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Investigation of Herb-Drug Interactions between Korean Red Ginseng Extract and five CYP Substrates by LC-MS/MS

Jung Jae Jo and Sangkyu Lee*

BK21 Plus KNU Multi-Omics based Creative Drug Research Team, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

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Abstract : Ginseng (*Panax ginseng* Meyer) is a well-known health functional food used as a traditional herbal drug in Asian countries owing to its diverse pharmacological effects. Herb-drug interactions may cause unexpected side effects of co-administered drugs by the alteration of pharmacokinetics through effects on cytochrome P450 activity. In this study, we investigated the herb-drug interactions between Korean red ginseng extract (KRG) and five CYP-specific probes in mice. The pharmacokinetics of KRG extract induced-drug interactions were studied by cassette dosing of five CYP substrates for CYP1A, 2B, 2C, 2D, and 3A and the LC-MS/MS analysis of the blood concentration of metabolites of each of the five probes. The linearity, precision, and accuracy of the quantification method of the five metabolites were successfully confirmed. The plasma concentrations of five metabolites after co-administration of different doses of the KRG extract (0, 0.5, 1, and 2 g/kg) were quantified by LC-MS/MS and dose-dependent pharmacokinetic parameters were determined. The pharmacokinetic parameters of the five metabolites were not significantly altered by the dose of the KRG extract. In conclusion, the single co-administration of KRG extract up to 2 g/kg in vivo did not cause any significant herb-drug interactions linked to the modulation of CYP activity.

Keywords : Korean red ginseng extract; cocktail substrates; herb-drug interaction; pharmacokinetics

Introduction

Ginseng (*Panax ginseng* Meyer), a health functional food used as traditional herbal drug in Asian countries, is known to exert pharmacological and biological effects, including anti-inflammatory, anti-diabetic, and anti-tumor effects.[1,2] Furthermore, it can affect the blood pressure and immune system.[3,4] Among the different plants used as medicines, the popularity of ginseng products has recently increased, with red ginseng products accounting for more than 50% of the total consumed ginseng products.[5]

Herb-drug interactions (HDIs) refer to changes in the pharmacokinetic (PK) interactions, which occur through antagonism, enhancement, and synergism between the herb and any co-administered drugs,[6] and may cause unexpected side effects that lead to increased toxicity or loss of drug efficacy.[7] HDIs can result from interactions with chemicals, binding to proteins, or the modulation of biotransformation; the main cause is the modulation of cytochrome P450 (CYP) enzyme activities by chemicals.[8]

The CYP family of enzymes is one of the most important, because the metabolism of approximately 75% of currently used drugs is affected by CYP enzymes.[9] Therefore, investigation of the activity of CYP enzymes is important in the study of HDIs. Among the 57 CYPs used in many studies, the CYP1A, CYP2B, CYP2C, CYP2D, and CYP3A subfamilies are of greater importance; in particular, CYP3A is known to be involved in the metabolism of approximately 50% of drugs.[10] CYP2C consists of four isoforms, CYP2C8, 2C9, 2C18 and 2C19, which share a sequence homology of >82% and influence the metabolism of 25% of clinically used drugs.[11] CYP1A is one of the major CYPs in human liver (~13%) and CYP2D affects the metabolism of approximately 25% of marketed drugs.[12,13]

The modulation of CYP activity by the herbs may be associated with variations in the PK of their substrates, which may lead to the alteration of the pharmacodynamics of co-administered drugs.[14] In a previous study, we investigated the inhibitory effects of 12 ginsenosides on seven CYP isoforms in human liver microsomes by LC-MS/MS in conjunction with a cassette assay.[15] In the present study, we investigated the HDIs of Korean red ginseng extract (KRG) with co-administered drugs through

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*Reprint requests to Sangkyu Lee
E-mail: sangkyu@knu.ac.kr

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monitoring of the PK parameters of five CYP-specific substrates in mice to confirm the in vitro HDIs.

