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Effects of morin on the pharmacokinetics of nicardipine after oral and intravenous administration of nicardipine in rats

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Abstract

This study investigated the effects of orally administered morin, an inhibitor of cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp), on the pharmacokinetics of orally and intravenously administered nicardipine in rats. Nicardipine is reportedly a substrate for CYP3A4 and P-gp. Nicardipine was administered orally (12 mg kg^{-1}) with or without orally administered morin (1.5, 7.5 and 15 mg kg^{-1}), and intravenously (4 mg kg^{-1}) with or without orally administered morin (7.5 and 15 mg kg^{-1}). In the presence of morin, the pharmacokinetic parameters of nicardipine were significantly altered in the oral group but not in the intravenous group, suggesting that CYP3A-mediated metabolism of nicardipine in the liver is not significantly inhibited by morin. The presence of 7.5 and 15 mg kg^{-1} of morin significantly increased ($P < 0.01$, 67.8–112%) the area under the plasma concentration–time curve and the peak plasma concentration ($P < 0.01$, 53.5–93.1%) of orally administered nicardipine. The presence of 7.5 and 15 mg kg^{-1} of morin significantly decreased ($P < 0.01$, 40.4–52.8%) the total body clearance of orally administered nicardipine compared with the control group. The enhanced oral bioavailability of nicardipine suggests that intestinal-mediated CYP3A4 metabolism and P-gp-mediated efflux of nicardipine are inhibited by morin. Based on these results, concomitant use of morin or morin-containing dietary supplements with nicardipine may require close monitoring for potential drug interactions.

Introduction

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 ischaemia 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). Nicardipine is primarily a substrate of cytochrome P450 3A (CYP3A) subfamily enzymes, especially CYP3A4 in humans, and is metabolized to pharmacologically inactive forms (Higuchi & Shiobara 1980; Guengerich et al 1986; Guengerich 1991). In addition, nicardipine is also a P-glycoprotein (P-gp) substrate (Hu et al 1996; Wang et al 2000).

Flavonoids are the most abundant polyphenolic compounds in the human diet (e.g. fruits, vegetables, tea and red wine). Many reports claim various beneficial pharmacological properties for flavonoids, including antioxidation, antiviral, antimutagenesis and anti-inflammatory activities (Middleton et al 2000).

Morin (3,5,7,2′,4′-pentahydroxyflavone) is a flavonoid constituent of many herbs and fruits. Morin inhibits P-gp-mediated cellular efflux of P-gp substrates (Zhang & Morris 2003; Kitagawa et al 2005) and could modulate the activity of metabolic enzymes, including CYP (Hodek et al 2002). Morin significantly increased the bioavailability of diltiazem in rats, which may be due to the inhibition of CYP3A-mediated metabolism of diltiazem (Choi & Han 2005). Morin, an inhibitor of CYPs and P-gp, may improve the bioavailability of orally and intravenously administered nicardipine. Three doses (1.5, 7.5 and 15 mg kg^{-1}) of morin were

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selected based on previous reports (Choi & Burm 2006; Choi et al 2006; Shin et al 2006).

The bioavailability of orally and intravenously administered nicardipine is mainly affected by CYP3A4 and P-gp during first-pass metabolism. When morin is administered with nicardipine, it may influence the bioavailability of nicardipine. However, the effects of morin on the pharmacokinetics of nicardipine have not been reported in rats. The purpose of this study was to investigate the effects of morin on the pharmacokinetics and bioavailability of orally and intravenously administered nicardipine in rats.

Materials and Methods

Materials

Nicardipine, morin and nimodipine, an internal standard for the high-performance liquid chromatography (HPLC) analysis of nicardipine, were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). HPLC-grade acetonitrile was acquired from Merck Co. (Darmstadt, Germany). All other chemicals for this study were of reagent grade and were used without further purification.

The HPLC apparatus used in this study was 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), an 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 microcentrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague–Dawley rats, 7–8 weeks old (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 a 12-h light–dark cycle. 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). 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 before beginning the experiments but had free access to tap water. Each animal was anaesthetized before the surgery. 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 intravenous injection, respectively.

