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Pharmacokinetic Interaction of Chrysin with Caffeine in Rats

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Abstract

Pharmacokinetic interaction of chrysin, a flavone present in honey, propolis and herbs, with caffeine was investigated in male Sprague-Dawley rats. Because chrysin inhibited CYP1A-selective ethoxyresorufin *O*-deethylase and methoxyresorufin *O*-demethylase activities in enriched rat liver microsomes, the pharmacokinetics of caffeine, a CYP 1A substrate, was studied following an intragastric administration with 100 mg/kg chrysin. In addition to the oral bioavailability of chrysin, its phase 2 metabolites, chrysin sulfate and chrysin glucuronide, were determined in rat plasma. As results, the pharmacokinetic parameters for caffeine and its three metabolites (i.e., paraxanthine, theobromine and theophylline) were not changed following chrysin treatment *in vivo*, despite of its inhibitory effect on CYP 1A *in vitro*. The bioavailability of chrysin was found to be almost zero, because chrysin was rapidly metabolized to its sulfate and glucuronide conjugates in rats. Taken together, it was concluded that the little interaction of chrysin with caffeine might be resulted from the rapid metabolism of chrysin to its phase 2 metabolites which would not have inhibitory effects on CYP enzymes responsible for caffeine metabolism.

Key Words: Chrysin, Caffeine, Drug interaction, Pharmacokinetics, *in vivo*

INTRODUCTION

Chrysin (5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one, Fig. 1) is a naturally present flavone contained in propolis and honey (Siess *et al*., 1996), and herbs, such as *Passiflora coerulea* and *Passiflora incarnata* (Wolfman *et al*., 1994; Brown *et al*., 2007). It showed several pharmacological effects, such as hepatoprotective (Pushpavalli *et al*., 2010), anti-oxidant (Villar *et al*., 2002), anti-inflammatory (Cho *et al*., 2004), and anti-aromatase activity (Kellis and Vickery, 1984). Because chrysin has recently been used as a nutritional supplement to promote anabolic hormones, the possibility of drug interaction has also been increasing. Nevertheless, drug interaction study with chrysin has rarely been reported.

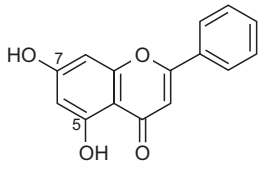

Fig. 1. Structure of chrysin.

Cytochrome P450 (CYP) enzymes play important roles in drug interaction, because they are either induced or inhibited by many flavonoid compounds (Hodek *et al*., 2002). In this regard, the effects of chrysin on CYP enzymes were studied elsewhere. For examples, chrysin inhibited CYP1A1/2 and CYP3A activities in rat and human liver microsomes, respectively (Moon *et al*., 1998; Tsujimoto *et al*., 2009). In contrast, ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD) activities, which represent CYP1A and CYP2B activities, respectively, were significantly induced *in vivo* following repeated dose of chrysin for 2 weeks in rats (Breinholt *et al*., 1999). Nevertheless, *in vivo* study to evaluate the effect of chrysin on the pharmacokinetics of certain drugs has not been conducted until to date. Likewise, understanding

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pharmacokinetics of chrysin would also be important to understand whether the systemic exposure of chrysin is enough to show its pharmacological effects or not. Unfortunately, however, the absolute bioavailability of chrysin has not been investigated extensively.

In this study, we evaluated the effects of chrysin on CYP enzymes *in vitro* and pharmacokinetics of caffeine *in vivo*. Furthermore, pharmacokinetic characteristics of chrysin with its phase 2 metabolism were also investigated to understand the possible mechanism of less interaction with caffeine.

MATERIALS AND METHODS

Reagents

Chrysin, caffeine, paraxanthine, theobromine, theophylline, ethoxyresorufin, methoxyresorufin, benzyloxyresorufin, resorufin, *p*-nitrophenol, erythromycin, 4-dimethylaminoantipyrine, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, β-glucuronidase (type H-3 from *Helix pomatia*), sulfatase (type H-1 from *Helix pomatia*) and D-saccharic acid 1,4-lactone monohydrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). The reduced form of β-nicotinamide adenine dinucleotide phosphate (β-NADPH) was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan), and methanol and acetonitrile were HPLC-grade from J.T.Baker (Center Valley, PA, USA). All other chemicals were of analytical grade and used as received.