Experimental Methods

Materials

KRG was obtained from Punggi Ginseng Cooperative Association (Punggi, Korea). The NADPH regeneration system (NGS) was purchased from Promega Co. (Madison, WI, USA). Caffeine, 1,7-dimethylxanthine, bupropion hydrochloride, OH-bupropion, omeprazole, OH-omeprazole, dextromethorphan, dextrorphan, OH-midazolam, and reserpine were purchased from Sigma-Aldrich Co., LLC (St. Louis, MO, USA). Midazolam was purchased from Bukwang Pharmaceutical Co., Ltd (Seoul, Korea). HPLC-grade water and acetonitrile (ACN) were purchased from Fisher Scientific Korea (Seoul, Korea). Reserpine, used as an internal standard (IS), was purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA).

Animals

Five-week-old male ICR mice were purchased from Orient Co. (Seongnam, Korea). Four mice were randomly chosen and housed per cage. For 1 week prior to experimental conditions, the mice were acclimatized to a controlled environment (relative humidity, 60%; temperature, maintained at 25°C, light/dark cycle, 12/12 h), fed standard rodent chow, and given free access to tap water. All animal procedures were conducted in compliance with the guidelines issued by the Society of Toxicology (USA; 1989) and the study was approved by Institutional Review Board of Kyungpook National University (KNU-2017-0089-1).

Single administration of KRG with CYP substrate cocktail

The mice were divided into four groups, which were each administered a different dosage of KRG extract (0, 0.5, 1.0, and 2.0 g/kg). Prior to administration of KRG and CYP substrate cocktail, the mice were fasted for 12 h, but given access to water. First, the KRG extract was orally administered to mice, and after 30 min, a CYP cocktail, containing caffeine for CYP1A (2 mg/kg), bupropion for CYP2B (30 mg/kg), omeprazole for CYP2C (4 mg/kg), dextromethorphan for CYP2D (40 mg/kg), and midazolam for CYP3A (2 mg/kg), was administered.

Plasma sample preparation

Approximately 20 μL of blood was obtained from the tail vein at 0, 5, 10, 15, 30, 60, 120, 240 and 360 min after CYP substrates administration, respectively, and collected in heparinized tubes after each administration. Each blood sample was centrifuged (4,000 ×g, 15 min, 4°C) and 10 μL of the obtained plasma was mixed with 90 μL ACN containing 0.1% formic acid (FA) and 1 μM reserpine (the IS). The sample was vortexed for 1 min and centrifuged (13,000 g, 10 min, 4°C). Ninety microliters of the supernatant was used in the LC-MS/MS analysis.

Instruments

The LC-MS/MS system comprised LC-20AD pump, SIL-20A autosampler, CTO-20A column oven (Shimadzu, Kyoto, Japan). A Shim-pack GIS column (3 mM ODS, 100 mm × 3 mm) (Shimadzu, Kyoto, Japan) were used for LC separation. The mobile phase was a combination of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in ACN) and the flow rate was 500 mL/min. The following gradient program was employed: 5–5% B (0-1 min), 5-95% B (1-3 min), 95-95% B (3-5 min), 95-5% B (5-5.5 min), and 5-5% B (5.5-10 min). During the analysis, the column oven was maintained at 40°C. The samples were analyzed by using an LC-MS/MS system coupled to a TSQ vantage (Thermo Scientific Inc., MA, USA) with an electrospray ionization (ESI) source. The mass spectrometer was operated in positive ESI mode with nitrogen as the auxiliary gas and sheath gas pressure set at 10 and 35 psi, respectively. The ESI spray voltage was adjusted to 4,000 V, the vaporizer temperature was set at 300°C, and the capillary temperature was set to 350°C. The instrument was operated in selective reaction monitoring (SRM) mode with transitions; paraxanthine for CYP1A (m/z 181.3 → 124.0, CE20), 6-OH bupropion for CYP2A m/z (m/z 256.1 → 238.0, CE15), 5-OH omeprazole for CYP2C (m/z 362.3 → 214.0, CE10), dextrorphan for CYP2D (m/z 258.3 → 157.1, CE50), 1'-OH midazolam for CYP3A (m/z 342.2 → 324.2, CE20), and reserpine as the internal standard (m/z 609.3 → 195.2, CE35). Data procurement was conducted by Xcalibur software (Version 3.0.63).

Accuracy and precision

The precision and accuracy of the methodology were evaluated through repeated analysis of mouse plasma spiked with known concentrations of substrates and metabolites. The accuracy and precision of substrates and metabolites were determined by repetition of the assay at each concentration.

