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Original Article

Effects of Korean traditional herbal formula for common cold on the activities of human CYP450 isozymes

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Objectives: Most drug interactions are attributed to the inhibition or induction of the activity of cytochrome P450s (CYP450). Although the regulation of CYP450s by drugs has been widely reported, there have been few studies on influence of traditional herbal formulas on the drug-metabolizing enzymes. Because herbal formulas have been used traditionally to treat various diseases and because herb-drug interactions are crucial factors determining therapeutic efficacies, a systematic evaluation of the effects of herbal formulas is important.
Methods: The effects of *Galgeun-tang* (GGT, *gegen tang*), *Gumiganghwal-tang* (GMGHT, *jiuweiqianghuo tang*), *Insampaedok-san* (ISPDS, *renshenbaidu powder*), *Samsoeum* (SSE, *shensu drink*), *Socheongryong-tang* (SCRT, *xiaoqinglong-tang*) and *Sosiho-tang* (SSHT, *xiaochaihu tang*) that are traditional herbal formulas used to treat common cold, on drug-metabolizing enzymes were evaluated through an in vitro CYP3A4, CYP2D6, CYP2C19 and CYP2E1 inhibition assay to assess its interaction potential with synthetic drugs. The inhibitory effects of herbal formulas were characterized with IC_{50} values.
Results: These six herbal formulas inhibited the activities of CYP3A4, 2C19, 2D6 and 2E1, in a concentration-dependent manner. Among the six herbal formulas, GGT critically inhibited CYP2C19, CYP2D6 and CYP2E1. GMGHT also inhibited CYP2D6 and CYP2E1 to a greater extent than the other CYP450 isozymes. Additionally, SSE and SSHT may change the effects of medicines that depend primarily on the CYP2C19 and CYP2E1 pathways. On the other hand, ISPDS and SCRT were not inhibited CYP3A4, CYP2C19, CYP2D6 and CYP2E1-mediated metabolism.
Conclusions: These findings provide useful information regarding the safety and effectiveness of herbal formulas.

Key Words : Cytochrome P450s, Herb-drug interactions, Herbal formulas

Introduction

Cytochrome P450s (CYP450), the most important drug-metabolizing enzyme system in humans, are expressed predominantly in the liver.[1] Since herbal formulas modulate the metabolism of drugs by controlling the activities of specific CYP450 isozymes, herb-drug interactions can occur when herbal formulas and synthetic drugs are co-administered.[2] In recent years, many studies reported that co-administration of herbal medicine and synthetic drug can lead to herb-drug interactions through regulation of the drug-metabolizing enzymes.[3] Although several studies have evaluated the risk and safety for combination of herb and drug, study on effect of herbal formulas on the drug-metabolizing enzyme CYP450s are still rare.

Among the traditional herbal formulas, *Galgeun-tang* (GGT, *gegen tang*, *kakkon-to*), *Gumiganghwal-tang*

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(GMGHT, *jiuweiqianghuo tang*, *kumi-kyokatsu-to*), *Insampaedok-san* (ISPDS, *renshenbaidu powder*, *ninjin-haidoku-san*), *Samsoeum* (SSE, *shensu drink*, *jinsoin*), *Socheongryong-tang* (SCRT, *xiaoqinglong-tang*, *sho-seiryu-to*) and *Sosiho-tang* (SSHT, *xiaochaihu tang*, *shosaika-to*) are frequently used to treat common cold in Korea.[4,5] These herbal formulas composed of different crude herbs (Table 1) and commonly exhibits various biochemical activities such antipyretic[6-11] and anti-inflammatory[12] effects in common. In addition, GGT has antioxidant[13] and antivirus[14] effects, and SSE and SCRT have antiallergic[15] and antiasthma[16] effects. SCRT also exhibits biological activities such as antiobesity and improvement of lung damage[16] as well as induction of CYP2B, CYP2C and CYP3B activation effects.[17] SSHT has immunoregulation, anti-hepatitis, antivirus and antioxidant effects.[18] These herbal formulas can be used in combination with conventional drugs, and thus herb-drug interactions should be considered for proper administration of herb and drug. Therefore, we investigated the possible effects of six herbal

Table 1. The Compositions of Herbal Formulas

Crude drugs	Amount (g)					
	Galgeun-tang	*Gumiganghwal-tang*	*Insampaedok-san*	*Samsoeum*	*Socheongryong-tang*	*Sosiho-tang*
Puerariae Radix	9.00			3.75		
Cinnamomi Ramulus	6.00				3.75	
Ephedrae Herba	6.00				5.63	
Paeoniae Radix alba	6.00				5.63	
Glycyrrhizae Radix	6.00	1.88	3.75	2.81	3.75	1.88
Zingiberis Rhizoma	6.00		3.75	3.75		3.75
Zizyphi Fructus	5.00			3.75		3.75
Osterici Radix		5.63	3.75			
Saposhnikoviae Radix		5.63				
Cnidii Rhizoma		4.50	3.75			
Angelicae Dahuricae Radix		4.50				
Atractylodis Rhizoma		4.50				
Scutellariae Radix		4.50				3.75
Rehmanniae Radix		4.50				
Asiasari Radix et Rhizoma		1.88			3.75	
Bupleuri Radix			3.75			11.25
Ginseng Radix			3.75	3.75		3.75
Pinelliae Tuber				3.75	5.63	7.50
Angelicae Gigantis Radix						
Citri Unshius Pericarpium				2.81		
Magnoliae Cortex						
Platycodonis Radix			3.75	2.81		
Aurantii Fructus				2.81		
Zingiberis Rhizoma					3.75	
Poria Sclerotium			3.75	3.75		
Cinnamomi Cortex						
Perillae Herba				3.75		
Anthrisci Radix			3.75	3.75		
Schizandrae Fructus					5.63	
Araliae Cordatae Radix			3.75			
Menthae Herba			3.75			
Total (g)	44.00	37.52	41.25	41.24	37.52	35.63
Yield (%)	12.60	22.76	24.30	12.60	21.70	16.37

