



와파린의 약물동태학에 니모디핀의 영향

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Effects of Nimodipine on the Pharmacokinetics of Warfarin in Rats: A Possible Role of P-glycoprotein and CYP3A4 Inhibition by Nimodipine

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Purpose: The aim of this study was to investigate the effect of nimodipine on the pharmacokinetics of warfarin after oral and intravenous administration of warfarin in rats. **Methods:** Warfarin was administered orally (0.2 mg/kg) or intravenously (0.05 mg/kg) without or with oral administration of nimodipine (0.5 or 2 mg/kg) in rats. The effect of nimodipine on the P-glycoprotein as well as cytochrome P450 (CYP) 3A4 activity was also evaluated. **Results:** Nimodipine inhibited CYP3A4 enzyme activity with 50% inhibition concentration (IC₅₀) of 10.2 μM. Compared to those animals in the oral control group (warfarin without nimodipine), the area under the plasma concentration-time curve (AUC) of warfarin was significantly greater (0.5 mg/kg, P<0.05; 2 mg/kg, P<0.01) by 31.3-57.6%, and the peak plasma concentration (C_{max}) was significantly higher (2 mg/kg, P<0.05) by 29.4% after oral administration of warfarin with nimodipine, respectively. Consequently, the relative bioavailability of warfarin increased by 1.31- to 1.58-fold and the absolute bioavailability of warfarin with nimodipine was significantly greater by 64.1-76.9% compared to that in the control group (48.7%). In contrast, nimodipine had no effect on any pharmacokinetic parameters of warfarin given intravenously. **Conclusion:** Therefore, the enhanced oral bioavailability of warfarin may be due to inhibition of CYP 3A4-mediated metabolism rather than P-glycoprotein-mediated efflux by nimodipine.

□ Key words - nimodipine, warfarin, pharmacokinetics, P-glycoprotein, CYP3A4

Nimodipine is a dihydropyridine calcium channel blocker that has been shown to selectively dilate cerebral arteries and increase cerebral blood flow in animals and humans.¹⁾ Its major therapeutic indication is for the prevention and treatment of delayed ischemic neurological disorders that often occur in patients with subarachnoid hemorrhages.^{2,3)} Nimodipine is rapidly absorbed after oral administration 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.^{4,5)} The reduced bioavailability of nimodipine after administering nimodipine orally might not only be due to the metabolizing enzyme CYP3A4, but also to the P-glycoprotein efflux transporter in the small intestine. Saeki *et al.*⁶⁾ reported that nimodipine is a substrate for the efflux of P-glycoprotein and Wachter *et al.*⁷⁾ reported that nimodipine is both a CYP3A4 and P-glycoprotein substrate. P-glycoprotein is found in the secretory epithelial tissues, including the brush border of the renal proximal tubules, the canalicular mem-

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branes 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 CYP3A4.⁸⁾ P-glycoprotein and CYP3A4 may act synergistically to presystemic drug metabolism to make the substrate of P-glycoprotein circulate between the lumen and epithelial cells, leading to prolonged exposure to CYP3A4, resulting in a reduced absorption of the drug.⁹⁻¹¹⁾

Warfarin is the most extensively used oral anticoagulant for the prevention and treatment of thromboembolic complications in cardiovascular diseases such as arterial fibrillation, venous thrombosis and pulmonary embolism.¹²⁾ Warfarin's anticoagulant effect is due to its interference with the cyclic interconversion of vitamin K and its 2, 3 epoxide, and to its limitation of the synthesis of the vitamin K-dependent clotting factors, II, VII, IX and X.¹³⁾ Warfarin is readily absorbed from the gastrointestinal tract, extensively bound to plasma proteins. Warfarin is used as a racemic mixture of roughly equal amounts of R and S enantiomers yet S-warfarin has been reported to be more potent.¹⁴⁾ Warfarin is metabolized by CYPs and is converted to inactive metabolites through selective hydroxylation.¹⁵⁾ R-warfarin is metabolized primarily by CYP3A4 to 10-hydroxywarfarin and S-warfarin is metabolized primarily by CYP2C9 to 7-hydroxywarfarin.¹⁶⁾ Potential warfarin-drug interactions could occur with any of a very wide range of drugs that are metabolized by these CYPs, and a number of such interactions have been reported.¹⁷⁾

However, there have been no reports regarding the effects of nimodipine on the bioavailability or pharmacokinetics of warfarin in rats. Moreover, nimodipine and warfarin could be prescribed for the prevention or treatment of thromboembolism and ischemic stroke in some patients with arterial fibrillation, valvular heart disease and a myocardial infarction as a combination therapy. Warfarin has a narrow therapeutic range and its efficacy can be influenced by drug-drug interactions. Therefore, the present study aimed to investigate the effects of nimodipine on the pharmacokinetics of warfarin after oral and intravenous administration in rats.

