BIOMOLECULES & THERAPEUTICS

**Original Article**
*Biomol Ther 23(2), 201-206 (2015)*

# Effects of Baicalin on Oral Pharmacokinetics of Caffeine in Rats

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## Abstract

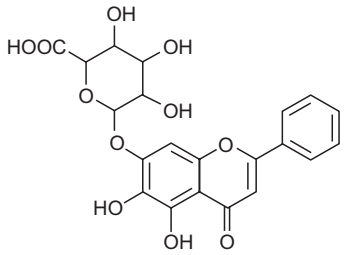
*Scutellaria baicalensis* is one of the most widely used herbal medicines in East Asia. Because baicalein and baicalin are major components of this herb, it is important to understand the effects of these compounds on drug metabolizing enzymes, such as cytochrome P450 (CYP), for evaluating herb-drug interaction. The effects of baicalin and baicalein on activities of ethoxyresorufin *O*-deethylase (EROD), methoxyresorufin *O*-demethylase (MROD), benzyloxyresorufin *O*-debenzylase (BROD), *p*-nitrophenol hydroxylase and erythromycin *N*-demethylase were assessed in rat liver microsomes in the present study. In addition, the pharmacokinetics of caffeine and its three metabolites (i.e., paraxanthine, theobromine and theophylline) in baicalin-treated rats were compared with untreated control. As results, EROD, MROD and BROD activities were inhibited by both baicalin and baicalein. However, there were no significant differences in the pharmacokinetic parameters of oral caffeine and its three metabolites between control and baicalin-treated rats. When the plasma concentration of baicalin was determined, the maximum concentration of baicalin was below the estimated $IC_{50}$ values observed *in vitro*. In conclusion, baicalin had no effects on the pharmacokinetics of caffeine and its metabolites *in vivo*, following single oral administration in rats.

**Key Words:** Baicalin, Baicalein, Caffeine, Drug interaction, Pharmacokinetics

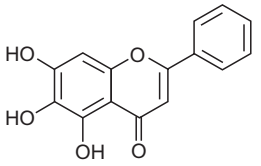
## INTRODUCTION

Herbal medicines have widely been used all over the World. It has been estimated that 70% of all medical doctors in France and Germany regularly prescribe herbal preparations (Murray, 2012). For the reason, the possibility of interaction between herbal medicines and conventional drugs is increasing, and several cases of herb-drug interactions have been reported (Alsanad *et al*., 2014).

*Scutellaria baicalensis* is one of the important medicinal herbs widely used for the treatment of various inflammatory diseases, hepatitis, tumors and diarrhea in East Asian countries, including Korea, China and Japan (Ishimaru *et al*., 1995). Baicalin (baicalein 7-*O*-glucuronide) and its aglycone, baicalein, are flavone compounds, and they are major components of *Scutellaria baicalensis* (Fig. 1). So, understanding the effects of these compounds on drug-metabolizing enzymes seems to be of importance for evaluating drug interaction with *Scutellaria baicalensis*. Especially, because cytochrome P450 (CYP) enzymes act a critical role in drug metabolism, the




Baicalin




Baicalein

**Fig. 1.** Structures of baicalin and baicalein.

**Open Access** **http://dx.doi.org/10.4062/biomolther.2014.134**



**Received** Dec 2, 2014 **Revised** Dec 23, 2014 **Accepted** Dec 23, 2014
**Published online** Mar 1, 2015

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modulation of these enzymes could affect not only the pharmacokinetics of certain drugs but also plasma concentration-dependent effects including toxicity.

Although several studies have been conducted regarding the effects of baicalin and baicalein on CYP enzymes, some discrepancies were shown between *in vitro* and *in vivo* experiments. For an example, baicalein significantly increased the CYP3A4 activity *in vitro*, whereas the pharmacokinetics of certain drugs metabolized by CYP3A4 were inhibitedly altered in the presence of baicalein (Cho *et al*., 2011; Li *et al*., 2011). The effects of baicalin on drug metabolism were also evaluated through *in vivo* studies (Gao *et al*., 2013; Gao *et al*., 2014). Considering these results, the effects of baicalin and baicalein on CYP isozymes and pharmacokinetics of certain drugs needed to be re-evaluated.