Oral and intravenous administration of nicardipine

The rats were divided into six groups (n = 6 in each group): the oral group (12 mg kg^{-1} of nicardipine dissolved in water, homogenized at 36°C for 30 min; 3.0 mL kg^{-1}) without (control) or with 1.5, 7.5 and 15 mg kg^{-1} of oral morin, and the intravenous group (4 mg kg^{-1} of nicardipine, dissolved in 0.9% NaCl solution, homogenized at 36°C for 30 min; 1.5 mL kg^{-1}) without (control) or with 7.5 and 15 mg kg^{-1} of oral morin. Oral nicardipine was administered intragastrically using a feeding tube, and morin was intragastrically administered 30 min before oral administration of nicardipine. Nicardipine for intravenous administration was injected through the femoral vein within 0.5 min. A 0.45-mL blood sample was collected into heparinized tubes from the femoral artery at 0 h (to serve as a control), 0.017 h (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after intravenous infusion, and 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h for the oral study. The blood samples were centrifuged at 13 000 rev min^{-1} for 5 min, and the plasma samples were stored at –40°C until HPLC analysis of nicardipine. Approximately 1 mL of whole blood collected from untreated rats was infused via the femoral artery at 0.25, 1, 3 and 8 h to replace the blood loss due to blood sampling.

HPLC assay

The plasma concentrations of nicardipine were determined by the HPLC assay method reported by Eastwood et al (1990), with a slight modification. Briefly, 50 μL of 2 μg mL^{-1} nimodipine, as an internal standard, 20 μL of 2 M sodium hydroxide solution and 1.2 mL of *tert*-butylmethylether/hexane (75:25) were added to 0.2 mL of the plasma sample. The mixture was then stirred for 2 min and centrifuged at 13 000 rev min^{-1} for 10 min. Then, 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 at 13 000 rev min for 5 min. A total of 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^{-1}. Chromatography was performed at a temperature of 30°C, which 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 7.5 and 4.4 min, respectively (Figure 1). The detection limit of nicardipine in rat plasma was 10 ng mL^{-1}. The coefficients of variation for nicardipine were below 14.1%.

Pharmacokinetic analysis

The plasma concentration data were analysed by a non-compartmental 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 (t½) 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

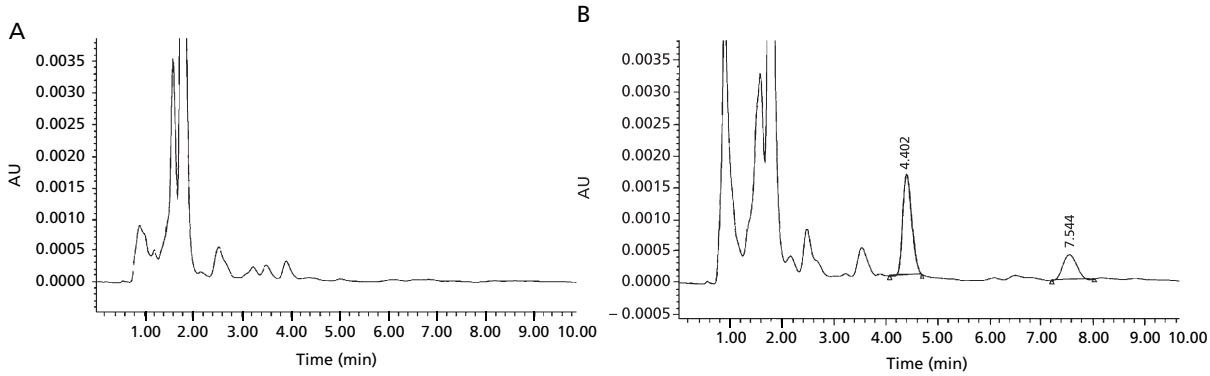

Figure 1 Chromatograms of blank plasma (A) and plasma with the internal standard (4.4 min) and nicardipine (7.5 min) added (B).

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 infinity ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. Total body clearance (CL/F) was calculated by dose/AUC. The relative bioavailability of nicardipine was estimated by $AUC_{combined}/AUC_{control}$.

Statistical analysis

Results are presented as mean ± s.d. Statistical analysis was conducted using a one-way analysis of variance followed by *a posteriori* testing with Dunnett's correction. Differences were considered significant at a level of $P < 0.05$.

