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Enhanced Nimodipine Bioavailability After Oral Administration of Nimodipine with Morin, a Flavonoid, in Rabbits

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The aim of this study was to investigate the effect of morin on the bioavailability of nimodipine after administering nimodipine (15 mg/kg) orally to rabbits either co-administered or pretreated with morin (2, 10 and 20 mg/kg). The plasma concentrations of nimodipine in the rabbits pretreated with morin were increased significantly ($p<0.05$ at 10 mg/kg, $p<0.01$ at 20 mg/kg) compared with the control, but the plasma concentrations of nimodipine co-administered with morin were not significant. The areas under the plasma concentration-time curve (AUC) and the peak concentrations (C_{max}) of the nimodipine in the rabbits pretreated with morin were significantly higher ($p<0.05$ at 10 mg/kg, $p<0.01$ at 20 mg/kg), but only the C_{max} of nimodipine co-administered with morin 10 mg/kg was increased significantly ($p<0.05$). The absolute bioavailability (A.B%) of nimodipine in the rabbits pretreated with morin was significantly ($p<0.05$ at 10 mg/kg, $p<0.01$ at 20 mg/kg) higher (54.1-65.0%) than the control (36.7%). The increased bioavailability of nimodipine in the rabbits pretreated with morin might have been resulted from the morin, which inhibits the efflux pump P-glycoprotein and the first-pass metabolizing enzyme by cytochrome P-450 3A4 (CYP 3A4).

Key words: Nimodipine, Morin, Bioavailability, P-Glycoprotein, Cytochrome P-450 3A4

INTRODUCTION

Nimodipine is a dihydropyridine calcium channel blocker which has been shown to selectively dilate cerebral arteries and increase cerebral blood flow in animals and humans (Kazda *et al.*, 1982). Its major therapeutic indication is for the prevention and treatment of delayed ischemic neurological disorders that often occur in patients with subarachnoid hemorrhages (Scholz *et al.*, 1997; Epstein *et al.*, 1990). Nimodipine is rapidly absorbed after being administered orally and is widely distributed throughout the body. Orally administered nimodipine is subject to an extensive first-pass hepatic metabolism from the portal circulation, resulting in a low systemic bioavailability (Maruhn *et al.*, 1985; Suwelack *et al.*, 1985). Usually only the parent compound is active and most of the metabolic steps involve reactions catalyzed by cytochrome P-450 (CYP) enzymes. CYP enzymes have been shown to catalyzed pyridine formation, methyl hydroxylation, and various modes of side-chain oxidation (Ramsch *et al.*, 1985; Scherling *et al.*, 1991; Guengerich *et al.*, 1991).

The reduced bioavailability of nimodipine after administering nimodipine orally might not only be due to the metabolizing enzyme CYP 3A4 but also to the P-glycoprotein efflux transporter in the small intestine. Saeki *et al.* (1993) reported that nimodipine is a substrate for the efflux of P-glycoprotein and also Wacher *et al.* (2001) reported that nimodipine is both a CYP 3A4 and P-glycoprotein substrate. P-glycoprotein is found in the secretory epithelial tissues, including the brush border of the renal proximal tubules, the canalicular membranes in the liver and the apical membranes lining the gut. In the small intestine, P-glycoprotein is co-localized at the apical membrane of the cells with CYP 3A4 (Gottesman *et al.*, 1993). P-glycoprotein and CYP3A4 might act synergistically to the presystemic drug metabolism (Gan *et al.*, 1996; Watkins, 1996; Wacher *et al.*, 1998; Ito *et al.*, 1999) to make the substrate of P-glycoprotein circulate between the lumen and epithelial cells, leading to prolonged exposure to CYP 3A4, resulting in a reduced absorption of the drug.

