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Pharmacokinetic interaction between lovastatin or pravastatin and nifedipine in rats

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흰쥐에서 로바스타틴 및 프라바스타틴과 니페디핀과의 약물동태학적 상호작용

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이 논문을 약학박사학위신청 논문으로 제출함.

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위원장	강원대학교	교수	이범진	인
위 원	서울대학교	교수	김대덕	인
위 원	서울대학교	교수	신완균	인
위 원	조선대학교	교수	유진욱	인
위 원	조선대학교	교수	최준식	인

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조선대학교 대학원

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임 태 환

지 도 교 수: 최 준 식

조선대학교대학원 약학과

항콜레스테롤제와 니페디핀의 병용투여가 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 로바스타틴 (0.3, 1.0 mg/kg) 또는 프라바스타틴 (0.3, 1.0 mg/kg)을 병용 경구투여한 후 니페디핀 및 그 활성대사체인 디하이드로니페디핀의 약물동태학적 변수들을 대조군과 비교 검토하였다.

로바스타틴 또는 프라바스타틴과 병용 투여시 니페디핀의 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 로바스타틴 (1.0 mg/kg) 또는 프라바스타틴 (1.0 mg/kg)과 병용투여군에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 유의성 ($P < 0.05$) 있게 증가되었으며, 전신클리어런스 (CL/F)는 유의성 ($P < 0.05$) 있게 각각 감소되었다.

절대적생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$) 있게 증가되었다. 아울러 로바스타틴 (1.0 mg/kg)과 니페디핀을 병용투여한군에서 대조군에 비해 활성대사체인 디하이드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 ($P < 0.05$) 있게 증가되었다.

그리고 로바스타틴은 니페디핀의 대사율 (MR)을 유의성 있게 감소시켰다.

정맥투여군에서는 로바스타틴 또는 프라바스타틴은 니페디핀의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 항콜레스테롤제인 로바스타틴 또는 프라바스타틴을 각각 고혈압치료제인 니페디핀과 병용투여 하였을 때 경구투여된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 로바스타틴 및 프라바스타틴에 의해서 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 간장에 존재하는 CYP3A 억제에 의한 니페디핀의 초회통과효과 (대사)감소와 전신클리어런스 감소에 기인한 것으로 사료된다.

Part I. Pharmacokinetic interaction between lovastatin and nifedipine in rats

국문초록

항콜레스테롤제와 니페디핀의 병용투여가 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 로바스타틴 (0.3, 1.0 mg/kg)을 병용 경구투여한 후 니페디핀 및 그 활성대사체인 디하이드로니페디핀의 약물동태학적 변수들을 대조군과 비교 검토하였다.

로바스타틴 병용 투여시 니페디핀의 약물동태학적 변수는 유의성 있게 변화하였다. 니페디핀 경구투여군에서는 대조군에 비해 로바스타틴 (1.0 mg/kg)과 병용투여 했을 때 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max}) 는 각각 45, 41%로 유의성 ($P < 0.05$) 있게 증가되었으며, 전신클리어런스 (CL/F)는 29.7% 유의성 ($P < 0.05$) 있게 감소되었다.

절대적생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$) 있게 증가되었다. 아울러 로바스타틴 (1.0 mg/kg)과 니페디핀을 병용투여한군에서 대조군에 비해 활성대사체인 디하이드로니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)이 유의성 ($P < 0.05$) 있게 16.4% 증가되었다. 그리고 로바스타틴은 니페디핀의 대사율 (MR)을 유의성 있게 감소시켰다.

정맥투여군에서는 로바스타틴은 니페디핀의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 항콜레스테롤제인 로바스타틴 각각 고혈압치료제인

니페디핀과 병용투여 하였을 때 경구투여 된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 로바스타틴에 의해서 주로 소장에 존재하는 P-gp 억제에 의한 흡수증가와 주로 소장 및 간장에 존재하는 CYP3A 억제에 의한 니페디핀의 초회통과효과 (대사) 감소와 전신클리어런스 감소에 기인한 것으로 사료된다.

Introduction

Nifedipine (dimethyl2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel-blocking agent that is widely used for the treatment of essential hypertension, coronary artery spasm, and angina pectoris [1]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload [2, 3].

In humans, nifedipine is predominantly metabolized by CYP3A4 to its primary pyridine metabolite, dehydronifedipine [4, 5]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs [6]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin [7, 8]. There are some reports that nifedipine is a substrate of CYP3A4 in human [9-11]. P-gp is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney [12, 13] and capillary endothelial cells in the central nervous system [14, 15]. Because of such tissue localized and its broad

substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs [16, 17]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other [18]. Dorababu et al. [19] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption of drugs.

Lovastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, is widely used in preventing the progression of atherosclerosis by lowering plasma LDL levels in patients with hypercholesterolemia [20, 21]. Lovastatin is an enzyme that catalyzes the conversion of HMG-CoA to mevalonate [22]. Mevalonate is a required building block for cholesterol biosynthesis and lovastatin interferes with its production by acting as a reversible competitive inhibitor for HMG-CoA, which binds to the HMG-CoA reductase. Lovastatin, being inactive in the native form in which it is administered, is hydrolysed to the active β-hydroxy acid form in the body.

Lovastatin is mainly metabolized by CYP 3A4 to a number of active metabolites in human liver microsomes [23-25]. Cytochrome P-450 oxidation is the primary route of phase I metabolism for lovastatin in humans and dogs [26]. Wang et al. [27] reported that HMG-CoA reductase inhibitors (statins) are inhibitors of P-gp in the rodent system, but the effects of lovastatin on P-gp-inhibition and CYP3A4-inhibition are partially ambiguous. Thus, we attempted to evaluate P-gp activity

using rhodamine-123 retention assay in P-gp-overexpressed MCF-7/ADR cells, and furthermore, to evaluate CYP3A4 activity of lovastatin.

Antihypertensive agents are commonly co-administered with cholesterol-lowering agents in clinics. There are some reports on the effects of calcium channel antagonists on the pharmacokinetics of HMG-CoA reductase inhibitors. Calcium-channel blockers increased plasma concentrations of some statins (lovastatin, pravastatin and simvastatin), possibly through the inhibition of CYP 3A4 and P-gp [28, 29]. But there are fewer reports about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [30, 31]. Moreover, lovastatin and nifedipine could be prescribed for the prevention or treatment of cardiovascular diseases as a combination therapy. However, the effect of lovastatin on the pharmacokinetics of nifedipine in vivo has not yet been reported. Thus, the purpose of this study was to investigate the possible effects of lovastatin on the CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with lovastatin in rats.

Materials and Methods

Materials

Nifedipine, dehydronifedipine and amlodipine [internal standard for the high-performance liquid chromatographic (HPLC) analysis of nifedipine] were purchased from the Sigma–Aldrich Co. (St. Louis, MO, USA). Methanol, isooctane, methyl-*tert*-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were products from Merck Co. (Darmstadt, Germany). Rhodamine was from Calbiochem (USA), the CYP inhibition assay kit was from GENTEST (Woburn, MA, US). Other chemicals were of reagent or HPLC grade.

Apparatus used in this study included an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters^{TM} 2487 scanning UV 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 studies

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague-Dawley rats

(270–300 g) were purchased from Dae Han Laboratory Animal Research Co. (Eumsung, Republic of Korea), and were given free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. Throughout the experiments, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2°C, 50-60% relative humidity, under a 12 h light-dark cycle. The rats were acclimated under these conditions for at least 1 week. Each rat was fasted for at least 24 h prior to the experiment. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using a polyethylene tube (SP45; i.d., 0.58 mm, o.d., 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under light ether anesthesia.

Intravenous and oral administration of nifedipine

The rats were divided into six groups (n = 6, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 0.3 and 1.0 mg/kg of lovastatin (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups (2.5 mg/kg of nifedipine; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.0 mL/kg) without (control) or with 0.3 and 1.0 mg/kg of lovastatin. A feeding tube was used to administer nifedipine and lovastatin intragastrically. Lovastatin was administered 30 min prior to oral administration of nifedipine. A blood sample (0.5-mL aliquot) was collected into heparinized tubes via the femoral artery at 0.017 (at the end of

infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h for the intravenous study, and 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1.2 mL) collected from untreated rats was infused via the femoral artery at 0.75, 4, and 8 h, respectively, to replace blood loss due to blood sampling. The blood samples were centrifuged (13,000 rpm, 3 min), and a 200-μL aliquot of plasma samples was stored in the deep freezer at -40^{o}C until the HPLC analysis.

HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay by a modification of the method reported by Grundy et al. [32]. Briefly, 50-μL of amlodipine (3 μg/mL), as the internal standard and 50-μL of 1.0 M sodium hydroxide were added to 0.2-mL of the plasma sample. It was then mixed for 3 s and 5-mL MTBE-isooctane (75 : 25, v/v) was added. The resultant mixure was vortex-mixed for 1 min and centrifuged at 3,000 rpm for 5 min. The organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas (no heat applied). The dried extract was reconstituted with 200 μl of mobile phase vortex-mixed for 1 min and aliquots of 160 μl were transferred to a clean autosampler vial. A 70-μL aliquot of the supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a Nova-pack C_8 (100mm × 8 mm I.D., 4 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol : water (62 : 38, v/v, pH 4.5, adjusted with acetic acid, 320 μL TEA/1000 mL mixture was added)

was used as the mobile phase at a flow rate of 1.0 mL/min. The retention times were: internal standard at 16.8 min, nifedipine at 8.2 min, and dehydronifedipine at 6.5 min (Figure 1). The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng/mL. The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0%.

CYP3A4 inhibition assay

The assay of inhibition on human CYP3A4 enzyme activity was performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [33]. Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without test compounds in the enzyme/substrate buffer with 1 pmol of P450 enzyme and an NADPH-generating system (1.3 mM NADP, 3.54mM 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 incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. All experiments were done in duplicate, and the results were expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method [34]. MCF-7/ADR cells were seeded in 24-well plates. 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 in the presence or absence of lovastatin (1, 3 and 10 μM) and verapamil (positive control) 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 EBC lysis buffer. 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 were presented as the ratio to control.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by a trapezoidal rule. The peak concentration (C_{max}) of nifedipine in plasma and time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The terminal half-

life ($t_{1/2}$) was calculated by $0.693/K_{el}$. Total body clearance (CL/F) was calculated by dose/AUC. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability (RB) of nifedipine were calculated by $AUC_{nifedipine\ with\ lovastatin}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{dehydronifedipine}/AUC_{nifedipine}$.