Results and Discussion

With the increase in the consumption of herbal products as alternative medicines, more preclinical and clinical investigation of interactions between herbal constituents and drugs needs to be performed to prevent potential adverse reactions. The present study evaluated the effect of morin on the pharmacokinetics of nicardipine in rats to determine a potential interaction between morin and nicardipine. In particular, given that both morin and nicardipine can interact with CYP3A4 and P-gp, the concomitant use of morin may affect the pharmacokinetics of nicardipine (Saeki et al 1993; Wacher et al 2001).

Figure 2 shows the plasma concentration–time profiles of nicardipine after oral administration at a dose of 12 mg kg^{-1} of nicardipine in the presence or absence of morin (1.5, 7.5 and 15 mg kg^{-1}). The pharmacokinetic parameters of oral nicardipine are summarized in Table 1. The presence of morin (7.5 and 15 mg kg^{-1}) significantly ($P < 0.01$) increased the AUC of nicardipine by 67.8–112%. The presence of morin (7.5 and 15 mg kg^{-1}) significantly ($P < 0.01$) increased the C_{max} of nicardipine by 53.5–93.1%. The presence of morin (7.5 and 15 mg kg^{-1}) significantly ($P < 0.01$) decreased the CL/F of nicardipine by 40.4–52.8%. Consequently, the

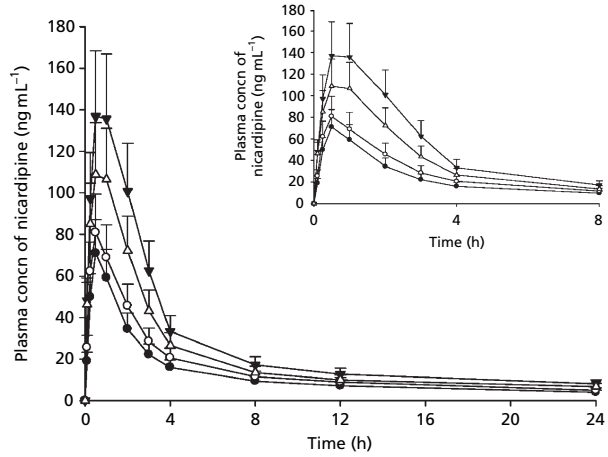

Figure 2 Mean plasma concentration–time profiles of nicardipine after oral administration of nicardipine (12 mg kg^{-1}) to rats in the presence or absence of morin (1.5, 7.5 and 15 mg kg^{-1}). Bars represent the standard deviation (n = 6); •, 12 mg kg^{-1} nicardipine; ○, in the presence of 1.5 mg kg^{-1} morin; ▼, in the presence of 7.5 mg kg^{-1} morin; △, in the presence of 15 mg kg^{-1} morin.

relative bioavailability of nicardipine was increased by 1.68- to 2.12-fold. The presence of morin did not significantly change the K_{el}, T_{max} and t½ of oral nicardipine. Orally administered nicardipine is a substrate for CYP3A-mediated metabolism and P-gp-mediated efflux. As with its isomer quercetin, orally administered morin is easily absorbed in the intestine, but it is mainly metabolized to glucuronides and sulfates, and the amount of the parent form in systemic circulation is quite low (Hsiu et al 2001; Hou et al 2003). Therefore, the enhanced oral bioavailability of nicardipine in the presence of morin might be due to the decreased presystemic extraction of nicardipine in the intestine and/or liver. These results are consistent with a previous report from Choi & Han (2005) in which morin significantly enhanced the oral bioavailability of diltiazem in rats. The effect of morin on the oral pharmacokinetics of nicardipine was not dose proportional. This may be because flavonoids can either inhibit or induce metabolic enzymes depending on their structure, dosage and the experimental

Table 1 Pharmacokinetic parameters of nicardipine after oral administration of nicardipine (12 mg kg^{-1}) to rats in the absence or presence of morin