Caffeine is metabolized to *N*-demethylated metabolites, such as paraxanthine, theobromine and theophylline, by CYP1A2 and 2E1 (Schneider *et al*., 2003). For this reason, caffeine has been widely used as a probe drug to evaluate drug interaction modulated by these CYP isozymes. In this study, we evaluated the effect of baicalin and baicalein on CYP enzymes in rat liver microsomes and the effect of baicalin on pharmacokinetics of caffeine, a probe drug of CYP1A2 and 2E1, in rats, because *in vitro* inhibition studies indicated the possible modulation of these isozymes by baicalin.

## MATERIALS AND METHODS

### Materials

Baicalin (purity, >90.0%) was supplied form Tokyo Chemical Industry. Baicalein, caffeine, paraxanthine, theobromine, theophylline, ethoxyresorufin, methoxyresorufin, benzyloxyresorufin, resorufin, *p*-nitrophenol, erythromycin, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Sigma (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 (Central Valley, PA, USA). All other chemicals were of analytical grade and used as received.

### Animals

Male Sprague-Dawley (SD) rats (7 weeks, 240-270 g) were obtained from Samtako Bio 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/h, 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). All animals used in this study were cared in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Preparation of enriched rat liver microsomes

Rats were pretreated with either 3-methylcholanthrene (CYP1A inducer), phenobarbital sodium (CYP2B inducer), dexamethasone (CYP3A inducer) or acetone (CYP2E1 inducer). 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 sodium dissolved in saline was also intraperitoneally administered to rats at a dose of 80 mg/kg for 3 days. Acetone was given once only to rats by oral administration at 5 mL/kg. Two days after acetone administration, rats were sacrificed to remove 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 9,000×*g* for 20 min at 4°C, and the resulting supernatants were centrifuged again 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 -70°C until use. The content of protein in rat liver microsomes was determined using bovine serum albumin as a standard (Lowry *et al*., 1951).

### Assay of monooxygenase activities

Ethoxyresorufin *O*-deethylase (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 fluorometrically measured at λEx/λEm of 550/585 nm. Methoxyresorufin *O*-demethylase (MROD) and benzyloxyresorufin *O*-debenzylase (BROD) activities were determined by the method of Lubet *et al*. (1985) with a slight modification. The reactions were conducted under the same condition for EROD, except that the substrates were used 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 λmax 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. 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 λmax of 412 nm.

### Effect of baicalin on the pharmacokinetics of caffeine

Rats were fasted overnight, and divided into two groups (n=5). Baicalin was suspended in corn oil and vortexed before treatment. Either vehicle (corn oil at 10 ml/kg) or baicalin (200 mg/kg/10 ml corn oil) were administered orally 8 hr prior to the caffeine administration, because oral baicalin reached its maximum concentration in plasma 8 hr after administration (Lu *et al*., 2007). Caffeine was given to rats by an oral administration at 1 mg/kg. Blood samples (200 μL), collected into heparinized centrifuge tubes, were taken at 5 min before and 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 14 and 24 hr after caffeine administration via subclavian vein. After centrifugation at 10,000×*g* for 5 min, the plasma was separated. The obtained

plasma samples were stored at -70°C until analysis.

### Sample preparation for 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 vortexing for 30 sec, plasma samples were centrifuged at 10,000×*g* for 10 min. Then, the supernatant was transferred into a vial, and 5 μL of supernatant was injected for analysis.

**Table 1.** Inhibitory effects of baicalin and baicalein on CYP enzyme activities in rat liver microsomes

| CYP activity | CYP | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | Baicalin | Baicalein |
| EROD | 1A | 24.2 | 6.4 |
| MROD | 1A | 9.3 | 0.5 |
| BROD | 2B | 22.9 | 35.9 |
| PNPH | 2E1 | > 100 | > 100 |
| ERDM | 3A | > 100 | > 100 |

EROD and MROD were determined in the microsome induced by 3-methylcholanthrene.
BROD was determined in the microsome induced by phenobarbital.
PNPH was determined in the microsome induced by acetone.
ERDM was determined in the microsome induced by dexamethasone.