Animals

Male Sprague-Dawley rats (7 weeks, 246~260 g) were obtained from Samtako Bio Korea (Osan, Korea), and randomized and housed three per cage. The animal room was maintained at a temperature of 22 ± 2°C, relative humidity of 50 ± 10% with 10-20 air changes/hr, and light intensity of 150-300 Lux with a 12-hr light/dark cycle. This study was approved by the Yeungnam University Animal Care and Use Committee (approved No., 2014-008).

Preparation of rat liver microsomes

Rats were pretreated with either 3-methylcholanthrene, phenobarbital, dexamethasone or acetone to enrich CYP 1A, CYP 2B, CYP 3A or CYP 2E1 in livers, respectively. By enrichment, the inhibitory effects of chrysin on specific CYP isozymes could be more selectively determined (Noh *et al*., 2015). Dexamethasone and 3-methylcholanthrene suspended in corn oil were intraperitoneally given to rats at a dose of 50 mg/kg and 40 mg/kg for 3 consecutive days, respectively. Phenobarbital suspended in saline was intraperitoneally administered to rats at a dose of 80 mg/kg for 3 days, and acetone was given once only to rats by intragastric administration at 5 mL/kg. Twenty-four hr after last dose (or two days after acetone administration), rats were sacrificed to isolate livers.

Then, rat liver microsomes were prepared as described previously (Kim *et al*., 2014). In brief, livers removed from each treatment group were homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The liver homogenates were centrifuged at 9,000xg for 20 min at 4°C, and the supernatants were centrifuged at 105,000×g for 60 min at 4°C. Then, the microsomal pellets were resuspended in 0.1 M potassium phosphate buffer, pH 7.4, containing 20% glycerol, and aliquots were stored at -80°C until use. The content of protein in rat liver microsome was determined using bovine serum albumin as a standard (Lowry *et al*., 1951).

Assay of monooxygenase activities

EROD activity was determined, as previously described, with a slight modification (Blank *et al*., 1987). The reaction mixture (1 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 2 mg/mL of bovine serum albumin, 5 mM glucose-6-phosphate, 1 U of glucose-6-phosphate dehydrogenase, 5 μM β-NADPH, and 2.5 μM 7-ethoxyresorufin as a substrate. The formation of resorufin was measured fluorometrically at $\lambda_{Ex}/\lambda_{Em}$ of 550/585 nm. Methoxyresorufin *O*-demethylase (MROD) and benzyloxyresorufin *O*-debenzylase (BROD) activities were determined by the method of Lubet *et al*. with a slight modification (Lubet *et al*., 1985). These reactions were conducted under the same condition for EROD, except that the substrates used were 2.0 μM methoxyresorufin and benzyloxyresorufin for MROD and BROD, respectively. *p*-Nitrophenol hydroxylase (PNPH) activity was determined as described previously (Koop, 1986). The reaction mixture (1 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 100 μM *p*-nitrophenol, 1 mM NADPH, and an enzyme source. The reaction was terminated by adding 0.6 N perchloric acid, and the amount of 4-nitrocatechol formed was measured spectrophotometrically at λ_{max} of 512 nm. Erythromycin N-demethylase (ERDM) activity was determined by measuring the amount of formaldehyde formed, as described previously (Nash, 1953). Erythromycin at 400 μM was used as a substrate for assaying ERDM. The reaction mixture (1.5 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 3 mM glucose-6-phosphate, 1 U glucose-6-phosphate dehydrogenase, 0.8 mM β-NADPH, 7.5 mM semicarbazide, and 5.0 mM $MgCl_2$. The reaction was terminated by adding 20% trichloroacetic acid, and the amount of formaldehyde formed was measured spectrophotometrically at λ_{max} of 412 nm. Aminopyrine *N*-demethylase (APDM) reaction was conducted under the same condition for ERDM, except that the substrate used was 400 μM 4-dimethylaminoantipyrine.