Parameter	Control	Nicardipine + morin		
		1.5 mg kg^{-1} Morin	7.5 mg kg^{-1} Morin	15 mg kg^{-1} Morin
AUC (ng mL^{-1} h)	365 ± 91.2	447 ± 106	773 ± 169**	612 ± 142**
C_{max} (ng mL^{-1})	70.9 ± 15.5	80.8 ± 19.5	137 ± 32.8**	109 ± 27.1**
T_{max} (h)	0.5	0.5	0.5	0.5
CL/F (mL min^{-1} kg^{-1})	549 ± 125	447 ± 113	258 ± 62.3**	327 ± 78.3**
K_{el} (h^{-1})	0.064 ± 0.016	0.065 ± 0.016	0.064 ± 0.015	0.061 ± 0.014
t½ (h)	10.9 ± 2.40	10.6 ± 2.63	10.9 ± 2.73	11.4 ± 2.89
Relative bioavailability (%)	100	123	212	168

AUC, area under the plasma concentration–time curve from time 0 h to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach peak concentration; CL/F, total plasma clearance; K_{el}, elimination rate constant; t½, terminal half-life. Data are mean ± s.d., n = 6. $^{**}P < 0.01$, significantly different compared with the control group.

conditions (Hodek et al 2002; Choi et al 2006; Shin et al 2006).

Figure 3 shows the plasma concentration–time profiles of nicardipine after intravenous injection (4 mg kg^{-1}) in the presence or absence of morin (7.5 and 15 mg kg^{-1}). We omitted the 1.5-mg kg^{-1} dose of morin in the intravenous studies because it appeared to be ineffective in the oral studies. As shown in Table 2, the presence of morin (7.5 and 15 mg kg^{-1}) enhanced the AUC of nicardipine and decreased CL_t and K_{el}, but the effect was not statistically significant.

The presence of morin significantly enhanced the oral bioavailability of nicardipine, whereas it did not affect the intravenous pharmacokinetics of nicardipine, suggesting that the enhanced bioavailability of nicardipine might be mainly due to the inhibitory effect of morin on the intestinal extraction of nicardipine.

In conclusion, the presence of morin (7.5–15 mg kg^{-1}) enhanced the oral bioavailability of nicardipine. Therefore, concomitant use of morin or morin-containing dietary supplements with nicardipine may require close monitoring for potential drug interactions.

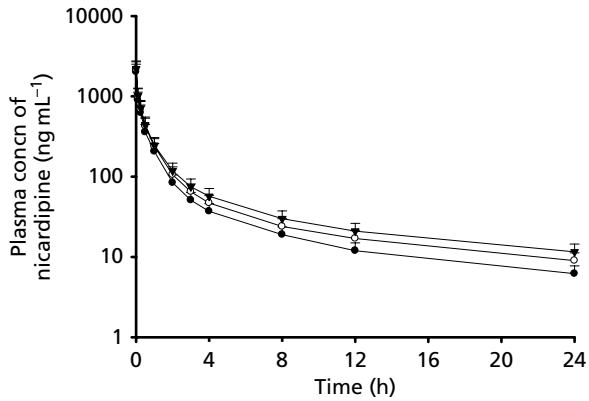

Figure 3 Mean plasma concentration–time profiles of nicardipine after intravenous administration of nicardipine (4 mg kg^{-1}) to rats in the presence or absence of morin. Bars represent the standard deviation (n = 6); •, 4 mg kg^{-1} nicardipine; ○, in the presence of 7.5 mg kg^{-1} morin; ▼, in the presence of 15 mg kg^{-1} morin.

Table 2 Pharmacokinetic parameters of nicardipine after intravenous administration of nicardipine (4 mg kg^{-1}) to rats in the absence or presence of morin

Parameter	Control	Nicardipine + morin	
		7.5 mg kg^{-1} Morin	15 mg kg^{-1} Morin
AUC (ng mL^{-1} h	1138 ± 292	1402 ± 369	1580 ± 391
CL_t (mL min^{-1} kg^{-1})	58.6 ± 14.1	47.6 ± 10.9	42.2 ± 9.78
K_{el} (h^{-1})	0.084 ± 0.021	0.077 ± 0.019	0.074 ± 0.018
t ½ (h)	8.3 ± 2.01	9.1 ± 2.23	9.4 ± 2.34

AUC, area under the plasma concentration–time curve from time 0 h to infinity; CL_t, total plasma clearance; K_{el}, elimination rate constant; t½, terminal half-life. Data are mean ± s.d., n = 6.

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