Pharmacokinetic parameters

A non-compartmental model was used to evaluate the PK parameters of substrates and metabolites by using WinNonlin software (Version 2.1, Scientific Consulting, KY, USA). The included parameters were the area under the plasma concentration-time curve for 360 min (AUC_{0-360}), time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), and half-life ($T_{1/2}$).

Results and Discussion

To determine the activity dynamics of CYP isoforms, we monitored the plasma concentration of five specific

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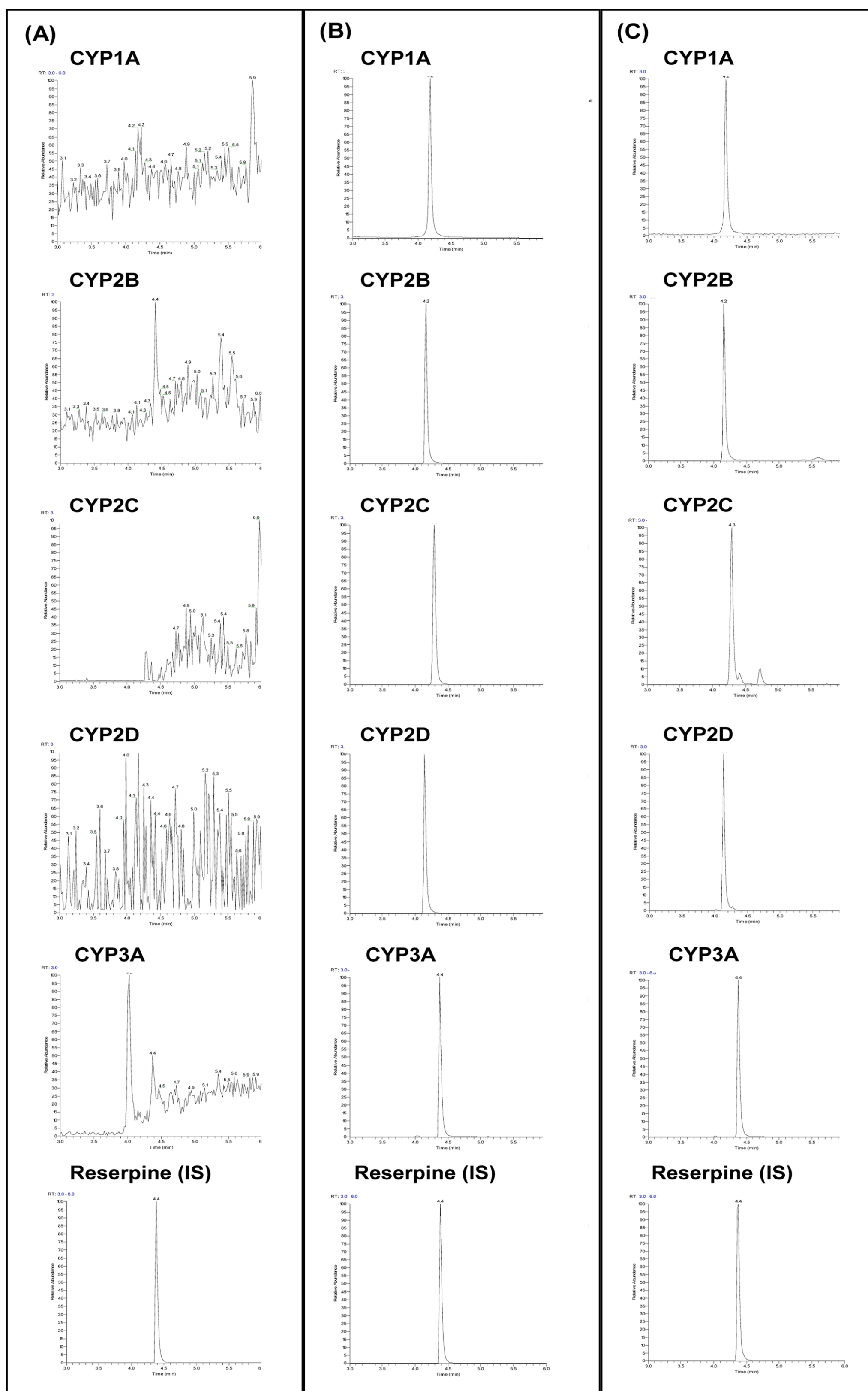