Effect of chrysin on the pharmacokinetics of caffeine

Rats were fasted overnight, and randomized into two groups of five rats. Chrysin was suspended in corn-oil and vortexed immediately before treatment. Thirty min before caffeine administration, corn oil or chrysin (100 mg/kg/2 mL corn oil) were orally given to each group. Caffeine dissolved in saline was given to rats by an intragastric administration at 5 mg/kg. Blood samples (200 μL) were taken via subclavian vein up to 24 hr after caffeine administration, and collected into heparinized centrifuge tubes. Plasma samples were prepared by a centrifugation at 3,000×g for 10 min, and stored at -80°C until analysis.

Pharmacokinetics of chrysin

Before experiment, rats were fasted overnight. Chrysin was dissolved in the mixture of dimethyl sulfoxide, polyethylene glycol 200, saline and ethanol (3:3:2:2, v/v/v/v) for intravenous injection, and suspended in corn oil for the intragastric administration. Then, chrysin was given to rats by a single intravenous injection into tail vein at 2 mg/kg/0.5 mL, and by an intragastric administration at 100 mg/kg/2 mL. Blood samples (200 μL) were taken via subclavian vein up to 24 hr following chrysin administration, and collected into heparinized centrifuge tubes. Plasma samples were prepared and stored as

mentioned above.

Sample preparation for the determination of caffeine and its metabolites

Sixty microliter of methanol containing 1 ng/mL methaqualone (internal standard, IS) was added to 20 μL of plasma sample. After vortex for 30 sec, plasma samples were centrifuged at 3,000×g for 10 min. Then, the supernatant was transferred into a vial, and 5 μL of supernatant was injected for analysis.

Sample preparation for the determination of chrysin

One hundred microliter of methanol containing 1 ng/mL IS was added to 20 μL of plasma sample. After vortex for 30 sec, plasma samples were centrifuged at 3,000×g for 10 min. Then, the supernatant was transferred into a vial, and 3 μL of supernatant was injected for analysis.

Enzymatic hydrolysis of chrysin conjugates in plasma

Chrysin glucuronide and sulfate in rat plasma were hydrolyzed by previously reported methods with some modifications (Galijatovic *et al*., 1999; Wen *et al*., 2008). Fifteen microliter of each plasma sample was incubated with either 15 μL of 5,000 units/mL β-glucuronidase in 0.5 M phosphate buffer, pH 4.5, for 1 hr at 37°C, or 15 μL of 200 units/mL sulfatase and 20 mM D-saccharic acid 1,4-lactone in 0.1 M sodium acetate buffer (pH 5.0) for 2 hr at 37°C. Reaction was terminated by adding 150 μL of IS, and samples were prepared as mentioned above.

Analytical method of caffeine and its metabolites

Plasma concentrations of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, were determined by the previous reported method with some modifications (Noh *et al*., 2011; Choi *et al*., 2013). Caffeine and its metabolites were analyzed using high performance liquid chromatography (1260 system, Agilent) with mass spectrometry (API-4000, AB SCIEX). Analytes were separated using ZORBAX Bonus-RP column (2.1×150 mm, 5 μm; Agilent). The mobile phase was composed of 0.2% formic acid (A) and methanol (B), and eluted with gradient condition as follows: initial time at 90% mobile phase A and 10% mobile phase B; from 90% mobile phase A to 5% mobile phase A, and from 10% mobile phase B to 95% mobile phase B from 0.5~4 min; 5% mobile phase A and 95% mobile phase B holding for 2 min (4~6 min); from 5% mobile phase A to 90% mobile phase A, and from 95% mobile phase B to 10% mobile phase B from 6~7 min; and 90% mobile phase A and 10% mobile phase B holding for 7 min (7~14 min). The flow rate and temperature were maintained at 0.4 mL/min and 35°C during analysis, respectively. Caffeine, paraxanthine, theobromine, theophylline, and IS were detected in the positive ion mode. Mass transitions used in the analysis were *m/z* 195.2→138.1 for caffeine, *m/z* 181.1→124.0 for paraxanthine and theophylline, *m/z* 181.1→138.1 for theobromine, and *m/z* 251→132.1 for IS. Quantitative analysis for caffeine and its metabolites were performed by multiple reaction monitoring of the precursor ion and the related product ion using the ratio of the area under the peak for each sample.