Statistical analysis

All the means were presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A P value < 0.05 was considered statistically significant.

Results

Inhibition of CYP3A4

The inhibitory effect of lovastatin on CYP3A4 activity is shown in Figure 4. Lovastatin inhibited CYP3A4 activity in a concentration-dependent manner. Lovastatin inhibited CYP3A4 activity with an IC_{50} value of 5.9 μM.

Rhodamine-123 retention assay

Accumulation of rhodamine-123, a P-glycoprotein substrate, was increased in MCF-7/ADR cells overexpressing P-glycoprotein compared to that in MCF-7 cells lacking P-glycoprotein, as shown in Figure 5. The concurrent use of lovastatin (3-10 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner. This result suggests that lovastatin significantly inhibited P-gp activity.

Effect of lovastatin on the pharmacokinetics of oral nifedipine

The mean plasma concentration–time profiles of nifedipine in the presence and absence of lovastatin (0.3 and 1.0 mg/kg) are shown in Figure 6. The pharmacokinetic parameters of nifedipine are summarized in Table 1. Lovastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the area under the plasma concentration – time curve from time zero to time infinity ($AUC_{0-\infty}$) of nifedipine by 45.0%, and peak concentration (C_{max}) of nifedipine by 41.0%. The total body

clearance (CL/F) was significantly decreased (1.0 mg/kg, $P < 0.05$) by lovastatin. Accordingly, the absolute bioavailability (AB) values of nifedipine in the presence of lovastatin (1.0 mg/kg) were significantly ($P < 0.05$) higher (44.9%) than that of the control group. Lovastatin increased the relative bioavailability (RB) of nifedipine by 1.18- to 1.45-fold. There were no significant differences in the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$) of nifedipine in the presence of lovastatin.

Effect of lovastatin on the pharmacokinetics of dehydronifedipine

The plasma concentration – time profiles of dehydronifedipine are shown in Figure 7. The pharmacokinetic parameters of dehydronifedipine are summarized in Table 2. The $AUC_{0-\infty}$ of dehydronifedipine was increased, but was not statistically significant compared to that in the control. The MR ratios were significantly ($P < 0.05$, 1.0 mg/kg) decreased (22.4%) by lovastatin, suggesting that the formation of dehydronifedipine was considerably altered by lovastatin. Thus, the increased bioavailability of nifedipine by lovastatin may be mainly due both to the inhibition of P-gp activity in the small intestine and to the inhibition of CYP3A4 activity in the small intestine and/or in the liver by lovastatin.

Effect of lovastatin on the pharmacokinetics of intravenous nifedipine

Mean arterial plasma concentration-time profiles of nifedipine following an intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence or

absence of lovastatin (0.3 and 1.0 mg/kg) are shown in Figure 8, while the corresponding pharmacokinetic parameters are shown in Table 3. The $AUC_{0-\infty}$ and CL_t of nifedipine were changed, but was not statistically significant compared to those in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not statistically significant. The pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of lovastatin in contrast to those of oral nifedipine. Accordingly, the enhanced oral bioavailability in the presence of lovastatin, while there was no significant change in the pharmacokinetics of intravenous nifedipine, may be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver by lovastatin rather than renal elimination of nifedipine.

Discussion

Based on the broad overlap in the substrate specificities as well as their co-localization in the small intestine, the primary site of absorption for orally administered drugs, cytochrome P450 (CYP) 3A4 and P-glycoprotein (P-gp), have been recognized as a concerted barrier to drug absorption [35, 36]. The prescription of more than one drug as a combination therapy is increasingly common in current medical practice. Cholesterol-lowering agents such as HMG-CoA reductase inhibitors could be co-administered with calcium channel blockers in the treatment of hypertension [37].

Considering that the drugs used in combination therapy often share the same metabolic pathways or cellular transport pathways, there exist high potential for pharmacokinetic as well as pharmacodynamic drug interactions between calcium channel antagonists and HMG-CoA reductase inhibitors. Indeed, some studies have reported that calcium-channel blockers increased the plasma concentrations of lovastatin or simvastatin [28, 29]. Similarly, as the dual substrates of CYP 3A and P-gp, nifedipine and lovastatin may undergo the same metabolic pathways and/or cellular transport pathways after co-administration. Therefore, lovastatin could affect the bioavailability or pharmacokinetics of nifedipine in rats.

In Figure 4, the inhibitory effect of lovastatin on CYP3A4 activity is shown and lovastatin inhibited CYP3A4 activity with an IC_{50} value of 5.9 μM. In Figure 5, the

concurrent use of lovastatin (3-10 μM) enhanced the cellular uptake of rhodamine-123 in a concentration-dependent manner, it is suggested that lovastatin significantly inhibited P-gp activity. These results were consistent with reports showing that lovastatin is an effective inhibitor of P-gp and CYP 3A transport [26, 27]. Some in-vitro and in-vivo studies have indicated that nifedipine is metabolized to dehydronifedipine mainly by CYP3A4 enzymes [4, 5]. Furthermore, Dorababu et al. [19] suggested that nifedipine is a substrate of P-gp. Considering that nifedipine is a substrate of both CYP enzymes and P-gp, lovastatin, as a dual inhibitor of both CYP3A4 and P-gp, may significantly impact the pharmacokinetics and bioavailability of nifedipine.

As CYP3A9 expressed in rat is corresponding to the ortholog of CYP3A4 in human [38], CYP3A2 of rats are similar to those of human [39, 40]. Human 3A4 and rat 3A1 have 73% protein homology [41]. Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there should be some extent of difference in enzyme activity between rat and human [42].

As shown in Table 1, the presence of lovastatin significantly enhanced the $AUC_{0-\infty}$ and C_{max} of nifedipine in rats. Subsequently, the relative bioavailability (RB) of nifedipine was increased by 118 to 145% in the presence of lovastatin (0.3 and 1.0 mg/kg). Those results were similar to reports by Hong et al. [31] in that lovastatin significantly enhanced the $AUC_{0-\infty}$ and C_{max} of diltiazem, a substrate of both CYP enzymes and P-gp in rats, and by Chung et al. [30] in that lovastatin

significantly enhanced the AUC and C_{max} of nicardipine in rats. Results of these studies were also consistent with the report that oral diallyl trisulfide (major organosulfur compounds derived from garlic) significantly increased the bioavailability of nifedipine by inhibition of CYP3A4 in rats [43]. These results are consistent with the report by Kuroha et al. in that ketoconazole, a CYP3A4 inhibitor, significantly increased the $AUC_{0-\infty}$ and C_{max} of nifedipine [44].

The $AUC_{0-\infty}$ of dehydronifedipine was increased by the presence of 1.0 mg/kg of oral lovastatin (Table 2), but was not statistically significant. The metabolite-parent ratio (MR) in the presence of lovastatin (1.0 mg/kg) was significantly ($P < 0.05$) decreased compared to that of the control group. Those results were similar to reports by Hong et al. [31] in that the metabolite-parent ratio (MR) of diltiazem in the presence of lovastatin (1.0 mg/kg) was significantly ($P < 0.05$) decreased compared to that of the control group.

After intravenous administration of nifedipine with lovastatin, the AUC of nifedipine increased, but was not statistically significant (Table 3). The CL_t and $t_{1/2}$ values of nifedipine tend to decrease, but was not statistically significant. This suggests that the effects of oral lovastatin on the inhibition of hepatic metabolism of nifedipine via CYP3A4 were almost negligible. In contrast to those of oral nifedipine, the pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of lovastatin.

Since the present study raised awareness of potential drug interactions by concomitant use of lovastatin with nifedipine, this finding has to be further

evaluated in clinical studies. The increased bioavailability of nifedipine in the presence of lovastatin might be due to an inhibition of the P-gp-mediated efflux transportor and CYP 3A-mediated metabolism by lovastatin.

Conclusion

The increased bioavailability of nifedipine in the presence of lovastatin might be due to an inhibition of the P-gp-mediated efflux transportor in the small intestine and CYP 3A-mediated metabolism in the small intestine and/or in the liver rather than renal elimination by lovastatin.

Concomitant use of nifedipine with lovastatin may require close monitoring for potential drug interactions. However, the clinical importance of these findings should be further investigated in clinical trials.

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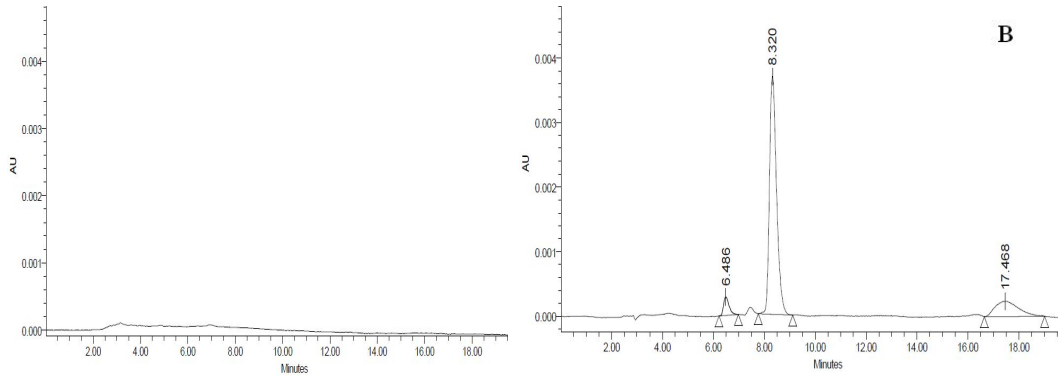

Figure 1. HPLC chromatograms of the rat blank plasma (A) and the plasma spiked with nifedipine (8.320 min), dehydronifedipine (6.486 min) and amlodipine (internal standard; 17.468 min) (B).