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Table 2. The Contents of Marker Compounds in Herbal Formulas

Compound	Contents in extract (mg/g)											
	Galgeun-tang		*Gumiganghwal-tang*		*Insampaedok-san*		*Samsoeum*		*Socheongryong-tang*		*Sosiho-tang*	
Puerarin	10.26	0.030					5.30	0.026				
Albiflorin	2.54	0.010							2.123	0.010		
Daidzin	1.94	0.012					1.25	0.012				
Paeoniflorin	3.54	0.012							12.224	0.016		
Liquiritin	6.60	0.084	1.78	0.007	2.33	0.022	2.30	0.023	3.131	0.034	2.80	0.04
Glycyrrhizin	12.13	0.045	1.87	0.001	3.28	0.008	4.00	0.040	2.025	0.006	2.30	0.010
Cinnamic acid	2.00	0.021							0.338	0.000		
Cinnamaldehyde	7.26	0.055							0.236	0.002		
Ferulic acid			1.69	0.001	0.72	0.002						
Baicalin			37.90	0.267							60.90	0.040
Baicalein			0.26	0.007								
Wogonin			0.03	0.000								
Naringin					9.88	0.033	10.45	0.076				
Hesperidin					1.42	0.034	5.69	0.081				
Neohesperidin					6.82	0.028	5.98	0.031				
Gallic acid									0.993	0.004		
Benzoic acid									0.974	0.013		
Isoliquiritin									0.240	0.000		
Coumarin									0.215	0.000		
6-Gingerol									0.456	0.001		
Schizandrin									0.596	0.003		
Methyl eugenol									0.123	0.000		

The data are presented as means±SD from three independent experiments in triplicate.

formulas on the four major human CYP450 isozymes (CYP3A4, CYP2D6, CYP2C19 and CYP2E1) by an *in vitro* CYP450 isozyme inhibition assay.

Materials and methods

1. Chemicals and materials

Vivid®CYP450 Screening Kits (Vivid® CYP3A4 Green, Vivid® CYP2D6 Blue, Vivid® CYP2C19 Blue, and Vivid® CYP2E1 Blue) were purchased from Invitrogen Co. (Camarillo, CA, USA). The kits use di(benzyloxymethoxy)fluorescein (DBOMF) as a substrate for CYP3A4 and 7-ethoxy-methyloxy-3-cyanocoumarin (EOMCC) as a substrate for CYP2D6, CYP2C19 and CYP2E1. Ketoconazole, quinidine, miconazole, and sodium diethyldithiocarbamate trihydrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2. Preparation of herbal formula extracts

The herbs of each herbal formulation (Table 1) were chopped, mixed and extracted in water at 100℃ for 120 min in an herb extractor (COSMOS660, Kyung Seo Machine Co., Incheon, Korea) and then filtered. All herbs were taxonomically confirmed by Professor Je-Hyeun Lee, Dongguk University, Korea. Voucher specimens have been deposited at the Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine. The extracts were lyophilized and powdered extracts were stored at 4℃. The yields of herbal formula extracts are shown in Table 1. The HPLC profile of each extract was is same as in the previous study announced (Table 2).[18-23]

3. Cytochrome P450 isozyme assay

The assays were performed using the Vivid® CYP450 Screening Kits with the protocol provided by the manufacturer. The Vivid® CYP450 Screening Kits are designed to assess the metabolic activity and inhibition of the predominant human CYP450 isozymes involved in hepatic drug metabolism: CYP3A4, CYP2D6, CYP2C19 and CYP2E1. Vivid® Substrate and Fluorescent Standards were reconstituted, and a standard curve was prepared. A test sample of 40 μL diluted in solvent, a positive inhibition control (a compound that inhibits the respective CYP450 isozyme) or a solvent control was added to each well. The solutions were mixed after adding 50 μL of Master Pre-Mix containing P450 BACULOSOMES in Vivid® CYP450 Reaction Buffer and Regeneration System (consisting of glucose-6-phosphate and glucose-6-phosphate dehydrogenase), and the plate was incubated for 20 min to allow the samples to