Analytical method of chrysin

Chrysin and chrysin metabolites (i.e., chrysin sulfate and chrysin glucuronide) after hydrolysis were determined using the same instruments aforementioned. Atlantis dC18 column (2.1×150 mm, 3 μm; Waters) was used for separation, and column oven was maintained at 40°C. The mobile phase was composed of 0.1% formic acid (A) and acetonitrile (B), and eluted with gradient condition as follows: initial time at 90% mobile phase A; from 90% mobile phase A to 20% mobile phase A from 0~3 min; 20% mobile phase A holding for 3 min (3~6 min); from 20% mobile phase A to 90% mobile phase A from 6~8 min; and 90% mobile phase A holding for 7 min (8~15 min). The flow rate was maintained at 0.25 mL/min during analysis. Chrysin and IS were detected in the positive ion mode. Mass transitions used in the analysis were *m/z* 254.9 →153.1 for chrysin, and *m/z* 251→132.1 for IS. Quantitative analysis for chrysin was performed by multiple reaction monitoring of the precursor ion and the related product ion using the ratio of the area under the peak for each sample. The concentrations of chrysin sulfate and chrysin glucuronide were calculated by subtraction from chrysin concentration before and after the hydrolysis with either β-glucuronidase or sulfatase.

Table 1. Inhibitory effects of chrysin on CYP enzyme activities in rat liver microsomes

Enzymes	CYPs responsible	IC_{50} (μM)
EROD	1A	28.5
MROD	1A	2.9
BROD	2B	>200
PNPH	2E1	>200
ERDM	3A	>200
APDM	2C	>200

Pharmacokinetic analysis

Pharmacokinetic parameters of caffeine, chrysin and their corresponding metabolites were obtained from time course plasma concentrations and the peak area ratios of analyte to IS in rats. Standard methods were used to calculate the following pharmacokinetic parameters using non-compartmental analysis (WinNonlin; version 2.1; Scientific Consulting): maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}), area under the plasma concentration-time curve from the time of dosing extrapolated to infinity (AUC_{∞}), total body clearance (CL), volume of distribution based on the terminal phase (V_d), apparent volume of distribution based on the terminal phase (V_d/F) and terminal half-life ($t_{1/2}$). AUC was calculated using the trapezoidal rule-extrapolation method (Chiou, 1978). The pharmacokinetic parameters were expressed as mean ± S.D. The statistical significance of the results was analyzed using Student's *t*-test, with *p*-values less than 0.05 as considered statistically significant.

RESULTS

Assay of monooxygenase activities

Prior to conduct the pharmacokinetic and drug interaction studies, inhibitory effects of chrysin on certain CYP enzymes were determined in enriched rat liver microsomes. Chrysin strongly inhibited MROD with calculated IC_{50} value of 2.9 μM (Table 1). Likewise, EROD activity was also inhibited by chry-

