 enzymes. The Woohwangcheongsimwon suspensions showed strong inhibition of CYP2B6 activity. To identify individual constituents with inhibitory activity, the suspension was partitioned using hexane, ethyl acetate, and dichloromethane, and each fraction was tested for its inhibitory effect on CYP2B6-catalyzed bupropion hydroxylation. The hexane fraction possessed inhibitory activity, and gas chromatography/mass spectrometry analysis identified borneol and isoborneol as major constituents of the hexane fraction. These two terpenoids moderately inhibited CYP2B6-catalyzed bupropion hydroxylase activity in a competitive manner, with K_i values of 9.5 and 5.9 μM, respectively, as well as efavirenz 8-hydroxylase activity, with K_i values of 22 and 26 μM, respectively. Additionally, reconstituted mixtures of borneol and isoborneol, at the same concentrations as in the Woohwangcheongsimwon suspension, had comparable potency in inhibiting bupropion hydroxylation. These in vitro data indicate that Woohwangcheongsimwon preparations contain constituents that can potently inhibit the activity of CYP2B6 and suggest that these preparations should be examined for potential pharmacokinetic drug interactions in vivo.

Herbal medicines have received much attention as alternatives to conventional clinical therapy, and consumption of herbal medicines in Asian, North American, and European countries has increased dramatically in recent years (Eisenberg et al., 1998; De Smet and Debeer, 2002). A recent report indicates that as many as 16% of prescription drug users consume herbal dietary supplements (Kaufman et al., 2002). With the widespread use of herbal medicines, the risk of herb-drug interactions is a growing medical issue, and physicians and pharmacists are concerned about adverse effects such as hepatotoxicity and drug interactions (Kaplowitz, 1997; Suchard et al., 2004). Several medicinal herbs, including St. John's wort (Henderson et al., 2002; Mills et al., 2004), ginkgo biloba (Yin et al., 2004), and kava (Anke and Ramzan, 2004), have been reported to cause herb-drug interactions. Interactions among therapeutic drugs as well as interactions of drugs with food and herbal medicines have attracted attention.

The modulation of drug-metabolizing enzymes is one of the main mechanisms of drug interactions (Guengerich, 1997). Cytochrome P450 (P450) monooxygenases are probably the most important enzymes in the detoxification and bioactivation of a number of therapeutic drugs. The P450 family comprises a group of enzymes with broad substrate specificity, which leads to herb-induced drug interactions with some P450 substrates (Ueng et al., 2002). In recent years, in vitro systems using human liver microsomes or recombinant P450 enzymes with tandem mass spectrometry have been established as tools for evaluating potential inhibitory effects of drugs on P450 enzyme activity (Dierks et al., 2001; Kim et al., 2005). Accordingly, in vitro evaluation systems are now widely used in screening procedures to exclude candidate drugs with potent P450-inhibiting effects (Baranczewski et al., 2006).

This study was supported by the Korea Research Foundation Grant funded by the Korean Government (MODHRD, Basic Research Promotion Fund) (KRF-2006-311-E00238) and by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (R13-2007-023-00000-0), Korea.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.107.019612.

ABBREVIATIONS: P450, cytochrome P450; thiotepa, triethylenethiophoramide; LC, liquid chromatography; MS/MS, tandem mass spectrometry; GC, gas chromatography; MS, mass spectrometry.

TABLE 1

Woohwangcheongsimwon suspensions used in this study

Code	Pharmaceutical Company	Content[a]		Dosage[b]
		Isoborneol	Borneol	
		ppm		*ml*
A	Kwang-Dong Pharmaceutical Co., Ltd.	586.3	919.6	30
B	Dae-Han New Pharm Co., Ltd.	246.3	419.8	50
C	Ik-Su Pharmaceutical Co., Ltd.	559.8	946.4	50
D	SamSung Pharmaceutical Industry Co., Ltd.[c]	969.3	1624.1	20
E	Cho-Seon Pharmaceutical & Trading Co., Ltd.	440.3	694.9	50
F	SamSung Pharmaceutical Industry Co., Ltd.[d]	848.3	1433.2	20

[a] Content in Woohwangcheongsimwon suspension measured using GC/MS.
[b] Dosage filled in one bottle of each product, and one or two bottles are available per day for an adult.
[c] Contains *l*-muscone.
[d] *Acori graminei* rhizoma is substituted for *l*-muscone.

In a previous study, we screened the P450-inhibiting effects of 20 herbal medications commonly used in Korea. This screening revealed that Woohwangcheongsimwon suspension showed a potent inhibitory effect on CYP2B6-mediated bupropion hydroxylase activity (Kim and Liu, 2007). Woohwangcheongsimwon is one of the most widely used traditional Chinese medicines for the emergency and acute treatment of stroke, numbness, hypertension, epilepsy, and arteriosclerosis. It contains 29 herbs; the main components are *Calculus bovis*, *Moschus*, *Borneolum syntheticum*, *Radix ginseng*, and *Rhizoma dioscoreae* (Lee et al., 2005). Woohwangcheongsimwon suspension is commercially available and has been commonly used in China, Taiwan, and Korea. Simply because of its widespread use, Woohwangcheongsimwon is highly likely to be used in combination with various drugs.

In this study, we investigated the effects of Woohwangcheongsimwon suspension on human P450 activity, using human liver microsomes, to assess the probability of herb-drug interactions. Through this study, we have identified components having a CYP2B6-inhibiting effect comparable with that of thioTEPA and have shown that these components inhibit CYP2B6 activity in a competitive manner.

