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Effects of HMG-CoA reductase inhibitors on the pharmacokinetics of nifedipine in rats: Possible role of P-gp and CYP3A4 inhibition by HMG-CoA reductase inhibitors

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ABSTRACT

Background: This study aimed to investigate the effects of HMG-CoA reductase inhibitors on the pharmacokinetics of nifedipine in rats.
Methods: We determined the pharmacokinetic parameters of nifedipine and dehydronifedipine in rats after oral and intravenous administration of nifedipine without and with HMG-CoA reductase inhibitors.

We evaluated the effect of HMG-CoA reductase inhibitors on the activity of P-glycoprotein (P-gp) and cytochrome P450 (CYP)3A4.
Results: Atorvastatin, fluvastatin, pravastatin and simvastatin inhibited CYP3A4 activities; inhibitory concentration (IC_{50}) values were 47.0, 5.2, 15.0 and 3.3 μM, respectively. Simvastatin and fluvastatin increased the cellular uptake of rhodamine-123.

The area under the plasma concentration–time curve ($AUC_{0-\infty}$) and the peak plasma concentration (C_{max}) of oral nifedipine were significantly increased by fluvastatin and simvastatin, respectively, compared to control group. The total body clearance (CL/F) of nifedipine after oral administration with fluvastatin and simvastatin were significantly decreased compared to those of control. The metabolite–parent AUC ratio (MR) of nifedipine with fluvastatin and simvastatin were significantly decreased, which suggested that fluvastatin and simvastatin inhibited metabolism of nifedipine, respectively. The $AUC_{0-\infty}$ of intravenouse nifedipine with fluvastatin and simvastatin was significantly higher than that of the control group.
Conclusion: The increased bioavailability of nifedipine may be mainly due to inhibition of both P-gp in the small intestine and CYP3A subfamily-mediated metabolism of nifedipine in the small intestine and/or in the liver and to the reduction of the CL/F of nifedipine by fluvastatin and simvastatin.

Introduction

HMG-CoA reductase inhibitor such as atorvastatin, fluvastatin, pravastatin and simvastatin inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which leads to a decrease in circulating total cholesterol and low density lipoprotein cholesterol (LDL-C) concentrations [1]. They are widely used to treat hypercholesterolemia by lowering plasma low density lipoprotein (LDL) levels and also have antioxidant, anti-inflammatory and antithrombotic properties [1]. Hypercholesterolemia is one of the risk factors in patients with cardiovascular diseases such as hypertension and ischemic heart disease. Recent studies indicate the beneficial effects of statins on regulating blood pressure and improving the prognosis of patients with hypertension and dyslipidemia [1,2].

The oxidative biotransformations of simvastatin [3], fluvastatin [4] and pravastatin [5] is primarily mediated by cytochrome P450 (CYP) 3A4. Atorvastatin has been identified as a substrate of CYP3A4, which could be a controlling factor for the low systemic availability of atorvastatin [6]. Moreover, it was reported that statins are inhibitors of P-glycoprotein (P-gp) in a rodent system [7]. However, the effects of atorvastatin, fluvastatin, pravastatin and simvastatin on the inhibition of activities of CYP3A4 and P-gp are somewhat ambiguous. Thus, we evaluated P-gp activity using

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the rhodamine-123 retention assay in P-gp-overexpressing MCF-7/ADR cells and assessed the CYP3A4 activity.

Nifedipine (dimethyl 2,6-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 [8]. Nifedipine inhibits the influx of extracellular calcium through myocardial and vascular membrane pores by physically blocking the channel which results 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 [9,10]. Nifedipine is predominantly metabolized by CYP3A4 to its primary pyridine metabolite, dehydronifedipine [11,12]. CYP enzymes are responsible for the oxidative metabolism of many xenobiotics and play a major role in the phase I metabolism of many drugs [13]. CYP3A4 is the most abundant CYP enzyme (30–40%) in the adult liver and metabolizes more than 50% of the clinically used drugs including nifedipine, cyclosporine, midazolam, and erythromycin [14,15]. Some studies indicate that nifedipine is a substrate of CYP3A4 [16–18]. P-gp is an adenosine-5-triphosphate (ATP)-dependent efflux drug transporter that is constitutively expressed in normal tissues including the gastrointestinal epithelium, canalicular membrane of the liver and kidney [19,20] and capillary endothelial cells in the central nervous system [21,22]. Because of its tissue localization and its broad substrate specificity, P-gp appears to play a key role in the absorption, distribution, and elimination of many drugs [23,24]. The substrates and/or inhibitors of CYP3A4 and P-gp overlap with each other [25]. Dorababu et al. [26] reported that nifedipine belongs to a group of P-gp substrates. Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically to promote presystemic drug metabolism which may result in the limited absorption of drugs.

Some studies have reported the effects of calcium channel antagonists on the pharmacokinetics of HMG-CoA reductase inhibitors. Calcium-channel blockers increase the plasma concentrations of some statins, possibly through the inhibition of CYP 3A4 and P-gp [27,28]. However, few studies have reported about the effects of HMG-CoA reductase inhibitors on the bioavailability or pharmacokinetics of antihypertensive agents [29–32]. Although a combination of nifedipine and statins have been clinically prescribed for treatment of hypertension, the pharmacokinetic interaction between a HMG-CoA reductase inhibitor and nifedipine *in vivo* has not been reported thus far. Therefore, in this study we aimed to investigate the effect of HMG-CoA reductase inhibitors (atorvastatin, fluvastatin, pravastatin and simvastatin) on the activities of CYP3A4 and P-gp and the bioavailability and pharmacokinetics of nifedipine and its active metabolite, dehydronifedipine, after oral and intravenous administration of nifedipine with HMG-CoA reductase inhibitors in rats.

Materials and methods

Materials

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

Apparatus used in this study included an HPLC system 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 they 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 before 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 ten groups ($n = 6$, each): oral groups [10 mg/kg of nifedipine dissolved in distilled water (1.0 mL/kg)] without (control) or with 0.8 mg/kg of atorvastatin, fluvastatin, pravastatin and simvastatin (mixed in distilled water; total oral volume of 1.0 mL/kg), and intravenous groups (2.5 mg/kg of nifedipine in 0.9% NaCl-injectable solution; total injection volume of 1.0 mL/kg) without (control) or with oral 0.8 mg/kg of atorvastatin, fluvastatin, pravastatin and simvastatin (dissolved in distilled water, total oral volume of 1.0 mL/kg). A feeding tube was used to administer nifedipine and HMG-CoA reductase inhibitors intragastrically. A blood sample (0.3-mL aliquot) was collected from the femoral artery into heparinized tubes 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 to replace blood loss caused by blood sampling. The blood samples were centrifuged (13,000 rpm for 3 min), and a 150-μL aliquot of each plasma sample was stored in the deep freezer at −40 °C until the HPLC analysis.

HPLC assay

The plasma concentrations of nifedipine were determined using an HPLC assay with a modification to the method reported by Grundy et al. [33]. Briefly, 50 μL of amlodipine (3 μg/mL), as the internal standard and 50 μL of 1.0 M sodium hydroxide were added to 0.15 mL of the plasma sample. The sample was then mixed for 3 s and 1 mL of MTBE:isooctane (75:25, v/v) was added. The resultant mixture was vortex-mixed for 1 min and centrifuged at 3000 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 the mobile phase vortex-mixed for 1 min and 160-μL aliquots were transferred to a clean autosampler vial. A 70-μL aliquot of each supernatant was injected into the HPLC system. The UV detector wavelength was set to 350 nm; and the column, a