Flavonoids represent a group of phytochemicals that are

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produced by various plants in high quantities (Dixon *et al.*, 1999). Among flavonoids, morin (3, 5, 7, 2', 4'-pentahydroxyflavone) exhibits various biological activities including antioxidation, anti-mutagenesis and anti-inflammation (Hanasaki *et al.*, 1994; Francis *et al.*, 1989; Fang *et al.*, 2003). Furthermore, morin was reported to modulate metabolic enzymes as well as P-glycoprotein efflux pump (Zhang *et al.*, 2003; Hodek *et al.*, 2002). Choi *et al.* (2005) reported that the co- and pre-administration of morin significantly increased the C_{max} and AUC of diltiazem, other calcium channel blocker (substrate for P-glycoprotein and CYP 3A4), in rabbits. Nguyen *et al.* (2003) also reported that morin significantly increased the cellular accumulation of vinblastine, a P-glycoprotein substrate in Panc-1 cells. Therefore, morin seems to be an effective dual inhibitor of CYP3A4 and P-glycoprotein.

The bioavailability of oral nimodipine is mainly affected by CYP 3A4 and P-glycoprotein at the first-pass metabolism. When morin administered with nimodipine orally, it might influence the bioavailability of nimodipine. However, there has been no report about if the morin effluences the bioavailability of nimodipine in rabbits. The aim of this study was to examine the bioavailability of nimodipine when nimodipine was either co-administered or pretreated with morin.

MATERIALS AND METHODS

Materials

Nimodipine, nitrendipine(interal standard) and morin were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethyl acetate and methanol were purchased from Merck Co. (Darmstadt, Germany). The other chemicals were of reagent grade and were used without further purification. The apparatuses used HPLC (Model LC-10A, Shimadzu Co., Kyoto, Japan), a syringe pump (Model 341B, Sage Co., Kyoto, Japan), a vortex mixer (Scientific Industries, Seoul, Korea) and a centrifuge (Abbot Co., TM, U.S.A.).

Animal experiments and drug administration

The male New Zealand white rabbits were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and were given access to a normal standard chow diet and tap water *ad libitum*. Throughout the experiment, the animals were housed, two per cage, in laminar flow cages maintained at 22±2°C, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were kept in these facilities for at least one week prior to the experiment. This experiments were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (U.S.A.) in July 1989 and revised in March 1999. The animal care committee in our institution (Chosun University) approved this study.

Rabbits were divided in eight groups of eight each: one of control group (nimodipine 15 mg/kg, oral), three of co-administration groups (15 mg/kg nimodipine co-administered orally with morin 2, 10 and 20 mg/kg, respectively), three of pretreatment groups (15 mg/kg nimodipine pretreated orally with 2, 10 and 20 mg/kg morin 0.5 h before, respectively), and one of IV group (intravenous administration of 4 mg/kg nimodipine).

The rabbits were fasted for at least 24 h prior to experiments and given free access to water. Each rabbit was anaesthetized by an injection of 25% urethane saline solution (4 mL/kg). The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, U.S.A.) for blood sampling. Nimodipine solutions were prepared by adding nimodipine (15 mg/kg) to distilled water (10 mL) and stirring for 1 h, and then administered orally through a catheter for the control. The mixtures for co-administered group were prepared by adding nimodipine (15 mg/kg) and morin (2, 10 and 20 mg/kg) in distilled water (10 mL) and stirred for 1 h before administration. The morin suspensions for pretreated groups were prepared by adding morin (2, 10 and 20 mg/kg) to distilled water (5 mL) and stirring for 1 h, and morin suspensions were administered orally 30 minutes prior to administration of nimodipine solutions. In order to estimate the absolute bioavailability (A.B%), nimodipine (4 mg/kg) was injected through the ear vein by dissolving nimodipine in the saline solution. Blood samples (1.2 mL) were withdrawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after the oral administration of the nimodipine to each of all rabbits. The blood samples were centrifuged at 3,000 rpm for 10 min. The plasma samples (0.5 mL) were stored at -40 until analyzed by the HPLC.

HPLC Assay

The plasma nimodipine concentrations was determined by a HPLC assay using a modification of the method repored by Qian *et al.* (1992). Briefly, 50 μL of nitrendipine (1 μL/mL), as the internal standard and 50 μL of ethyl acetate were added to 0.5 mL of the plasma samples. The mixture was then stirred for 10 min and centrifuged at 3,000 rpm for 10 min. Five milliliters of the organic layer were transferred to a clean test tube and evaporated at 40°C under a stream of nitrogen. The residue was then dissolved in 300 μL of 65% methanol, which was centrifuged at 3,000 rpm for 10 min, and 50 μL of the solution was injected into the HPLC system.