Materials and Methods

Chemicals and Reagents. Borneol, bupropion, chlorpropamide, chlorzoxazone, coumarin, dextromethorphan, isoborneol, phenacetin, thioTEPA, tolbutamide, β-NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxybupropion and pooled human liver microsomes (H161) were obtained from BD Gentest (Woburn, MA). *S*-Mephenytoin and midazolam were purchased from Ultrafine Chemical Co. (Manchester, UK). Efavirenz and 8-hydroxyefavirenz were purchased from Toronto Research Chemicals, Inc. (Toronto, ON, Canada). All other chemicals and solvents were of the highest grade available.

Samples of Woohwangcheongsimwon Suspension. Woohwangcheongsimwon suspensions manufactured by various pharmaceutical companies including the product of Kwang-Dong Pharmaceutical Co., Ltd (lot 06004) were obtained from a local pharmacy (Table 1). Samples were stored at 4°C until use. All samples were tested soon after the package was opened. Kwang-Dong Woohwangcheongsimwon (aqueous solutions) was used for each experiment as a representative.

Inhibitory Effects of Woohwangcheongsimwon on P450 Activity. The inhibitory potency of Woohwangcheongsimwon suspension was determined with cytochrome P450 assays in the presence and absence of Woohwangcheongsimwon suspension, using pooled human liver microsomes. Phenacetin *O*-deethylase, coumarin 7-hydroxylase, bupropion hydroxylase, paclitaxel 6α-hydroxylase, tolbutamide 4-hydroxylase, *S*-mephenytoin 4-hydroxylase, dextromethorphan *O*-demethylase, chlorzoxazone 6-hydroxylase, and midazolam 1′-hydroxylase activities were determined as probe activities for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A, respectively, using cocktail incubation and tandem mass spectrometry, as described previously (Kim et al., 2005). Briefly, the incubation mixtures containing pooled human liver microsomes (0.25 mg/ml), P450-selective substrates, and Woohwangcheongsimwon suspension (0–1.7%, v/v; 0–1,000 μg/ml, dry weight basis) were preincubated for 10 min at 37°C. The reaction was initiated by adding a NADPH-generating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2$, and 1.0 unit/ml glucose-6-phosphate dehydrogenase) and was incubated for 15 min at 37°C in a shaking water bath. After incubation, the reaction was stopped by placing the tubes on ice and adding 100 μl of ice-cold acetonitrile. The incubation mixtures were then centrifuged (10,000*g* for 5 min at 4°C). Aliquots of the supernatants were injected into an LC/MS/MS system. All incubations were performed in triplicate, and mean values were used for analysis. To evaluate the effect of preincubation on inhibitory potency, Woohwangcheongsimwon suspension was preincubated for 20 min with the NADPH-generating system, buffer, and microsomes. The reaction was started by adding the P450-selective substrates. The substrates were used at the following concentrations: 50 μM phenacetin, 5 μM coumarin, 50 μM bupropion, 10 μM paclitaxel, 100 μM tolbutamide, 100 μM *S*-mephenytoin, 5 μM dextromethorphan, 50 μM chlorzoxazone, and 5 μM midazolam.

Fractionation of Woohwangcheongsimwon Suspension and Identification of the CYP2B6-Inhibitory Components. The Woohwangcheongsimwon suspension (100% 5 ml, v/v) was sequentially extracted with *n*-hexane, ethyl ether, ethyl acetate, and dichloromethane. In brief, Woohwangcheongsimwon suspension was extracted with the same volume of *n*-hexane, and then the remaining aqueous fraction was sequentially partitioned with ethyl ether, ethyl acetate, and dichloromethane. The *n*-hexane fraction exhibited a significant inhibitory effect on CYP2B6-catalyzed bupropion hydroxylase activity. The hexane-soluble fraction of Woohwangcheongsimwon suspension was subjected to GC/MS. The GC/MS analysis was carried out on a Shimadzu GC-2010 GC instrument, connected to a QP-5000 mass spectrometer (Shimadzu, Tokyo, Japan) with electron impact ionization (EI mode, 70 eV). The column was a DB-WAX capillary column (60 m × 0.32 mm i.d., 0.25-μm film thickness; Agilent Technologies, Wilmington, DE), and the oven temperature was raised from 80 to 250°C at a rate of 10°C/min with a 4-min hold at 80°C and a 2-min hold at 250°C. Helium was used as the carrier gas, at a flow rate of 1.5 ml/min. The temperatures of the injection port, ion source, and interface were 170, 200, and 270°C, respectively.

Assay of Bupropion Hydroxylase and Efavirenz 8-Hydroxylase Activity of Human CYP2B6. Assays for bupropion hydroxylase and efavirenz 8-hydroxylase activity of human CYP2B6 were performed according to the methods of Kim et al. (2005) and Ward et al. (2003), with minor modifications. Briefly, each incubation was performed with 0.25 mg/ml pooled human liver microsomes (H161) in 100 mM phosphate buffer (pH 7.4) at a final volume of 250 μl. The incubation mixtures, containing one of the CYP2B6 isoform-specific substrates, an inhibitor (Woohwangcheongsimwon suspension, 0–1.7% (v/v), 0–1,000 μg/ml, dry-weight basis; borneol or isoborneol dissolved in methanol, 0–50 μM), and human liver microsomes were preincubated for 5 min at 37°C. The final concentration of methanol for the incubation condition was 1.0%. The substrates were used at concentrations approximately equal to their respective K_m values (50 μM bupropion and 10 μM efavirenz) for the determination of IC_{50} values. For the determination of K_i values, various concentrations of substrates (20–100 μM bupropion and 2–10 μM efavirenz) were also used. After preincubation, the reactions were initiated by

