

## 티크로피딘이 니칼디핀의 생체이용률 및 약물동태에 미치는 영향

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<sup>1</sup>국립목포대학교 약학대학, <sup>2</sup>조선대학교 약학대학 & 임상약학대학원

(2011년 12월 30일 접수 · 2012년 1월 28일 수정 · 2012년 1월 30일 승인)

## Effects of Ticlopidine on the Bioavailability and Pharmacokinetics of Nicardipine after Oral and Intravenous Administration

Hong-Seop Moon<sup>1</sup>, Jun-Shik Choi<sup>2</sup>, and Joon Seok Bang<sup>2\*</sup>

<sup>1</sup>College of Pharmacy, Mokpo National University, Jeonnam, Korea

<sup>2</sup>College of Pharmacy & Graduate School of Clinical Pharmacy, Chosun University, Gwangju, Korea

(Received December 30, 2011 · Revised January 28, 2012 · Accepted January 30, 2012)

항혈소판제인 티크로피딘과 항고혈압제인 니칼디핀과의 약동학적 상호작용 연구를 위하여 티크로피딘 (3 또는 10 mg/kg) 과 니칼디핀의 경구 (4 mg/kg) 및 정맥 (12 mg/kg) 투여하여 본 연구를 시행하였다. 연구방법: 티크로피딘이 cytochrome P450 (CYP) 3A4 활성과 P-glycoprotein (P-gp)의 활성에 미치는 영향도 평가하였다. 결 과: 티크로피딘과 니칼디핀의 병용투여 시 티크로피딘이 니칼디핀의 약물동태 파라미터에 미치는 결과는 다음과 같다. 티크로피딘은 CYP3A4 효소의 활성을 저해 하였으나 P-gp활성에는 영향을 미치지 못하였다. 니칼디핀의 혈중농도곡선하면적 (AUC)는 대조군에 비해 티크로피딘 10 mg/kg 병용투여군에서 유의성 ( $p < 0.05$ )있게 증가되었다. 상대적 생체이용률 (RB)은 티크로피딘 병용 투여군에서 115-143%로 증가하였다. 결 론: 본 논문에서 흰쥐에 티크로피딘과 니칼디핀을 병용경구투여 시 니칼디핀의 생체이용률 (bioavailability)이 유의성 ( $p < 0.05$ )있게 증가된 것은 티크로피딘이 대사효소인 CYP3A4를 억제하여 소장과 간장에서 초회통과효과 (first-pass metabolism)를 감소 시켰기 때문인 것으로 사료된다. 본 실험결과를 토대로 인체에서 티크로피딘과 니칼디핀의 상호작용을 검토한 후 투여용량을 조절하는 것이 바람직하다고 사료된다.

□ Key words - nicardipine, ticlopidine, bioavailability, pharmacokinetics, CYP3A subfamily, P-gp, 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<sup>1,2</sup> 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.<sup>3</sup> The pharmacokinetic parameters of nicardipine are non-linear due to hepatic first-pass metabolism, thus, the extent of oral bioavailability (F)

was low about 35% following a 30 mg dose at steady state.<sup>4,5</sup> It is a substrate of cytochrome P450 (CYP) 3A subfamily, especially CYP3A4 in humans and forms to pharmacologically inactive metabolite.<sup>6-8</sup> In addition, nicardipine is also a P-glycoprotein (P-gp) substrate.<sup>9,10</sup>

Ticlopidine is extensively metabolized in the liver and is a potent inhibitor of platelet aggregation caused by adenosine diphosphate (ADP), whereas its ability to inhibit aggregation induced by collagen, thrombin, arachidonic acid, adrenaline, and platelet-activating factor varies.<sup>11</sup> It has been tried in a variety of platelet-dependent disease states.<sup>12-14</sup> Indeed, several recent reviews recommend ticlopidine as a valuable alternative when patients cannot tolerate aspirin.<sup>15-20</sup>

Ticlopidine is an antiplatelet drug reported to cause significant inhibition of several drugs metabolized by the hepatic cytochrome P-450 enzyme system, includ-

Correspondence to :Joon Seok Bang

College of Pharmacy & Graduate School of  
Clinical Pharmacy 309 Pilmundaero, Dong-gu,  
Gwangju, 501-759, Korea  
Tel: +82-62-230-6374, Fax: +82-62-222-5414  
E-mail: jsbang@chosun.ac.kr

ing theophylline and antipyrine. For example, ticlopidine co-medication results in a significant increase in mean warfarin concentrations.<sup>21)</sup> There is also report that the oral bioavailability of ticlopidine administered with meal was increased by 20% and the absorption of ticlopidine administered with antacid was approximately 20% lower than those under fasting conditions.<sup>22)</sup> Furthermore, we evaluated the inhibition of CYP enzyme activity and P-gp activity by ticlopidine using CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells.