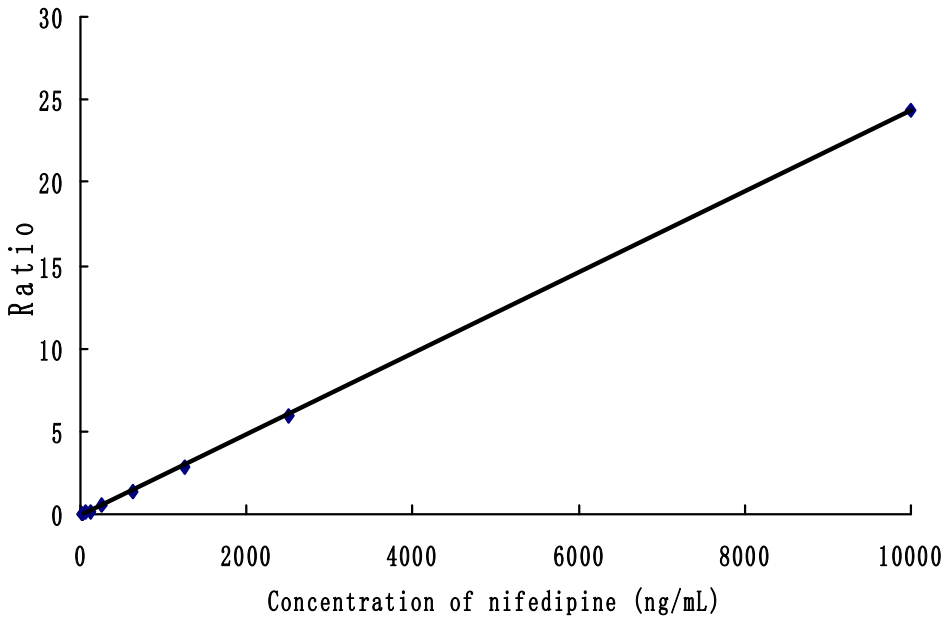

Figure 2. A calibration curve of nifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was y=0.0024x - 0.0983, where “y” is the peak area ratio of nifedipine to amlodipine and “x” is the concentration of nifedipine.

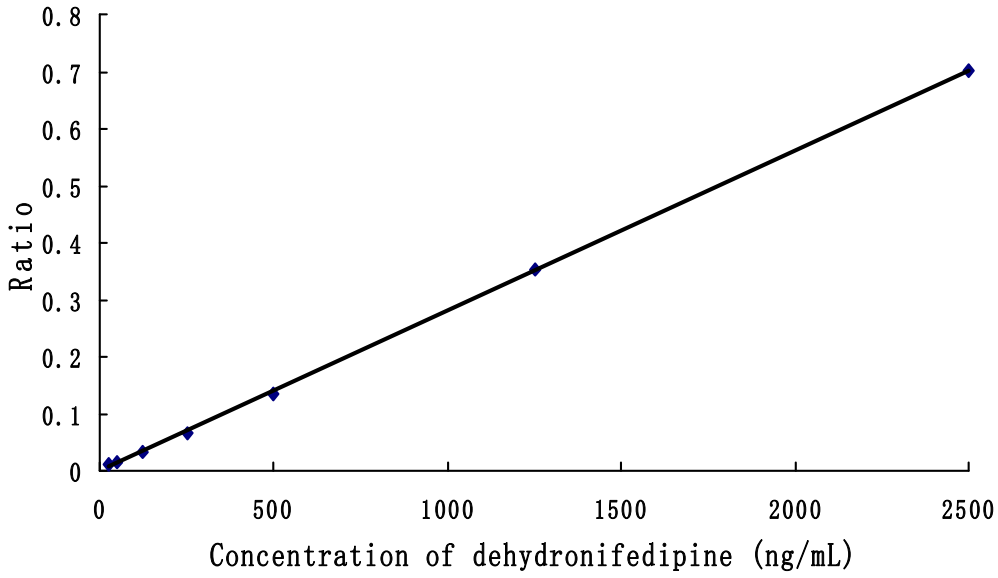

Figure 3. A calibration curve of dehydronifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was $y=0.0003x-0.0014$, where "y" is the peak area ratio of dehydronifedipine to amlodipine and "x" is the concentration of dehydronifedipine.

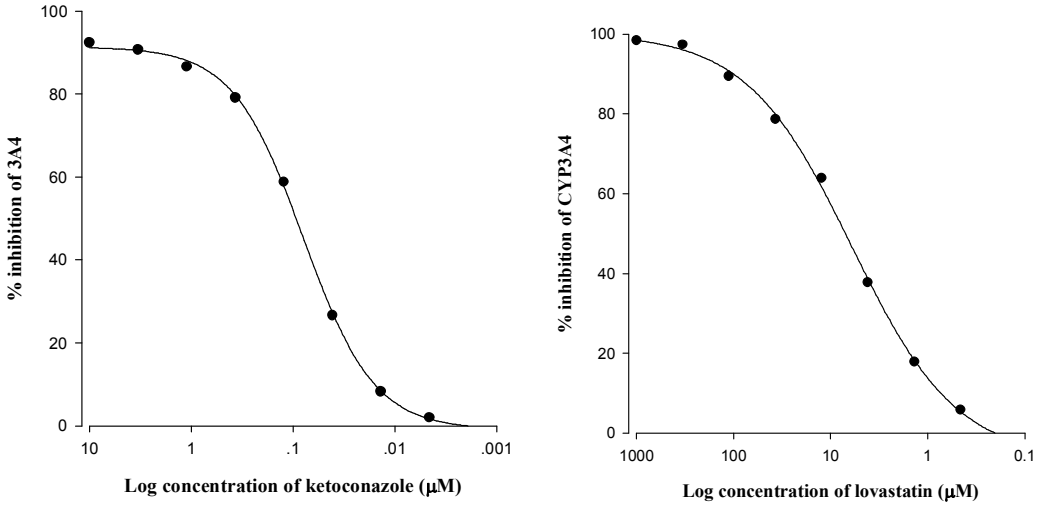

Figure 4. Inhibitory effect of ketokonazole and lovastatin on CYP3A4 activity. All experiments were done in duplicate, and the results were expressed as the percent of inhibition.

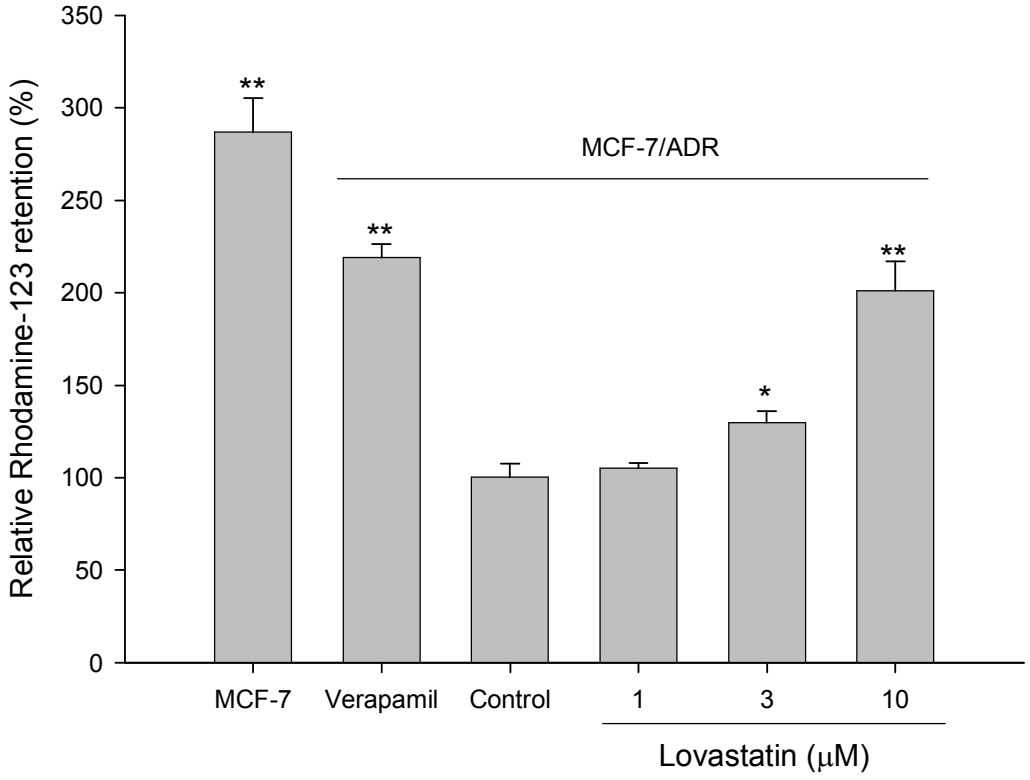

Figure 5. Effects of lovastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD (n = 6). *P < 0.05, **P < 0.01, significant difference compared to positive control (verapamil).

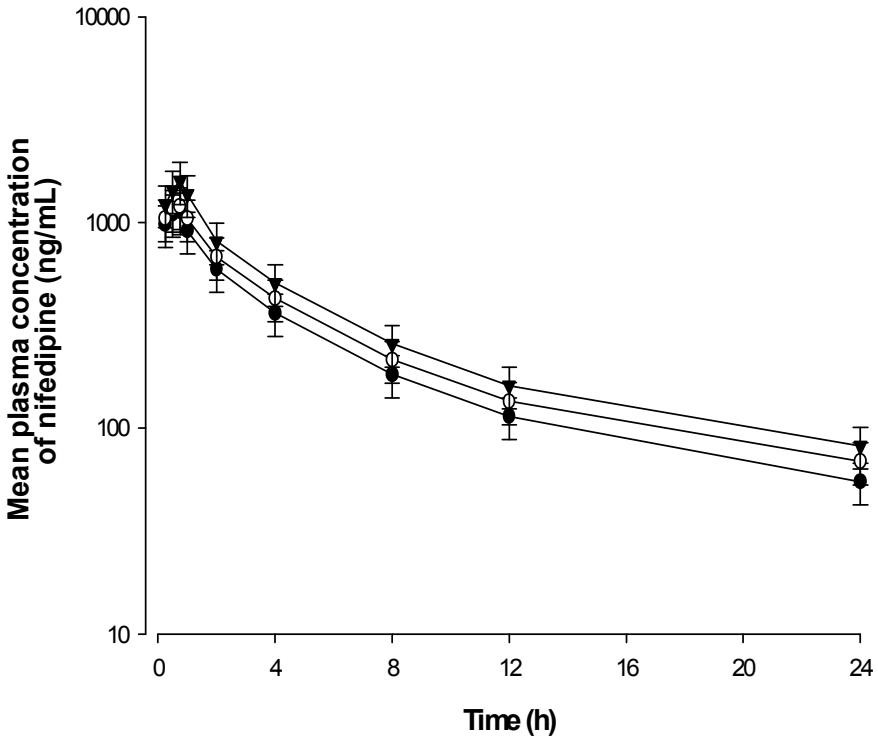

Figure 6. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine in the presence and absence of lovastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 10 mg/kg), ○ – with 0.3 mg/kg lovastatin, ▼ – with 1.0 mg/kg lovastatin.