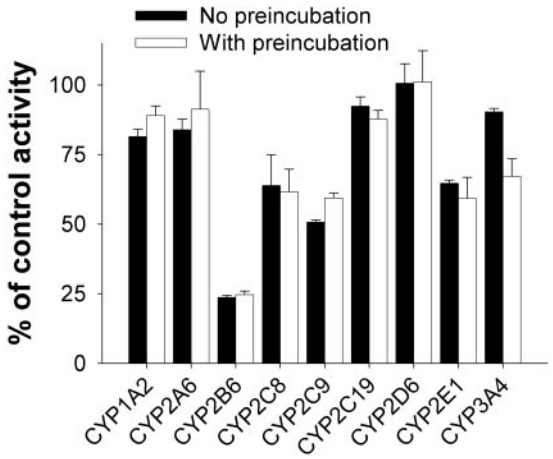

FIG. 1. Inhibitory effects of Woohwangcheongsimwon suspension (0.84%, v/v; 500 μg/ml dry-weight basis) on cytochrome P450 activity in pooled human liver microsomes (H161). The activity of each isoform was measured using the respective specific probe substrate reaction, as described previously (Kim et al., 2005). Results are shown as the mean ± S.D. of triplicate experiments (■, no preincubation; □, with preincubation). The representative control activities of phenacetin *O*-deethylation, coumarin 7-hydroxylation, bupropion hydroxylation, paclitaxel 6α-hydroxylation, tolbutamide 4-hydroxlation, *S*-mephenytoin 4′-hydroxylation, dextromethorphan *O*-demethylation, chlorzoxazone 6-hydroxylation, and midazolam 1′-hydroxylation were 3.8, 2.9, 2.6, 0.94, 0.74, 0.34, 1.6, 11, and 9.9 pmol/min/mg of protein, respectively.

addition of a NADPH-generating system (3.3 mM glucose 6-phosphate, 1.3 mM β-$NADP^+$, 3.3 mM $MgCl_2$, and 1.0 unit/ml glucose-6-phosphate dehydrogenase) and stopped after 15 min by placing the incubation tubes on ice and adding 100 μl of ice-cold acetonitrile, containing an internal standard (2 μM chlorpropamide for bupropion or 0.5 μg/ml ritonavir for efavirenz). The incubation mixtures were centrifuged (10,000*g* for 5 min at 4°C), and aliquots of the supernatants were analyzed by LC/MS/MS. The reaction rates were linear with incubation time and microsomal protein content under these conditions.

LC/MS/MS Analysis. All metabolites of the P450 isoform-specific substrates, excluding 8-hydroxyefavirenz, were measured by tandem mass spectrometry as described previously (Kim et al., 2005). The concentration of 8-hydroxyefavirenz was measured by LC/MS/MS as described elsewhere (Fan et al., 2002), with some modifications. Briefly, the system consisted of an API 3000 LC/MS/MS system (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization interface. The compounds were separated on a reversed-phase column (Luna C18, 2.0 mm i.d. × 30 mm, 3-μm particle size; Phenomenex, Torrance, CA) with an isocratic mobile phase consisting of acetonitrile and water (80/20, v/v) containing 0.1% formic acid. The mobile phase was eluted using an Agilent 1100 series pump (Agilent Technologies), at a flow rate of 0.2 ml/min. The TurboIonSpray interface was operated in the negative ion mode at −4500 V and 400°C. The operating conditions were as follows: nebulizing gas flow, 1.46 L/min; auxiliary gas flow, 4.0 L/min; curtain gas flow, 1.25 L/min; orifice voltage, −80 V; ring voltage, −350 V; collision energy, −25 V; and collision gas (nitrogen) pressure, 3.77×10^{-5} Torr. The mass transition used for quantitation of 8-hydroxyefavirenz was *m*/*z* 330 → 258. The analytical data were processed using Analyst software (version 1.2; Applied Biosystems).

Data Analysis. The cytochrome P450-mediated activities in the presence of inhibitors were expressed as a percentage of the corresponding control values in the presence of methanol alone. The apparent kinetic parameters for inhibitory potential (K_i values) were initially estimated by graphical methods such as Lineweaver-Burk plot, Dixon plot, and Eadie-Hosftee plot, but ultimately determined by nonlinear least-squares regression analysis from the best enzyme inhibition model (Segel, 1975) using WinNonlin software (version 4.0; Pharsight, Mountain View, CA). In our experiment, WinNonlin estimation consistently showed that inhibition data were best fitted by a competitive inhibition model via Akaike information criteria and Schwartz criteria among the models tested including pure and partial competitive inhibition, noncompetitive inhibition, mixed-type inhibition, and uncompetitive inhibition (Shin et al., 1999).