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV detector (Model SPD-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and a autoinjector

(SIL-10AD). The UV detector was set at a wavelength of 310 nm. The stationary phase used was a Hypersil ODs column (5 μm, 4.6×150 mm). The mobile phase consisted of methanol : water (65 : 35, v/v). The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. The retention times at a flow rate of 1.0 mL/min were as follows: internal standard, 7.6 min, and nimodipine, 9.1 min. Linear regression analysis using a least-square fit was performed. The calibration curve was obtained from the standard samples at the following concentration: 10, 20, 50, 100, 500, and 1000 ng/mL. The following regression equations were obtained: y = 206.0x + 18.1 (r=0.999).

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by the LAGRAN method using the LARGAN computer program (Rocci *et al.*, 1983). The area under the plasma concentration-time curve from time zero to infinity (AUC) was computed using the LAGRAN method in order to reduce the errors associated with using the trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{1/2}$) of the drug was obtained by 0.693/K_{el}. The absolute bioavailability of nimodipine after being administered orally compared to the nimodipine that is injected intravenlously was calculated as follows:

Absolute bioavailability (A.B%)

$$= \frac{AUC_{oral}}{AUC_{IV}} \times \frac{\text{IV dose}}{\text{Oral dose}} \times 100$$

The relative bioavailability of nimodipine administered orally was calculated as follows:

$$\text{Relative bioavailability (R.B\%)} = \frac{AUC_{combined}}{AUC_{control}} \times 100$$

Statistical analysis

All the means are presented with their standard deviation. The analysis of variance (ANOVA) with Scheffe's test was used to determine any significance difference between the control groups and co-administration or pretreatment groups. A p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The plasma concentrations of nimodipine after the oral administration of nimodipine (15 mg/kg), either co-administered or pretreated with morin (2, 10 and 20 mg/kg) to rabbits are shown in Fig. 1 and 2. The bioavailability and the pharmacokinetic parameters of nimodipine after administering nimodipine, either co-administered or pretreated with morin are shown in Table I and II. When nimodipine was pretreated with morin 10 mg/kg and 20 mg/kg, the plasma concentrations of nimodipine were increased significantly (p<0.05 at 10 mg/kg, p<0.01 at 20 mg/kg), but the plasma concentrations of nimodipine were not significant in the co-administered groups compared with

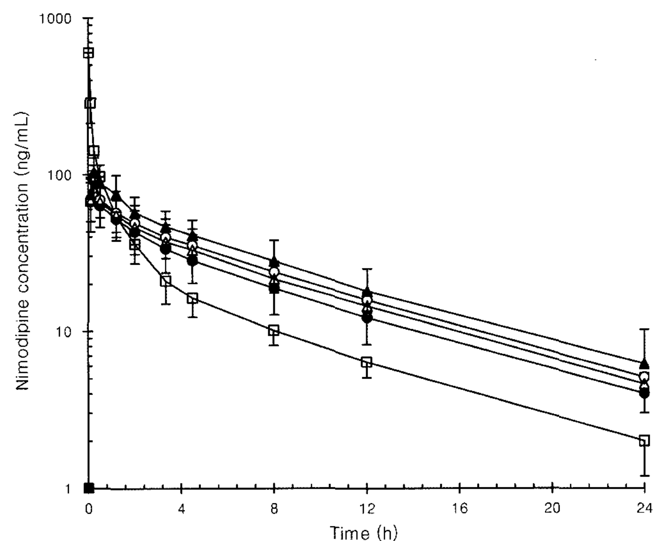

Fig. 1. Mean plasma concentration-time profiles of nimodipine after the oral co-administration of nimodipine (15 mg/kg) with morin to the rabbits. Bars represent the standard deviation. (n=8). (●) Nimodipine control, (○) Co-administered with morin 2 mg/kg, (▲) Co-administered with morin 10 mg/kg, (△) Co-administered with morin 20 mg/kg, (□) Nimodipine I.V. 4 mg/kg.