MATERIALS AND METHODS

Chemicals and apparatus

Warfarin, nimodipine and 7-ethoxycoumarin (internal standard) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade methanol was purchased from Merck Co. (Darmstadt, Germany). Other chemicals for this study were reagent grade.

The HPLC system used in this study was comprised of 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 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 per cage, maintained at 22±2°C and 50-60% relative humidity under a 12:12 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) 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 with light ether. The left femoral artery and vein were cannulated using polyethylene tubing (I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and drug administration, respectively.

Oral and intravenous administration of warfarin

The rats were randomly divided into four groups (n = 6, each); an oral group (0.2 mg/kg of warfarin dissolved in water; homogenized at 36°C for 30 min; 1.0 ml/kg) without (control) or with 0.5 or 2 mg/kg of oral nimodipine, and an intravenous group (0.05 mg/kg of warfarin, dissolved in 0.9% NaCl-injectable solution; homogenized at 36°C for 30 min; 0.3 ml/kg) without (control) or with 0.5 or 2 mg/kg of oral nimodipine. Warfarin was administered orally using a gastric gavage tube, and nimodipine was orally administered 30 min prior to oral or intravenous administration of warfarin. Warfarin for intravenous administration was injected through the femoral vein within 0.5 min. A blood sample (0.3 ml) was collected into heparinized tubes from the femoral artery at 0 (control), 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 h after intravenous infusion, and 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48 and 72 h after oral administration. The blood samples were centrifuged (13,000 rpm, 5 min), and the plasma samples (0.15 ml) were stored at -40°C until HPLC analysis of warfarin. An approximately 1 ml of whole blood collected from untreated rats was infused via the femoral artery at 0.25, 1, 3 and 8 h, respectively, to replace blood loss due to blood sampling.

HPLC assay

The plasma concentrations of warfarin were determined by a HPLC assay method reported by Zhu *et al.*¹⁸⁾ Briefly, 50 µl of 7-ethoxycoumarin (2 µg/ml dissolved in methanol), 50 ml of methanol, 200 ml distilled water, 0.5 ml of 2 M hydrochloric acid, and 0.8 ml of diethyl ether were added to 0.15 ml of plasma sample. The mixture was then stirred for 10 min and centrifuged (13,000 rpm, 10 min). 0.7 ml of the organic layer was transferred to a clean test tube and evaporated under a gentle stream of nitrogen at 35°C. The residue was dissolved in 150 µl of the phosphate buffer. Next, 70 µl of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a guard column packed with C₁₈ column (4×3.0 mm, 5 µm, Phenomenex), and a reversed-phase Luna[®] C₁₈ column (4.6×150 mm, 5 µm, Phenomenex). The mobile phase was 10 mM phosphate buffer–methanol (50:50, v/v),

which was run at a flow rate of 1.0 ml/min. Chromatography was performed at a temperature of 30°C that was set by an HPLC column temperature controller, while the UV detector was set to 300 nm. The retention times of warfarin and the internal standard were 16.7 and 9.1 min, respectively. The detection limit of warfarin in rat's plasma was 5 ng/ml. The coefficients of variation for warfarin were below 12.8%.

CYP inhibition assay

The inhibition assays of human CYP3A4 enzyme activity were performed in a multiwell plate using the CYP inhibition assay kit (BD Bioscience, San Jose, CA) as described previously.¹⁹⁾ Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. The CYP3A4 substrate, 7-Benzoyloxy-4-(trifluoromethyl) couamrin (7-BFC) was incubated with or without nimodipine in a reaction mixture containing 1 pmol of CYP3A4 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 MgCl₂) 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 control (1 µM ketoconazole) was run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and the results are expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures used for the rhodamine-123(a P-glycoprotein substrate) retention assay were similar to a previously reported method.²⁰⁾ NCI/ADR-RES cells was seeded in 24 well plates. At 80 % confluence, the cells were incubated in fetal bovine serum (FBS)-free Dulbecco's modified Eagle's medium (DMEM) for 18 h. The culture medium was changed with 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 in the presence or absence of nimodipine (10, 30 or 100 μM) or verapamil (100 μM , a positive control) for 90 min, the medium was completely aspirated. The cells were then washed three times with an 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 percentage ratio to control.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated using non-compartmental analysis (WinNonlin; software version 4.1; Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by log-linear regression of warfarin concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) of warfarin were directly read from the experimental data. 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} . Total body clearance (CL) was calculated by $Dose/AUC$. The absolute bioavailability (A.B.) of warfarin was calculated by $AUC_{oral}/AUC_{intravenous} \times Dose_{intravenous}/Dose_{oral} \times 100$, and the relative bioavailability (R.B.) of warfarin was estimated