Results

Effects of Woohwangcheongsimwon Suspension on P450 Activity. The effects of Woohwangcheongsimwon suspension (500 μg/ml) on nine P450 activities are shown in Fig. 1. Of the P450 isoform activities tested, CYP2B6-catalyzed bupropion hydroxylation was most strongly inhibited by Woohwangcheongsimwon suspension (IC_{50} 110 μg/ml) (Table 2); the hydroxybupropion formation rate was decreased to 14% of control activity at the highest concentration tested (1000 μg/ml). To determine whether the inhibition by Woohwangcheongsimwon suspension was substrate-specific, we examined the inhibitory effect of Woohwangcheongsimwon suspension on CYP2B6-catalyzed efavirenz hydroxylation and found that Woohwangcheongsimwon inhibited it too, with an apparent IC_{50} of 190 μg/ml (Table 2; Fig. 2). Woohwangcheongsimwon suspension showed weak inhibition of CYP2C9 and CYP2E1, with IC_{50} values of 620 and 700 μg/ml, respectively (Table 2) and minimal or negligible inhibition of the other P450s tested (Fig. 1). The inhibitory potency of Woohwangcheongsimwon suspension was not reduced significantly after preincubation with microsomes in the presence of a NADPH-generating system (Fig. 1). The reproducibility of the inhibitory effects of different Woohwangcheongsimwon suspensions on CYP2B6 activity was examined. Regardless of manufacturer, the Woohwangcheongsimwon suspensions inhibited CYP2B6 activity to the same extent (Fig. 3).

Identification of the CYP2B6-Inhibiting Components in Woohwangcheongsimwon Suspension. To identify the major components responsible for the inhibitory effect on CYP2B6 activity, Woohwangcheongsimwon suspension was sequentially partitioned with various organic solvents. Of the fractions partitioned, the *n*-hexane fraction exhibited a strong inhibitory effect on CYP2B6 activity (Fig. 4). The *n*-hexane fraction was subjected to GC/MS analysis, and two major peaks were detected (Fig. 5). These two peaks (P1 and P2) were

TABLE 2

Effect of Woohwangcheongsimwon suspension on cytochrome P450 metabolic activity in pooled human liver microsomes with IC_{50} values

Data are mean ± S.D.

	CYP1A2	CYP2A6	CYP2B6	CYP2B6	CYP2C8
Substrate	Phenacetin	Coumarin	Bupropion	Efavirenz	Rosiglitazone
IC_{50} (%)	>1.5	>1.5	0.18 ± 0.0018	0.32 ± 0.034	>1.5
IC_{50} (μg/ml)[a]	>1000	>1000	110 ± 1.1	190 ± 20	>1000
	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Substrate	Tolbutamide	S-Mephenytoin	Dextromethorphan	Chlorzoxazone	Midazolam
IC_{50} (%)	1.0 ± 0.18	>1.5	>1.5	1.2 ± 0.087	>1.5
IC_{50} (μg/ml)[a]	620 ± 110	>1000	>1000	700 ± 53	>1000

[a] IC_{50} values are based on dry weight of Woohwangcheongsimwon suspension.

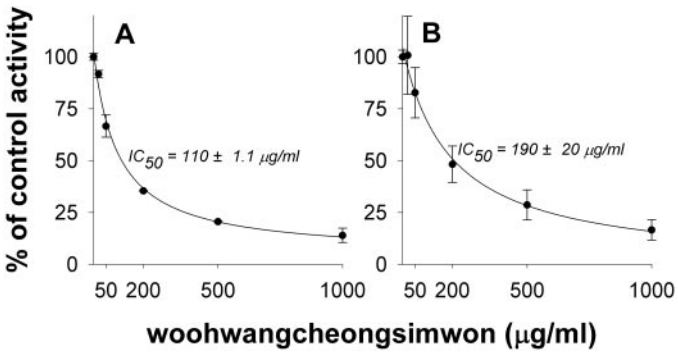

FIG. 2. Inhibitory effects of Woohwangcheongsimwon suspension on CYP2B6-catalyzed bupropion hydroxylation (A) and efavirenz 8-hydroxylation (B) in pooled human liver microsomes. Pooled human liver microsomes (0.25 mg/ml, H161) were incubated with bupropion (50 μM) or efavirenz (10 μM) in the presence or absence of various concentrations of Woohwangcheongsimwon suspension (0–1.7%, v/v; 0–1,000 μg/ml, dry-weight basis) at 37°C for 15 min. The activity was calculated as the percentage of control sample activity and plotted versus the Woohwangcheongsimwon concentration. Each data point is the average ± S.D. of triplicate experiments. The representative control activities of bupropion hydroxylation and efavirenz 8-hydroxylation were 2.2 and 0.88 pmol/min/mg of protein, respectively.

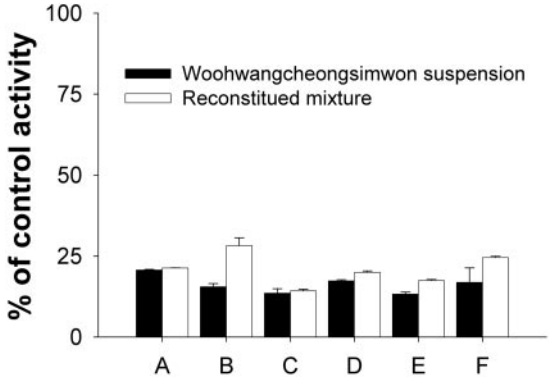

FIG. 3. Reproducibility of CYP2B6 inhibition by several Woohwangcheongsimwon suspensions (■) and their corresponding reconstituted mixtures of borneol and isoborneol (□). Pooled human liver microsomes (0.25 mg/ml, H161) were incubated with bupropion (50 μM) in the absence or presence of several manufacturers' Woohwangcheongsimwon suspensions (500 μg/ml) or the corresponding reconstituted solutions of borneol and isoborneol. The reconstituted mixtures contained the same borneol and isoborneol concentrations as in the Woohwangcheongsimwon suspensions, which were determined by GC/MS. Data represent the mean ± S.D. of triplicate experiments. A, Kwang-Dong Pharmaceutical Co., Ltd. B, Dae-Han New Pharm Co., Ltd. C, Ik-Su Pharmaceutical Co., Ltd. D, SamSung Pharmaceutical Industry Corp., Ltd. E, Cho-Seon Pharmaceutical & Trading Co., Ltd. F, SamSung Pharmaceutical Industry Corp., Ltd. Table 1 lists the Woohwangcheongsimwon suspensions tested. The representative control activity of bupropion hydroxylation was 2.3 pmol/ min /mg of protein.

