Pharmacogn. Mag.
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ORIGINAL ARTICLE

Expression of Cytochrome P450s in the Liver of Rats Administered with *Socheongryong-tang*, a Traditional Herbal Formula

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Submitted: 02-04-2015 Revised: 20-05-2015 Published: 14-07-2016

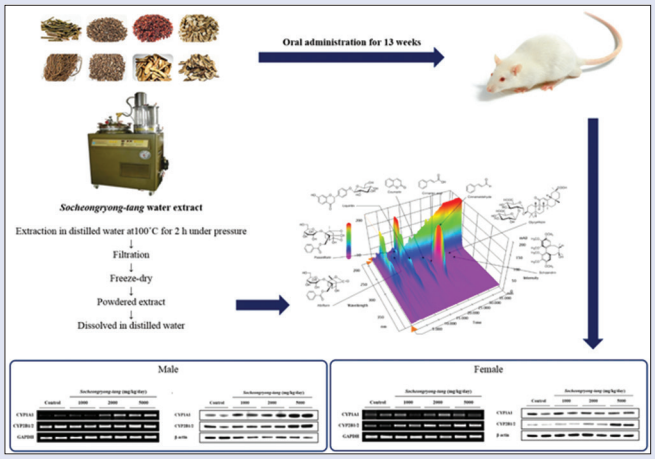
ABSTRACT

Objective: The purpose of this study was to investigate the potential influences of *Socheongryong-tang* (SCRT) on the messenger ribonucleic acid (mRNA) and protein expression of cytochrome P450 (CYP450) *in vivo*. **Materials and Methods:** SCRT was orally administered to either male or female Sprague-Dawley rats once daily at doses of 0, 1000, 2000, or 5000 mg/kg/day for 13 weeks. The mRNA expression of CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) in liver tissues was measured by reverse transcription polymerase chain reaction. And then, the protein expression of CYP1A1 and CYP2B1/2 in liver tissues was analyzed by the Western blot. **Results:** We found no significant influence in the mRNA expression of hepatic CYP1A2, 2C11, 2E1, 3A1, 3A2, and 4A1 after repeated administration of SCRT for 13 weeks. By contrast, the mRNA and protein expression of hepatic CYP1A1 was increased by repeated SCRT treatment in male rats, but not in female rats. The mRNA and protein expression of hepatic CYP2B1/2 in both genders was increased by administration of SCRT. **Conclusion:** A caution is needed when SCRT is co-administered with substrates of CYP2B1/2 for clinical usage. In case of male, an attention is also required when SCRT and drugs metabolized by CYP1A1 are taken together. Our findings provide information regarding the safety and effectiveness of SCRT when combined with conventional drugs.

Key words: Cytochrome P450, herb-drug interactions, metabolism, rat liver, *Socheongryong-tang*

SUMMARY

- Oral administration of *Socheongryong-tang* for 13 weeks did not affect the mRNA expression of hepatic CYP1A2, 2C11, 2E1, 3A1, 3A2, and 4A1
- In male rats, oral administration of *Socheongryong-tang* for 13 weeks induced the mRNA and protein expression of hepatic CYP1A1 and CYP2B1/2
- In female rats, oral administration of *Socheongryong-tang* for 13 weeks induced the mRNA and protein expression of hepatic CYP2B1/2.



Abbreviations used: SCRT: *Socheongryong-tang*, CYP450: Cytochrome P450, HPLC: High performance liquid chromatography, RT-PCR: Reverse transcription polymerase chain reaction.

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DOI: 10.4103/0973-1296.186340

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INTRODUCTION

Herbal medicines have been used in Eastern Asian countries including Korea, Japan, China, and India for the prevention and treatment of various diseases. In recent years, with the increased use of herbal medicines globally, the frequency of co-administration of herbs and conventional drugs has also increased.[1] Cytochrome P450s (CYP450s), the most important drug-metabolizing enzymes in the liver, metabolize most clinical drugs.[2] The bioavailability of drugs can be enhanced or reduced by induction or inhibition of CYP450s when herbal medicines are co-administered with conventional drug[3] because of the potential of herbal medicines to induce or inhibit CYP450s.[4] In particular, herbal formulas have the potential to interact with various classes of drugs because they are complex mixtures that include multiple active herbs and ingredients. However, there is insufficient information regarding specific herb-drug interactions.

Socheongryong-tang (SCRT, *xiaoqinglong-tang*, *sho-seiryu-to*) is a traditional oriental herbal formula comprising eight medicinal herbs [Table 1]. SCRT has been used for treating the common cold,[5] and reported pharmacological activities including antiallergy,[6] antiasthma,[7]

Cite this article as: Jin SE, Ha H, Seo CS, Shin HK, Jeong SJ. Expression of cytochrome P450s in the liver of rats administered with *Socheongryong-tang*, a traditional herbal formula. Phcog Mag 2016;12:211-8.