Figure 1. Representative chromatograms for metabolites of five CYP probe drugs. Chromatograms of blank (A), spiked sample (3,000 ng/ml) (B) and plasma sample at 30 min after administration of CYP cocktail with KRG extract (C).

metabolites, instead of the substrates, in blood. The metabolites represent a specific product of each CYP isoform. The plasma concentrations of the metabolites of five specific substrates for CYP1A, CYP2B, CYP2C, CYP2D and CYP3A were simultaneously determined by LC-MS/MS (Figure 1). The metabolites of all probe drugs in the present cocktail were evaluated for linearity, precision, and accuracy. The stock solutions of metabolites of CYP substrates used in this study were prepared at 30 mg/mL in dimethyl sulfoxide. Ten microliters of each stock solution was added to 90 µL of drug-free mouse plasma. The final concentrations of all metabolites, except 1'-OH midazolam, in the calibration standards were 10-3,000 ng/mL; 1'-OH midazolam was set within the range

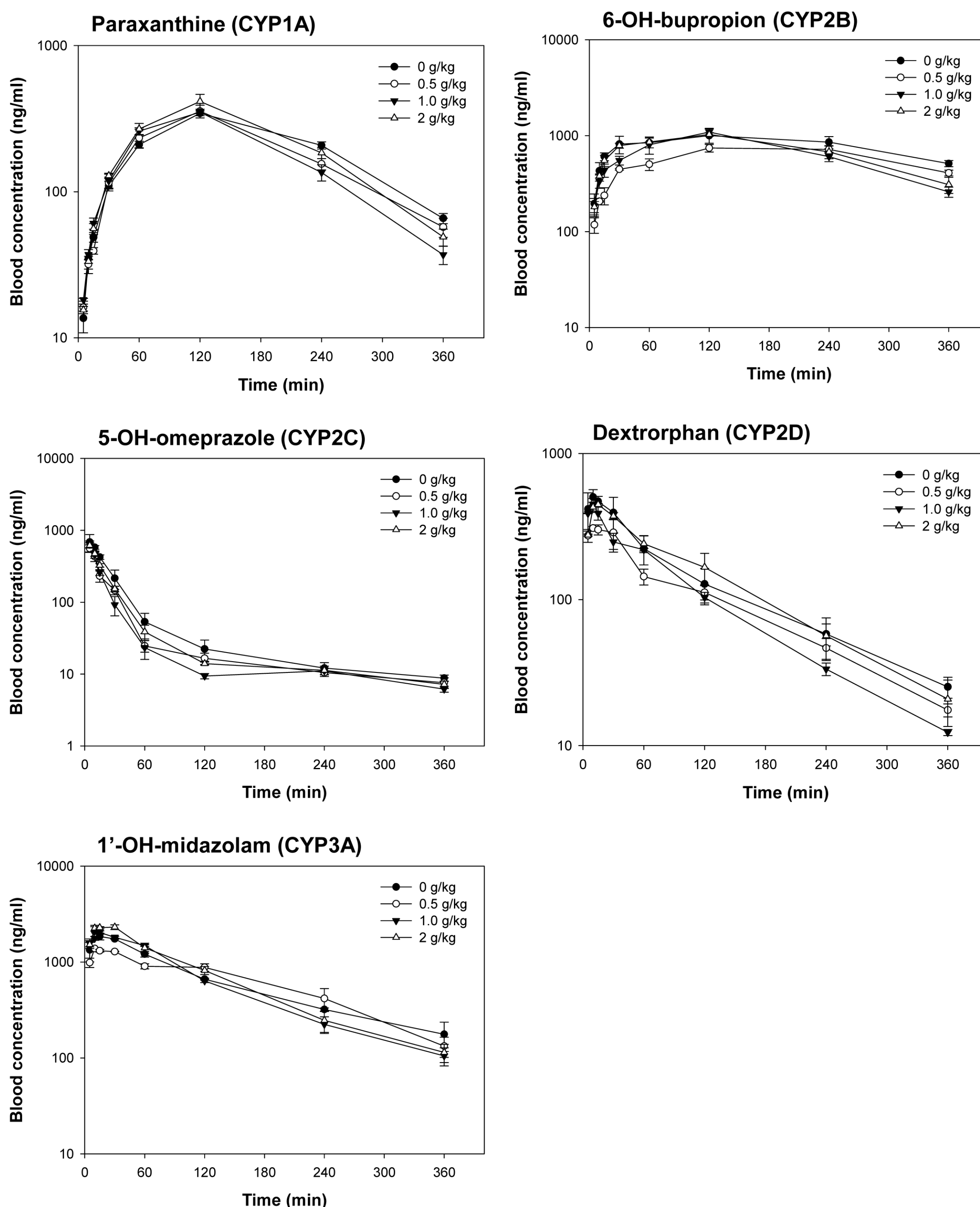

Figure 2. Plasma concentration of five CYP metabolites after a single administration of KRG (0, 0.5, 1, or 2 g/kg) in mice.

of 50-3,000 ng/mL. Aliquots (10 μL) of the standard plasma samples were processed as described above. The calibration curves were constructed by plotting the peak area ratios of the analytes or the IS versus the concentrations of each substrate and metabolite in mouse plasma. Linear calibration curves with correlation coefficients greater than 0.9988 were observed for analytes in the calibration range. The validation of the analytical methods for this study was performed to evaluate the standard mixture of five CYP metabolites. As

Table 1. Validation data of five CYP metabolites

CYP isoforms	Metabolites	Spiked concentration (ng/mL)	Measured concentration (ng/mL)	RSD (%)	Accuracy (%)
CYP1A	Paraxanthine	300	294.2 ± 10.9	3.7	102.0