identified as isoborneol and borneol, respectively, by cochromatography and GC/MS spectral data of authentic compounds (Fig. 6). Electron impact spectra of P1 and P2 revealed a molecular ion (M^+) at m/z 154 and a base peak at m/z 95.1. Applying this to the mass spectra of borneol and isoborneol, we can identify fragment peaks resulting from the loss of H_2O at m/z = 136 and the loss of ethane at m/z = 110, which corresponds to a reverse Diels-Alder reaction. The fragment peak at m/z = 95 is due to the loss of a methyl group (Donald et al., 2001).

Inhibitory Effects of Borneol and Isoborneol on P450 Activity. The inhibitory effects of borneol and isoborneol from Woohwangcheongsimwon suspension toward nine major human P450 isoforms were investigated to clarify the selectivity of inhibition. Borneol and isoborneol strongly inhibited microsomal CYP2B6 activity but showed little or no inhibition of the other eight P450s tested (Fig. 7).

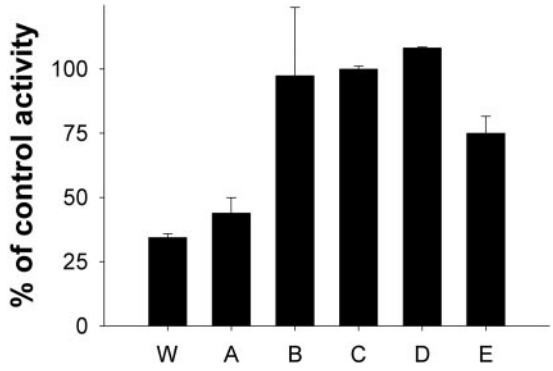

FIG. 4. Hexane extracts of Woohwangcheongsimwon suspension inhibit CYP2B6-catalyzed bupropion hydroxylase activity. Woohwangcheongsimwon suspension (100% 5 ml, v/v) was sequentially partitioned with the same volume of *n*-hexane, ethyl ether, ethyl acetate, and dichloromethane, and the inhibitory effect of each fraction on CYP2B6-catalyzed bupropion hydroxylase activity was determined in pooled human liver microsomes (H161). Data represent the mean ± S.D. of triplicate experiments. W, Woohwangcheongsimwon suspension; A, hexane extracts; B, ethyl ether extracts; C, ethyl acetate extracts; D, dichloromethane extracts; E, residuals. The representative control activity of bupropion hydroxylation was 2.3 pmol/min/ mg of protein.

The Lineweaver-Burk plots, Dixon plots, and secondary reciprocal plots indicated that borneol and isoborneol competitively inhibited CYP2B6-catalyzed bupropion hydroxylase activity, with apparent K_i values of 9.5 and 5.9 μM, respectively (Fig. 8; Table 3). The K_i value of thioTEPA, a typical CYP2B6 inhibitor, was simultaneously determined (1.8 μM) (Fig. 8; Table 3). To determine whether the inhibition by these monoterpenes was substrate-specific, we examined their inhibitory effects on CYP2B6-catalyzed efavirenz 8-hydroxylation and found that borneol and isoborneol competitively inhibited the activity, with K_i values of 22 and 26 μM, respectively (Table 3).

Discussion

Some difficulties have occurred in patients taking prescription medicines and herbal preparations, owing at least in part to a lack of information on herb-drug interactions arising from drug metabolism or absorption (Kim and Liu, 2007). Herbal preparations are taken as over-the-counter products in many Asian countries, including China, Japan, and Korea. To reduce the number of adverse interactions, it is necessary to study drug-herbal preparation interactions.

In the present study, we evaluated the P450-inhibitory effect of Woohwangcheongsimwon suspensions, a traditional Chinese medicine widely used for emergency and acute treatment of stroke and numbness. In human liver microsomes with a NADPH-generating system, Woohwangcheongsimwon suspension moderately inhibited metabolic CYP2B6-catalyzed bupropion hydroxylase activity, regardless of preincubation with microsomes, indicating that a mechanism-based inhibitory component was not present in Woohwangcheongsimwon suspension (Fig. 1) (Iwata et al., 2005). Woohwangcheongsimwon also inhibited CYP2B6 activity with IC_{50} values of 110 and 190 μg/ml for bupropion hydroxylase and efavirenz 8-hydroxylase activity, respectively.