Table 1: Composition of SCRT

Scientific name	Latin name	Family	Amount (g)	Origin
Ephedra sinica	Ephedrae Herba	Ephedraceae	5.625	China
Paeonia lactiflora	Paeoniae Radix	Paeoniaceae	5.625	Uiseong, Korea
Schisandra chinensis	Schisandrae Fructus	Schisandraceae	5.625	Samcheok, Korea
Pinellia ternata	Pinelliae Tuber	Araceae	5.625	China
Asiasarum sieboldii	Asiasari Radix	Aristolochiaceae	3.750	China
Zingiber officinale	Zingiberis Rhizoma Crudus	Zingiberaceae	3.750	Ulsan, Korea
Cinnamomum cassia	Cinnamomi Ramulus	Lauraceae	3.750	Vietnam
Glycyrrhiza uralensis	Glycyrrhizae Radix et Rhizoma	Leguminosae	3.750	China
Total			37.500	

Table 2: Oligonucleotide sequences of primers used for the RT-PCR analysis and PCR conditions

		Primer sequence (5′→3′)	Product size (bp)	PCR conditions				
				Predenaturation	Denaturation	Annealing	Extension	Elongation
CYP1A1	F	CTG GTT CTG GAT ACC CAG CTG	331	95°C, 5 min	95°C, 10 sec	57°C, 30 sec	72°C, 30 sec	72°C, 7 min
	R	CCT AGG GTT GGT TAC CAG G			(35 cycles)			
CYP1A2	F	GTC AGC AGT ATG GGG ACG TG	272		95°C, 10 sec	63°C, 30 sec	72°C, 30 sec	
	R	TGA TGT GGG GTC TGA GGC TA			(28 cycles)			
CYP2B1/2	F	GAG TTC TTC TCT GGG TTC CTG	549		95°C, 10 sec	62°C, 30 sec	72°C, 30 sec	
	R	ACT GTG GGT CAT GGA GAG CTG			(32 cycles)			
CYP2C11	F	CTG CTG CTG CTG AAA CAC GTG	248		95°C, 10 sec	60°C, 30 sec	72°C, 30 sec	
	R	GGA TGA CAG CGA TAC TAT CAC			(40 cycles)			
CYP2E1	F	TAC CCC ATG AAG CAA CCA GA	205		95°C, 10 sec	57°C, 30 sec	72°C, 30 sec	
	R	CCT GCA GAA AAT GCC TTG AA			(35 cycles)			
CYP3A1	F	ATC CGA TAT GGA GAT CAC	579		95°C, 10 sec	45°C, 30 sec	72°C, 30 sec	
	R	GAA GAA GTC CTT GTC TGC			(35 cycles)			
CYP3A2	F	AGG GAT GGA CCT GCT TTC AG	116		95°C, 10 sec	58°C, 30 sec	72°C, 30 sec	
	R	TGT CCA TGA TGG CAA ACA CA			(35 cycles)			
CYP4A1	F	GGT GAC AAA GAA CTA CAG C	344		95°C, 10 sec	53°C, 30 sec	72°C, 30 sec	
	R	AGA GGA GTC TTG ACC TGC CAG			(35 cycles)			
GAPDH	F	CAA GAT GGT GAA GGT CGG TG	277		95°C, 10 sec	57°C, 30 sec	72°C, 30 sec	
	R	CAC CCC ATT TGA TGT TAG CG			(30 cycles)			

Table 3: Contents of the 8 compounds of SCRT in 0, 1, and 13 weeks by high performance liquid chromatography (*n*=3)

Analyte	0 week			1 week			13 week		
	Mean (mg/g)	SD ×10^{-2}	RSD (%)	Mean (mg/g)	SD ×10^{-2}	RSD (%)	Mean (mg/g)	SD ×10^{-2}	RSD (%)
Albiflorin	1.11	0.67	0.61	1.18	1.99	1.69	1.18	1.44	1.22
Paeoniflorin	11.83	3.51	0.30	12.32	15.72	1.28	12.72	5.42	0.43
Liquiritin	3.07	0.23	0.07	3.48	1.75	0.50	3.46	2.20	0.64
Coumarin	0.98	0.41	0.42	1.02	0.10	0.10	1.06	0.30	0.28
Cinnamic acid	0.23	0.30	1.30	0.25	0.02	0.07	0.25	0.03	0.10
Cinnamaldehyde	1.58	0.95	0.61	1.53	0.51	0.34	1.63	0.52	0.32
Glycyrrhizin	0.31	0.11	0.35	0.33	0.86	2.59	0.33	0.95	2.88
Schizandrin	0.23	0.22	0.94	0.22	0.19	0.86	0.24	0.55	2.27

Table 4: Hepatic CYP450s mRNA expression in male or female rats treated with SCRT (5000 mg/kg/day)

	Male			Female		
	Control	5000 (mg/kg/day)		Control	5000 (mg/kg/day)	
CYP1A1	0.085±0.008	0.158±0.033	*	0.082±0.010	0.113±0.011	
CYP1A2	0.493±0.027	0.593±0.039		0.679±0.038	0.778±0.015	
CYP2B1/2	0.480±0.043	0.803±0.038	**	0.372±0.073	0.707±0.035	**
CYP2C11	0.580±0.036	0.623±0.037		0.057±0.002	0.051±0.002	
CYP2E1	0.720±0.023	0.701±0.024		0.836±0.031	0.783±0.021	
CYP3A1	0.667±0.012	0.733±0.040		0.492±0.046	0.449±0.057	
CYP3A2	0.814±0.030	0.797±0.032		0.412±0.016	0.405±0.050	
CYP4A1	0.266±0.009	0.256±0.011		0.639±0.031	0.563±0.014	