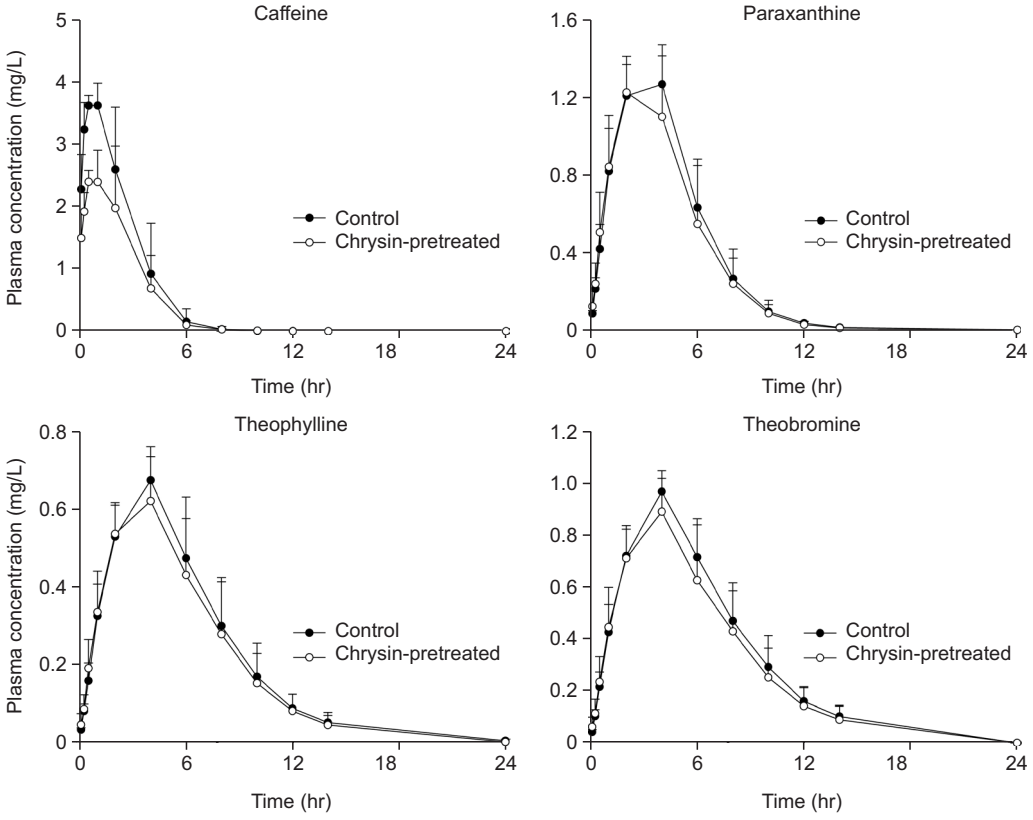

Fig. 2. Time courses of the plasma concentrations of caffeine, paraxanthine, theobromine, and theophylline following an intragastric administration with 5 mg/kg caffeine following pretreatment with chrysin in rats. Each value represents the mean ± S.D. of five animals.

Table 2. Pharmacokinetic parameters of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, following an intragastric administration with 5 mg/kg caffeine to rats pretreated either with or without chrysin

Parameter	Caffeine		Paraxanthine		Theobromine		Theophylline	
	Control	Pretreatment	Control	Pretreatment	Control	Pretreatment	Control	Pretreatment
C_{max} (mg/L)	3.8 ± 0.2	2.6 ± 0.4*	1.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
T_{max} (hr)	0.9 ± 0.7	0.9 ± 0.7	2.8 ± 1.1	3.2 ± 1.1	4.0 ± 0.0	3.2 ± 1.1	4.0 ± 0.0	3.2 ± 1.1
AUC_{∞} (mg·hr/L)	11.2 ± 3.9	7.9 ± 3.0	7.2 ± 0.6	6.7 ± 1.3	7.2 ± 0.8	6.7 ± 1.3	4.8 ± 0.7	4.5 ± 1.1
V_d/F (L/kg)	0.5 ± 0.1	1.1 ± 0.8	-	-	-	-	-	-
$t_{1/2}$ (hr)	0.8 ± 0.1	1.0 ± 0.3	1.4 ± 0.1	1.4 ± 0.3	2.9 ± 0.3	2.9 ± 0.7	2.6 ± 0.4	2.5 ± 0.5

Mean ± S.D. of 5 rats. *p<0.05 vs. control

sin in a concentration dependent manner with IC_{50} of 28.5 μM. In contrast, chrysin had no inhibitory effects on BROD, PNPH, ERDM and APDM with the estimated IC_{50} values of above 200 μM (Table 1). The results indicated that CYP 1A would be selectively inhibited by chrysin *in vitro*. Thereby, the effect of chrysin on caffeine pharmacokinetics were studied *in vivo* because caffeine is a well-known substrate for CYP 1A (Noh *et al*., 2015).

