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Effects of Baicalein on the Bioavailability of Nicardipine in Rats

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ABSTRACT – This study was to investigate the effect of baicalein, an antioxidant, on the bioavailability of nicardipine after orally or intravenously administered nicardipine in rats. Nicardipine was administered orally (12 mg/kg) or intravenously (4 mg/kg) with or without orally administered baicalein (0.4, 2 or 10 mg/kg) to rats. In the inhibitory effect of baicalein on CYP3A4 activity, baicalein inhibited CYP3A4 activity with IC_{50} values of 9.2 μM. The cell-based P-gp activity test using rhodamine-123 also showed that baicalein (30-10 μM, $p<0.01$) significantly inhibited P-gp activity. Compared with the control group (given nicardipine alone), the area under the plasma concentration-time curve (AUC) was significantly (2 mg/kg, $P<0.05$; 10 mg/kg, $P<0.01$) increased by 25.9-60.0%, and the peak concentration (C_{max}) was significantly (10 mg/kg, $P<0.01$) increased by 40.0% in the presence of baicalein after orally administration of nicardipine. Consequently, the relative bioavailability (R.B.) of nicardipine was increased by 1.26- to 1.60-fold and the absolute bioavailability (A.B.) was significantly (2 mg/kg, $P<0.05$; 10 mg/kg, $P<0.01$) increased by 26.0-59.9%. Compared to the i.v. control, baicalein did not significantly change pharmacokinetic parameters of nicardipine in i.v. administration. Accordingly, the enhanced oral bioavailability of nicardipine might be mainly due to increased intestinal absorption caused by P-gp inhibition rather than to reduced elimination of nicardipine by baicalein. The increase in the oral bioavailability might be mainly attributed to enhanced absorption in the small intestine via the inhibition of P-gp and reduced first-pass metabolism of nicardipine via the inhibition of the CYP3A subfamily in the small intestine and/or in the liver by baicalein. Based on these results, nicardipine dosage should be adjusted when given concomitantly with baicalein.

Key words – Nicardipine, Baicalein, CYP3A4, P-gp, Bioavailability, Pharmacokinetics, Rats

Nicardipine, a dihydropyridine calcium channel antagonist, causes coronary and peripheral vasodilatation by blocking the influx of extracellular calcium across cell membranes. Nicardipine is arterioselective and effective for the treatment of hypertension, myocardial ischemia, and vasospasm in surgical patients (Kishi et al., 1984; Hysing et al., 1986). Nicardipine has also been used experimentally as a probe to study the effects of calcium channel antagonists on the role of sympathetic nervous system activity in the development of cardiovascular risk (Van Zwieten et al., 1997). The pharmacokinetics of nicardipine are non-linear due to hepatic first-pass metabolism, and show a bioavailability of about 35% following a 30 mg dose at steady state (Graham et al., 1984, 1985). They are primarily substrates of CYP3A subfamily enzymes, especially CYP3A4 in humans, and metabolized to pharmacologically inactive forms (Higuchi and Shiobara, 1980; Guengerich, 1991; Guengerich et al., 1986). In addition, nicardipine is also a P-glycoprotein (P-gp) substrate (Wang et al., 2000; Hu et al., 1996).

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Flavonoids represent a group of phytochemicals that are produced by various plants in high quantities (Dixon and Steele, 1999). They exhibit a wide range of beneficial biological activities including antioxidative, radical scavenging, antiatherosclerotic, antitumor and antiviral effects (Nijveldt et al., 2001).

Baicalein are the major flavonoids of Scutellariae radix and are mainly present as their glucuronide forms. Baicalein glucuronides can constitute up to 20% of the dry weight of Scutellariae radix, respectively (Sagara et al., 1985; Takino et al., 1987). After digestion, the glucuronides are readily hydrolyzed by intestinal bacteria (Manach et al., 1996). The evidence suggests that baicalein and related flavonoids are the major components responsible for the pharmacological effects of Scutellariae radix (Lin and Shieh, 1996; Matsuzaki et al., 1996). Baicalein inhibits testosterone 6bâ-hydroxylation (CYP3A4) activity with an IC_{50} of 17.4 ìM. Baicalein is the inhibitor of P-gp in the KB/MDR cell system (Lee et al., 2004), but the effect of baicalein on P-gp inhibition is partially ambiguous. Thus, we reevaluated P-gp activity using rhodamine-123 retention assay in P-gp-overexpressing MCF-7/ADR cells. The effect of baicalein was similar to that of quercetin (Kitagawa et al., 2005). Baicalein and nicardipine

could be prescribed for the treatment or prevention of cardiovascular diseases as a combination therapy. Baicalein might affect the bioavailability and pharmacokinetics of nicardipine when baicalein and nicardipine were used concomitantly. The low bioavailability of oral nicardipine is mainly due to pre-systemic metabolism and P-gp mediated efflux in the intestine. However, the effect of baicalein on the bioavailability of nicardipine has not been reported in vivo.