Moreover, ticlopidine and nicardipine could be prescribed for the prevention or treatment of cardiovascular diseases as a combination therapy.

The low bioavailability of oral nicardipine is mainly due to pre-systemic metabolism by CYP3A4 and P-gp mediated efflux in the intestine. Therefore, the present study aimed to investigate the effect of ticlopidine on the intravenous and oral pharmacokinetics of nicardipine in rats.

## MATERIALS AND METHODS

### Chemicals and apparatus

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

HPLC system used in this study were 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 Branson® 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 Ani-

mal 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 *ad libitum*. The animals were housed (two rats per cage) in a clean- room maintained at a temperature of  $22\pm 2^{\circ}\text{C}$  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 with light 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 drug administration, respectively.

### Oral and intravenous administration of nicardipine

The rats were randomly divided into four groups (n = 6, each); an oral group (12 mg/kg of nicardipine dissolved in water; homogenized at  $36^{\circ}\text{C}$  for 30 min; 3.0 mL/kg) without (control) or with 3 or 10 mg/kg of oral ticlopidine, and an i.v. group (4 mg/kg of nicardipine, dissolved in 0.9% NaCl-injectable solution; homogenized at  $36^{\circ}\text{C}$  for 30 min; 1.5 mL/kg) without (control) or with 3 or 10 mg/kg of oral ticlopidine. Nicardipine was administered orally using a gastric gavage tube, and ticlopidine was orally 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 blood sample (0.45 mL) was collected into heparinized tubes from the femoral artery at 0 (control), 0.017 (end of the 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, 6, 8, 12 and 24 h after oral administration. The blood samples were centrifuged (13,000 rpm, 5 min), and the plasma samples were stored at  $-40^{\circ}\text{C}$  until HPLC analysis of nicardipine. 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 the blood loss due to blood sampling.

### HPLC assay

The plasma concentrations of nicardipine were determined by a HPLC assay method reported by Eastwood et al.<sup>23</sup> Briefly, a 50- $\mu$ L aliquot of nimodipine (2  $\mu$ g/mL), a 20-mL aliquot of 2 N sodium hydroxide solution and 1.2-mL of tert-butylmethylether:hexane (75:25) were added to 0.2-mL aliquot of plasma sample. The mixture was then stirred for 2 min and centrifuged (13,000 rpm, 10 min). A 1.0 mL aliquot 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 200-mL of the mobile phase and centrifuged (13,000 rpm, 5 min). A 50-mL aliquot of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Symmetry<sup>®</sup> C<sub>18</sub> column (4.6 $\times$ 150 mm, 5  $\mu$ m, Waters), and a iBondapak<sup>™</sup> C<sub>18</sub> HPLC Precolumn (10  $\mu$ m, Waters). The mobile phase was acetonitrile: 0.015 M KH<sub>2</sub>PO<sub>4</sub> (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. The detection limit of nicardipine in rat's plasma was 5 ng/mL. The coefficients of variation for nicardipine were below 14.1%.

### 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). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP3A4 substrate (7-Benzyloxy-4-(trifluoromethyl)couamrin (BFC)) was incubated with or without ticlopidine in a reaction mixture containing 1 pmol of CYP3A4 enzyme and the NADPH-generating system (1.3 mM NADP, 3.54 mM glucose 6-