Effect of chrysin on the pharmacokinetics of caffeine

Time-plasma concentration profiles of caffeine and its three metabolites in rats pretreated with or without chrysin were depicted in Fig. 2, and the obtained pharmacokinetic parameters were summarized in Table 2. In control group, caffeine reached the C_{max} of 3.8 ± 0.2 mg/L at 0.9 ± 0.7 hr, and the estimated AUC_{∞} and $t_{1/2}$ were 11.2 ± 3.9 mg·hr/L and 0.8 ± 0.1 hr, respectively. The highest plasma concentrations of metabolites were 1.3 ± 0.1 mg/L for paraxanthine, 1.0 ± 0.1 mg/L for theobromine, and 0.7 ± 0.1 mg/L for theophylline, respectively. The T_{max} of them were achieved at 2.8~4.0 hr after caffeine administration. The AUC_{∞} and $t_{1/2}$ of paraxanthine, theobromine and theophylline were 7.2 ± 0.6 mg·hr/L and 1.4 ± 0.1 hr, 7.2 ± 0.8 mg·hr/L and 2.9 ± 0.3 hr, and 4.8 ± 0.7 mg·hr/L and 2.6 ± 0.4 hr, respectively.

In chrysin-pretreated rats, the C_{max} of caffeine was significantly decreased by 32% of that in control group (p<0.05). Although the AUC_{∞} of caffeine showed a decreasing trend when compared with that of control group, the difference was not

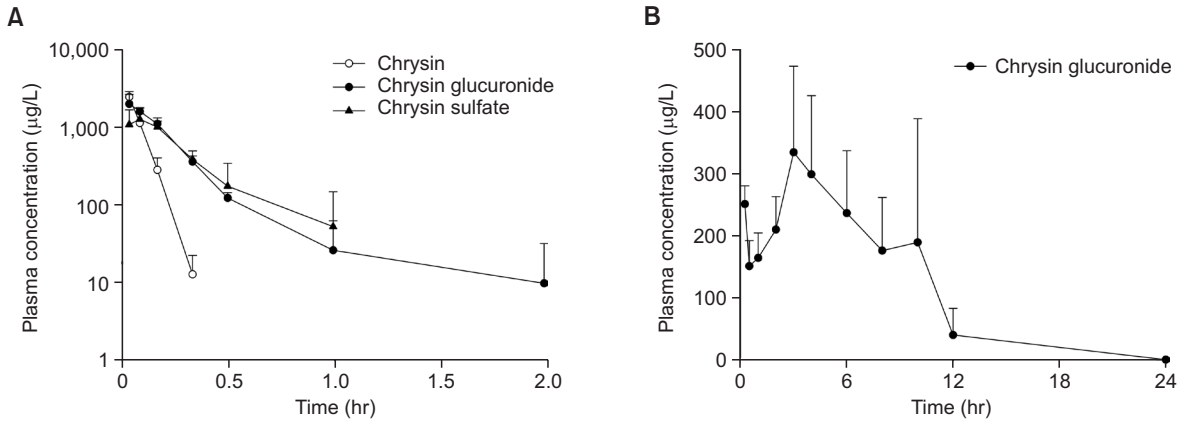

Fig. 3. Time course of the plasma concentrations of chrysin, chrysin sulfate and chrysin glucuronide following an intravenous (A) and intragastric (B) administration with 2 and 100 mg/kg chrysin in rats, respectively. Each value represents the mean ± S.D. of five animals.

Table 3. Pharmacokinetic parameters of chrysin and its metabolites following an intravenous injection with 2 mg/kg chrysin and an intragastric administration with 100 mg/kg chrysin in rats

	Intravenous administration			Intragastric administration
	Chrysin	Chrysin sulfate	Chrysin glucuronide	Chrysin glucuronide
C_{max} (μg/L)	-	1,367.0 ± 468.8	2,310.2 ± 1,069.2	364.6 ± 118.7
T_{max} (hr)	-	0.10 ± 0.07	0.03 ± 0.05	3.6 ± 0.6
AUC_{∞} (μg·hr/L)	275.9 ± 50.1	418.4 ± 189.5	472.9 ± 177.7	2,701.4 ± 962.9
CL (L/hr/kg)	7.4 ± 1.3	-	-	-
V_d (L/kg)	0.4 ± 0.1	-	-	-
$t_{1/2}$ (hr)	0.04 ± 0.01	0.2 ± 0.1	0.4 ± 0.6	3.0 ± 1.9

Mean ± S.D. of 5 rats.

statistically significant. The other pharmacokinetic parameters for caffeine and three metabolites in chrysin-pretreated rats were comparable with those in control group.