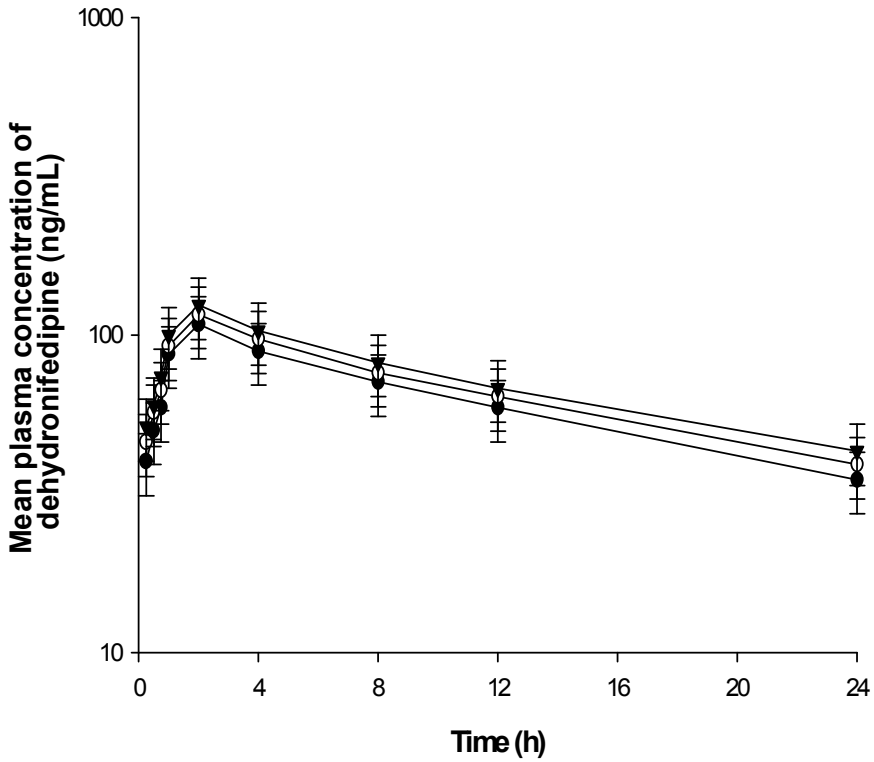

Figure 7. Mean plasma concentration-time profiles of dehydronifedipine after oral administration of nifedipine (10 mg/kg) in the presence and absence of lovastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 10 mg/kg), ○ – with 0.3 mg/kg lovastatin, ▼ – with 1.0 mg/kg lovastatin.

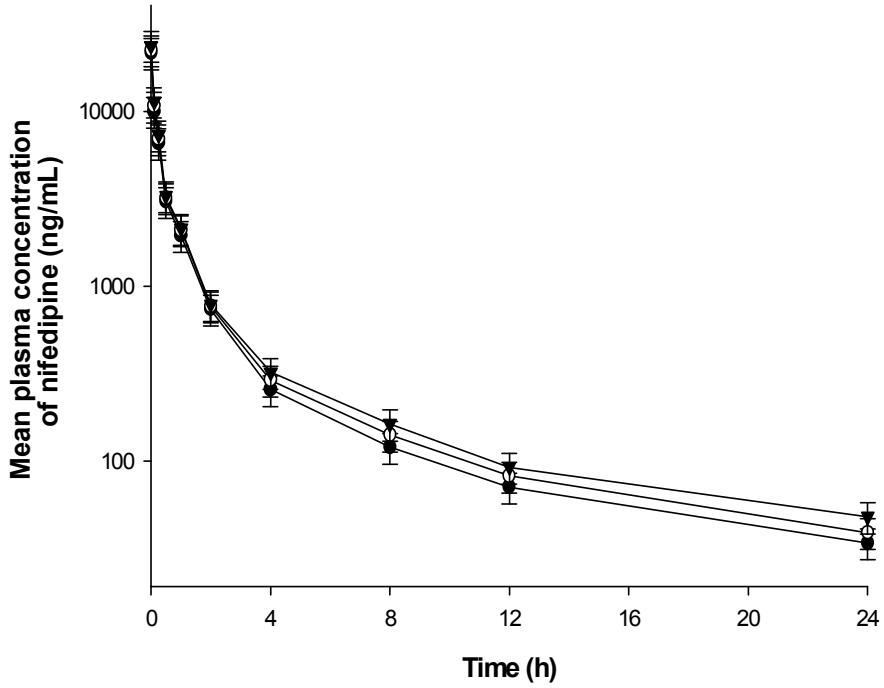

Figure 8. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine in the presence and absence of lovastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 2.5 mg/kg), ○ – with 0.3 mg/kg lovastatin, ▼ – with 1.0 mg/kg lovastatin.

Table 1. Mean (± SD, n = 6) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration of nifedipine in the presence and absence of lovastatin in rats

parameters	Control	Lovastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	5964 ± 1074	7048 ± 1269	8647 ± 1533*
C_{max} (ng/mL)	1130 ± 226	1201 ± 240	1593 ± 318*
T_{max} (h)	0.71 ± 0.10	0.79 ± 0.10	0.83 ± 0.13
$t_{1/2}$ (h)	9.8 ± 2.1	10.2 ± 2.2	10.3 ± 2.2
CL/F (mL/min)	27.9 ± 3.3	23.6 ± 2.8	19.6 ± 2.3*
AB (%)	15.8 ± 3.2	18.7 ± 3.7	22.9 ± 4.5*
RB (%)	100	118	145

* $P < 0.05$ (Significant difference compared to the control)

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; CL/F: total body clearance; AB: absolute bioavailability; RB: relative bioavailability.

Table 2. Mean (± SD, n = 6) pharmacokinetic parameters of dehydronifedipine following an oral administration of nifedipine (10 mg/kg) in the presence and absence of lovastatin in rats

Parameters	Control	Lovastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	2267 ± 385	2537 ± 431	2719 ± 474*
C_{max} (ng/mL)	108.0 ± 19.4	116.0 ± 20.9	124.0 ± 22.3
T_{max} (h)	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
$t_{1/2}$ (h)	15.5 ± 2.6	16.5 ± 2.8	17.4 ± 2.9
RB (%)	100	112	120
MR	38.1 ± 6.6	35.9 ± 6.4	31.2 ± 5.6*

* $P < 0.05$ (Significant difference compared to the control)

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; RB: relative bioavailability; MR: metabolite–parent AUC ratio.

Table 3. Mean (± SD, n = 6) pharmacokinetic parameters of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine with lovastatin in rats

Parameters	Control	Lovastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	9426 ± 1414	10181 ± 1527	10970 ± 1646
CL_t (mL/min)	4.4 ± 0.5	4.1 ± 0.5	3.8 ± 0.4
$t_{1/2}$ (h)	9.2 ± 1.6	9.3 ± 1.7	9.7 ± 1.8
RB (%)	100	108	116

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; CL_t: total body clearance; $t_{1/2}$: terminal half-life; RB: relative bioavailability.

Abstract

The purpose of this study was to investigate the possible effects of lovastatin on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. The effect of lovastatin on P-glycoprotein (P-gp) and cytochrome P450 (CYP) 3A4 activity was evaluated. Lovastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 5.9 μM. In addition, lovastatin significantly enhanced the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-glycoprotein.

The pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after oral and intravenous administration of nifedipine to rats in the presence and absence of lovastatin (0.3 and 1.0 mg/kg). The areas under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were significantly (1.0 mg/kg, $P < 0.05$) increased by 45.0% and 41.0%, repectively, in the presence of lovastatin compare to those of control. The total body clearance (CL/F) was significantly (1.0 mg/kg, $P < 0.05$) decreased by lovastatin (29.7%). Consequently, the absolute bioavailability (AB) of nifedipine in the presence of lovastatin (1.0 mg/kg) was significantly ($P < 0.05$) higher (44.9%) than that of the control group. Moreover, the relative bioavailability (RB) of nifedipine was 1.18- to 1.45-fold greater than that in the control group. The metabolite-parent AUC ratio (MR) in the presence of lovastatin (1.0 mg/kg) significantly decreased compared to the control group. This result implied that

lovastatin effectively inhibited the metabolism of nifedipine.

The increased bioavailability of nifedipine in the presence of lovastatin might be due to an inhibition of the P-gp-mediated efflux transportor in the small intestine and CYP3A-mediated metabolism in the small intestine and/or in the liver or to a reduction of total body clearance rather than to a reduction of renal elimination of nifedipine by lovastatin.

Key words: Nifedipine, Dehydronifedipine, Lovastatin, CYP3A4, P-gp, Pharmacokinetics, Bioavailability, Rats

Part II. Pharmacokinetic interaction between pravastatin and nifedipine in rats

국문초록

항콜레스테롤제와 니페디핀의 병용투여가 순환기 질환 예방 및 치료를 위해서 처방되는 경우가 있으므로 이에 대한 상호작용을 알아보고자, 흰쥐에 니페디핀 (경구; 10 mg/kg, 정맥; 2.5 mg/kg)과 프라바스타틴 (0.3, 1.0 mg/kg)을 병용 경구투여한 후 니페디핀 및 그 활성대사체인 디하이드로니페디핀의 약물동태학적 변수들을 대조군과 비교 검토하였다.

프라바스타틴과 병용 투여시 경구투여한 니페디핀의 약물동태학적 변수는 유의성 있게 변화하였다. 대조군에 비해 프라바스타틴 (1.0 mg/kg)과 병용투여군에서 니페디핀의 혈장농도곡선하면적 ($AUC_{0-\infty}$)과 최고혈중농도 (C_{max})는 각각 34.2, 33.7%로 유의성 ($P < 0.05$) 있게 증가되었으며, 전신클리어런스 (CL/F)는 28.1% 유의성 ($P < 0.05$) 있게 감소되었다.