### Analytical method of caffeine and its metabolites

Plasma concentrations of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, were determined by a previously reported method with some modifications (Choi *et al*., 2013). Caffeine and its metabolites were analyzed by using an HPLC (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 a gradient condition as follows: initially at 90% mobile phase A; from 90% mobile phase A to 5% mobile phase A from 0.5 to 4 min; 5% mobile phase A holding for 2 min (4-6 min); from 5% mobile phase A to 90% mobile phase A during 6-7 min; and 90% mobile phase A 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 precur-

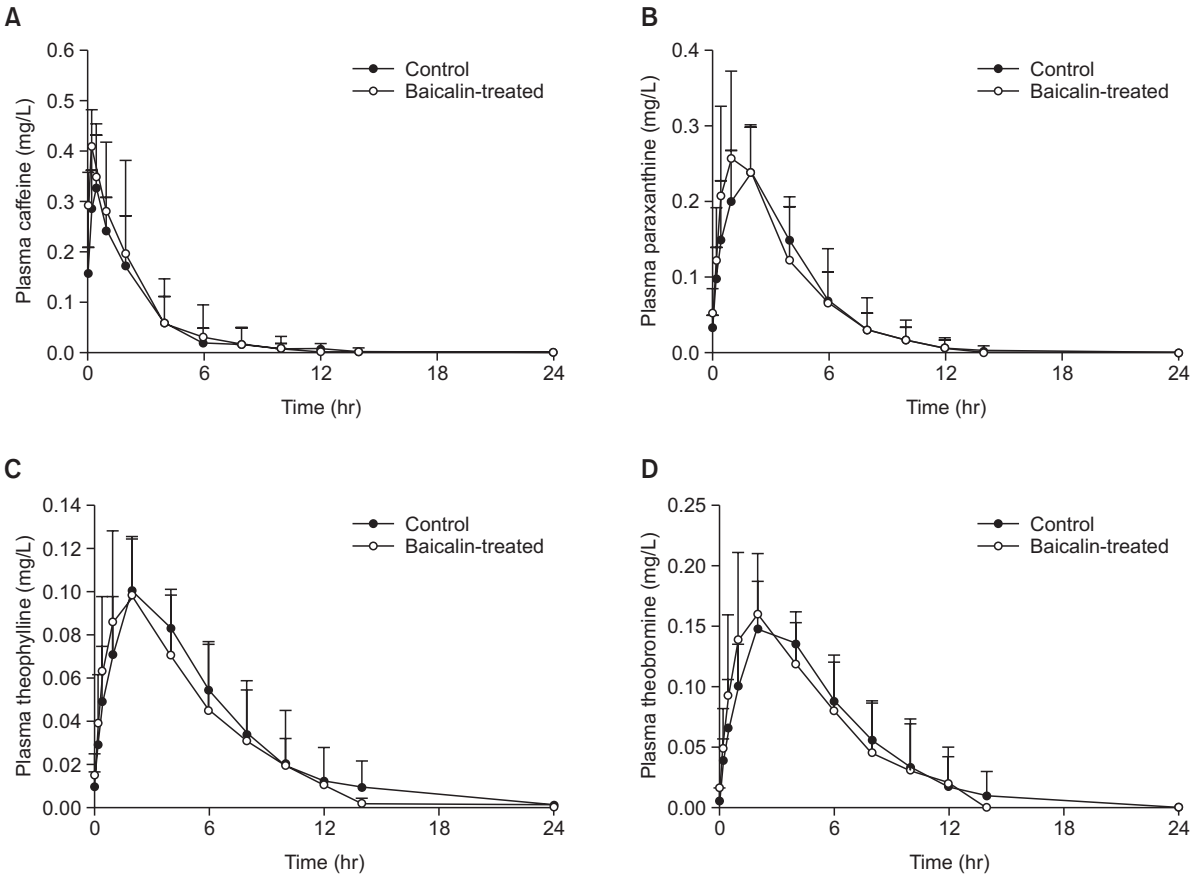


**Fig. 2.** Time courses of the plasma concentrations of caffeine (A), paraxanthine (B), theobromine (C), and theophylline (D) following an oral administration of 1 mg/kg caffeine in the presence and absence of baicalin in rats. Each value represents the mean ± S.D. of five rats.