Therefore, the aim of this study was to investigate the pharmacokinetics or bioavailability of nicardipine in the presence of baicalein in rats.

Materials and Methods

Chemicals and apparatus

Nicardipine, baicalein and nimodipine [an internal standard for high-performance liquid chromatograph (HPLC) analysis for nicardipine] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile were acquired from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade.

Apparatus used in this study were a HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA), a Bransonic® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats of 7-8 weeks of age (weighing 270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Co., Gangwon, Republic of Korea) and tap water. The animals were housed (two rats per cage) in a clean room maintained at a temperature of 22 ± 2°C and relative humidity of 50-60%, with 12 h light and dark cycles. The rats were acclimated under these conditions for at least 1 week. All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA) and the Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the protocol of this animal study. The rats were fasted for at least 24 h prior to beginning the experiments and had free access to tap water. Each animal was anaesthetized lightly with ether. The left femoral artery and vein were cannulated using polyethylene tubing (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and i.v. injection, respectively.

Oral and intravenous administration of nicardipine

The rats were divided into six groups (n = 6, each); an oral group (12 mg/kg of nicardipine dissolved in water; homogenized at 36°C for 30 min; 3.0 mL/kg) without (control) or with 0.4, 2 or 10 mg/kg of oral baicalein, and an i.v. group (4 mg/kg of nicardipine, dissolved in 0.9% NaCl solution; homogenized at 36°C for 30 min; 1.5 mL/kg) without (control) or with 0.4, 2 or 10 mg/kg of oral baicalein. Oral nicardipine was using a feeding tube, and baicalein was intragastrically administered 30 min prior to oral or intravenous administration of nicardipine. Nicardipine for i.v. administration was injected through the femoral vein within 0.5 min. A 0.4 mL blood sample was collected into heparinized tubes from the femoral artery at 0 (to serve as a control), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after intravenous infusion, and 0.1, 0.25, 0.5, 1, 2, 3, 6, 8, 12 and 24 h for oral study. The blood samples were centrifuged at 13,000 rpm for 5 min, and the plasma samples were stored at -40°C until HPLC analysis of nicardipine.

HPLC assay

The plasma concentrations of nicardipine were determined by a HPLC assay method reported by Eastwood et al. (1990). Briefly, a 50 μL of nimodipine (2 μg/mL), a 20 μL of 2 N sodium hydroxide solution and 1.2 mL of tert-butylmethylether: Hexane (75:25) were added to a 0.2 mL of the plasma sample. The mixture was then stirred for 2 min and centrifuged at 13,000 rpm for 10 min. A 1.0 mL of the organic layer was transferred to a clean test tube and evaporated at 35°C under a stream of nitrogen. The residue was dissolved in 200 μL of the mobile phase and centrifuged (13,000 rpm, 5 min). A 50 μL of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Symmetry® C_{18} column (4.6×150 mm, 5 μm, Waters Co.), and a μBondapak™ C_{18} HPLC Precolumn (10 μm, Waters Co.). The mobile phase was acetonitrile: 0.015 M KH_2PO_4 (60:40, v/v, PH 4.5) with 2.8 mM triethylamine, which was run at a flow rate of 1.5 mL/min. Chromatography was performed at a temperature of 30°C that was set by a HPLC column temperature controller. The UV detector was set to 254 nm. The retention times of nicardipine and the internal standard were 7.8 and 4.2 min, respectively (Figure 1). The detection limits of nicardipine in rat's plasma was 10 ng/mL. The coefficients of variation for nicardipine were below 14.1%.

CYP inhibition assay

The inhibition assays on the human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi et al., 1997). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates 50 mM [7-Benzyloxy-4-(trifluoromethyl) coumarin (7-BFC) and 150 mM 7-Methoxy-4-trifluoromethyl coumarin (7-MFC) for CYP3A4 and 2C9, respectively] were incubated with or without test compounds in a reaction mix containing 1 pmol of P450 enzyme and the NADPH generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/mL glucose 6-phosphate dehydrogenase and 3.3 mM $MgCl_2$) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45 min. Metabolite concentrations were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive controls (1 μM ketoconazole for CYP3A4) were run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

Rhodamine-123 retention assay

MCF-7/ADR cells were seeded on 24-well plates at a seeding density of 10^5 cells. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to control values.