Male or female rats were treated orally with SCRT at 5000 mg/kg/day or vehicle alone, once a day 13 weeks. The mRNA expression was analyzed by RT-PCR. The mRNA levels of hepatic CYP450s were normalized to that of GAPDH. Values were expressed as mean±SEM (*n*=8). *P<0.05 and **P<0.01 versus vehicle control group

and improvement of lung damage.[8] Although SCRT exerts these biological effects, there is no information regarding the effects of SCRT on the expression of hepatic CYP450s.

In the previous study, we demonstrated that SCRT has comparatively weak inhibitory activity on human CYP3A4, 2C19, 2D6, and 2E1 *in vitro*.[9] Further studies are required to clarify the effects of SCRT on the expression of CYP450s *in vivo* and to provide greater insight into the guidelines for the rational administration of this herbal formula. Therefore, we investigated the influences of SCRT on the messenger ribonucleic acid (mRNA) expression of CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) in Sprague-Dawley (SD) rat liver.

MATERIALS AND METHODS

Plant materials

Eight raw materials of SCRT were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Republic of Korea) as dried herb. Each herbal

medicine was authenticated taxonomically by Professor Je-Hyun Lee, College of Oriental Medicine, Dongguk University, Gyeongju, Republic of Korea. A voucher specimen (2012–KE13–1 ~ KE13–8) has been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine.

Chemicals and reagents

Albiflorin, paeoniflorin, cinnamaldehyde, glycyrrhizin, and schizandrin were purchased from Wako (Osaka, Japan). Coumarin and cinnamic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). Liquiritin was purchased from NPC BioTechnology Inc. (Yeongi, Republic of Korea). The purity of these compounds was ≥98.0% by high performance liquid chromatography-photodiode array analysis (HPLC-PDA). HPLC-grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA), and glacial acetic acid was obtained from Junsei (Tokyo, Japan).

Preparation of *Socheongryong-tang* decoction

A SCRT decoction is consisting of 8 medicinal herbs as shown Table 1 were prepared in K-herb Research Center, Korea Institute of Oriental Medicine (Daejeon, Republic of Korea). Namely, the mixtures (72.0 kg. i.e., about 1920 times of composition of single dose) of 8 medicinal herbs were extracted in distilled water (720 L) at 100°C for 2 h under pressure (98 kPa) by an electric extractor (COSMOS–660, Kyung Seo Machine Co., Incheon, Republic of Korea). The extract solution was filtered using a standard sieve (no. 270; 53 μm; Chung Gye Sang Gong Sa, Seoul, Republic of Korea) and freeze-dried (IlShinBioBase, Dongducheon, Republic of Korea). The amount of lyophilized extract was about 9.3 kg (12.9%). The powdered extracts were used in the experiment and stored at 4°C.

High performance liquid chromatography analysis of *Socheongryong-tang*

The SCRT extract was analyzed for quality evaluation at 0, 1, and 13 weeks using the Shimadzu Prominence LC-20A series HPLC system (Kyoto, Japan). This HPLC system is consisting of the solvent delivery unit (LC-20AT), on-line degasser (DGU-20A_3), column oven (CTO-20A), auto sample injector (SIL-20AC), and PDA detector (SPD-M20A). Data were collected and processed by LC solution software (Version 1.24, Shimadzu, Kyoto, Japan). Column for separation of eight compounds was a Phenomenex Gemini C_{18} column (250 × 4.6 mm; 5 μm, Torrance, CA, USA) and maintained at 40°C. The mobile phases consisted of 1.0% (v/v) aqueous acetic acid (A) and 1.0% (v/v) acetic acid in acetonitrile (B). The gradient flow was as follows: 15–65% B for 0–35 min, 65–100% B for 35–45 min, 100% B for 45–50 min, and 100–15% B for 50–55 min. The re-equilibrium time was 15 min. The flow rate and injection volume were 1.0 mL/min and 10 μL, respectively. For HPLC simultaneous determination, 200 mg of the lyophilized SCRT sample was dissolved in 20 mL of distilled water. The solution was filtered through a 0.2 μm syringe filter (Woongki Science, Seoul, Republic of Korea) before HPLC injection.

Animals

Each of 64 female and 64 male specific pathogen-free SD rats (5 weeks old upon receipt, Orient Bio, Gyeonggi-do, Seongnam, Republic of Korea) was used after acclimatization for 14 days before initiating the study with an evaluation of health status. The animals were maintained in environmentally controlled rooms at 22 ± 3°C under a relative humidity of 50 ± 20% with a 12 h light-dark cycle and 12–15 air changes per hour.