In this study, two chemicals, borneol and isoborneol, identified from the Woohwangcheongsimwon suspension were shown to inhibit microsomal CYP2B6 activity. Of the P450-catalyzed reactions tested here, borneol and isoborneol most significantly inhibited CYP2B6-catalyzed bupropion hydroxylation, with K_i values (9.5 and 5.9 μM, respectively) (Fig. 8) comparable to that of thioTEPA (K_i = 2.8 μM) (Turpeinen et al., 2004), a well known CYP2B6-selective inhibitor. Borneol and isoborneol only moderately inhibited (K_i = 22 and 26 μM, respectively) (Table 3) the metabolism of efavirenz, a different

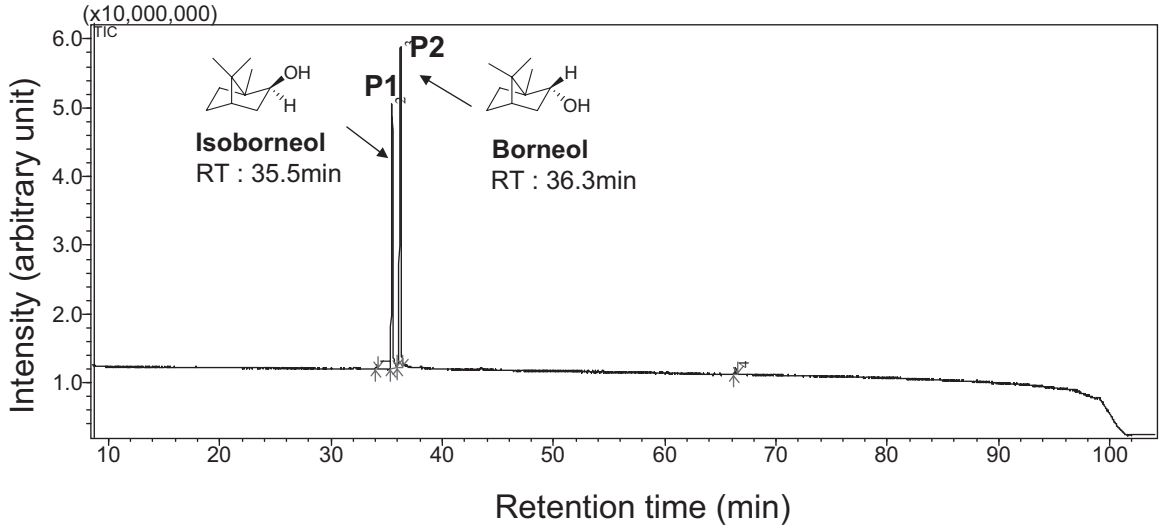

FIG. 5. GC/MS total ion chromatogram obtained from the hexane-soluble fraction of Woohwangcheongsimwon suspension. Woohwangcheongsimwon suspension (100% 5 ml, v/v) was partitioned with the same volume of *n*-hexane, and the hexane extracts were subjected to GC/MS (see *Materials and Methods*).

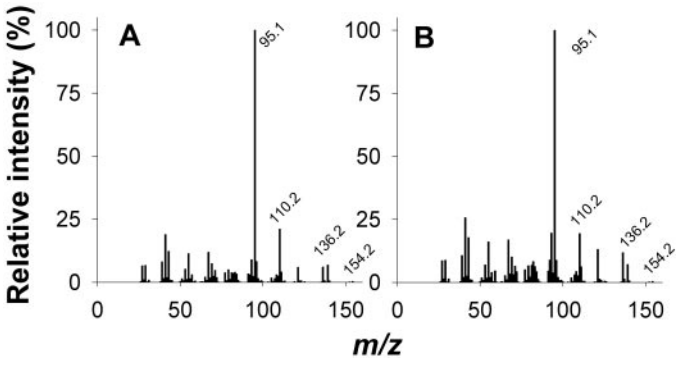

FIG. 6. Mass spectra of borneol (A) and isoborneol (B) obtained by GC/MS analysis of the hexane extract of Woohwangcheongsimwon suspension.

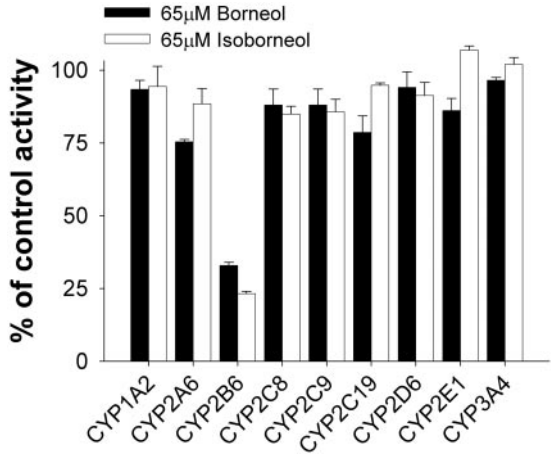

FIG. 7. Inhibitory effects of borneol (65 μM, ■) and isoborneol (65 μM, □) on cytochrome P450 activity in pooled human liver microsomes (H161). The activity of each isoform was measured using the respective specific substrate reaction probes, as described previously (Kim et al., 2005). Data are the means ± S.D. of triplicate experiments. The representative control activities of phenacetin *O*-deethylation, coumarin 7-hydroxylation, bupropion hydroxylation, paclitaxel 6α-hydroxylation, tolbutamide 4-hydroxlation, *S*-mephenytoin 4′-hydroxylation, dextromethorphan *O*-demethylation, chlorzoxazone 6-hydroxylation, and midazolam 1′-hydroxylation were 3.8, 2.9, 2.6, 0.94, 0.74, 0.34, 1.6, 11, and 9.9 pmol/min/mg of protein, respectively.

CYP2B6 substrate (Ward et al., 2003). Borneol and isoborneol showed negligible inhibitory effects on the other P450s tested (Fig. 7).