by $AUC_{with\ nimodipine}/AUC_{control} \times 100$.

Statistical analysis

All data were expressed with their standard deviation (mean \pm S.D.). Statistical analysis was conducted using a one-way analysis of variance (ANOVA) followed by a posteriori testing with Dunnett's correction. Differences were considered significant at a level of $p < 0.05$.

RESULTS

Inhibitory effect of nimodipine on CYP3A4 activity

The inhibitory effect of nimodipine on CYP3A4 activity is shown in Fig. 1. Nimodipine inhibited CYP3A4 enzyme activity and the 50% inhibition concentration (IC_{50}) value of nimodipine on CYP3A4 activity was 10.2 μM .

Rhodamine-123 retention assay

As shown in Fig. 2. The concurrent use of nimodipine did not enhance the cellular uptake of rhodamine-123 in a concentration-dependent manner ranging from 10-100 μM . This result suggests that nimodipine did not significantly inhibit P-glycoprotein activity.

Effect of nimodipine on the pharmacokinetics of warfarin after oral administration

The mean arterial plasma concentration-time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nimodipine (0.5 and 2 mg/kg) are shown in Fig. 3. The relevant pharmacokinetic parameters of warfarin are also listed in Table 1. The AUC of war-

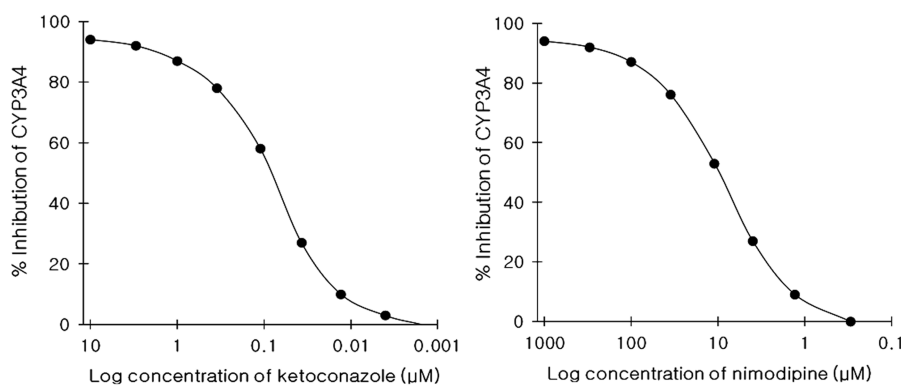


Fig. 1. Inhibitory effect of ketoconazole and nimodipine on CYP3A4 activity. All experiments were performed in duplicate, and results were expressed as the percent of inhibition (IC_{50} : 10.2 μM). Ketoconazole (1 μM) was used as a positive control.

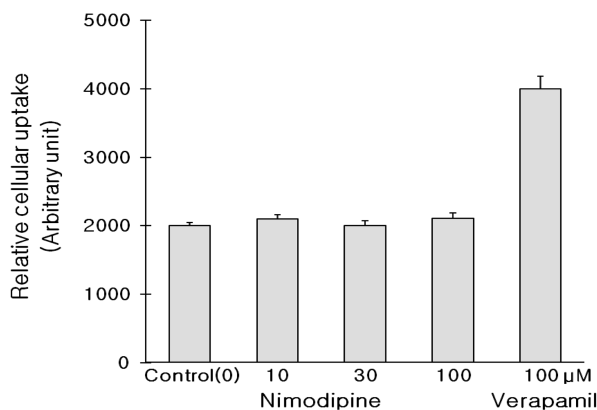


Fig. 2. Rhodamine-123 retention. NCI/ADR-RES cells were preincubated with nimodipine (0, 10, 30 and 100 μM) for 30 min, and after incubation of NCI/ADR-RES cells with 20 μM rhodamine-123 for 90 min. The values were divided by total protein contents of each sample. Verapamil (100 μM) was used as a positive control. Data represents mean \pm SD of 6 separate samples.