Pharmacokinetic analysis

The plasma concentration data were analyzed by noncompartmental method using WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of nicardipine concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of nicardipine in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The total body clearance for the i.v. route (CL_t) was calculated from D/AUC, where D is the dose of nicardipine. The absolute bioavailability (A.B.) was calculated by $AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral}$, and the relative bioavailability (R.B.) of nicardipine was estimated by $AUC_{with\ baicalein}/AUC_{control}$.

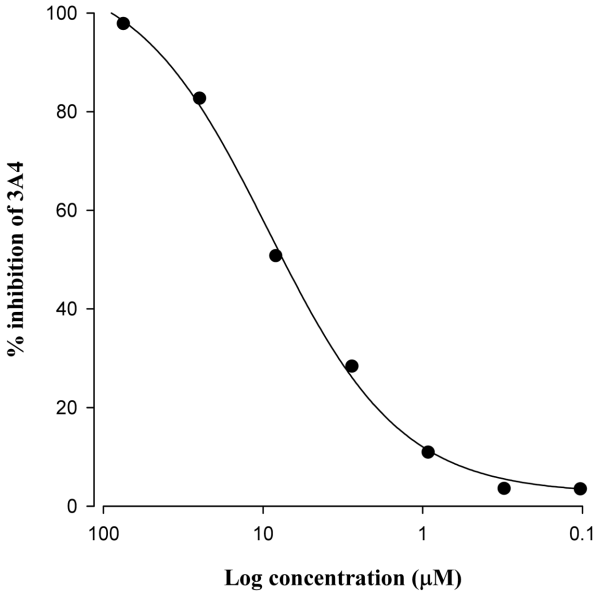

Figure 1. Inhibitory effect of baicalein on CYP3A4 activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.

Statistical analysis

All mean values are presented with their standard deviation (Mean ± S.D.). Statistical analysis was conducted using a one-way ANOVA followed by *a posteriori* testing with Dunnett's correction. Differences were considered significant at a level of $P<0.05$.

Results

Inhibition of CYP3A4

The inhibitory effect of baicalein on CYP3A4 activity is shown in Fig 1. Baicalein inhibited CYP3A4 activity with IC_{50} values of 9.2 μM.

Rhodamine-123 retention assay

In this study, the cell-based P-gp activity test using

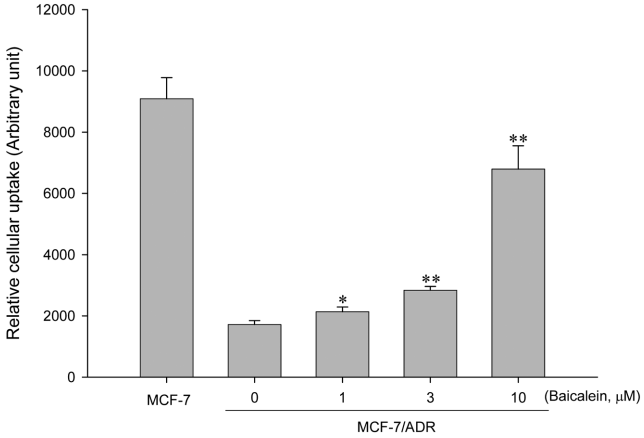

Figure 2. Effect of baicalein on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean±S.D. of 6 separate samples (significant versus control MCF-7 cells, **p<0.01).

rhodamine-123 also showed that baicalein (30-10 μM, p<0.01) significantly inhibited P-gp activity (Fig. 2).

Effect of baicalein on the pharmacokinetics of oral nicardipine

Figure 3 shows the plasma concentration–time profiles of nicardipine after oral administration at a dose of 12 mg/kg of nicardipine in rats with or without baicalein (0.4, 2 or 10 mg/kg), and the pharmacokinetic parameters of oral nicardipine are summarized in Table I. The area under the plasma concentration-time curve (AUC) was significantly (2 mg/kg, P<0.05; 10 mg/kg, P<0.01) increased by 25.9-60.0%, and the peak concentration (C_{max}) was significantly (10 mg/kg, P<0.01) increased by 40.0% in the presence of baicalein after orally administration of nicardipine. Consequently, the relative bioavailability (R.B.) of nicardipine was increased by 1.26- to 1.60-fold. The absolute bioavailability (A.B.) was significantly (2 mg/kg, P<0.05; 10 mg/kg, P<0.01) increased by 26.0-59.9%. The $t_{1/2}$ of nicardipine was significantly prolonged by baicalein. However, there was no significant change in the T_{max} in the presence of baicalein.