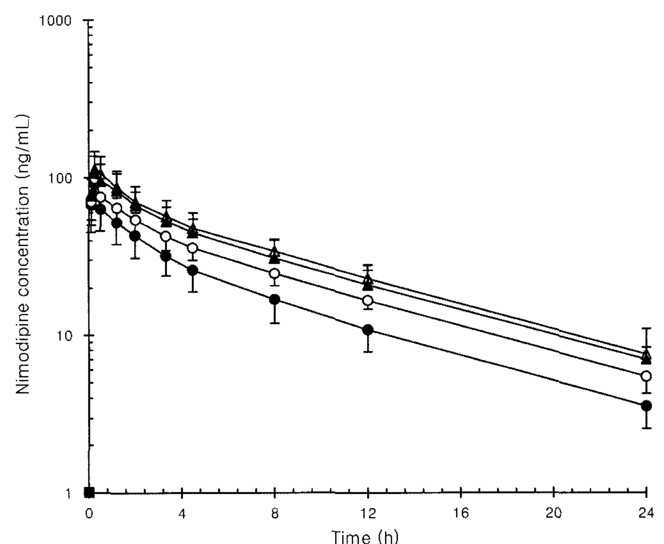

Fig. 2. Mean plasma concentration-time profiles of nimodipine after oral administration of nimodipine (15 mg/kg) pretreated with morin to the rabbits. Bars represent the standard deviation (n=8). (●) Nimodipine control, (○) Pretreated with morin 2 mg/kg, (▲) Pretreated with morin 10 mg/kg, (△) Pretreated with morin 20 mg/kg.

Table I. Pharmacokinetic parameters of nimodipine after the oral co-administration of nimodipine (15 mg/kg) with morin to the rabbits

Parameters	Nimodipine Control	Morin co-administration			i.v. (4 mg/kg)
		2 mg/kg	10 mg/kg	20 mg/kg	
AUC (ng/mL·h)	505.1 ±131.3	579.0 ±150.5	665.3±272.9	546.5±141.7	366.8±95.2
C_{max} (ng/mL)	91.3 ± 23.6	97.8 ± 25.2	103.2±26.8*	95.5±34.7	-
T_{max} (h)	0.25	0.25	0.25	0.25	-
K_{el} (h^{-1})	0.099± 0.025	0.092± 0.033	0.092±0.020	0.094±0.018	0.092±0.031
$t_{1/2}$ (h)	7.1 ± 2.8	7.5 ± 3.2	7.4±2.4	7.3±2.6	7.5±1.4
A.B (%)	36.7 ± 19.2	42.1 ± 22.7	48.4±18.5	39.7±15.7	100.0
R.B (%)	100.0	114.6	131.7	108.2	-

Mean°æS.D. (n=8), *p<0.05, **p<0.01, significant difference compared to control
AUC: area under the plasma concentration-time curve from 0 h to 24 h
C_{max}: peak concentration
T_{max}: time to reach peak concentration
K_{el} (h^{-1}): elimination rate constant
$t_{1/2}$: terminal half-life
R.B: AUC rate compared to $AUC_{control}$
A.B: absolute bioavailability.

Table II. Pharmacokinetic parameters of nimodipine after the oral administration of nimodipine (15 mg/kg) pretreated with morin to the rabbits.

Parameters	Nimodipine Control	Morin pretreatment			i.v. (4 mg/kg)
		2 mg/kg	10 mg/kg	20 mg/kg	
AUC (ng/mL·h)	505.1 ±131.3	599.8 ±155.7	743.5 ±193.0*	894.6 ±206.0**	366.8 ±95.2
C_{max} (ng/mL)	91.3 ± 21.6	98.3 ± 25.2	107.8 ± 27.8*	123.3 ± 19.4*	-
T_{max} (h)	0.25	0.25	0.30	0.30	-
K_{el} (h^{-1})	0.099± 0.025	0.093± 0.028	0.092± 0.018	0.093± 0.013	0.092± 0.031
$t_{1/2}$ (h)	7.1 ± 2.8	7.5 ± 2.1	7.6 ± 1.9	7.5 ± 2.3	7.5 ± 1.4
A.B (%)	36.7 ± 19.2	43.6 ± 10.6	54.1 ± 16.8*	65.0 ± 14.1**	100.0
R.B (%)	100	118.7	147.2	177.1	-