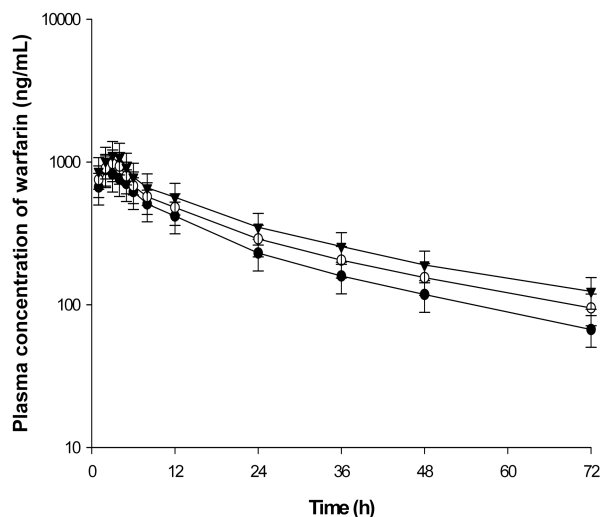


Fig. 3. Mean arterial plasma concentration-time profiles of warfarin after oral administration of warfarin (0.2 mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nimodipine in rats. Bars represent the standard deviation (n = 6).

warfarin was significantly greater (0.5 mg/kg, $p < 0.05$; 2 mg/kg, $p < 0.01$) by 31.3-57.6%, and the C_{max} was significantly higher (2 mg/kg, $p < 0.05$) by 29.4% after oral administration of warfarin with nimodipine. Consequently, the relative bioavailability of warfarin was increased 1.31- to 1.58-fold and the absolute bioavailability of warfarin with nimodipine was significantly greater by 64.1-76.9% compared to that in the control group (48.7%). The half-

Table 1. Mean pharmacokinetic parameters of warfarin after oral administration of warfarin (0.2 mg/kg) with or without nimodipine (0.5 and 2 mg/kg) in rats.

Parameters	Control without Nimodipine	with Nimodipine	
		0.5 mg/kg	2 mg/kg
AUC (ng·h/ml)	19810 \pm 3358	26018 \pm 4118*	31211 \pm 5992**
C_{max} (ng/ml)	882 \pm 152	992 \pm 288	1141 \pm 211*
T_{max} (h)	2.17 \pm 0.98	2.50 \pm 1.22	2.83 \pm 1.32
$t_{1/2}$ (h)	27.6 \pm 3.12	32.1 \pm 5.61	34.8 \pm 5.03*
A.B. (%)	48.7 \pm 10.1	64.1 \pm 13.1*	76.9 \pm 15.8**
R.B. (%)	100	131	158

Mean \pm S.D. (n=6), * $p < 0.05$, ** $p < 0.01$, significant difference compared to controls, AUC: area under the plasma concentration-time curve from 0 h to time infinity, C_{max} : peak plasma concentration, T_{max} : time to reach peak concentration, $t_{1/2}$: terminal half-life, A.B. (%): absolute bioavailability, R.B. (%): relative bioavailability.

life of warfarin was significantly longer (2 mg/kg, $p < 0.05$) and there was no significant change in the T_{max} .

Effect of nimodipine on the pharmacokinetics of warfarin after intravenous administration

The mean arterial plasma concentration-time profiles of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nimodipine (0.5 or 2 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters of warfarin are listed in Table 2. Nimo-

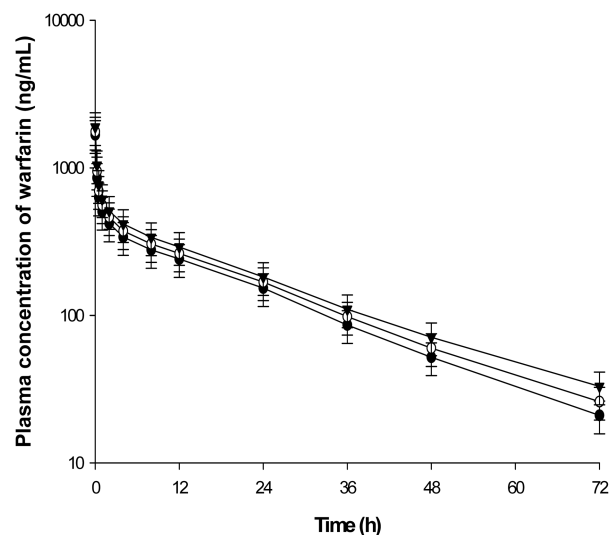


Fig. 4. Mean arterial plasma concentration-time profiles of warfarin after intravenous administration of warfarin (0.05 mg/kg) without (●) or with 0.5 mg/kg (○) and 2 mg/kg (▼) of nimodipine in rats. Bars represent the standard deviation (n = 6).