		3,000	3012.2 ± 134.4	4.5	99.6
CYP2B	6-OH-bupropion	300	281.4 ± 7.1	2.5	106.6
		3,000	3016.1 ± 94.6	3.1	99.5
CYP2C	5-OH-omeprazole	300	301.6 ± 12.8	4.2	99.5
		3,000	2988.6 ± 96.2	3.2	100.4
CYP2D	Dextrorphan	300	310.0 ± 10.1	3.3	96.8
		3,000	3000.8 ± 76.7	2.6	99.7
CYP3A	1'-OH-midazolam	300	301.8 ± 3.9	1.3	99.4
		3,000	2999.2 ± 77.1	2.6	100.0

Table 2. Pharmacokinetic parameters of five CYP metabolites with a single administration of KRG

CYP isoforms	Parameters	KRG (g/kg)			
		0	0.5	1.0	2.0
		Paraxanthine			
CYP1A	T_{max} (min)	120.0 ± 0.0	120.0 ± 0.0	120.0 ± 0.0	120.0 ± 0.0
	Cmax (ng/mL)	344.2 ± 11.4	355.5 ± 30.7	350.5 ± 6.0	415.4 ± 41.9
	half-life (min)	95.4 ± 3.7	91.8 ± 6.9	74.1 ± 4.7	78.2 ± 4.7
	AUC (μg.min/mL)	72.1 ± 2.3	67.7 ± 4.0	65.3 ± 1.2	78.2 ± 5.9
		6-OH-bupropion			
CYP2B	T_{max} (min)	100.0 ± 17.3	120.0 ± 0.0	120.0 ± 0.0	90.0 ± 26.0
	C_{max} (ng/mL)	1,009.9 ± 143.2	743.6 ± 57.8	1,089.9 ± 90.4	977.9 ± 53.8
	half-life (min)	323.7 ± 70.8	285.0 ± 23.1	121.3 ± 17.1	184.6 ± 51.1
	AUC (μg.min/mL)	289.5 ± 32.5	214.7 ± 15.9	241.4 ± 20.9	252.3 ± 12.2
		5-OH-omeprazole			
CYP2C	T_{max} (min)	6.7 ± 1.4	5.0 ± 0.0	5.0 ± 0.0	6.7 ± 1.4
	C_{max} (ng/mL)	736.8 ± 128.2	561.8 ± 57.8	641.7 ± 60.3	619.0 ± 42.7
	half-life (min)	139.0 ± 71.0	244.6 ± 81.9	121.5 ± 54.1	203.8 ± 65.4
	AUC (μg.min/mL)	21.9 ± 3.2	14.9 ± 1.3	13.8 ± 1.4	17.0 ± 0.7
		Dextrorphan			
CYP2D	T_{max} (min)	18.3 ± 5.2	20.0 ± 7.1	18.3 ± 5.2	11.7 ± 1.4
	C_{max} (ng/mL)	526.8 ± 41.9	345.0 ± 18.2	376.0 ± 82.9	464.4 ± 3.5
	half-life (min)	101.9 ± 14.8	100.9 ± 7.1	75.8 ± 4.2	78.5 ± 4.0
	AUC (μg.min/mL)	37.7 ± 0.8	36.6 ± 0.1	34.4 ± 4.3	50.3 ± 7.1
		1'-OH-midazolam			
CYP3A	T_{max} (min)	16.7 ± 5.8	16.7 ± 5.8	15.0 ± 0.0	18.3 ± 5.2
	C_{max} (ng/mL)	2,147.5 ± 166.9	1,355.4 ± 23.6	2,036.5 ± 71.9	2,353.9 ± 80.3
	half-life (min)	107.6 ± 15.3	94.9 ± 8.7	85.5 ± 10.7	75.6 ± 5.1
	AUC (μg.min/mL)	239.6 ± 2.8	224.5 ± 21.4	232.7 ± 9.2	299.8 ± 38.7