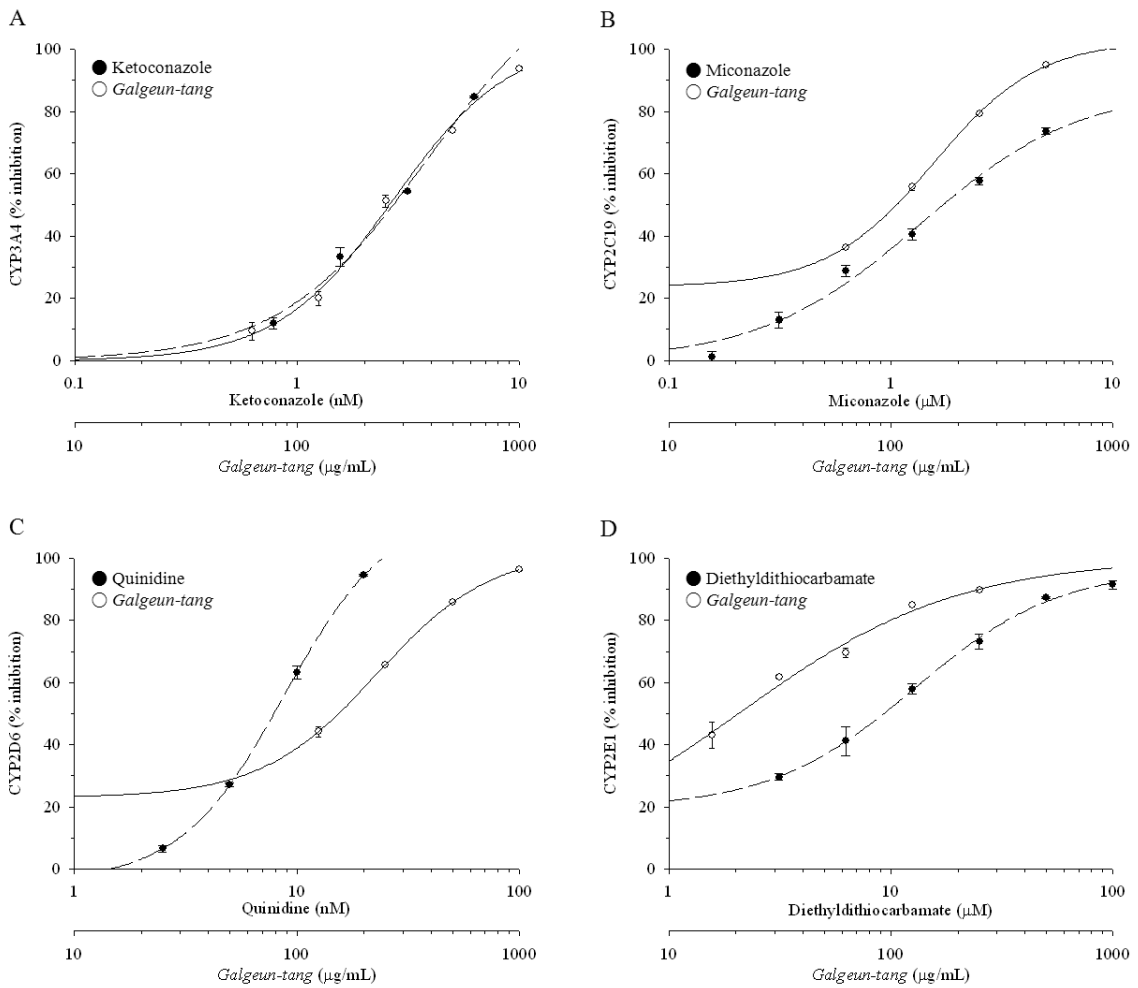

Fig. 1. Effects of *Galgeun-tang* on the CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established *in vitro*. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

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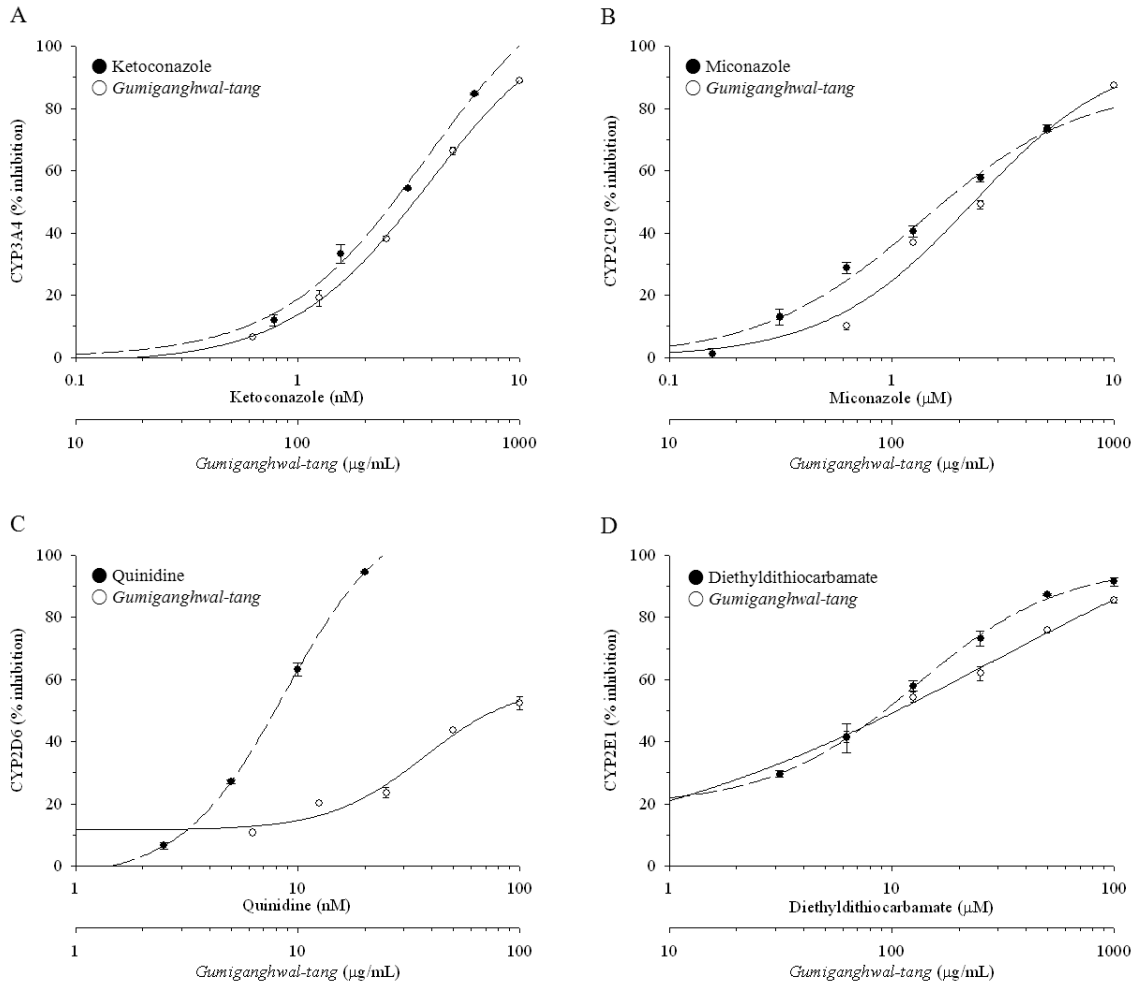