Mean ± S.D. (n = 8), *p<0.05, **p<0.01, significant difference compared to control
AUC: area under the plasma concentration-time curve from 0 h to 24 h
C_{max}: peak concentration
T_{max}: time to reach peak concentration
K_{el} (h^{-1}): elimination rate constant
$t_{1/2}$: terminal half-life
R.B: AUC rate compared to $AUC_{control}$
A.B: absolute bioavailability.

the control. The AUC and the C_{max} of the nimodipine in the rabbits pretreated with morin were significantly higher ($p<0.05$ at 10 mg/kg, $p<0.01$ at 20 mg/kg) than the control, but only the C_{max} of nimodipine co-administered with morin 10 mg/kg were increased significantly ($p<0.05$). The absolute bioavailability (A.B%) of the nimodipine control was 36.7%, which was increased significantly ($p<0.05$ at 10 mg/kg, $p<0.01$ at 20 mg/kg) from 54.1% to 65.0% by pretreatment. The relative bioavailability (R.B%) of nimodipine pretreated with morin was 1.47 to 1.77 times higher than the control.

It was reported that nimodipine is metabolized by cytochrome P-450 (CYP3A) both in the liver and small intestine (Ramsch *et al.*, 1985; Scherling *et al.*, 1991; Guengerich *et al.*, 1991) and the absorption of nimodipine in the intestinal mucosa is inhibited by P-glycoprotein efflux pump (Saeki *et al.*, 1993). P-glycoprotein and CYP 3A4 are believed to act synergistically to the first-pass metabolism. The increased AUC and C_{max} of nimodipine by pretreatment of morin might have been resulted from the inhibition of the P-glycoprotein efflux pump and the metabolizing enzyme, CYP 3A4, in the intestinal mucosa. Morin could inhibit the P-glycoprotein efflux pump and restrain the metabolizing enzyme, CYP3A4 (Zhang *et al.*,

2003; Hodek *et al.*, 2002). Therefore, it might increase the bioavailability of nimodipine by inhibiting CYP 3A4 to reduce the metabolism and inhibiting P-glycoprotein to increases the absorption through the intestinal mucosa. This is consistent with the report by Choi et al. (2005a; 2005b), in that the pre-administration of morin significantly increased the C_{max} and AUC of diltiazem, other calcium channel blocker (substrate for P-glycoprotein and CYP 3A4), in rats. It is also consistent with the report by Wang *et al.* (2004), in that the co-administration of morin significantly increased the C_{max} and AUC of digoxin (substrate for P-glycoprotein and CYP 3A4) in pigs.

But, Hsiu *et al.* (2002) reported that significantly decreased cyclosporin AUC by oral concomitant administration of morin (50 mg/kg) with cycolosporin, a substrate for CYP 3A4 and P-glycoprotein, to pigs and rats, on the other hand, shown significant inhibition of P-glycoprotein function by morin at the everted intestine sac. It might be considered that the morin interact with nimodipine in the gastrointestinal lumen to form the complex by co-administration of the high dose of morin, or the absorption of morin in the gastrointestinal mucosa is early enough to inhibit nimodipine metabolizing enzyme CYP3A4 and efflux pump P-glycoprotein by pretreatment of morin 30 min before nimodipine, but not by coadministration.

Although it is need to investigate further, it appears that nimodipine dose, which is widely used in clinics, should be taking into consideration when nimodipine is administered with diets or supplements containing morin over a long period. As flavonoids like morin are widely distributed in the daily diet as glycosides, it is necessary to investigate the effect of flavonoid glycosides on the bioavailability of many drugs act as substrate for CYP 3A4 and P-glycoprotein in the future study.

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