절대적생체이용률 (AB)도 대조군에 비해 각각 유의성 ($P < 0.05$) 있게 증가되었다. 아울러 프라바스타틴과 니페디핀을 병용투여한군에서 대조군에 비해 활성대사체인 디하이드로니페디핀의 약동학적 파라미터에는 변화가 없었다.

정맥투여군에서도 프라바스타틴은 니페디핀의 약동학적 변수에는 거의 영향을 주지 못하였다.

본 연구에서 항콜레스테롤제인 프라바스타틴을 각각 고혈압치료제인 니페디핀과 병용투여 하였을 때 경구투여된 니페디핀의 생체이용률이 유의성 있게 증가된 것은 프라바스타틴에 의해서 주로 소장에 존재하는

P-gp 억제에 의한 흡수증가와 주로 소장과간장에 존재하는 CYP3A 억제에 의한 니페디핀의 초회통과효과 (대사) 감소와 전신클리어런스 감소에 기인한 것으로 사료된다.

Introduction

Nifedipine (dimethyl2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) is a calcium channel-blocking agent that is widely used for the treatment of essential hypertension, coronary artery spasm, and angina pectoris [1]. It inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically plugging the channel, resulting in decreased intracellular calcium levels, inhibition of the contractile processes of smooth muscle cells, dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, and decreased total peripheral resistance, systemic blood pressure, and afterload [2, 3].

In humans, nifedipine is predominantly metabolized by CYP3A4 to its primary pyridine metabolite, dehydronifedipine [4, 5]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs [6]. CYP3A4 is the most abundant CYP enzyme (30-40%) in adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam and erythromycin [7, 8]. There are some reports that nifedipine is a substrate of CYP3A4 in human [9-11]. P-gp is an adenosine-50-triphosphate (ATP) dependent efflux drug transporter that is constitutively expressed in normal tissues that includes gastrointestinal epithelium, canalicular membrane of the liver, kidney [12, 13] and capillary endothelial cells in the central nervous system [14, 15]. Because of such tissue localized and its broad

substrate specificity, P-gp appears to play a key role in absorption, distribution, and elimination of many drugs [16, 17]. It is generally known that the substrate and/or inhibitors of CYP3A4 and P-gp overlap with each other [18]. Dorababu et al. [19] reported that nifedipine belonged to a group of P-gp substrate. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism, resulting in the limited absorption of drugs.

Pravastatin, one of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) widely used in the management of hypercholesterolaemia [20]. Pravastatin is rapidly but incompletely absorbed from the gastrointestinal tract and undergoes extensive first-pass metabolism in the liver, its primary site of action [21]. 3″-Hydroxy pravastatin and 3′α, 5′β-dihydroxy-pravastatin are the major metabolites of pravastatin [20]. Cytochrome P450 (CYP) 3A is mainly responsible for 3″-hydroxy pravastatin formation, whereas members of the CYP enzymes other than the CYP3A subfamily involved in the formation of 3′α, 5′β-dihydroxy-pravastatin [22-24].

There is no clinically important pharmacokinetic interaction of pravastatin with a number of common CYP3A inhibitors. Itraconazole, diltiazem and grapefruit juice have no statistically significant effect on pharmacokinetics of pravastatin [25-27]. The contribution of CYP-dependent biotransformation to pravastatin elimination is minor. Pharmacokinetic interaction of pravastatin with other drugs are rare compared with those of other statins, which may be due to the dual routes of

elimination and low plasma protein binding of pravastatin. Pravastatin seems to be more favourable in the management of hypercholesterolaemia compared with the other statins.

The organic anion transporting polypeptide 1B1 (OATP1B1) and multidrug resistance-associated protein 2 (MRP2) are thought to be the major transporters involved in the pharmacokinetics of pravastatin in humans [28]. Kato et al. [29] reported that cyclosporine transport could be competitively inhibited by pravastatin via MRP 2.

There are several reports on the effects of calcium channel antagonists on the pharmacokinetics of HMG-CoA reductase inhibitors. Calcium-channel blockers increased the plasma concentrations of some statins, possibly through the inhibition of CYP 3A4 and P-gp [30]. However, there are a few reports on the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of calcium channel antagonists in rats [31-34]. Moreover, pravastatin and nifedipine could be prescribed as a combination therapy for the prevention or treatment of cardiovascular diseases. However, little information is available regarding the effects of pravastatin on the pharmacokinetics of nifedipine. Therefore, the purpose of this study was to investigate the possible effects of pravastatin on the CYP3A4 and P-gp activity and bioavailability or the pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with pravastatin in rats.

Materials and Methods

Materials

Nifedipine, dehydronifedipine, pravastatin and amlodipine [internal standard for the high-performance liquid chromatographic (HPLC) analysis of nifedipine] were purchased from the Sigma–Aldrich Co. (St. Louis, MO, USA). Methanol, isooctane, methyl-tert-butyl ether (MTBE), analytical grade acetic acid and triethylamine (TEA) were products from Merck Co. (Darmstadt, Germany). Rhodamine was from Calbiochem (USA), the CYP inhibition assay kit was from GENTEST (Woburn, MA, US). Other chemicals were of reagent or HPLC grade.

Apparatus used in this study included an HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus auto sampler and a Waters™ 2487 scanning UV 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 studies

All animal study protocols were approved by the Animal Care Committee of Chosun University (Gwangju, Republic of Korea). Male Sprague-Dawley rats (270–300 g) were purchased from Dae Han Laboratory Animal Research Co.

(Eumsung, Republic of Korea), and were given free access to a normal standard chow diet (No. 322-7-1; Superfeed Co., Wonju, Republic of Korea) and tap water. Throughout the experiments, the animals were housed, four or five per cage, in laminar flow cages maintained at 22 ± 2°C, 50-60% relative humidity, under a 12 h light-dark cycle. The rats were acclimated under these conditions for at least 1 week. Each rat was fasted for at least 24 h prior to the experiment. The left femoral artery (for blood sampling) and left femoral vein (for drug administration in the intravenous study) were cannulated using a polyethylene tube (SP45; i.d., 0.58 mm, o.d., 0.96 mm; Natsume Seisakusho Company, Tokyo, Japan) while each rat was under light ether anesthesia.

Intravenous and oral administration of nifedipine

The rats were divided into six groups (n = 6, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 0.3 and 1.0 mg/kg of pravastatin (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups (2.5 mg/kg of nifedipine; the same solution used: 0.9% NaCl-injectable solution; total injection volume of 1.0 mL/kg) without (control) or with 0.3 and 1.0 mg/kg of pravastatin. A feeding tube was used to administer nifedipine and pravastatin intragastrically. Pravastatin was administered 30 min prior to oral administration of nifedipine. A blood sample (0.5-mL aliquot) was collected into heparinized tubes via the femoral artery at 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h for the intravenous study, and 0.25,

0.5, 0.75, 1, 2, 4, 8, 12, and 24 h for the oral study. Whole blood (approximately 1.2 mL) collected from untreated rats was infused via the femoral artery at 0.75, 4, and 8 h, respectively, to replace blood loss due to blood sampling. The blood samples were centrifuged (13,000 rpm, 3 min), and a 200-μL aliquot of plasma samples was stored in the deep freezer at -40^{o}C until the HPLC analysis.

HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay by a modification of the method reported by Grundy et al. [35]. Briefly, 50-μL of amlodipine (3 μg/mL), as the internal standard and 50-μL of 1.0 M sodium hydroxide were added to 0.2-mL of the plasma sample. It was then mixed for 3 s and 5-mL MTBE-isooctane (75 : 25, v/v) was added. The resultant mixure was vortex-mixed for 1 min and centrifuged at 3,000 rpm for 5 min. The organic layer (0.8 mL) was transferred into a clean test tube and evaporated under a gentle stream of nitrogen gas (no heat applied). The dried extract was reconstituted with 200 μl of mobile phase vortex-mixed for 1 min and aliquots of 160 μl were transferred to a clean autosampler vial. A 70-μl aliquot of the supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a Nova-pack C_8 (100mm × 8 mm I.D., 4 μm; Waters Co., Milford, MA, USA), was used at room temperature. A mixture of methanol : water (62 : 38, v/v, pH 4.5, adjusted with acetic acid, 320 μL TEA/1000 mL mixture was added) was used as the mobile phase at a flow rate of 1.0 mL/min. The retention times

were: internal standard at 17.5 min, nifedipine at 8.3 min, and dehydronifedipine at 6.5 min (Figure 9). The detection limits of nifedipine and dehydronifedipine in rat plasma were all 5 ng/mL. The coefficients of variation for nifedipine and dehydronifedipine were all below 5.0%.

CYP3A4 inhibition assay

The assay of inhibition on human CYP3A4 enzyme activity was performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously [36]. Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without test compounds in the enzyme/substrate buffer with 1 pmol of P450 enzyme and an NADPH-generating system (1.3 mM NADP, 3.54mM 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 incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. All experiments were done in duplicate, and the results were expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a reported method [37]. MCF-7/ADR cells were seeded in 24-well plates. 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 in the presence or absence of pravastatin (10, 30 and 100 μM) and verapamil (positive control) 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 EBC lysis buffer. 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 were presented as the ratio to control.

Pharmacokinetic analysis

The plasma concentration data were analyzed by the non-compartmental method using Thermo Kinetica Software Version 5.0 (Thermo Fisher Scientific Inc., Miami, OK, USA). The parameter values were obtained by fitting to the pharmacokinetic model using the simplex algorithm. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by a trapezoidal rule. The peak concentration (C_{max}) of nifedipine in plasma and time to reach C_{max} (T_{max}) were obtained by visual inspection of the data from the concentration–time curve. The terminal half-

life ($t_{1/2}$) was calculated by $0.693/K_{el}$. Total body clearance (CL/F) was calculated by dose/AUC. The absolute bioavailability (AB) was calculated by $AUC_{oral}/AUC_{i.v.} \times dose_{i.v.}/dose_{oral}$, and the relative bioavailability (RB) of nifedipine were calculated by $AUC_{nifedipine\ with\ pravastatin}/AUC_{control}$. The metabolite–parent AUC ratio (MR) was calculated by $AUC_{dehydronifedipine}/AUC_{nifedipine}$.

Statistical analysis

All the means were presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A P value < 0.05 was considered statistically significant.

Results

Inhibition of CYP3A4

The inhibitory effect of pravastatin on CYP3A4 activity is shown in Figure 12. Pravastatin inhibited CYP3A4 activity in a concentration-dependent manner. Pravastatin inhibited CYP3A4 activity with an IC_{50} value of 14 μM.