Fig. 2. Effects of *Gumiganghwal-tang* on the CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established *in vitro*. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

interact with the CYP enzymes. After pre-incubation, the reaction was started by adding 10 μL of Vivid® Substrate and $NADP^+$. The Regeneration System converts $NADP^+$ into NADPH, which is required to start the CYP450 reaction. The enzymatic reaction is initiated by the addition of a mixture of $NADP^+$ and the appropriate Vivid® Substrate. The fluorescence intensity was measured using an EnVision2103 Multilabel Reader (PerkinElmer Inc., MA, USA) for 15 min at excitation and emission wavelengths of 485 and 535 nm for CYP3A4. For CYP2D6, CYP2C19 and CYP2E1, the fluorescence intensity was measured for 60 min at excitation and emISPDSion wavelengths of 405 and 450 nm, respectively.

The percent inhibition (%) was obtained by the following equation: % Inhibition = $[1 - (S_1 - S_0)/(C_1 - C_0)]$ 100, where C_1 is the fluorescence of the control after incubation, C_0 is the initial fluorescence of the control, S_1 is the fluorescence of the test

sample after incubation, and S_0 is the initial fluorescence of the test sample in the linear section.

The background fluorescence of herbal formulas was corrected by subtracting the values obtained from incubation without substrates. The CYP450s inhibition of each sample was in term of IC_{50}, as calculated from the log-dose inhibition curve. Data were expressed as the meanSEM (n = 3). Ketoconazole, miconazole, quinidine and sodium diethyldithiocarbamate trihydrate were used as positive controls for CYP3A4, CYP2C19, CYP2D6 and CYP2E1, respectively.

Results

To evaluate whether six Korean traditional herbal formulas (GGT, GMGHT, ISPDS, SSE, SCRT and SSHT) can influence activities of CYP3A4, CYP2C19, CYP2D6 and CYP2E1, CYP450 isozyme inhibition assay was conducted. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate used as positive controls inhibited CYP3A4, CYP2C19, CYP2D6 and CYP2E1 in a dose-dependent manner, with IC_{50} values of 1.17 to 2.70 nM, 0.25 to 1.54 M, 4.33 to 7.90 nM and 5.87 to 9.83 μM, respectively.

1. Effects of GGT on the activities of CYP450s

As demonstrated by the data in Fig. 1 and Table 3, GGT exhibited the most potent inhibition of CYP2E1, with an IC_{50} value of 20.57 μg/mL. In contrast, GGT exerted inhibition of CYP3A4, CYP2C19 and CYP2D6, with IC_{50} values of 259.6, 105.6 and 151.0 μg/mL, respectively.

2. Effects of GMGHT on the activities of CYP450s

GMGHT showed the most potent inhibition of CYP2D6, with an IC_{50} value of 80.15 μg/mL (Fig. 2 and Table 3). Additionally, GMGHT inhibited CYP3A4, CYP2C19 and CYP2E1, with respective IC_{50} values of 334.0, 255.8, and 106.0 μg/mL.

3. Effects of ISPDS on the activities of CYP450s

As shown in Fig. 3 and Table 3, ISPDS inhibited of CYP3A4 and CYP2C19, with similar IC_{50} values of 286.6 and 231.6 μg/mL, respectively. Additionally, ISPDS relatively weak inhibition of CYP2D6 and CYP2E1, with IC_{50} values of 695.5 and 958.1 μg/mL, respectively.

4. Effects of SSE on the activities of CYP450s

As presented in Fig. 4 and Table 3, SSE potently repressed the activity of inhibited CYP2C19, with an IC_{50} value of 84.38 μg/mL, while having no effect on CYP2D6, with an IC_{50} value of over 1000 μg/mL. For CYP3A4 and CYP2E1, SSE exhibited inhibitory activities, with IC_{50} values of 308.9 and 137.0 μg/mL, respectively.