Compared to the i.v. control, baicalein did not significantly change pharmacokinetic parameters of nicardipine in i.v. administration. The AUC and the $t_{1/2}$ of nicardipine were increased but were not significant in i.v. administration. Accordingly, the enhanced oral bioavailability of nicardipine might be mainly due to increased intestinal absorption caused by P-gp inhibition rather than to reduced elimination of nicardipine by baicalein.

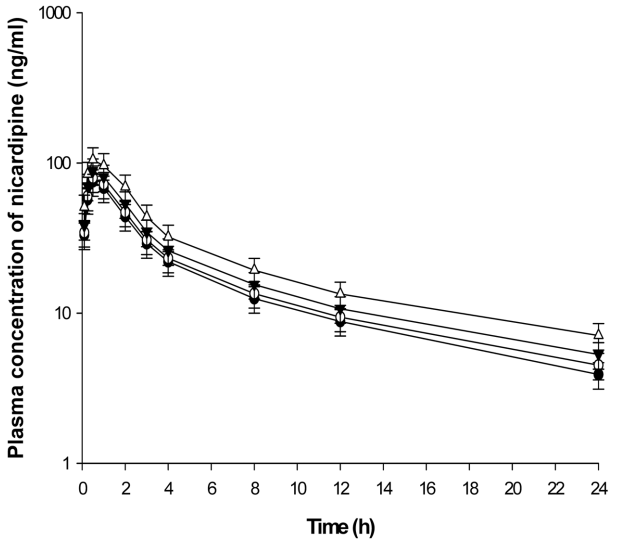

Figure 3. Mean plasma concentration–time profiles of nicardipine after oral administration of nicardipine (12 mg/kg) to rats with or without baicalein (0.4, 2 or 10 mg/kg). Bars represent the standard deviation (n = 6), (●) 12 mg/kg of oral nicardipine, (○) with 0.4 mg/kg of baicalein, (▼) with 2 mg/kg of baicalein, (▽) with 10 mg/kg of baicalein.

Table I. *Mean (±S.D.) Pharmacokinetic Parameters of Nicardipine after Oral Administration of Nicardipine (12 mg/kg) to Rat with or without Baicalein*

Paramater	Control	Nicardipine + baicalein		
		0.4 mg/kg	2 mg/kg	10 mg/kg
AUC(ng·h/mL)	413±78	452±81	520±111*	661±130**
C_{max} (ng/mL)	75±13	79±14	88±17	105±20**
T_{max} (h)	0.5	0.5	0.5	0.5
$t_{1/2}$(h)	9.6±1.7	10.3±2.0	10.7±2.1	11.6±2.1*
R.B.(%)	100	109	126	160
A.B.(%)	14.7±3.1	16.1±3.4	18.5±3.5*	23.5±4.3**

Mean ± S.D., n = 6. *P<0.05, **P<0.01, compared with the control group.
AUC: area under the plasma concentration–time curve from 0 h to infinity, C_{max}: peak plasma concentration, T_{max}: time to reach peak plasma concentration, $t_{1/2}$: terminal half-life, R.B.: relative bioavailability, A.B.: absolute bioavailability.

Table II. *Mean (±S.D.) Pharmacokinetic Parameters of Nicardipine after Intravenous Administration of Nicardipine (4 mg/kg) to Rats with or without Baicalein*

Paramater	Control	Nicardipine + baicalein	
		2 mg/kg	10 mg/kg
AUC(ng·h/mL)	937±183	1025±201	1114±211
CL_t(mL/h/kg)	53.4±13.6	48.7±11.5	44.9±10.2
$t_{1/2}$(h)	8.4±1.4	8.6±1.5	9.0±1.7
R.B.(%)	100	109	118

Mean ± S.D., n = 6.
AUC: area under the plasma concentration–time curve from 0 h to infinity; CL_t: total plasma clearance; $t_{1/2}$: terminal half-life; R.B.: relative bioavailability.

Discussion

CYPs enzymes make a contribution significantly to the first-pass metabolism and oral bioavailability of many drugs. The first-pass metabolism of compounds in the intestine limits absorption of toxic xenobiotics and may ameliorate side effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug and drug interactions when one agent decreases or increases the bioavailability and absorption rat constant of a concurrently administered drug (Kaminsky and Fasco, 1991).