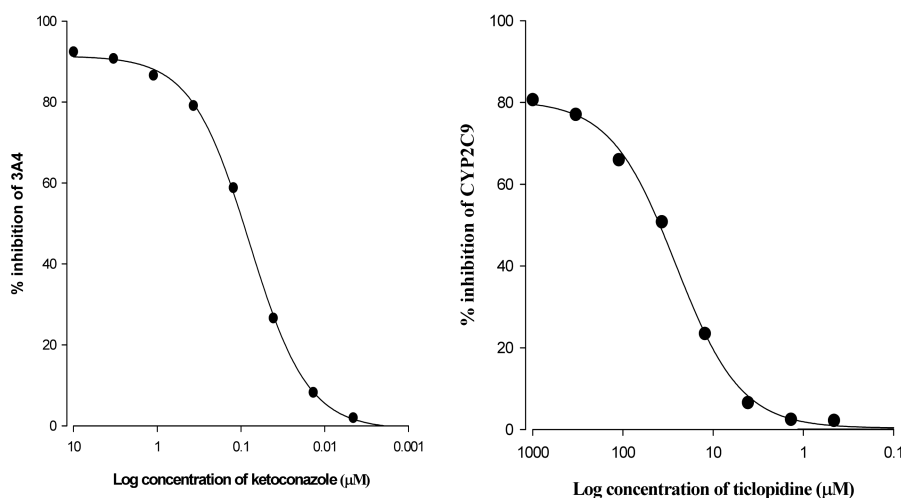
phosphate, 0.4 U/mL glucose 6-phosphate dehydrogenase and 3.3 mM MgCl<sub>2</sub>) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45 min. Metabolite concentrations were measured with a spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1  $\mu$ M 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 P-gp-overexpressed multidrug resistant human breast carcinoma cell line (MCF-7/ADR 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- $\mu$ M rhodamine-123 in the presence or absence of ticlopidine (1, 3 or 10  $\mu$ M) or verapamil (100  $\mu$ M) 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 a non-compartmental analysis (WinNonlin; software version 4.1; Pharsight Co., Mountain View, CA, USA). The elimination rate constant ( $K_{e1}$ ) 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  $0.693/K_{e1}$ . The peak plasma concentration ( $C_{max}$ ) and time to reach peak plasma concentration ( $T_{max}$ ) of nicardipine were directly read from the exper-



**Fig. 1. Inhibitory effect of ketoconazole and ticlopidine on CYP3A4 activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition.**

imental 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 ( $AUC_{0-∞}$ ) 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 (F) of nicardipine was calculated by  $AUC_{oral}/AUC_{iv} \times Dose_{i.v.}/Dose_{oral} \times 100$ , and the relative bioavailability (RB) of nicardipine was estimated by  $AUC_{with\ ticlopidine}/AUC_{control} \times 100$ .

### Statistical analysis

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

## RESULTS

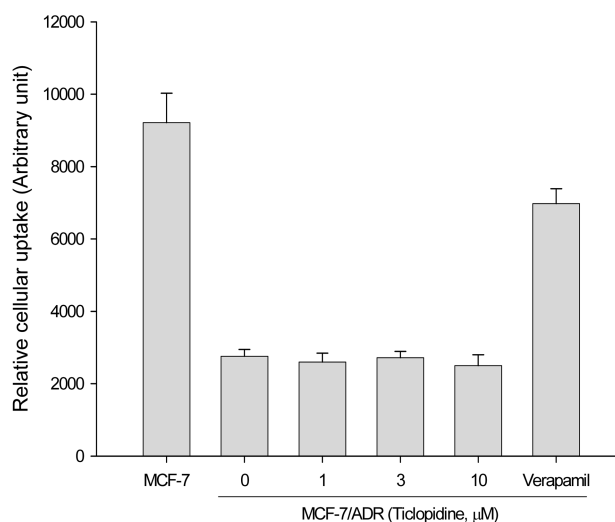
### Inhibitory effect of ticlopidine on CYP3A4

The inhibitory effect of ticlopidine on CYP3A4 activity is shown in Fig. 1. Ticlopidine inhibited CYP3A4 enzyme activity in a concentration-dependent manner and the 50% inhibition concentration ( $IC_{50}$ ) values of ticlopidine on

CYP3A4 activity was determined as 48  $\mu M$ .

### Rhodamine-123 retention assay

As shown in Fig. 2, accumulation of rhodamine-123, a P-gp substrate, was not reduced in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. This result suggests that ticlopidine did



**Fig. 2. Rhodamine-123 retention. MCF-7/ADR cells were preincubated with ticlopidine for 30 min. After incubation of MCF-7/ADR cells with 20  $\mu M$  R-123 for 90 min. The values were divided by total protein contents of each sample. Verapamil (100  $\mu M$ ) was used as a positive control. Data represents  $mean \pm SD$  of 6 separate samples.**