**Table 2.** Pharmacokinetic parameters of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, following an oral administration with 1 mg/kg caffeine in the presence and absence of baicalin in rats

| Parameter | Caffeine | | Paraxanthine | | Theobromine | | Theophylline | |
|---|---|---|---|---|---|---|---|---|
| | Control | Baicalin pretreated | Control | Baicalin pretreated | Control | Baicalin pretreated | Control | Baicalin pretreated |
| $C_{max}$ (mg/L) | 0.35 ± 0.10[a] | 0.43 ± 0.07 | 0.25 ± 0.05 | 0.30 ± 0.08 | 0.16 ± 0.02 | 0.18 ± 0.03 | 0.10 ± 0.02 | 0.11 ± 0.02 |
| $T_{max}$ (hr) | 0.40 ± 0.14 | 0.45 ± 0.33 | 2.20 ± 1.10 | 1.80 ± 1.30 | 2.80 ± 1.10 | 2.40 ± 2.07 | 2.40 ± 0.89 | 2.40 ± 2.07 |
| $AUC_{\infty}$ (mg·hr/L) | 0.87 ± 0.51 | 0.98 ± 0.76 | 1.13 ± 0.24 | 1.15 ± 0.29 | 1.12 ± 0.32 | 1.14 ± 0.41 | 0.70 ± 0.20 | 0.61 ± 0.19 |
| $V_d/F$ (L/kg) | 2.35 ± 0.84 | 1.58 ± 0.61 | - | - | - | - | - | - |
| $t_{1/2}$ (hr) | 1.37 ± 0.94 | 0.95 ± 0.71 | 1.69 ± 0.63 | 1.42 ± 0.58 | 3.13 ± 1.46 | 3.07 ± 1.81 | 2.79 ± 1.24 | 2.08 ± 0.34 |

[a]Mean ± SD, n=5

sor ion and the related product ion using the ratio of area under the peak for each sample.

### Analytical method for baicalin

Plasma concentration of baicalin was determined by using same instruments above mentioned. Atlantis T3 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 a gradient condition as follows: initially at 75% mobile phase A; from 75% mobile phase A to 10% mobile phase A from 0 to 2 min; 10% mobile phase A holding for 6 min (2-8 min); from 10% mobile phase A to 75% mobile phase A from 8 to 10 min; and 75% mobile phase A holding for 8 min (10-18 min). The flow rate was maintained at 0.2 mL/min during analysis. Baicalin was detected in the positive ion mode, and mass transition used in the analysis was *m/z* 447.1→271.1. The mass transition for IS was the same as in the analytical method for caffeine and its metabolites. Quantitative analysis for baicalin was performed by multiple reaction monitoring of the precursor ion and the related product ion using the ratio of area under the peak for each sample.

### Pharmacokinetic analysis

Pharmacokinetic parameters of caffeine and its 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 by 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 ($AUC_{\infty}$), 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 ± SD obtained from 5 rats (Noh *et al*., 2011). The statistical significance of the results was analyzed using Student's *t*-test, with *p*-values of less than 0.05 considered statistically significant.

## RESULTS

### Inhibitory effects of baicalin and baicalein on monooxygenase activities

The half maximal inhibitory concentration ($IC_{50}$) of baicalin and baicalein on CYP enzymes were shown in Table 1. Baicalin inhibited EROD, MROD and BROD activities in rat liver microsomes with the $IC_{50}$ values of 24.2, 9.3 and 22.9 μM, respectively. Baicalein also showed inhibitory effects on EROD, MROD and BROD activities. Especially, the inhibition was much more potent on EROD and MROD, when compared to the inhibition by baicalin. There were no inhibitory effects on PNPH and ERDM activities by neither baicalin nor baicalein.