Rhodamine-123 retention assay

Accumulation of rhodamine-123, a P-glycoprotein substrate, was increased in MCF-7/ADR cells overexpressing P-glycoprotein compared to that in MCF-7 cells lacking P-glycoprotein, as shown in Figure 13. The concurrent use of pravastatin did not enhance the cellular uptake of rhodamine-123 in a concentration-dependent manner ranging from 10-100 μM. This result suggests that pravastatin did not significantly inhibit P-gp activity.

Effect of pravastatin on the pharmacokinetics of oral nifedipine

The mean plasma concentration–time profiles of nifedipine in the presence and absence of pravastatin (0.3 and 1.0 mg/kg) are shown in Figure 14. The pharmacokinetic parameters of nifedipine are summarized in Table 4. Pravastatin (1.0 mg/kg) significantly ($P < 0.05$) increased the area under the plasma concentration–time curve from time zero to time infinity ($AUC_{0-\infty}$) of nifedipine by 34.2%, and peak concentration (C_{max}) of nifedipine by 33.7%. The total body

clearance (CL/F) was significantly decreased (1.0 mg/kg, $P < 0.05$) by pravastatin. Accordingly, the absolute bioavailability (AB) values of nifedipine in the presence of pravastatin (1.0 mg/kg) were significantly ($P < 0.05$) higher (35.0%) than that of the control group. Pravastatin increased the relative bioavailability (RB) of nifedipine by 1.07- to 1.34-fold. There were no significant differences in the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$) of nifedipine in the presence of pravastatin.

Effect of pravastatin on the pharmacokinetics of dehydronifedipine

The plasma concentration–time profiles of dehydronifedipine are shown in Figure 15. The pharmacokinetic parameters of dehydronifedipine are summarized in Table 5. The $AUC_{0-\infty}$ of dehydronifedipine was increased, but was not statistically significant compared to that in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not significant. The MR ratios were decreased by pravastatin, but there have no statistical difference, and then, the C_{max}, T_{max} and RB of dehydronifedipine were also not significant differences with those of control in the presence of pravastatin.

Effect of pravastatin on the pharmacokinetics of intravenous nifedipine

Mean arterial plasma concentration-time profiles of nifedipine following an intravenous administration of nifedipine (2.5 mg/kg) to rats in the presence or absence of pravastatin (0.3 and 1.0 mg/kg) are shown in Figure 16, while the

corresponding pharmacokinetic parameters are shown in Table 6. The $AUC_{0-\infty}$, CL_t and RB of nifedipine were changed, but were not statistically significant compared to those in the control. The $t_{1/2}$ of nifedipine was also prolonged, but this increase was not significant. The pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of pravastatin in contrast to those of oral nifedipine. Accordingly, the enhanced oral bioavailability in the presence of pravastatin, while there was no significant change in the pharmacokinetics of intravenous nifedipine, may be mainly due to inhibition of the CYP3A-mediated metabolism of nifedipine in the small intestine and/or in the liver by pravastatin rather than renal elimination of nifedipine.

Discussion

The importance of first-pass metabolism for limiting systemic drug availability is well established; however, intestinal drug metabolism can further decrese systemic availability. Through functional enzyme activity studies and immunoblot analyses, CYP3A expression in mature enterocytes, located mainly in the villi tips of jejunal mucosa, was shown to be comparable to or even exceed the expression of CYP3A in hepatocytes [38]. Total CYP P450 content increased slightly proceeding from the duodenum to the jejunum and then decreased sharply to the ileum [39]. Using in situ hybridization with a probe specific for CYP3A4, McKinnon confirmed CYP3A expression throughout the entire small intestine, with highest levels in the proximal regions [40]. The most abundant CYP isoenzyme in the intestine is 3A4 [41].

Based on their broad overlap in substrate specificities as well as their 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 drug absorption [42-44]. The prescription of more than one drug as a combination therapy is increasingly common in current medical practice. Cholesterol-lowering agents such as HMG-CoA reductase inhibitors could be co-administered with calcium channel blockers for the treatment of hypertension [45]. Antihypertensive agents are commonly co-administered with cholesterol-lowering agents in clinics. It is possible that the efficacy of nifedipine would be increased

when coadministered with pravastatin. Orally administered pravastatin would affect the pharmacokinetics of nifedipine because it is a co-substrate of CYP3A4 and P-gp.

In the present study, cell-based P-gp activity tests using rhodamine-123 showed that pravastatin (10, 30 and 100 μM) did not inhibit P-gp activity, but pravastatin significantly inhibited CYP3A4 activity (Figs. 12 and 13). These results are consistent with a report showing that pravastatin effectively inhibited CYP3A4 activity [46]. Therefore, pravastatin, an inhibitor of CYP3A4 may significantly impact the bioavailability of nifedipine, a substrate of CYP3A4. As CYP3A9 expressed in rat is corresponding to the ortholog of CYP3A4 in human [47], CYP3A2 of rats are similar to those of human [48-49]. Human 3A4 and rat 3A1 have 73% protein homology [50]. Rats were selected as an animal model in this study to evaluate the potential pharmacokinetic interactions mediated by CYP3A4, although there should be some extent of difference in enzyme activity between rat and human [51].

This study evaluated the influence of pravastatin, an HMG-CoA reductase inhibitor, on the pharmacokinetics of nifedipine in rats in order to assess the potential drug interactions between pravastatin and nifedipine.

As shown in Table 4, pravastatin significantly (1 mg/kg, $P < 0.05$) increased area under the plasma concentration-time curve ($AUC_{0-\infty}$) of nifedipine. Pravastatin also significantly (1 mg/kg, $p < 0.05$) increased the absolute bioavailability (AB) of nifedipine by 35.0% compared to the oral control group, and the relative

bioavailability (RB) of nifedipine was increased by 1.07- to 1.34-fold.

Jacobson reported that clarithromycin, cytochrome P450-3A4 inhibitors, significantly increased the $AUC_{0-\infty}$ and C_{max} of pravastatin in healthy subjects [52]. These results were consistent with reports that simvastatin significantly increased the $AUC_{0-\infty}$ and C_{max} of verapamil in rats [34], and atorvastatin and fluvastatin also significantly increased the bioavailability of diltiazem in rats [38-39].

The $AUC_{0-\infty}$ of dehydronifedipine was not significantly increase by the presence of oral pravastatin (Table 5). The metabolite-to-parent ratio (MR) in the presence of pravastatin (1.0 mg/kg) also was not significantly decreased compared to that of the control group. Those results were similar to reports by Yang et al. [46] in that the metabolite-to-parent ratio (MR) of nifedipine in the presence of pravastatin (1.0 mg/kg) was not significantly decreased compared to that of the control group.

The pharmacokinetic profiles of intravenous nifedipine were also evaluated in the presence and absence of pravastatin (Table 6). The AUC and C_{max} of nifedipine were not significantly increase by pravastatin, suggesting that the presence of pravastatin did not affect renal elimination in rats. These results were similar to reports by Yang et al. [46], which showed that pravastatin did not affect pharmacokinetic parameters of intravenous losartan in rats. The pharmacokinetics of intravenous nifedipine was not affected by the concurrent use of pravastatin in contrast to those of oral administration of nifedipine. Accordingly, the enhanced oral bioavailability in the presence of pravastatin may be mainly due to reduced first-pass metabolism of nifedipine via the inhibition of the CYP3A subfamily in

the small intestine and/or in the liver rather than to reduced renal elimination of nifedipine by pravastatin.

Concomitant use of pravastatin and nifedipine will require close monitoring of potential drug interaction for safe therapy of cardiovascular diseases. Furthermore, the clinical importance of these findings should be investigated in clinical trials.

Conclusion

The increased bioavailability of nifedipine in the presence of pravastatin might be due to the inhibition of CYP 3A-mediated metabolism in the small intestine and/or in the liver rather than both inhibition of P-gp and renal elimination by pravastatin. Concomitant use of nifedipine with pravastatin may require close monitoring for potential drug interactions. However, the clinical importance of these findings should be further investigated in clinical trials.

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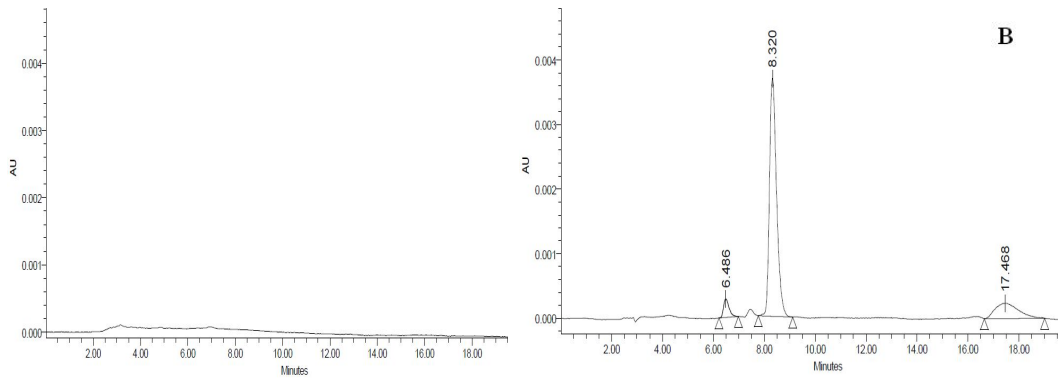

Figure 9. HPLC chromatograms of the rat blank plasma (A) and the plasma spiked with nifedipine (8.320 min), dehydronifedipine (6.486 min) and amlodipine (internal standard; 17.468 min) (B).