This study was performed at the Korea Testing and Research Institute and the study protocol was approved by the Institutional Animal Care and Use Committee according to the Guidelines for Toxicity Tests of Drugs and Related Materials (Document No. 2012–86) as prepared by the Korea Food and Drug Administration (KFDA; 2012). These experiments were performed according to the Organization for Economic Cooperation and Development Principles of Good Laboratory Practice (1997).

Experimental groups and treatment

Healthy male and female rats were randomly assigned to eight experimental groups. Each group consisted of 10 rats of each gender. Because the oral administration is the clinically intended route for SCRT, the extract was administered by oral gavage in this study. SCRT (1000, 2000, or 5000 mg/kg/day) dissolved in distilled water was administered orally to rats for 13 weeks. Distilled water was given to the rats in a vehicle control group. At the end of the experimental period (13 weeks), the rats were sacrificed after overnight fasting and liver tissues were removed. Livers were immediately frozen and stored at −81°C.

Total ribonucleic acid extraction and cDNA synthesis

The total RNA was isolated using Trizol reagent (Invitrogen, Camarillo, CA, USA) according to the manufacturer's instructions. The RNA pellet was dissolved in RNase-free water. Total RNA concentration and purity were determined by measuring the absorbance at 260 nm and 280 nm by using NanoDrop 2000 (Thermo Scientific, Rockford, IL, USA). One microgram of total RNA was converted to cDNA using an iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). The obtained cDNA was stored at −20°C or used directly for the reverse transcription polymerase chain reaction (RT-PCR).

Reverse transcription polymerase chain reaction

To confirm the level of mRNA expression in rat liver, eight rats from each group were randomly selected. The level of mRNA expression in rat hepatic tissues was analyzed by RT-PCR. The primer sequences and PCR conditions used for CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, 4A1, and GAPDH amplifications are described in Table 2.[10] All primers were synthesized by Bioneer (Daejeon, Republic of Korea). GAPDH was used as a housekeeping gene for normalization of target gene expression. Reaction mixtures comprised 1 μL of cDNA, 0.1 μL of rTag polymerase, 0.4 μL of 2.5 mMdNTPs, and 0.3 μL of each of forward and reverse primers 10 pmol. Amplicon size and reaction specificity were confirmed by electrophoresis through 1–2% agarose gels stained with loading star (Dynebio, Gyeonggi-do, Seongnam, Republic of Korea). The gels were photographed and the band intensities were quantified using a commercially available ChemiDoc™ XRS+ imaging system (Bio-Rad Laboratories, Hercules, CA, USA).

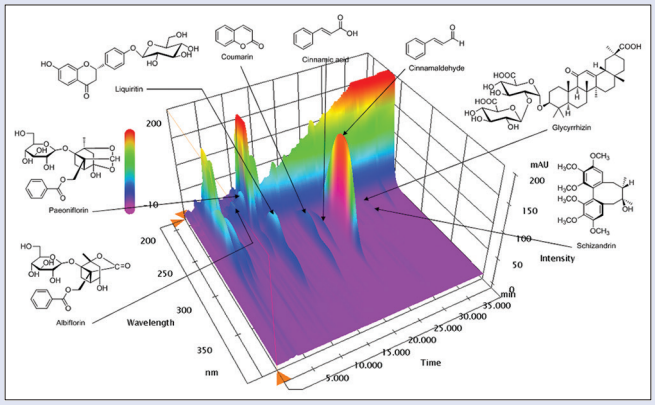

Figure 1: Three-dimensional chromatogram of *Socheongryong-tang* by High performance liquid chromatography-photodiode array