Based on the broad overlap in the substrate specificities as well as co-localization in the small intestine, the primary site of absorption for orally administered drugs, CYP3A4 and P-gp have been recognized as a concerted barrier to the drug absorption (Benet et al., 2003; Cummins et al., 2002). Therefore, dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailability of many drugs where CYP3A4 metabolism as well as P-gp mediated efflux is the major barrier to the systemic availability. Besides the extensive metabolism by CYP3A4, nicardipine appeared to be the substrate of P-gp, suggesting that P-gp and CYP3A4 should act synergistically to limit the oral bioavailability of nicardipine (Wacher et al., 2001; Saeki et al., 1993).

Studies on drug interactions with grapefruit juice have provided much understanding of the role of intestinal CYP450 in the absorption of orally administered drugs. CYP3A4 is the predominant P450 present in the small intestine (Kolars, 1992) Oral administered nicardipine is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux. As shown in Table 1, the area under the plasma concentration-time curve (AUC) was significantly increased by 25.9-60.0%, and the peak concentration (C_{max}) was significantly increased by 40.0% in the presence of baicalein after orally administration of nicardipine. Consequently, The absolute bioavailability (A.B.) was significantly (2 mg/kg, $P<0.05$; 10 mg/kg, $P<0.01$) increased by 26.0-59.9%.

The enhanced oral bioavailability of nicardipine might be due to decreased P-gp efflux and CYP3A metabolism of nicardipine in the intestine and/or liver. 0.6 mg/kg of baicalein did not significantly change pharmacokinetic parameters of nicardipine, possibly it can either inhibit or stimulate rat CYPs depending upon their structures, concentrations, and experimental conditions. This result appeared to be consistent with a previous report that oral administration of morin or resveratrol significantly enhanced the oral bioavailability of nicardipine in rats (Piao and Choi, 2008; Choi et al., 2009).

Figure 4 shows the plasma concentration-time profiles of nicardipine after i.v. injection (4 mg/kg) in rats with or without baicalein (2 or 10 mg/kg). As shown in Table II, baicalein did not significantly change pharmacokinetic parameters of nicardipine in i.v. administration, suggesting that baicalein may improve the oral bioavailability of nicardipine by increasing the absorption or reducing gut wall metabolism rather than elimination of nicardipine.

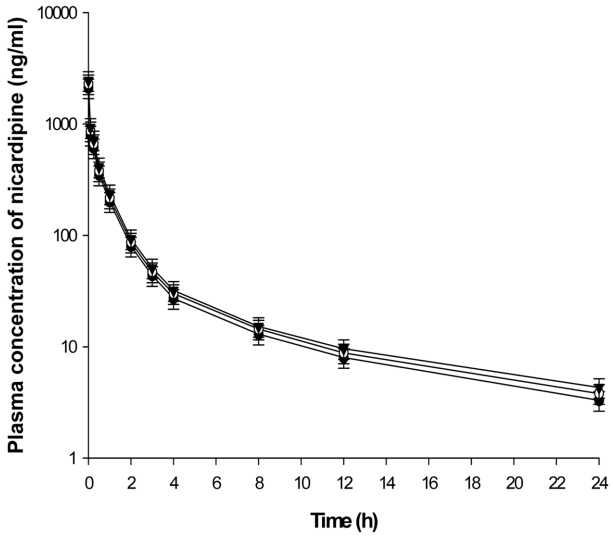

Figure 4. Mean plasma concentration–time profiles of nicardipine after i.v. administration of nicardipine (4 mg/kg) to rats with or without baicalein (2 or 10 mg/kg). Bars represent the standard deviation (n = 6), (●) 12 mg/kg of oral nicardipine, (○) with 2 mg/kg of baicalein, (▼) with 10 mg/kg of baicalein.

The increased bioavailability of orally administered nicardipine might be due to competitive inhibition of CYPs and P-gp in the intestine by baicalein, since the inhibition of CYP isoenzyme and P-gp in the liver and kidney was not marked after intravenous administration as mentioned above. The increase in the oral bioavailability might be mainly attributed to enhanced absorption in the small intestine via the inhibition of P-gp and reduced first-pass metabolism of nicardipine via the inhibition of the CYP3A subfamily in the small intestine and/or in the liver by baicalein.

Conclusion

The increase in the oral bioavailability might be mainly attributed to enhanced absorption in the small intestine via the inhibition of P-gp and reduced first-pass metabolism of nicardipine via the inhibition of the CYP3A subfamily in the small intestine and/or in the liver by baicalein. Therefore, concomitant use of baicalein or baicalein-containing dietary supplements with nicardipine will require close monitoring for potential drug interactions.

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