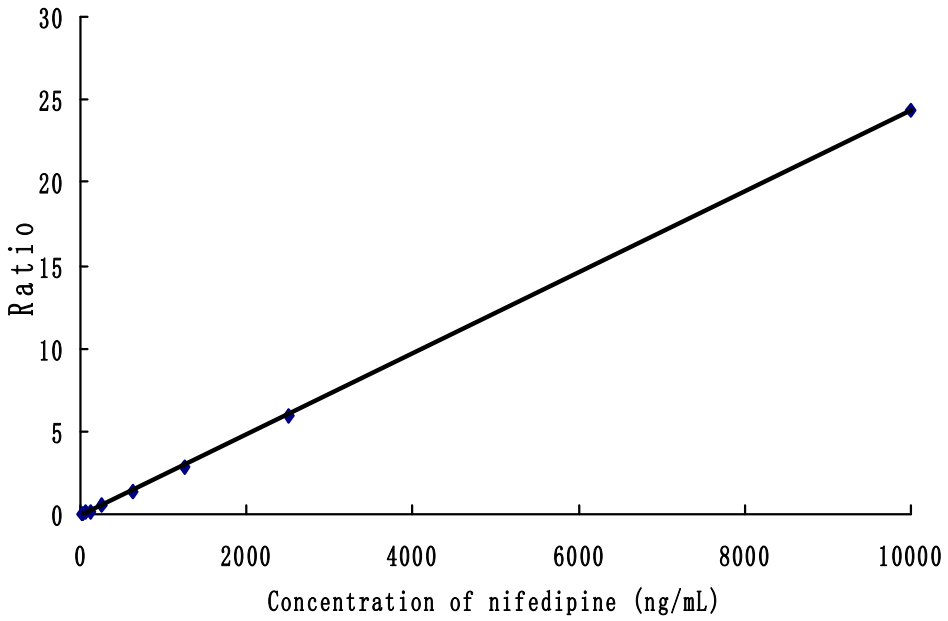

Figure 10. A calibration curve of nifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was $y=0.0024x-0.0983$, where "y" is the peak area ratio of nifedipine to amlodipine and "x" is the concentration of nifedipine.

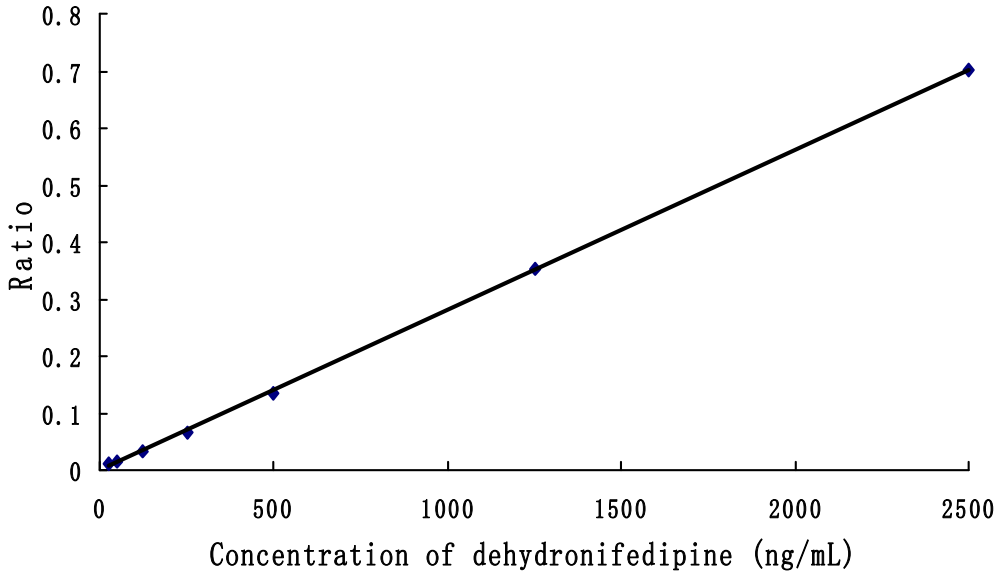

Figure 11. A calibration curve of dehydronifedipine when spiked into the rat blank plasma. The typical equation describing the calibration curve in rat plasma was $y=0.0003x-0.0014$, where "y" is the peak area ratio of dehydronifedipine to amlodipine and "x" is the concentration of dehydronifedipine.

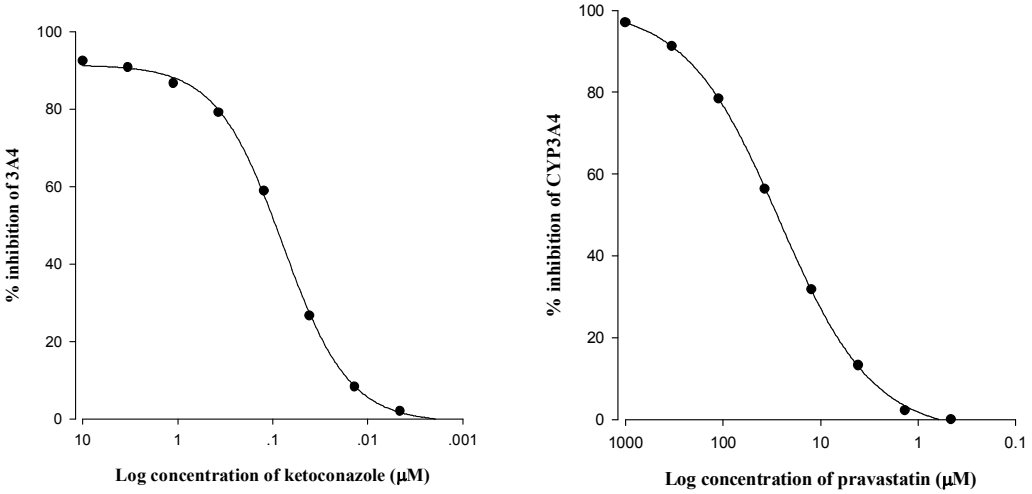

Figure 12. Inhibitory effect of ketokonazole and pravastatin on CYP3A4 activity. All experiments were done in duplicate, and the results were expressed as the percent of inhibition.

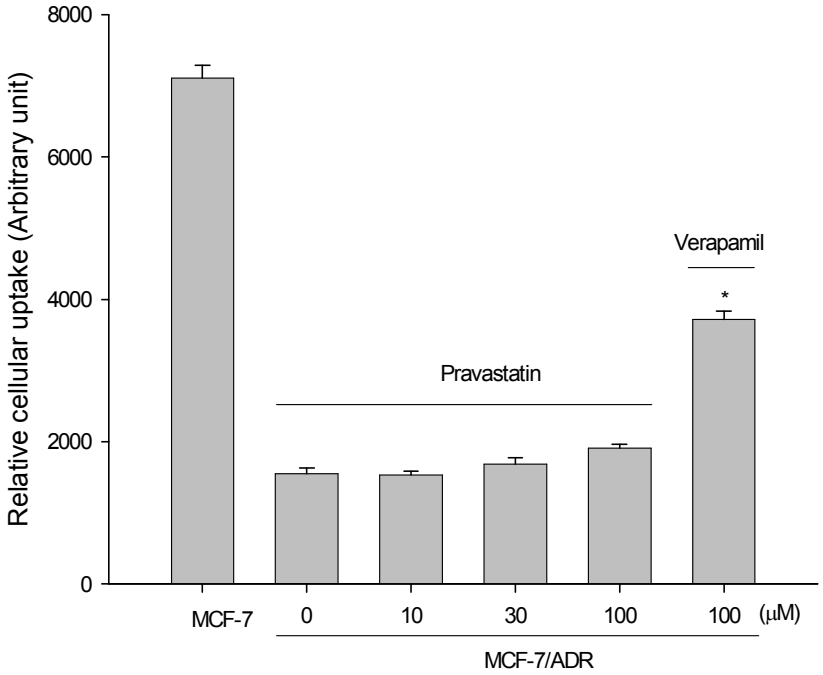

Figure 13. Effects of pravastatin on the cellular accumulation of rhodamine-123 in MCF-7 and MCF-7/ADR cells. Data represents mean ± SD (n = 6). *P < 0.05, significant difference compared to positive control (verapamil).

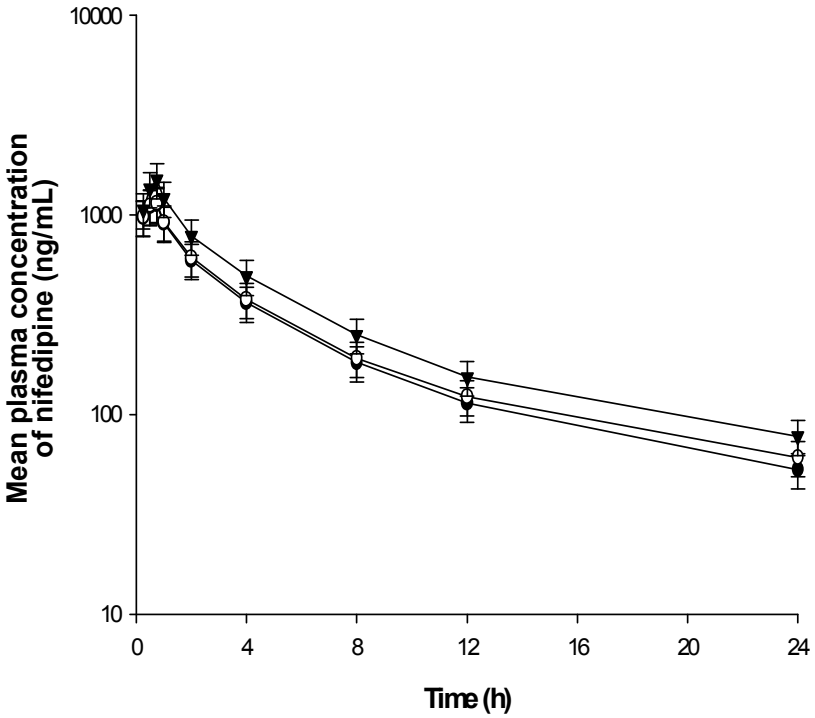

Figure 14. Mean plasma concentration-time profiles of nifedipine after oral (10 mg/kg) administration of nifedipine in the presence and absence of pravastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 10 mg/kg), ○ – with 0.3 mg/kg pravastatin, ▼ – with 1.0 mg/kg pravastatin.