Table 3. IC_{50} Values (μg/mL) of Herbal Formulas on CYP450 Isozyme Activities

Herbal formula	CYP3A4	CYP2C19	CYP2D6	CYP2E1
Galgeun-tang	259.6	105.6	151.0	20.57
Gumiganghwal-tang	334.0	255.8	80.15	106.0
Insampaedok-san	286.6	231.6	695.5	958.1
Samsoeum	308.9	84.38	> 1000	137.0
Socheongryong-tang	596.3	578.9	378.2	270.2
Sosiho-tang	373.8	35.44	497.4	193.1
Positive Control	1.17-2.70 nM	0.25-1.54 μ M	4.33-7.90 nM	5.87-9.83 μ M

Ketoconazole, miconazole, quinidine and diethyldithiocarbamate were used as positive controls for CYP3A4, CYP2C19, CYP2D6 and CYP2E1, respectively.

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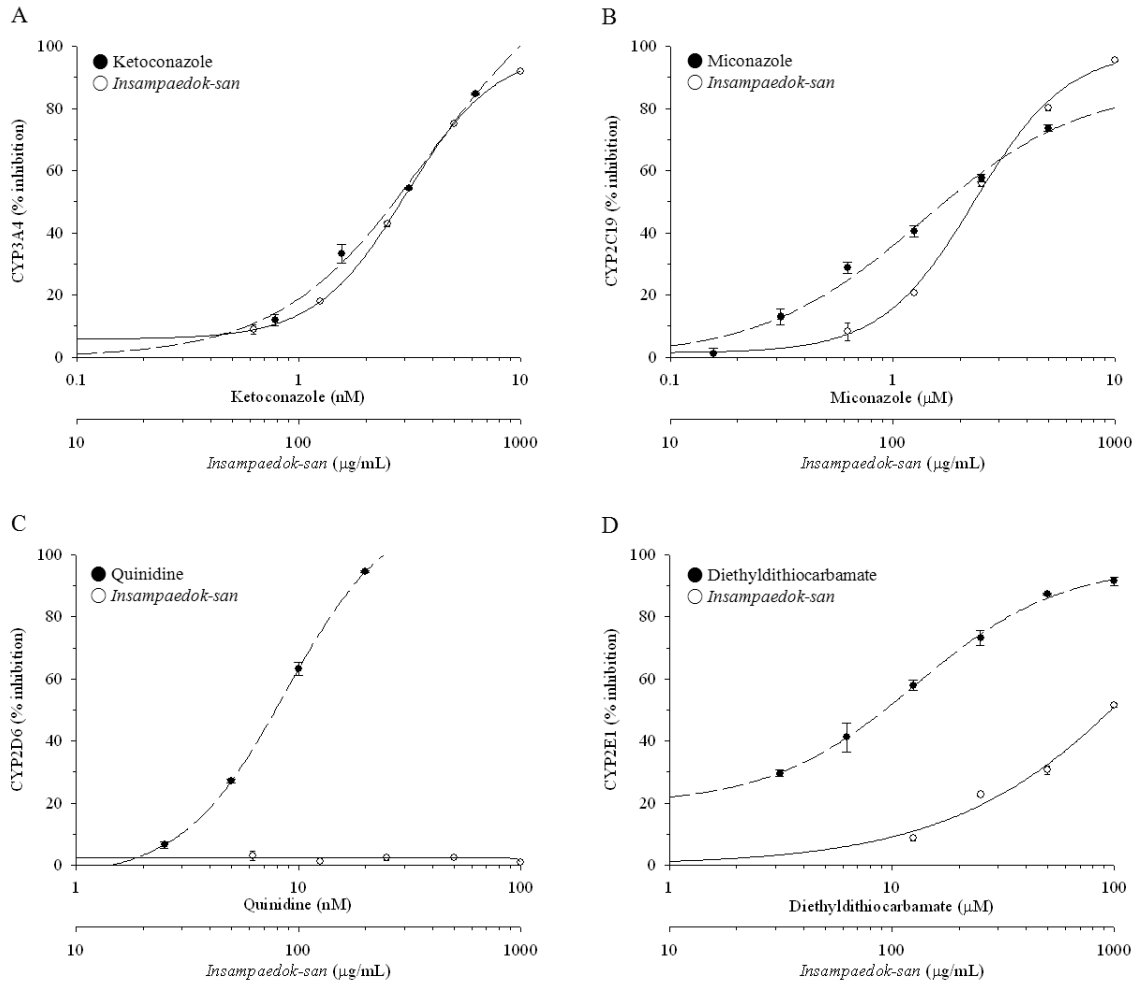

Fig. 3. Effects of *Insampaedok-san* on the CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established in vitro. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

5. Effects of SCRT on the activities of CYP450s

As demonstrated by the data in Fig. 5 and Table 3, SCRT showed the most potent inhibition of CYP2E1 (IC_{50} = 270.2 μg/mL), followed by CYP2D6 (IC_{50} = 378.2 μg/mL). In addition, SCRT displayed similar inhibition against CYP3A4 and CYP2C19, with IC_{50} values of 596.3 and 578.9 μg/mL, respectively.