demonstrated in Table 1, the LC-MS/MS method showed good reproducibility for the quantification of the metabolites of CYP probe drugs and the IS. The overall accuracy was 99.4-106.6%, with relative standard deviations between 1.3% and 4.2%. Therefore, the proposed method was suitable for the determination of the metabolites of CYP probe drugs.

The plasma concentration profiles of five metabolites after the co-administration of KRG extract with the CYP substrate cocktail are shown in Figure 2. The PK parameter values are presented in Table 2. The C_{max} values (in ng/mL) were 344.2-415.4 for paraxanthine for CYP1A, 743.6-1,089.9 for OH-bupropion for CYP2B, 561.8-736.8 for OH-omeprazole for CYP2C, 345.0-526.8 for dextrorphan for CYP2D, and 1,355.4-2147.5 for OH-midazolam for CYP3A, respectively. In addition, the AUC_{0-360} values (in mg.min/mL) were 65.3-72.1 for paraxanthine, 214.7-289.5 for OH-bupropion, 13.8-21.9 for OH-omeprazole, 34.4-50.3 for dextrorphan, and 224.5-299.8 for OH-midazolam. In present study, no significant differences in any PK parameters were found after treatment of different concentrations of KRG (Table 2).

The present study indicated that the single co-administration of KRG extract up to 2 g/kg in mice did not result in any significant herb-drug interactions linked to the modulation of CYP activity. The present result is consistent with our earlier findings, in which the most of ginsenosides exerted a weak inhibitory effect on CYP enzymes, with IC_{50} values greater than 50 mM.[15] Generally, it is known that ginsenosides are poorly absorbed after oral administration, because they are mainly destroyed in the gastrointestinal tract or metabolized by intestinal microflora and then excreted.[16,17] Although the treated concentration in the present study was two-fold higher than the dose generally recommended by the manufacturing company, the blood concentration might not be sufficient to inhibit CYP activities owing to a poor absorption rate after single administration.

However, this was not confirmation that the administration of KRG extracts was perfectly safe and did not result in HDIs. For example, the long-term administration of KRG was previously shown to influence CYP3A activity.[18] Healthy volunteers were administered ginseng 500 mg twice per day and then orally administered midazolam. In this PK study, the C_{max} was decreased from 39 to 29 ng/mL; furthermore, the AUC was also decreased from 120 to 79 ng·h/mL, which indicated that ginseng can induce CYP3A activity after repeated administration. In addition, the content of ginsenosides in ginseng products can be altered by processing methods and ginsenosides can be changed to other ginsenosides by biotransformation.[19,20] As ginsenosides can be improperly accumulated in products during processing, the contents of each ginsenside may be quantified to predict significant KRG-induced drug interactions.

Conclusion

This study investigated the effects of KRG extract on the changes in CYP activities in an in vivo PK study. Although it was determined that ginseng products could influence CYP activity after long-term administration, the PK parameters of five CYP probes were not significantly changed by the single co-administration of KRG extract in vivo.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with the publication of this manuscript and that there has been no significant financial support for this work that may have influenced the outcome.

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