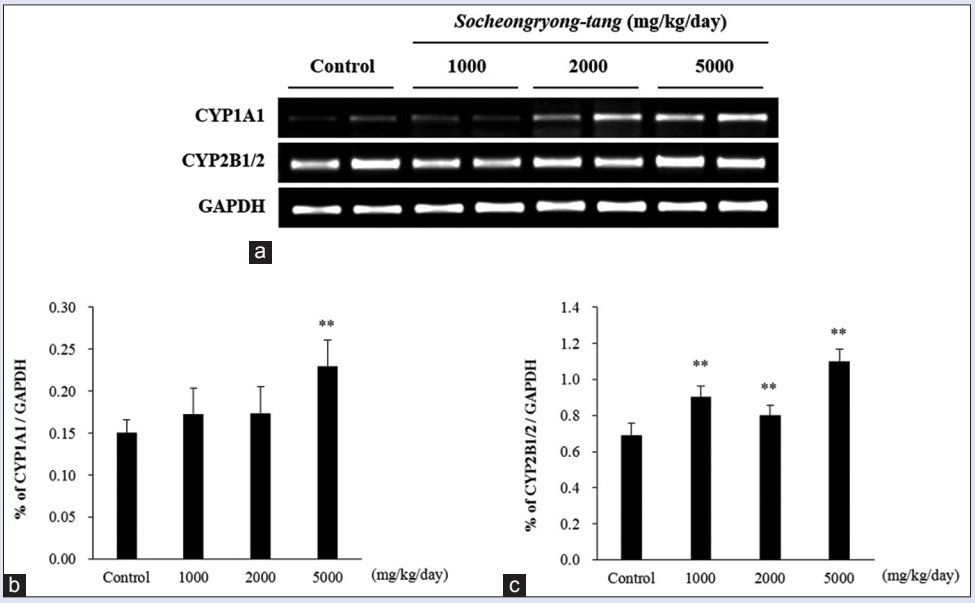

Figure 2: The messenger ribonucleic acid expression of hepatic CYP1A1 and CYP2B1/2 in male Sprague-Dawley rats treated with *Socheongryong-tang*. (a) Adult male rats were treated orally with *Socheongryong-tang* (1000, 2000, or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The messenger ribonucleic acid expression of CYP1A1 and 2B1/2 was analyzed by reverse transcription polymerase chain reaction. The messenger ribonucleic acid levels of hepatic CYP1A1 (b) and CYP2B1/2 (c) were normalized to that of GAPDH. Values express as a mean ± standard deviation ($n = 8$). $^{**}P < 0.01$ versus vehicle control group

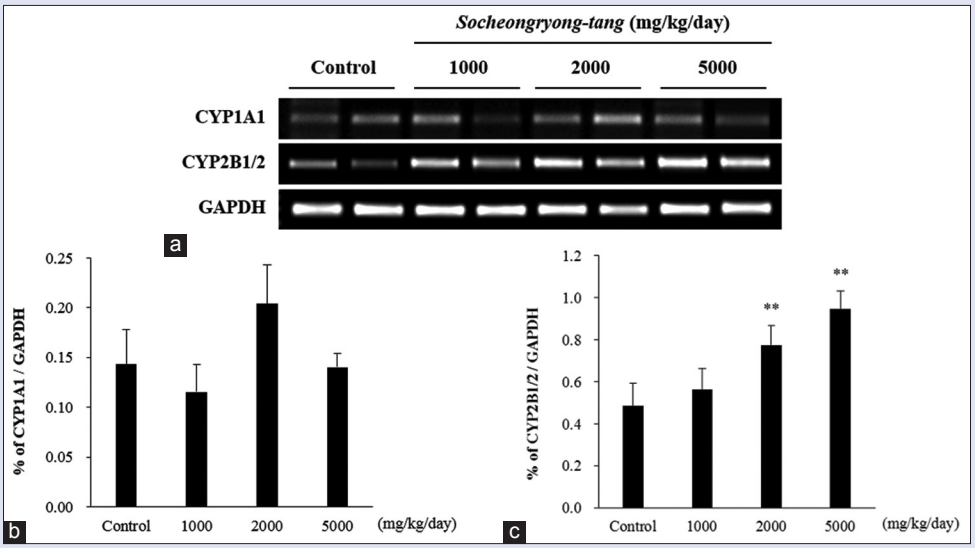

Figure 3: The messenger ribonucleic acid expression of hepatic CYP1A1 and CYP2B1/2 in female Sprague-Dawley rats treated with *Socheongryong-tang*. (a) Adult female rats were treated orally with *Socheongryong-tang* (1000, 2000 or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The messenger ribonucleic acid expression of CYP1A1 and 2B1/2 was analyzed by reverse transcription polymerase chain reaction. The messenger ribonucleic acid levels of hepatic CYP1A1 (b) and CYP2B1/2 (c) were normalized to that of GAPDH. Values express as a mean ± standard deviation ($n = 8$). $^{**}P < 0.01$ versus vehicle control group

Western blot

To confirm the expression profile of CYP1A1 and CYP2B1/2 at the protein level, the level of protein expression in rat hepatic tissues was analyzed by Western blot. Liver tissues were homogenized in CelLytic™ MT Cell Lysis Reagent (Sigma-Aldrich) containing protease inhibitors to obtain extracts of liver tissues. After centrifugation (12,000 g, 4°C, 10 min), the protein concentration in the supernatant was determined using a Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Total proteins (30 μg) were resolved by 4–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were immediately blocked with 5% skim milk in tris-buffered saline containing 0.1% Tween-20 (pH 7.4) (TBST), followed by incubation with primary antibodies against CYP1A1

(Santa Cruz Biotechnology, Santa Cruz, CA, USA), CYP2B1/2 (Santa Cruz Biotechnology), and β-actin (Santa Cruz Biotechnology) at 4°C overnight. After washing three times with TBST, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Jackson Immuno Research, PA, USA) for 1 h at room temperature. The membranes were washed three times with TBST and then developed using an ECL kit. The membranes were photographed using a ChemiDoc™ XRS+ imaging system (Bio-Rad Laboratories).

Statistics

The data were expressed as the mean ± standard deviation ($n = 8$) and were analyzed using GraphPad InStat (GraphPad Software Inc., Version 3.05). One-way analysis of variance was used to detect significant differences between the control and treatment groups. Dunnett's test was used for multiple comparisons. The differences were considered significant at $P < 0.05$.