6. Effects of SSHT on the activities of CYP450s

As shown in Fig. 6 and Table 3, SSHT exhibited the maximum inhibition of CYP2C19, with an IC_{50} value of 35.44 μg/mL. SSHT also inhibited CYP3A4, CYP2D6 and CYP2E1, with IC_{50} values of 373.8, 497.4 and 193.1 μg/mL, respectively.

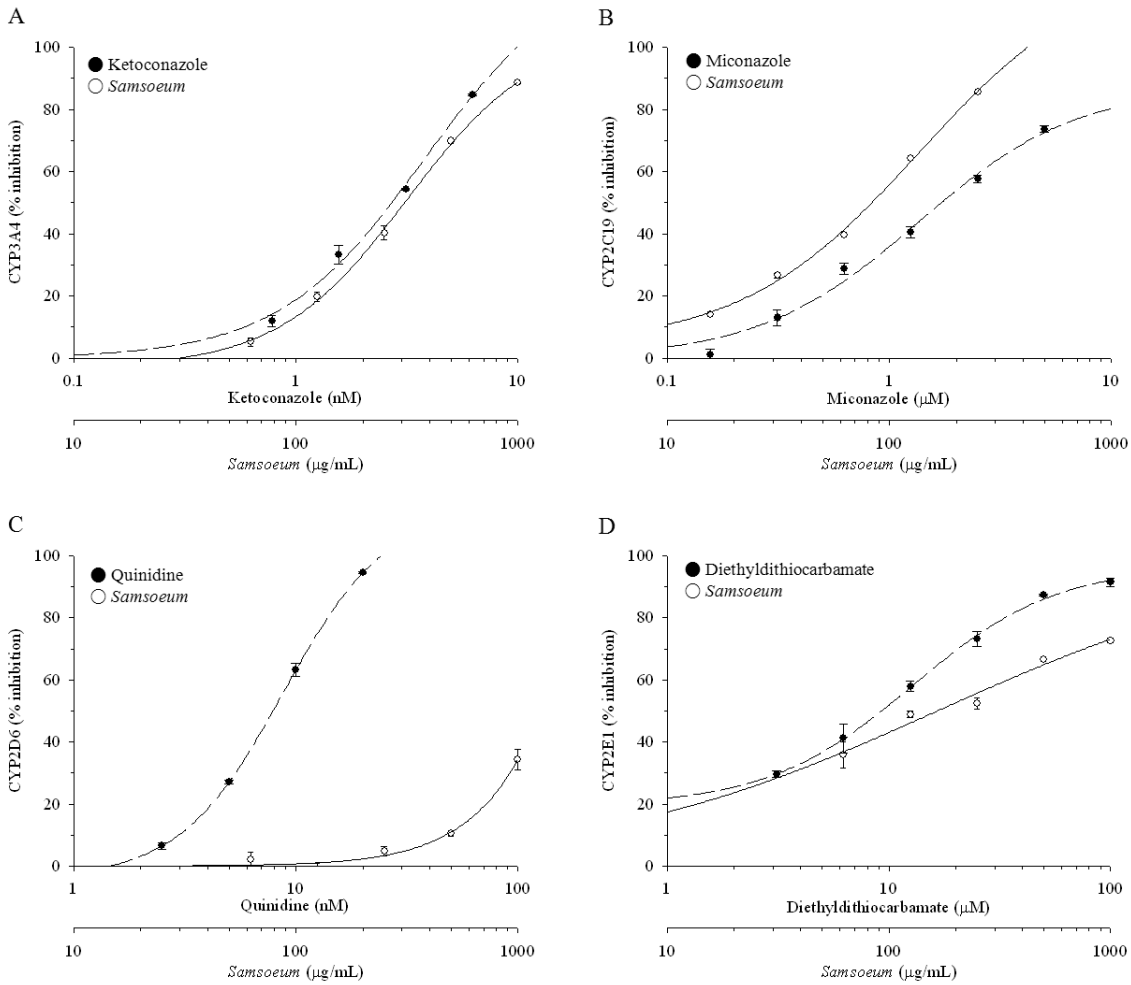

Fig. 4. Effects of *Samsoeum on the* CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established in vitro. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

Discussion

In recent years, many researchers have shown an increasing interest in herbal medicines, especially traditional herbal formulas, a combination of several different herbs. Although herbal formulas have been used continuously and extensively, there have been few clinical and preclinical data generated on the interactions between herbal formulas and synthetic drugs. CYP450s, drug-metabolizing enzymes in the liver were determined to be heme proteins, can be regulated by a wide variety of xenobiotics, including multiple active constituents of herbs.[24-26] The inhibition of CYP450s results in elevated drug concentrations in tissues, leading to various adverse reactions, particularly for drugs with a low therapeutic index.[27,28] Therefore, it is important to identify the potential herb-drug interactions to prevent adverse effects in patients taking combinations of herbal formulas and conventional drugs. The major