### Effect of baicalin on the pharmacokinetics of caffeine

Subsequently, possible drug interaction of baicalin with caffeine was studied in male SD rats, because baicalin and baicalein were inhibitory on CYP 1A activity in rat liver microsomes. Baicalin was administered orally, because baicalin could be metabolized to baicalein, the aglycone form, in the

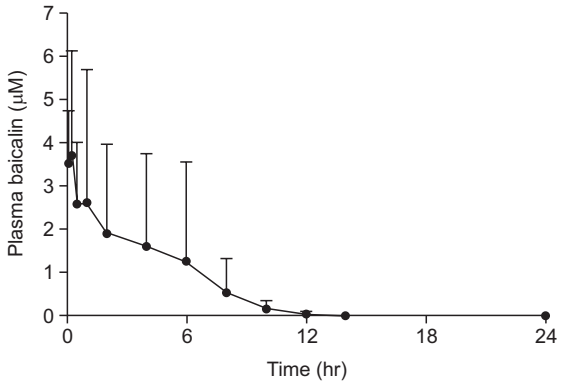


**Fig. 3.** Time course of the plasma concentrations of baicalin from 8 h after an oral administration of rats with 200 mg/kg baicalin in rats. Each value represents the mean ± S.D. of five rats.

intestine by intestinal microbiota prior to absorption (Kang *et al*., 2014). Time-concentration profiles of caffeine and its three metabolites in rats pretreated with or without baicalin were depicted in Fig. 2, and the relevant pharmacokinetic parameters of them were presented in Table 2. In control group, caffeine reached to the $C_{max}$ of 0.35 ± 0.10 mg/L at 0.40 ± 0.14 hr, and estimated $AUC_{\infty}$ and $t_{1/2}$ were 0.87 ± 0.51 mg·hr/L and 1.37 ± 0.94 hr, respectively. The $C_{max}$ of metabolites were 0.25 ± 0.05 mg/L for paraxanthine, 0.16 ± 0.02 mg/L for theobromine, and 0.10 ± 0.02 mg/L for theophylline, respectively, and the highest concentrations of them were achieved at 2.20-2.80 hr after caffeine administration. The obtained $AUC_{\infty}$ and $t_{1/2}$ of paraxanthine, theobromine and theophylline were 1.13 ± 0.24 mg·hr/L and 1.69 ± 0.63 hr, 1.12 ± 0.32 mg·hr/L and 3.13 ± 1.46 hr, and 0.70 ± 0.20 mg·hr/L and 2.79 ± 1.24 hr, respectively.

In baicalin-pretreated rats, the pharmacokinetic parameters of caffeine and its three metabolites, paraxanthine, theobromine, and theophylline, were comparable with those in the control group, and there were no significant differences of pharmacokinetic parameters between groups, as shown in Table 2. The present results indicated that baicalin might not interact with caffeine *in vivo*, although baicalin could inhibit some CYP enzyme activities *in vitro*.

### Pharmacokinetics of baicalin

Because baicalin at 200 mg/kg did not show any drug interaction with caffeine, the concentration of plasma baicalin was determined to explain the reason for inconsistency. Based on the dosing schedule for drug interaction study in Fig. 2, time-concentration profile of baicalin from 8 hr after oral administration of rats with 200 mg/kg baicalin were obtained, as depicted in Fig. 3. The plasma concentrations of baicalin were highly variable. The mean maximum concentration of baicalin was calculated to be 3.7 μM, which was below $IC_{50}$ values for EROD and MROD for baicalin that showed inhibition. The results clearly explained the reason why baicalin might not interact with caffeine in rats, and indicated that baicalin at the dose tested in the present study would be safe in terms of the possible drug interaction with certain drugs that are CYP1A2 and 2E1 substrates.