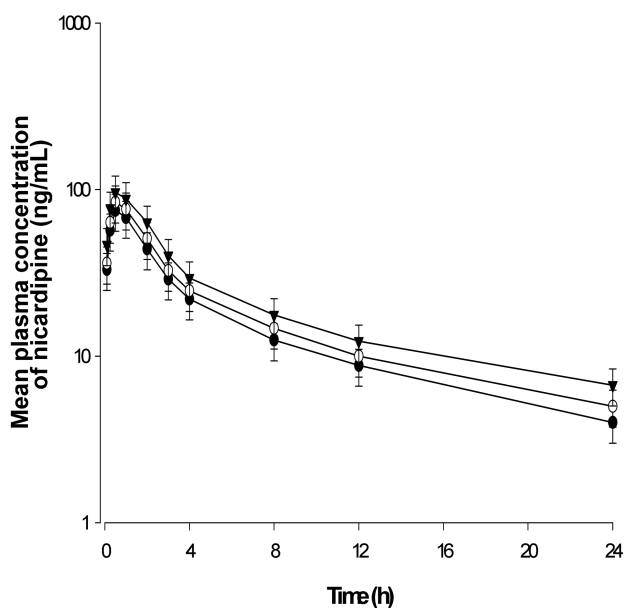


Fig. 3. Mean arterial plasma concentration–time profiles of nicardipine after its oral administration (12 mg/kg) without (control, ●) or with 3 mg/kg (○) and 10 mg/kg (▼) of ticlopidine to rats (Mean±SD, n=6).

not significantly inhibit P-gp activity.

#### Effect of ticlopidine on the pharmacokinetics of nicardipine

The mean arterial plasma concentration–time profiles of oral nicardipine (12 mg/kg) with or without ticlopidine (3 or 10 mg/kg) are shown in Fig. 3. The relevant pharmacokinetic parameters of nicardipine are also listed in Table 1. The area under the plasma concentration–time curve (AUC) was significantly ( $p < 0.05$ ) greater by 43.4%, and the peak concentration ( $C_{max}$ ) was significantly (10 mg/kg,  $p < 0.05$ ) higher by 26.7% with ticlopidine after oral administration of nicardipine. Consequently, the relative bioavailability (RB) of nicardipine was increased by 1.15- to 1.43-fold, and the absolute bioavailability (F) of nicardipine with ticlopidine was significantly (10 mg/kg,  $p < 0.05$ ) increased by 43.4%, compared to that of the controls. There was no significant change in the time to reach peak concentration ( $T_{max}$ ) and the half-life ( $t_{1/2}$ ) of nicardipine with ticlopidine.

The mean arterial plasma concentration–time profiles

Table 1. Mean pharmacokinetic parameters of nicardipine after its oral administration (12 mg/kg) with or without ticlopidine to rats (mean±SD, n=6).

Parameters	Control	Nicardipine+Ticlopidine	
		3 mg/kg	10 mg/kg
AUC (ng·h/mL)	415±84	478±105	595±143*
$C_{max}$ (ng/mL)	75±17	83±19	95±21*
$T_{max}$ (h)	0.46±0.10	0.46±0.10	0.58±0.20
$t_{1/2}$ (h)	8.5±1.6	9.1±1.8	9.8±2.3
F (%)	14.3±2.9	16.4±3.3	20.5±4.1*
RB (%)	100	115	143

\*  $p < 0.05$  significant difference compared to controls group.

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; F(%): absolute bioavailability; RB(%): relative bioavailability.

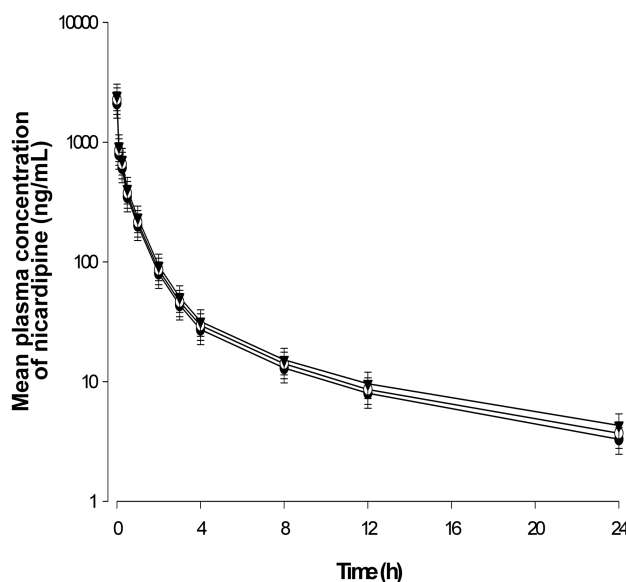


Fig. 4. Mean arterial plasma concentration–time profiles of nicardipine after its i.v. administration (4 mg/kg) without (control, ●) or with 3 mg/kg (○) and 10 mg/kg (▼) of ticlopidine to rats. Bars represent the standard deviation (Mean±SD, n=6). Table 1. Mean pharmacokinetic parameters of nicardipine after its oral administration (12 mg/kg) with or without ticlopidine to rats (mean±SD, n=6).