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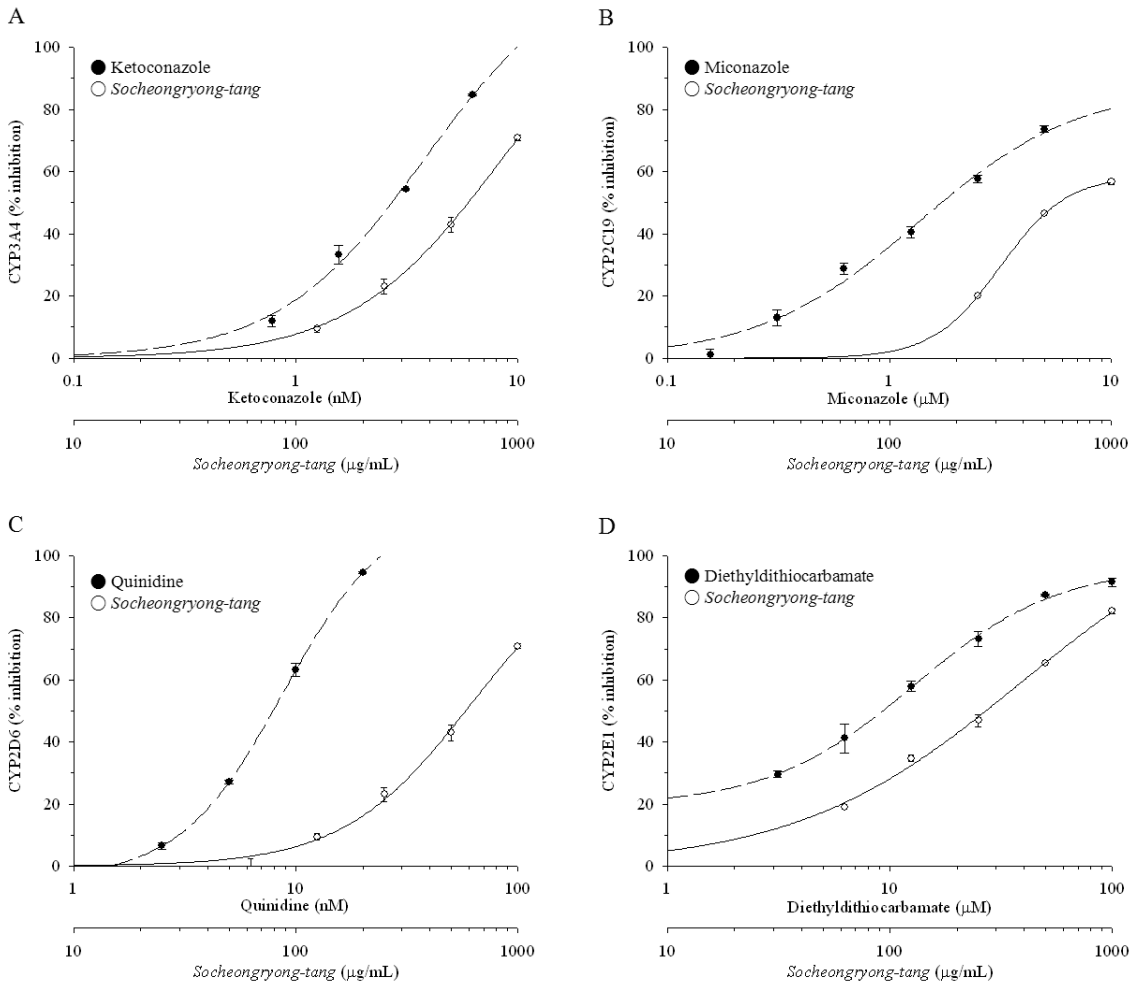

Fig. 5. Effects of *Socheongryong-tang* on the CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established in vitro. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

CYP450 isozymes that are important in human drug metabolisms are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.[25]

GGT, GMGHT, ISPDS, SSE, SCRT and SSHT are traditional herbal formulas used to treat common cold. These herbal formulas are consisted of respective different herbs (Table 1), and contain various active components. To date, several studies reported the influences of herbal extracts or chemicals in herbal formulas on the CYP450s activities. Puerariae Radix and its component puerarin of GGT and SSE were reported to inactivate the human CYP3A, CYP2B1 and CYP2E1.[29] In addition, pseudoephedrine in Ephedrae Herba of GGT and SCRT has inhibitory effects on CYP1A1/2 and CYP2E1 of rat liver microsomes,[30] and ginsenoside Rg1 and Rb1 in Ginseng Radix of ISPDS, SSE and SSHT have inductive effect on CYP1A1 expression in HepG2 cells.[31] Furthermore, GGT and SSHT of the six formulas inhibited the activities of CYP3A4