RESULTS

Quantification of the eight compounds in *Socheongryong-tang*

The established HPLC-PDA method was applied for the simultaneous determination of the eight components at 0, 1, and 13 weeks in SCRT decoction. The retention time of albiflorin, paeoniflorin, liquiritin,

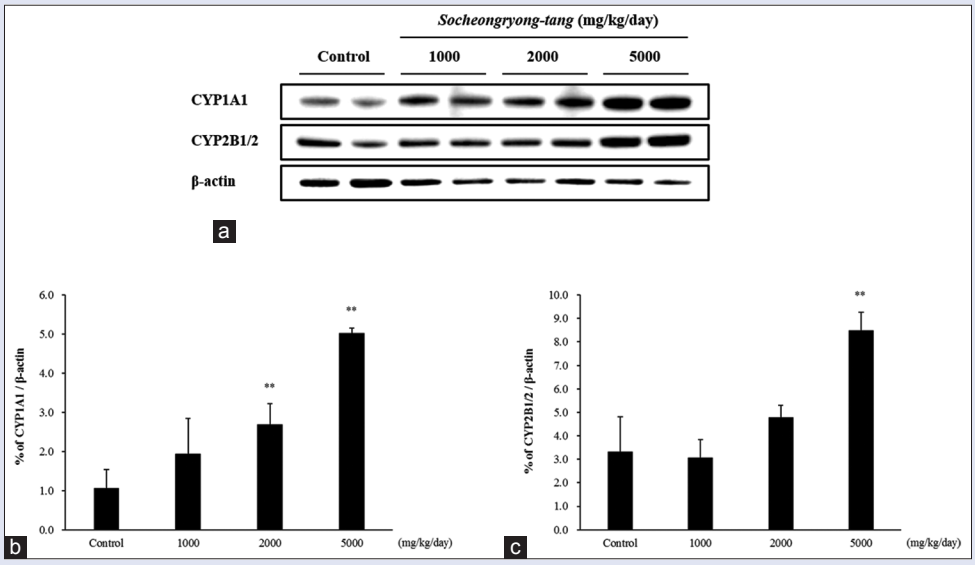

Figure 4: The protein expression of hepatic CYP1A1 and CYP2B1/2 in male Sprague-Dawley rats treated with *Socheongryong-tang*. (a) Adult male rats were treated orally with *Socheongryong-tang* (1000, 2000 or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The protein expression of CYP1A1 and 2B1/2 was analyzed by the Western blot. The protein levels of hepatic CYP1A1 (b) and CYP2B1/2 (c) were normalized to that of β-actin. Values express as a mean ± standard deviation ($n = 4$). $^{**}P < 0.01$ versus vehicle control group

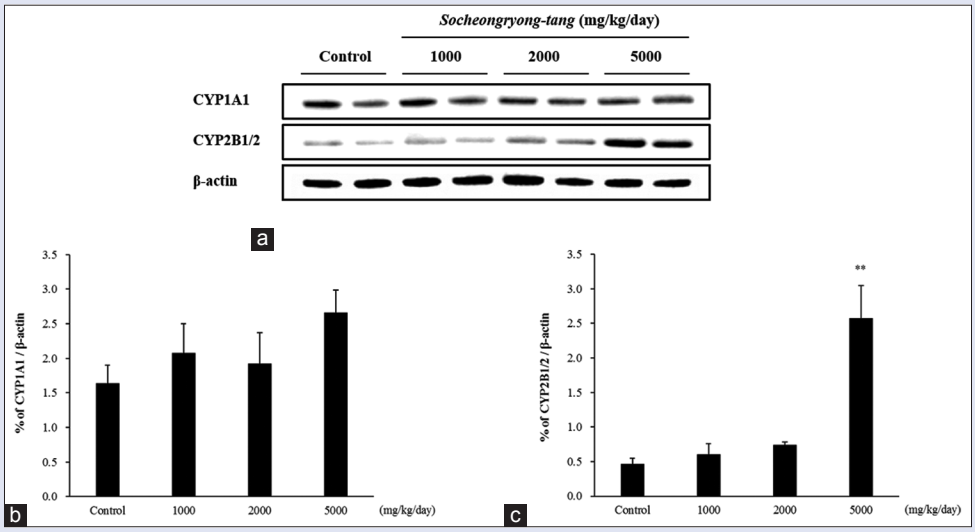

Figure 5: The protein expression of hepatic CYP1A1 and CYP2B1/2 in female Sprague-Dawley rats treated with *Socheongryong-tang*. (a) Adult female rats were treated orally with *Socheongryong-tang* (1000, 2000 or 5000 mg/kg/day) or vehicle once a day for 13 weeks. The protein expression of CYP1A1 and 2B1/2 was analyzed by Western blot. The protein levels of hepatic CYP1A1 (b) and CYP2B1/2 (c) were normalized to that of β-actin. Values express as a mean ± standard deviation ($n = 4$). $^{**}P < 0.01$ versus vehicle control group

coumarin, cinnamic acid, cinnamaldehyde, glycyrrhizin, and schizandrin were about 8.77, 9.63, 11.42, 17.86, 20.69, 23.49, 28.39, and 31.79 min, respectively [Figure 1]. The quantitative analysis of each compound was performed at 230 nm for albiflorin and paeoniflorin, 254 nm for glycyrrhizin and schizandrin, and 280 nm for liquiritin, coumarin, cinnamic acid, and cinnamaldehyde. The amounts of 8 compounds at 0, 1, and 13 weeks in SCRT decoction were summarized in Table 3.