## DISCUSSION

Because the microsomes isolated from control rats were not useful to study the effects of test compound on specific CYP isozymes, the rats were pretreated with specific CYP inducers to enrich CYP 1A, 2B, 2E1 and 3A by 3-methylcholanthrene, phenobarbital, acetone, and dexamethasone, respectively (Noh *et al*., 2011). Baicalin and baicalein showed inhibitory effects on EROD and MROD *in vitro*, the markers for CYP1A. Both compounds might not affect other CYPs, including CYP 2E1 and 3A, at the concentrations tested (~100 μM). These results were consistent with previous reports (Gao *et al*., 2013; Gao *et al*., 2014). Gao *et al*. (2014) reported that baicalin was an inhibitor of CYP1A2 in human liver microsomes, and the clearance of phenacetin, a probe drug of CYP1A2, was significantly decreased following an intravenous administration with baicalin. The authors also reported that CYP2E1 was inhibited by baicalin in rat liver microsomes, but the obtained $IC_{50}$ value on CYP2E1 inhibition was slightly higher than 100 μM (Gao *et al*., 2013). However, *in vivo* studies showed some discrepancies regarding the effects of baicalin on CYP enzyme activities when compared to *in vitro* results. Repeated doses of baicalin in human could induce CYP2B6, so that the formed metabolite of bupropion, a probe drug of CYP2B6, was increased about 2-fold (Fan *et al*., 2009). In other reports, baicalein inhibited CYP3A4, and the pharmacokinetics of the CYP3A4 probe drugs were changed by co-administration with baicalein (Cho *et al*., 2011; Li *et al*., 2011). Because the effects of baicalin and baicalein on CYP enzymes were not studied at the same time, *in vitro* inhibitory potential of them were studied in enriched rat liver microsomes in the present study. Due to the strong inhibitory effects of baicalin and baicalein on CYP1A *in vitro*, caffeine was used as a test drug to investigate the possible drug interaction *in vivo*.

Orally administered baicalin was reportedly to be metabolized to baicalein by intestinal microbiota for absorption (Kim *et al*., 2008). In addition, baicalein more strongly inhibited CYP1A activities than baicalin (Table 1). Nevertheless, baicalin was selected to evaluate the drug interaction with caffeine in the present study, because it was predominantly present in the blood when even baicalein was administered (Lai *et al*., 2003; Kim *et al*., 2007), and because baicalein was hardly detected in the blood due to the biotransformation (Kang *et al*., 2014). In fact, most baicalein absorbed could be metabolized to baicalin, a glucuronate on 7-hydroxy position, and to baicalin-6-glucuronide in our recent report (Kang *et al*., 2014). In a previous report on oral pharmacokinetics, baicalin showed not only a double-peak phenomenon but also the second peak as high as the first peak at 8 hr after drug administration in rats (Lu *et al*., 2007). Based on this time-concentration profile in rat plasma, baicalin was orally administered 8 hr prior to caffeine administration in the present study. As results, although the activity of CYP1A2, responsible for caffeine metabolism, was inhibited by baicalin *in vitro*, there were no significant differences in the pharmacokinetic parameters of caffeine and its three metabolites, regardless of pretreatment of rats with baicalin (Table 2). It suggested that the systemic exposure to baicalin following a single oral administration was not enough to affect caffeine pharmacokinetics. In fact, the observed plasma concentration of baicalin determined in the present study was below the $IC_{50}$ value for MROD (9.3 μM). The extensive metabolism of baicalin to other metabolite might also contrib-

ute to this result (Wang *et al*., 2012). Taken together, it could be concluded that the drug interaction of baicalin with caffeine would be clinically marginal. The dosage of baicalin in this study was to be reasonable in clinical situation, based on the human dosage of 1.5 g/day in the previous report (Fan *et al*., 2009), because the recalculated dose of baicalin equivalent to animal was approximately to be 155 mg/kg in rats according to the FDA guideline (FDA, 2005).

In conclusion, we evaluated the effects of baicalin and baicalein on CYP enzymes in rat liver microsomes, and found that CYP1A and 2B activities were significantly inhibited by these compounds. However, baicalin had no effects on the pharmacokinetics of caffeine and its metabolites, paraxanthine, theobromine and theophylline, following a single oral administration with baicalin in rats, possibly because the plasma concentration of baicalin was not higher enough to inhibit CYP enzymes at the dose tested.

## ACKNOWLEDGMENTS

This work was supported by a grant from Yeungnam University in 2013 (213-A-380-177).

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