Borneol and isoborneol are major components of borneolum (*Dryobalanops aromatica*), one of the major herbal extracts in Woohwangcheongsimwon suspension (Park et al., 2003). To reconfirm that the inhibition of CYP2B6 activity by Woohwangcheongsimwon suspension resulted from borneol and isoborneol, we quantified the borneol and isoborneol concentrations in Woohwangcheongsimwon suspensions from several manufacturers, prepared standard solutions with the same borneol and isoborneol concentrations, and showed that all of the reconstituted mixtures of borneol and isoborneol had similar inhibitory effects and that the extent of inhibition was comparable among the mixtures and their respective Woohwangcheongsimwon suspensions (Fig. 3). Thus, these newly identified monoterpenes probably contribute to the CYP2B6 inhibition caused by Woohwangcheongsimwon suspension. These results also suggest that Woohwangcheongsimwon suspensions from several manufacturers have similar inhibitory potencies although the concentration of the Woohwangcheongsimwon suspension tested is slightly high (500 μg/ml) in detecting differences between various formulations.

Borneol and isoborneol are monoterpenes; monoterpenes are found in the volatile essences of flowers and oils of various plants and in herbal medicines (Li Lin et al., 2006). Some are commonly used as food additives and as fragrance components in cosmetics, soaps, and cleaning products (Guitton et al., 1998). Borneol is often a major constituent of the essential oils of medicinal herbs such as the genus *Micromeria* (Sneyd et al., 1994) and rosemary (Tabanca et al., 2001). Thus, monoterpenes contained in those medicinal herbs may also affect CYP2B6 activity.

Woohwangcheongsimwon suspension is one of the most popular herbal medicines in Korea. It is commonly used in the treatment and prevention of apoplexy, hypertension, palpitations, convulsions, and unconsciousness and is composed of approximately 30 types of traditional drugs from herbs, animals, and even metals such as gold (Lee et al., 2005). In China, it is called Niuhuang Qinxin Wan and was recorded in the *Prescriptions of Taiping Benevolent Dispensary* during the Song dynasty. It is still produced at the Tong Ren Tang Pharmaceutical factory. Thus, because of its widespread use, Woohwangcheongsimwon presents a significant possibility for herb-drug interactions. The possibility of adverse herb-drug interactions

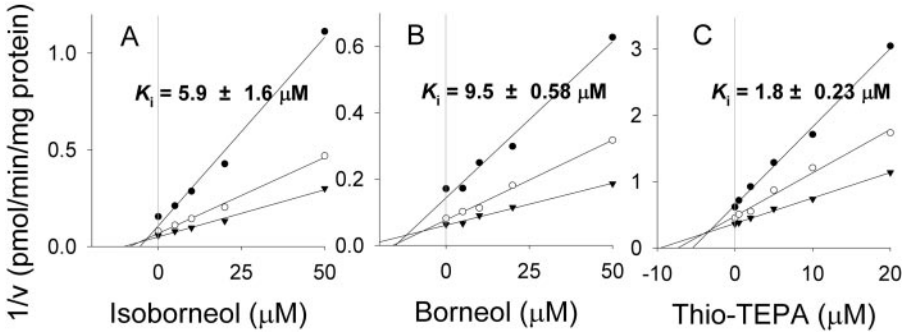

FIG. 8. Representative Dixon plots for the inhibition of CYP2B6-catalyzed bupropion hydroxylation to hydroxybupropion with (A) isoborneol (0–50 μM), (B) borneol (0–50 μM), and (C) thioTEPA (0–20 μM) in pooled human liver microsomes (H161). Each data point was obtained by incubation with 20 (●), 50 (○), or 100 (▼) μM bupropion in the presence or absence of inhibitor. Each point represents the mean of triplicate experiments.

TABLE 3

K_i values for the inhibition of CYP2B6-mediated metabolism by borneol, isoborneol, and thioTEPA in pooled human liver microsomes

Data are mean ± S.D.

Inhibitor	Type of Inhibition	K_i	
		Bupropion Hydroxylation	Efavirenz 8-Hydroxylation
		μM	
Borneol	Competitive	9.5 ± 1.6	22 ± 3.0
Isoborneol	Competitive	5.9 ± 0.58	26 ± 4.5
ThioTEPA	Competitive	1.8 ± 0.23	9.5 ± 1.0

between Woohwangcheongsimwon and drugs that are cleared primarily by CYP2B6-mediated pathways should be examined in vivo, especially given the interaction potential of St. John's wort in humans (Henderson et al., 2002).

In conclusion, by screening the inhibitory effects of Woohwangcheongsimwon suspensions on the activities of nine P450 isoforms, we identified two components in Woohwangcheongsimwon suspension, borneol and isoborneol, as potent inhibitors of CYP2B6. The inhibitory potencies of borneol and isoborneol were comparable with that of thioTEPA. These results suggest that the use of high amounts of this herbal preparation may cause an interaction with drugs metabolized by CYP2B6 in some individuals. It is important to note, however, that the inhibition of CYP2B6 activity in vitro does not necessarily translate into drug interactions in clinical situations. In vivo studies on the interactions between Woohwangcheongsimwon suspension and CYP2B6 substrates are required to determine the clinical relevance of CYP2B6 inhibition by Woohwangcheongsimwon.

References

Anke J and Ramzan I (2004) Pharmacokinetic and pharmacodynamic drug interactions with kava (*Piper methysticum* Forst. f.). *J Ethnopharmacol* **93:**153–160.