Effect of *Socheongryong-tang* on the hepatic messenger ribonucleic acid expression of cytochrome P450s

To determine the influence of SCRT on the expression of hepatic CYP450s, both male and female rats were orally treated with SCRT (1000, 2000, or 5000 mg/kg/day) once daily for 13 weeks. No significant differences in the relative liver weights between the vehicle control and SCRT-treated rats were identified.[11] In this study, the mRNA expression of hepatic CYP450s (CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1) was determined by RT-PCR.

Screening for effects of *Socheongryong-tang* on the messenger ribonucleic acid expression of hepatic cytochrome P450s

We firstly screened for the effect of SCRT on the mRNA expression of hepatic CYP450s using either male or female rats administered with SCRT at 5000 mg/kg/day. CYP1A2, 2C11, 2E1, 3A1, 3A2, and 4A1 were not significantly changed by SCRT in both male and female rats compared with the vehicle control [Table 4]. By contrast, the expression of hepatic CYP1A1 and 2B1/2 was induced in male rats treated with SCRT. In female rats, the expression of CYP2B1/2 was increased by SCRT. To confirm the effect of SCRT on CYP1A1 and 2B1/2 in more detail, we then compared the expression of CYP1A1 and 2B1/2 when SCRT was administered at 1000, 2000, or 5000 mg/kg/day to rats of both genders.

Effect of *Socheongryong-tang* on the messenger ribonucleic acid expression of hepatic CYP1A1 and 2B1/2 in male rats

Male rats treated with SCRT at 5000 mg/kg/day, but not 1000 or 2000 mg/kg/day, was significantly induced the mRNA expression of hepatic CYP1A1 compared with the vehicle control ($P < 0.01$) [Figure 2a and b]. Administration of SCRT at 1000, 2000, or 5000 mg/kg/day significantly increased the expression of hepatic CYP2B1/2 in male rats compared with the vehicle control ($P < 0.01$) [Figure 2a and c].

Effect of *Socheongryong-tang* on the messenger ribonucleic acid expression of hepatic CYP1A1 and 2B1/2 in female rats

In female rats, SCRT had no significant effect on the RNA expression of hepatic CYP1A1 [Figure 3a and b]. By contrast, the mRNA expression of hepatic CYP2B1/2 was markedly increased by SCRT at 2000 or 5000 mg/kg/day in a dose-dependent manner compared with the vehicle control [Figure 3a and c].

Effect of *Socheongryong-tang* on the hepatic protein expression of cytochrome P450s

To confirm the expression of hepatic CYP1A1 and CYP2B1/2 at the protein level, liver tissues were homogenized and then analyzed by the Western blot.

Effect of *Socheongryong-tang* on the protein expression of hepatic CYP1A1 and CYP2B1/2 in male rats

In male rats, SCRT at 2000 or 5000 mg/kg/day, but not 1000 mg/kg/day, markedly induced the expression of hepatic CYP1A1 at protein level compared with the vehicle control ($P < 0.01$) [Figure 4a and b]. In addition, the protein expression of hepatic CYP2B1/2 was significantly induced by SCRT of 5000 mg/kg/day in male rats compared with the vehicle control ($P < 0.01$) [Figure 4a and c].

Effect of *Socheongryong-tang* on the protein expression of hepatic CYP1A1 and CYP2B1/2 in female rats

Female rats administered with SCRT did not significantly affect the protein expression of hepatic CYP1A1 [Figure 5a and b]. By contrast, female rats administered with SCRT at 5000 mg/kg/day significantly induced the protein expression of hepatic CYP2B1/2 compared with the vehicle control ($P < 0.01$) [Figure 5a and c].

DISCUSSION

The major mechanism of herb-drug interactions involves induction or inhibition of metabolic enzymes such as CYP450s.[12] Changes in the levels of CYP450s or their activities can affect the concentration of a drug in the blood, its pharmacokinetics and its medicinal properties.[2] Therefore, it is important to study the modulation of CYP450s by herbal formulas to understand the mechanisms of herb-drug interactions.

Among the CYP450s, CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 are involved in the metabolism of more than 95% of available pharmaceuticals used clinically.[13] The relative abundance of different CYP450s in human hepatic smooth endoplasmic reticulum has been determined as 13% CYP1A2, 4% CYP2A6, 1% CYP2B6, 20% CYP2C, 2% CYP2D6, 7% CYP2E1, and 30% CYP3A4. In humans, the extent of drug metabolism varies with the individual CYPs, that is, 50% of drugs are metabolized by CYP3A4, followed by 25% and 20% by CYP2D6 and CYP2C family, respectively.[3]

The known major compounds of medicinal herbs contained in SCRT are as follows: Ephedrine and pseudoephedrine from *Ephedra sinica*,[14] albiflorin and paeoniflorin from *Paeonia lactiflora*[15] 6-gingerol from *Z. officinale*,[16] cinnamic acid, cinnamaldehyde, and coumarins from *Cinnamomum cassia*,[17] liquiritin and glycyrrhizin from *Glycyrrhiza uralensis*,[18] and schizandrin, gomisin A, and gomisin N from *Schisandra chinensis*.[19] Among them, we analyzed eight compounds such as albiflorin and paeoniflorin from *P. lactiflora*, coumarin, cinnamic acid and cinnamaldehyde from *C. cassia*, liquiritin and glycyrrhizin from *G. uralensis*, and schizandrin from *S. chinensis* using HPLC-PDA. As a result, there was no significant change in the content of the eight components for 13 weeks. It is indicate that the components of SCRT are stable for 13 weeks.