Pharmacokinetics of chrysin and its metabolites

The plasma concentration-time profile and obtained pharmacokinetic parameters of chrysin and its metabolites following chrysin administration were depicted in Fig. 3, Table 3, respectively. After an intravenous injection with chrysin in rats, it was rapidly eliminated in the systemic circulation and showed a mono-phasic decay. The calculated $t_{1/2}$ and CL of chrysin were 0.04 ± 0.01 hr and 7.4 ± 1.3 L/hr/kg, respectively. The AUC_{∞} was 275.9 ± 50.1 μg·hr/L, and estimated V_d was 0.4 ± 0.1 L/kg. The highest plasma concentrations of metabolites were 1,367.0 ± 468.8 μg/L at 0.10 ± 0.07 hr for chrysin sulfate, and 2,310.2 ± 1,069.2 μg/L at 0.03 ± 0.04 hr for chrysin glucuronide, respectively. The AUC_{∞} and $t_{1/2}$ of chrysin sulfate and chrysin glucuronide were 418.4 ± 189.5 μg·hr/L and 0.2 ± 0.1 hr, and 472.9 ± 177.7 μg·hr/L and 0.4 ± 0.6 hr, respectively.

Interestingly, however, chrysin and chrysin sulfate were not detected at all in plasma, and chrysin glucuronide could only be determined in the present study, following an intragastric administration with chrysin in rats. Therefore, the bioavailability of oral chrysin was to be almost 0%. The C_{max} of chrysin glucuronide was 364.6 ± 118.7 μg/L, and obtained AUC_{∞} was 2,701.4 ± 962.9 μg·hr/L. The T_{max} and $t_{1/2}$ of chrysin glucuronide were 3.6 ± 0.6 hr and 3.0 ± 1.9 hr, respectively. The results clearly indicated that, once absorbed in the intestine, chrysin might be conjugated very rapidly with glucuronic acid in rats.

DISCUSSION

In enriched liver microsomes, chrysin inhibited CYP1A1 and 1A2 activities *in vitro* (Table 1), as indicated in previous reports (Siess *et al*., 1995; Moon *et al*., 1998). It was also found that inhibitory effects of chrysin on other CYP enzymes were marginal. Although Tsujimoto *et al*. (2009) reported that CYP3A activity was significantly inhibited by chrysin in human liver microsomes with IC_{50} of 3.76 μM, there was no inhibitory effect in rat liver microsomes. In addition, EROD and PROD activities were significantly increased following the repeated intragastric administration of rats with chrysin for 2 weeks (Breinholt *et al*., 1999). From the results, the effects of chrysin on the drug metabolism need to be evaluated *in vivo*. In addition, chrysin is now commercially available as a nutritional supplement, so that the possible drug interaction should be considered further. Nevertheless, *in vivo* pharmacokinetic studies to evaluate the drug interaction between certain drugs and chrysin have not been reported yet.

Caffeine is a representative drug metabolized by CYP1A2,

and widely consumed as coffee, tea and drug around the world (Mandel, 2002). In addition, there are many chances that drugs are under taken with coffee instead of water. Because chrysin inhibited CYP1A-selective enzymes *in vitro*, caffeine was selected as a probe drug to evaluate the possible interaction with chrysin. By pretreatment of rats with chrysin, the C_{max} of caffeine was significantly reduced, and AUC_{∞} of that was also slightly decreased with no significant difference. In addition, the $t_{1/2}$ of caffeine and formed amounts of metabolites were not changed when compared with control group. The results indicated that chrysin might have a marginal effect on the pharmacokinetics of caffeine.