Baranczewski P, Stanczak A, Sundberg K, Svensson R, Wallin A, Jansson J, Garberg P, and Postlind H (2006) Introduction to in vitro estimation of metabolic stability and drug interactions of new chemical entities in drug discovery and development. *Pharmacol Rep* **58:**453–472.

De Smet L and Debeer P (2002) Fetal hydantoin syndrome with unilateral atypical cleft hand: additional evidence for vascular disruption. *Genet Couns* **13:**157–161.

Dierks EA, Stams KR, Lim HK, Cornelius G, Zhang H, and Ball SE (2001) A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. *Drug Metab Dispos* **29:**23–29.

Donald LP, Gary ML, and George SK (2001) *Introduction to Spectroscopy*, Brooks/Cole, Belmont, CA.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, and Kessler RC (1998) Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. *JAMA* **280:**1569–1575.

Fan B, Bartlett MG, and Stewart JT (2002) Determination of lamivudine/stavudine/efavirenz in human serum using liquid chromatography/electrospray tandem mass spectrometry with ionization polarity switch. *Biomed Chromatogr* **16:**383–389.

Guengerich FP (1997) Role of cytochrome P450 enzymes in drug-drug interactions. *Adv Pharmacol* **43:**7–35.

Guitton J, Buronfosse T, Desage M, Flinois JP, Perdrix JP, Brazier JL, and Beaune P (1998) Possible involvement of multiple human cytochrome P450 isoforms in the liver metabolism of propofol. *Br J Anaesth* **80:**788–795.

Henderson L, Yue QY, Bergquist C, Gerden B, and Arlett P (2002) St John's wort (*Hypericum perforatum*): drug interactions and clinical outcomes. *Br J Clin Pharmacol* **54:**349–356.

Iwata H, Tezuka Y, Kadota S, Hiratsuka A, and Watabe T (2005) Mechanism-based inactivation of human liver microsomal CYP3A4 by rutaecarpine and limonin from Evodia fruit extract. *Drug Metab Pharmacokinet* **20:**34–45.

Kaplowitz N (1997) Hepatotoxicity of herbal remedies: insights into the intricacies of plant-animal warfare and cell death. *Gastroenterology* **113:**1408–1412.

Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, and Mitchell AA (2002) Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. *JAMA* **287:**337–344.

Kim H and Liu KH (2007) Screening for inhibitory effect on nine CYP isoforms by 20 herbal medications. *Kor J Life Sci* **17:**334–339.

Kim MJ, Kim H, Cha IJ, Park JS, Shon JH, Liu KH, and Shin JG (2005) High-throughput screening of inhibitory potential of nine cytochrome P450 enzymes in vitro using liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* **19:**2651–2658.

Lee MS, Lim HJ, Lee MS, and Jang HS (2005) Perceptions, knowledge and misuse of an oriental herbal drug: a survey of 608 Korean female nursing college students. *Complement Ther Clin Pract* **11:**200–204.

Li Lin A, Shangari N, Chan TS, Remirez D, and O'Brien PJ (2006) Herbal monoterpene alcohols inhibit propofol metabolism and prolong anesthesia time. *Life Sci* **79:**21–29.

Mills E, Montori VM, Wu P, Gallicano K, Clarke M, and Guyatt G (2004) Interaction of St John's wort with conventional drugs: systematic review of clinical trials. *BMJ* **329:**27–30.

Park TJ, Park YS, Lee TG, Ha H, and Kim KT (2003) Inhibition of acetylcholine-mediated effects by borneol. *Biochem Pharmacol* **65:**83–90.

Segel IH (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems,* Wiley, New York.

Shin JG, Soukhova N, and Flockhart DA (1999) Effect of antipsychotic drugs on human liver cytochrome P-450 (CYP) isoforms in vitro: preferential inhibition of CYP2D6. *Drug Metab Dispos* **27:**1078–1084.

Sneyd JR, Simons PJ, and Wright B (1994) Use of proton NMR spectroscopy to measure propofol metabolites in the urine of the female Caucasian patient. *Xenobiotica* **24:**1021–1028.

Suchard JR, Suchard MA, and Steinfeldt JL (2004) Physician knowledge of herbal toxicities and adverse herb-drug interactions. *Eur J Emerg Med* **11:**193–197.

Tabanca N, Kirimer N, Demirci B, Demirci F, and Baser KH (2001) Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phrygia* and the enantiomeric distribution of borneol. *J Agric Food Chem* **49:**4300–4303.

Turpeinen M, Nieminen R, Juntunen T, Taavitsainen P, Raunio H, and Pelkonen O (2004) Selective inhibition of CYP2B6-catalyzed bupropion hydroxylation in human liver microsomes in vitro. *Drug Metab Dispos* **32:**626–631.

Ueng YF, Don MJ, Peng HC, Wang SY, Wang JJ, and Chen CF (2002) Effects of Wu-chu-yu-tang and its component herbs on drug-metabolizing enzymes. *Jpn J Pharmacol* **89:**267–273.

Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, and Desta Z (2003) The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther* **306:**287–300.

Yin OQ, Tomlinson B, Waye MM, Chow AH, and Chow MS (2004) Pharmacogenetics and herb-drug interactions: experience with *Ginkgo biloba* and omeprazole. *Pharmacogenetics* **14:**841–850.

Address correspondence to: Dr. Kwang-Hyeon Liu, Department of Pharmacology and PharmacoGenomics Research Center, Inje University College of Medicine, Busan 614-735, South Korea. E-mail: dstlkh@inje.ac.kr