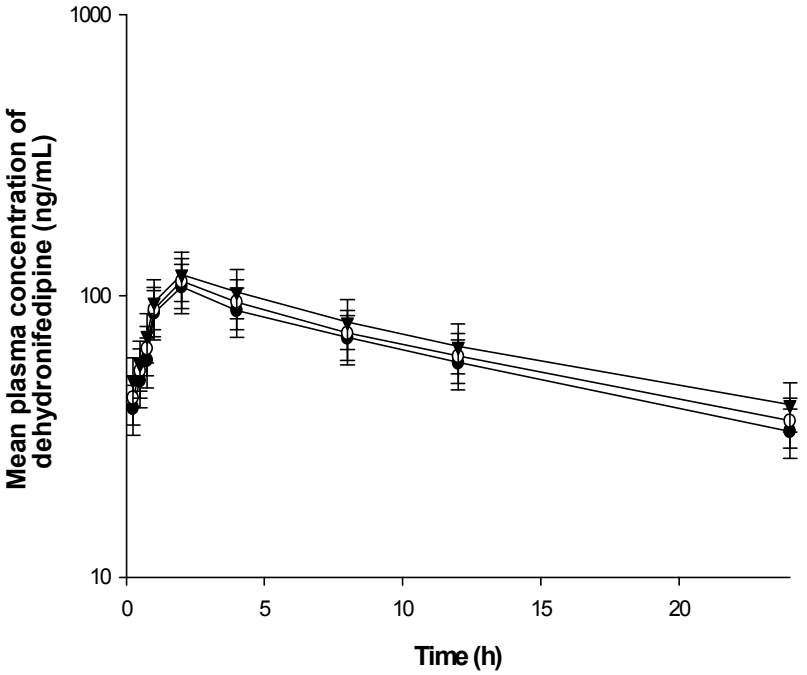

Figure 15. Mean plasma concentration-time profiles of dehydronifedipine after oral administration of nifedipine (10 mg/kg) in the presence and absence of pravastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 10 mg/kg), ○ – with 0.3 mg/kg pravastatin, ▼ – with 1.0 mg/kg pravastatin.

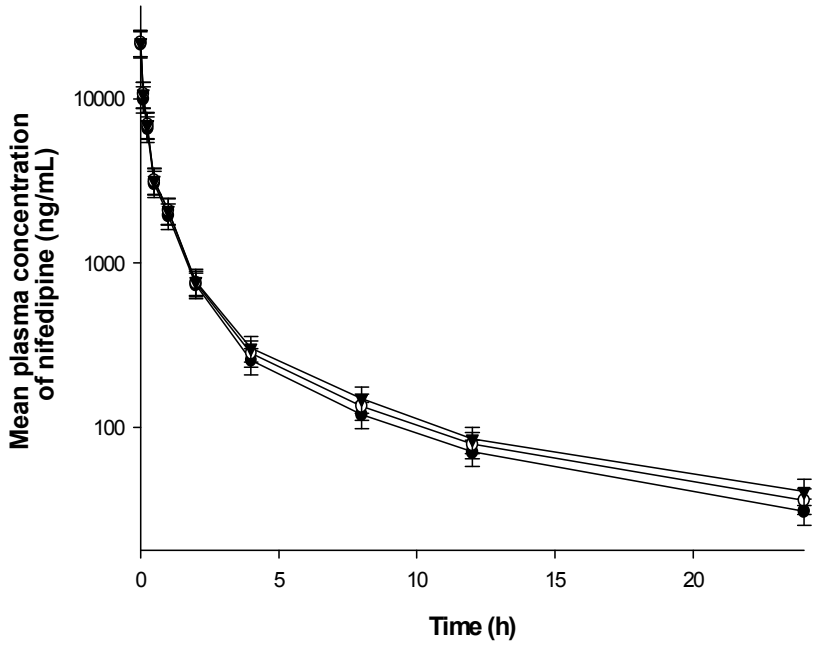

Figure 16. Mean plasma concentration-time profiles of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine in the presence and absence of pravastatin to rats (Mean ± SD, n = 6). ● – Control (nifedipine alone, 2.5 mg/kg), ○ – with 0.3 mg/kg pravastatin, ▼ – with 1.0 mg/kg pravastatin.

Table 4. Mean (± SD, n = 6) pharmacokinetic parameters of nifedipine after oral (10 mg/kg) administration of nifedipine in the presence and absence of pravastatin in rats

parameters	Control	Pravastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	5928 ± 889	6346 ± 952	7945 ± 1126*
C_{max} (ng/mL)	1130 ± 170	1146 ± 172	1511 ± 212*
T_{max} (h)	0.71 ± 0.10	0.79 ± 0.10	0.83 ± 0.13
$t_{1/2}$ (h)	9.3 ± 1.4	9.7 ± 1.5	9.9 ± 1.4
CL/F (mL/min)	28.1 ± 4.2	26.0 ± 3.9	20.2 ± 2.9*
AB (%)	15.7 ± 2.4	16.9 ± 2.5	21.2 ± 3.0*
RB (%)	100	107	134

* $p < 0.05$ (Significant difference compared to the control)

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; CL/F: total body clearance; AB: absolute bioavailability; RB: relative bioavailability.

Table 5. Mean (± SD, n = 6) pharmacokinetic parameters of dehydronifedipine following an oral administration of nifedipine (10 mg/kg) in the presence and absence of pravastatin in rats

Parameters	Control	Pravastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	2266 ± 340	2447 ± 367	2750 ± 413
C_{max} (ng/mL)	108.0 ± 16.2	113.0 ± 16.9	119.0 ± 17.9
T_{max} (h)	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.4
$t_{1/2}$ (h)	15.3 ± 2.3	15.8 ± 2.4	16.4 ± 2.5
RB (%)	100	108	121
MR	38.4 ± 5.7	38.4 ± 5.8	34.1 ± 5.1

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; RB: relative bioavailability; MR: metabolite–parent AUC ratio.

Table 6. Mean (± SD, n = 6) pharmacokinetic parameters of nifedipine after intravenous (2.5 mg/kg) administration of nifedipine with pravastatin in rats

Parameters	Control	Pravastatin	
		0.3 mg/kg	1.0 mg/kg
$AUC_{0-\infty}$ (ng·h/mL)	9393 ± 1315	10051 ± 1407	10385 ± 1454.
CL_t (mL/min)	4.5 ± 0.6	4.2 ± 0.5	4.0 ± 0.5
$t_{1/2}$ (h)	8.8 ± 1.2	9.0 ± 1.3	9.2 ± 1.3
RB (%)	100	107	111

$AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; CL_t: total body clearance; $t_{1/2}$: terminal half-life; RB: relative bioavailability.

Abstract

The purpose of this study was to investigate the possible effects of pravastatin on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats. The effect of pravastatin on cytochrome P450 (CYP) 3A4 and P-glycoprotein (P-gp) activity was evaluated. Pravastatin inhibited CYP3A4 enzyme activity in a concentration-dependent manner with a 50% inhibition concentration (IC_{50}) of 14 μM. In addition, pravastatin did not significantly enhance the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-glycoprotein.

The pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after orall and intravenous administration of nifedipine to rats in the presence and absence of pravastatin (0.3 and 1.0 mg/kg). The areas under the plasma concentration-time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were significantly ($P < 0.05$) increased by 34.2% and 33.7%, repectively, in the presence of pravastatin compare to those of control. The total body clearance (CL/F) was significantly decreased (1.0 mg/kg, $P < 0.05$) by pravastatin. Consequently, the absolute bioavailability (AB) of nifedipine in the presence of pravastatin (1.0 mg/kg) was significantly ($P < 0.05$) higher (35.0%) than that of the control group. Moreover, the relative bioavailability (RB) of nifedipine was 1.07- to 1.34-fold greater than that in the control group. Pravastatin did not affect the parameters of nifedipine in intravenous administration and the parameters of dehydronifedipine in rats.

The increased bioavailability of nifedipine in the presence of pravastatin might be due to an inhibition of the CYP 3A4-mediated metabolism in the small intestine and/or in the liver rather than both inhibition of P-gp and renal elimination by pravastatin.

Key words: Nifedipine, Dehydronifedipine, Pravastatin, CYP3A4, P-gp, Pharmacokinetics, Bioavailability, Rats

Abstract

Pharmacokinetic interaction between lovastatin or pravastatin and nifedipine in rats

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The purpose of this study was to investigate the possible effects of lovastatin or pravastatin, on the pharmacokinetics of nifedipine and its main metabolite, dehydronifedipine, in rats.

The pharmacokinetic parameters of nifedipine and dehydronifedipine were determined after oral (10 mg/kg) and intravenous (2.5 mg/kg) administration of nifedipine to rats in the presence and absence of lovastatin or pravastatin (0.3 and 1.0 mg/kg). The effect of lovastatin or pravastatin on the P-glycoprotein (P-gp) as well as CYP3A4 activity was also evaluated. Lovastatin or pravastatin significantly inhibited CYP3A4 enzyme. In addition, lovastatin significantly reduced rhodamine-123 efflux via P-gp in MCF-7/ADR cells overexpressing p-gp. However pravastatin did not significantly enhance the cellular accumulation of rhodamine-123 in MCF-7/ADR cells overexpressing P-gp.

Compared to the control (nifedipine alone), lovastatin or pravastatin significantly altered the pharmacokinetic parameters of nifedipine. The areas under the plasma

concentration-time curve ($AUC_{0-\infty}$) and the peak concentration (C_{max}) of nifedipine were significantly (1.0 mg/kg, $P < 0.05$) increased in the presence of lovastatin or pravastatin, repectively. The total body clearance (CL/F) was significantly decreased (1.0 mg/kg, $P < 0.05$) by lovastatin and pravastatin, respectively. Consequently, the absolute bioavailability (AB) values of nifedipine in the presence of lovastatin or pravastatin (1.0 mg/kg) were significantly higher than that of the control group, respectively. However, pharmacokinetics parameters of intravenous nifedipine were not affected by lovastatin or pravastatin in rats. The metabolite-to-parent AUC ratio (MR) in the presence of lovastatin (1.0 mg/kg) significantly decreased compared to the control group. This result implied that lovastatin effectively inhibited the metabolism of nifedipine.

The increase bioavailability of nifedipine in the presence of lovastatin might be due to an inhibition of the P-gp-mediated efflux transportor in the small intestine and CYP3A-mediated metabolism in the small intestine and/or in the liver or to a reduction of total body clearance rather than to a reduction of renal elimination of nifedipine by lovastatin. In addition, the increased bioavailability of nifedipine in the presence of pravastatin might be due to an inhibition of the CYP 3A4-mediated metabolism in the small intestine and/or in the liver or to a reduction of total body clearance rather than both inhibition of P-gp and renal elimination by pravastatin.

Key words: Nifedipine, Dehydronifedipine, Lovastatin, Pravastatin, CYP3A4, P-gp, Pharmacokinetics, Bioavailability, Rats

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