To understand the reason why chrysin did not significantly interact with caffeine, the pharmacokinetic characteristics of chrysin were also studied in the subsequent study. Until now, information on the pharmacokinetic behavior of chrysin has been very limited. The present results and literatures indicated that the low systemic exposure of chrysin following oral administration might explain the discrepancy of *in vitro* and *in vivo* results. For an example, Walle *et al*. (2001) predicted that the absolute bioavailability of chrysin might be 0.003~0.02% in human. Our present results also indicated that chrysin was not detected in systemic circulation following an oral administration, and thereby the absolute bioavailability was almost 0% in rats. Extensive and rapid presystemic, occurred in gastrointestinal tract, and systemic metabolism of chrysin might be responsible for the low bioavailability of chrysin. In the present study, chrysin rapidly disappeared in the systemic circulation following single oral and/or intravenous administration, and the $t_{1/2}$ was calculated to be about 2 min following an intravenous injection (Table 3). To understand the low bioavailability of chrysin, the possibility of conjugation was investigated subsequently in the present study. Like other flavonoid compounds, the major metabolites of chrysin were known as sulfate and glucuronide forms in both human- and rat-derived cell cultures (Galijatovic *et al*., 1999; Walle *et al*., 1999). In addition, in the study of oral administration with chrysin in human, chrysin sulfate was a predominant metabolite in plasma, and the concentrations of glucuronide conjugate of chrysin were very low (Walle *et al*., 2001). In contrast, only chrysin glucuronide was detected in rat plasma following an intragastric administration with chrysin in this study (Fig. 3). Meanwhile, these results were consistent with the *in vitro* studies. Whereas chrysin sulfate was formed two-fold higher than the amount of chrysin glucuronide formed in human Caco-2 and HepG2 cells, the production of chrysin glucuronide was about 2-fold greater than chrysin sulfate in rat hepatocyte suspension (Galijatovic *et al*., 1999). The results suggested that there might be a species difference in chrysin metabolism between rat and human, and that expression of enzymes responsible for chrysin metabolism in gastrointestinal tract could contribute to this difference. In fact, chrysin glucuronide and chrysin sulfate were mainly produced by UDP-glucuronosyltransferase (UGT) 1A1, and sulfotransferase (SULT) 1A3 and 1A1, respectively (Galijatovic *et al*., 1999; Walle *et al*., 2000). Among these enzymes, UGT1A1 and SULT1A3 are highly expressed in rat and human intestine, respectively (Shin *et al*., 2009). Although further studies are needed in the future, it would be considered as a main reason for difference in chrysin metabolism after oral administration between rat and human.

Furthermore, the patterns of plasma profile of chrysin glucuronide formed following intravenous and intrgastric administration with chrysin were significantly different (Fig. 3). Plasma concentration-time profile of chrysin glucuronide following an intravenous administration of chrysin reached the maximum at 5 min and declined bi-exponentially, whereas double peaks were observed following the intragastric administration with chrysin. The first and second peaks were observed at 0.25 and 3 hr after the dose, respectively (Fig. 3). Considering the chrysin metabolism both in liver and intestine (Galijatovic *et al*., 1999), intrinsic metabolism of chrysin in the liver following rapid absorption through gastrointestinal tract seems to contribute the first peak, and presystemic metabolism in the intestine might be responsible for the second peak at 3 hr after chrysin administration.

The rapid metabolism to its phase 2 conjugates would explain the low toxicity of chrysin. Most of the investigations with chrysin and propolis have been focused on their protective effects against many diseases, whereas the possible side effects reported would only be the risk of allergic effects and oxidative DNA damage which was related with their antioxidant effects (Hsu *et al*., 2004; Tsai *et al*., 2012). The rapid metabolism of chrysin to phase 2 conjugates also requires further studies on the active metabolite(s) responsible for its pharmacological activity in the near future.

Taken together, the present results clearly indicated that the rapid production of glucuronide conjugate from absorbed chrysin might be a critical reason why drug interaction of chrysin with caffeine could not be observed in the present study and that the most abundant metabolites of chrysin might be the glucuronide conjugate which would not have inhibitory effects on certain CYPs responsible for caffeine metabolism. Because the repeated dose of chrysin induced some CYP enzymes *in vivo* (Breinholt *et al*., 1999), the possible drug interaction of repeated chrysin with caffeine should be investigated in the near future.

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