Table 2. Mean pharmacokinetic parameters of warfarin after intravenous administration of warfarin (0.05 mg/kg) with or without nimodipine (0.5 and 2 mg/kg) in rats.

Parameters	Control without Nimodipine	with Nimodipine	
		0.5 mg/kg	2 mg/kg
AUC(ng·h/ml)	10149±2230	10921±2122	12648±2411
CL (ml/min/kg)	0.082±0.024	0.076±0.021	0.066±0.018
t _{1/2} (h)	18.1±3.71	19.4±4.12	21.0±4.62

Mean±S.D. (n=6), AUC: area under the plasma concentration-time curve from time 0 to infinity, CL: total body clearance, t_{1/2}: terminal half-life.

dipine had no effect on the pharmacokinetic parameters of warfarin given intravenously although it exhibited a significant effect on the bioavailability of warfarin given orally, suggesting that CYP3A4-mediated metabolism was inhibited by nimodipine in the rats.

DICUSSION

Warfarin is an anticoagulant that has been used to prevent thromboembolism including pulmonary embolism, cardiovascular disease and stroke. Scheduled monitoring and dosage adjustment are critical to maintain efficacy and to prevent bleeding events. Warfarin has a narrow therapeutic range and its efficacy can be influenced by drug-drug interactions, drug-food interactions, genetic factors and patient characteristics.^{12,17)}

CYPs enzymes make a considerable contribution to the first-pass metabolism and oral bioavailability of many drugs. Moreover, induction or inhibition of CYPs may be responsible for significant drug and drug interactions.^{21,22)} Modulators of P-glycoprotein can enhance or limit the permeability of a number of therapeutic agents that are considered substrates of this efflux pump protein.²³⁾ Therefore nimodipine, a dual inhibitor against both CYP3A4 and P-glycoprotein, should have a great impact on the bioavailability of many drugs where CYP3A4 metabolism as well as P-glycoprotein mediated efflux is the major barrier to the systemic bioavailability and thus could act synergistically to limit oral bioavailability of its substrates.^{6,24)}

As shown in Fig. 1, nimodipine exhibited inhibitory effect against CYP3A4-mediated metabolism with the

IC₅₀ values of 10.2 μM. A cell-based P-glycoprotein activity test using rhodamine-123 also showed that nimodipine did not significantly inhibit P-glycoprotein activity (Fig. 2). As nimodipine is an inhibitor of CYP3A4, concomitant use of the drug might play a role in the wide inter-individual variability in the response to drugs.²⁵⁻²⁷⁾ Most calcium channel blockers (verapamil, nifedipine, diltiazem, barnidipine, amlodipine) also have inhibitory effect on the drug transporter P-glycoprotein, which mediates drug's intestinal absorption.^{24,28)} However, Harmsze *et al.*²⁹⁾ reported that nimodipine have poor inhibitory effects on the drug transporter P-glycoprotein.

Compared to those animals in the oral control group (warfarin without nimodipine), the AUC of warfarin was significantly greater by 31.3-57.6%, and the C_{max} was significantly higher by 29.4% after oral administration of warfarin with nimodipine (Table 1). Consequently, the absolute bioavailability of warfarin with nimodipine was significantly greater by 64.1-76.9% compared to that in the control group (48.7%). In contrast, nimodipine had no effect on any pharmacokinetic parameters of warfarin given intravenously, implying that coadministration of nimodipine could inhibit CYP3A4-mediated metabolism of warfarin, resulting in reducing intestinal or hepatic first-pass metabolism.^{30,31)} These results were consistent with the results reported by Choi *et al.*²⁶⁾

In conclusion, the enhanced oral bioavailability of warfarin by nimodipine suggests that CYP3A4-mediated metabolism were inhibited rather than P-glycoprotein-mediated efflux or renal elimination of warfarin. In further clinical studies, the dosage regimen of warfarin might be readjusted when used concomitantly with nimodipine in the patients.

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