of i.v. nicardipine (4 mg/kg) with or without ticlopidine (3 or 10 mg/kg) are shown in Fig. 4. The relevant pharmacokinetic parameters of nicardipine are listed in Table 2. Ticlopidine did not significantly change pharmacokinetic parameters of i.v. administration of nicardipine, suggesting that the metabolism of nicardipine by ticlopidine via hepatic CYP3A subfamily and renal

**Table 2. Mean pharmacokinetic parameters of nicardipine after its intravenous administration (4 mg/kg) with or without ticlopidine to rats (mean±SD, n=6).**

Parameters	Control	Nicardipine+ Ticlopidine	
		3 mg/kg	10 mg/kg
AUC(ngh/mL)	969±204	1040±226	1133±259
CL(mL/min/kg)	51.6±12.5	47.9±10.4	43.9±9.3
t <sub>1/2</sub> (h)	6.9±1.4	7.1±1.6	7.4±1.8
RB (%)	100	107	117

AUC: area under the plasma concentration-time curve from time 0 to infinity; CL: total body clearance; t<sub>1/2</sub>: terminal half-life; RB(%): relative bioavailability.

excretion were almost negligible after intravenous administration in rats. Thus, enhanced oral bioavailability of nicardipine was due to increasing the intestinal absorption or reducing gut wall metabolism.

## 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 rate constant of a concurrently administered drug<sup>24</sup>.

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.<sup>25,26</sup> 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 and so could act synergistically to limit oral bioavailability of its substrates.<sup>27,28</sup>

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.<sup>29</sup> The inhibitory effect of ticlopidine on CYP3A4 activity is shown in Fig. 1. Ticlopidine inhibited CYP3A4 enzyme activity in a concentration-dependent manner and the 50 % inhibition concentration (IC<sub>50</sub>) values of ticlopidine on CYP3A4 activity was determined as 48 μM. These results are consistent with the report.<sup>30</sup>

A cell-based P-gp activity test using rhodamine-123 also showed that ticlopidine did not significantly inhibit P-gp activity (Fig. 2). These results are not consistent with the report.<sup>31</sup> The area under the plasma concentration-time curve (AUC) was significantly greater by 43.4%, and the peak concentration (C<sub>max</sub>) was significantly higher by 26.7% with ticlopidine (10 mg/kg) after oral administration of nicardipine. The absolute bioavailability (F) of nicardipine with ticlopidine was significantly increased by 43.4%, compared to that of the controls.

Orally administered nicardipine is a substrate for CYP3A-mediated metabolism. The enhanced oral bioavailability of nicardipine by ticlopidine could be mainly due to inhibition of CYP3A subfamily in the intestine and /or in the liver rather than P-gp efflux in the intestine. Ticlopidine did not significantly change pharmacokinetic parameters of i.v. nicardipine, suggesting that ticlopidine did not inhibit the metabolism of nicardipine via hepatic CYP3A subfamily and renal excretion in rats. This result appeared to be consistent with a previous report that oral administration of atorvastatin significantly increased the oral bioavailability of verapamil in rats.<sup>32</sup>

The increased in bioavailability of orally administered nicardipine by ticlopidine might be due to inhibition of CYP3A subfamily rather than P-gp in the intestine, since the metabolism of nicardipine by ticlopidine via hepatic CYP3A subfamily and renal excretion were almost negligible after intravenous administration. These results suggest enhanced bioavailability of nicardipine may be mainly inhibited CYP3A metabolism in the intestine and /or in the liver by ticlopidine.

## CONCLUSION

While there was no significant effect on the i.v. pharmacokinetics of nicardipine, ticlopidine significantly enhanced the oral bioavailability of nicardipine. Therefore, concomitant use of oral nicardipine and ticlopidine will require close monitoring for potential drug interactions.

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