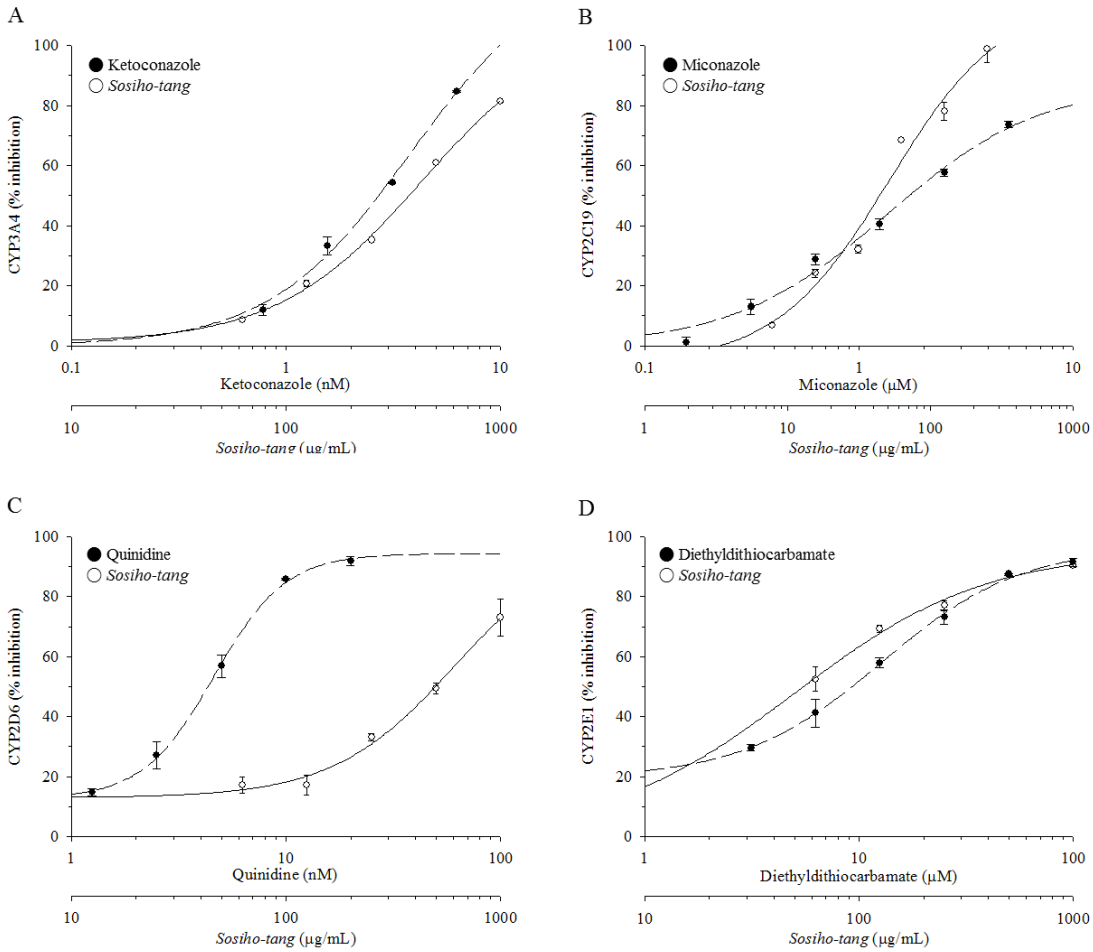

Fig. 6. Effects of *Sosiho-tang* on the CYP3A4 (A), CYP2C19 (B), CYP2D6 (C) and CYP2E1 (D).
The fluorescence-based enzyme assays on CYP450 isozymes were established in vitro. Ketoconazole, miconazole, quinidine and diethyldithiocarbamate was used as positive controls, respectively. Data are presented as mean ± SEM (n = 3).

in a dose-dependent manner but inhibition at 1000 g/mL did not reach 50%.[32] Among the marker compounds of these herbal formulas, glycyrrhizin in GGT, GMGHT, ISPDS, SSE and SSHT have been reported to inhibit the human CYP1A2.[33] Baicalin, containing of GMGHT and SSHT, was reported to increase the liver microsomal CYP1A1, CYP2B1 and CYP2C11 in mice.[34] According to Ueng et al.,[35] baicalein and wogonin in GMGHT have inhibitory effects on hepatic CYP3A4 and CYP2E1 in mice. In addition, it has been reported that the hepatic CYP1A2 protein level was increased by baicalein in mice,[35] and the activity of CYP1A2 and CYP2C19 were inhibited by wogonin in human liver microsomes.[36] Naringin of ISPDS and SSE reduce the CYP1A2 protein level in mice.[37] However, no studies have been conducted on the effects of six traditional herbal formulas used to treat common cold on the activities of human CYP450. Therefore, the present study focused on the effects of GGT, GMGHT, ISPDS, SSE, SCRT and SSHT, six traditional herbal formulas, on the activities of major

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human CYP450 isozymes: CYP3A4, CYP2D6, CYP2C19 and CYP2E1. Our results showed that GGT containing of Puerariae Radix, Cinnamomi Ramulus, Ephedrae Herba and Paeoniae Radix showed the most potent inhibition of CYP2E1. These results might be related to the inhibitory effect of Puerariae Radix on CYP2E1 based on Guerra et al's paper.[29] GMGHT likely interacts with drugs metabolized by CYP2D6 and CYP2E1 rather than the other isozymes. In contrast, ISPDS and SCRT were weak inhibited the activities of CYP3A4, CYP2C19, CYP2D6 and CYP2E1. These results suggest that ISPDS and SCRT are unlikely to inhibit the metabolism of drugs metabolized by CYP3A4, CYP2C19, CYP2D6 and CYP2E1. However, SSE and SSHT inhibited CYP2C19 and CYP2E1 at low concentrations. In addition, it is necessary to pay attention to herb-drug interactions when SSE or SSHT is used in combination with other cold medicine metabolized by CYP2C19.

Conclusions

By now the regulation of CYP450s by drugs has been widely reported, however there have been few studies on influence of traditional herbal formulas on the drug-metabolizing enzymes. Therefore, we investigated influence of six herbal formulas on the activities of CYP3A4, CYP2C19, CYP2D and CYP2E1 by *in vitro* CYP450 isozyme assay. As a result, the possibility of herb-drug interactions should be considered if a patient with colds consumes GGT, GMGHT, SSE or SSHT. Further studies regarding the importance of herb-drug interactions will be required because these reports provide limited information of herbal formulas on the CYP450 activities.

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