The effect of SCRT on the CYP2D6 genotype have been reported by Nakao *et al.*[20] However, no report has addressed the influences of SCRT on the different CYP450 members. Because some CYP450s (such as CYP1A, 2C11, 2E1, 3A2, and 4A1) in rats are similar to those in humans, rats are used to predict human CYP450s activities *in vivo*.[21] We evaluated the influence of oral administration of SCRT on the mRNA expression of hepatic CYP1A1, 1A2, 2B1/2, 2C11, 2E1, 3A1, 3A2, and 4A1, the relevant isozymes in rat, in this study.

SCRT was orally administered to male and female rats once a day for 13 weeks at doses of 0, 1000, 2000, or 5000 mg/kg/day, because herbal

formula is usually taken for long periods when used clinically. The mRNA expression of hepatic CYP450s was analyzed using RT-PCR. We found that the mRNA expression of hepatic CYP1A2, 2B1/2, 2E1, 3A1, 3A2, and 4A1 could be observed, while the mRNA expression of hepatic CYP1A1 was relatively weak in either gender. By contrast, the mRNA expression of hepatic CYP2C11 was verified in male rats, but not in female rats, consistent with previous findings.[22] Male rats administered with SCRT at 5000 mg/kg/day increased their mRNA and protein expression of hepatic CYP1A1 compared with the vehicle control group. By contrast, no significant differences were observed between the SCRT and vehicle control groups in the expression of mRNA and protein of hepatic CYP1A1 in female rats. These findings indicate that the CYP1A1 expression was more sensitive to SCRT in male rats than in female rats. The mRNA expression of hepatic CYP2B1/2 was increased in male rats given doses of SCRT at 1000, 2000, or 5000 mg/kg/day. In addition, male rats administered with SCRT at 5000 mg/kg/day also increased their protein expression of hepatic CYP2B1/2 compared with the vehicle control group. In female rats, administration of SCRT at 5000 mg/kg/day increased the expression of hepatic CYP2B1/2 at both mRNA and protein level. That is, administration of SCRT at high dose affects the expression of CYP2B1/2 in both genders. Meanwhile, SCRT at 1000, 2000, or 5000 mg/kg/day had no obvious influence on the mRNA expression of hepatic CYP1A2, 2C11, 2E1, 3A1, 3A2, and 4A1 in both genders.

Repeated administration of drugs can induce CYPs by enhancing the rate of enzyme synthesis. This induction leads to an increase in the rate of metabolite production and hepatic biotransformation, and decreases in serum half-life and drug response, and can result in pharmacokinetic tolerance.[23] Therefore, co-administration of SCRT and an inducer or substrate of CYP1A1 or 2B1/2 may cause reduced drug concentrations leading to decreased drug efficacy and treatment failure.

Metabolism by CYP1A1 often leads to the formation of reactive products resulting in the formation of DNA adducts with potential carcinogens, such as benzo(a)pyrene, arylamines and halogenated aromatic hydrocarbons.[24] There is only a 13 amino acid difference between rat CYP2B1 and CYP2B2, mostly in the C-flanking sequence of the enzyme protein, although CYP2B1 and CYP2B2 genes are non-allelic variants, which contain nine exons.[25] Rat hepatic CYP2B1/2 shows catalytic activity towards nicotine, activates carcinogens, and metabolizes some drugs.[26] Tobacco smoke induces the CYP1A1 and CYP2B1/2 expression in rats.[27] Therefore, the present studies suggest that smoking during the administration of SCRT may over induce CYP1A1 and 2B1/2.

CONCLUSION

Our present findings suggest that long-term (13 weeks) oral administration of SCRT at doses ≤5000 mg/kg/day did not affect metabolism by CYP1A2, 2C11, 2E1, 3A1, 3A2, and 4A1. By contrast, repeated oral administration of SCRT for 13 weeks induced expression of hepatic CYP2B1/2 in both genders. These results suggest that caution is necessary when SCRT is taken together with a substrate or inhibitor of CYP2B1/2. In addition, the level of hepatic CYP1A1 was significantly increased by 13-week repeat oral administration of SCRT in male rats, but not in female rats. Therefore, in the case of males, SCRT is likely to cause clinically relevant herb-drug interactions when co-administered with drugs metabolized by CYP1A1. These findings provide information regarding the safety and effectiveness of SCRT in clinical practice.

Acknowledgments

This research was supported by a grant for "Construction of Scientific Evidences for Herbal Medicine Formulas (K16251)" from the Korea Institute of Oriental Medicine.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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