永井　将弘 野元　正弘	パーキンソン病	臨床薬理	36（6）	273-276	2005
野元　正弘 中塚　晶子 永井　将弘 矢部　勇人 森豊　隆志 森豊浩代子 西川　典子	パーキンソン病治療の個人差と薬物動態	臨床神経学	45-11	895-898	2005
中塚　晶子 野元　正弘	健康食品による健康被害	日本医事新報	4225	23-25	2005
永井　将弘 野元　正弘	腎の薬物トランスポーターと薬物代謝	日本医事新報	4251	18-20	2005
永井　将弘 重松　裕二 野元　正弘	パーキンソン病におけるペルゴリド治療と拘束性心臓弁膜症	Parkinson's Disease Today	7	12-13	2005
Naito T. Uchida S. Shinno K. Miyamoto Y. Takamaya T. Suzuki K. Ozono S. Nakano M. Ohashi K. Hashimoto H.	Relationship between plasma mycophenolic acid and its glucuronide concentrations, and adverse effects in renal transplant patients	Journal of Pharmacological Sciences	97(suppl 1)	256	2005

Naito T. Shinno K. Maeda T. Kagawa Y. Hashimoto H. Otsuka A. Takayama T. Ushimyama T. Suzuki K. Ozomo S.	Effects of calcineurin inhibitors on pharmacokinetics of mycophenolic acid and its glucuronide metabolite during the maintenacne period following renal transplantation	Biol. Pharm. Bull.	29(2)	275-280	2006
内藤　隆文 新野　和子 前田　利夫 賀川　義之 橋本　久邦 大塚　篤志 牛山　知己 鈴木　和雄 大園誠一郎	腎移植患者におけるミコフェノール酸およびそのグルクロン酸抱合体の体内動態に及ぼす血液透析の影響	臨床薬理	36 (Suppl)	S168	2005
山本　知広 内田　信也 鈴木　吉成 寺田　肇 渡邉　裕司 林　秀晴 大橋　京一 橋本　久邦	ピルジカイニドの血漿中濃度と臨床検査値および心電図変化	第125年会日本薬学会要旨	2	181	2005
Ding Qunfang Hayashi T. Packiasamy AR J. Miyazaki A. Fukatsu A. Shiraishi H. Nomura T. Iguchi A.	The effect of high glucose on NO and Superoxide anion through endothelial GTPCHI and NADPH oxidase	Life Sciences	75	3185-3194	2004

Hayashi T. Rani P JA. Fukatsu A. Matsui-Hirai H. Osawa M. Miyazaki A. Tsunekawa T. Kano-Hayasi H. Iguchi A. Sumi D. Ignarro LJ.	A new HMG-CoA reductase inhibitor, pitavastatin remarkably retards the progression of high cholesterol induced atherosclerosis in rabbits	Atherosclerosis	176	255-263	2004
Hayashi T. Iguchi A. Ignarro LJ.	Gene transfer of endothelial NO synthase(eNOS), but not eNOS plus inducible NOS regressed atherosclerosis in rabbits	Cardiovascular Research	61	339-351	2004
Hayashi T. Juliet PA. Kano-Hayashi H. Tsunekawa T. Dingqunfang D. Sumi D. Matsui-Hirai H. Fukatsu A. Iguchi A.	NADPH oxidase inhibitor, apocynin, restores the impaired endothelial-dependent and -independent responses and scavenges superoxide anion in rats with type 2 diabetes complicated by NO dysfunction	Diabetes Obes Metab	7	334-43	2005

Hayashi T. Nomura H. Esaki T. Hattori A. Kano-Hayashi H. Iguchi A.	The treadmill exercise-tolerance test is useful for the prediction and prevention of ischemic coronary events in elderly diabetics	J Diabetes Complications	19	264-268	2005

Ⅳ. 研究成果の刊行物・別刷

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Pharmacokinetic and pharmacodynamic interactions between simvastatin and diltiazem in patients with hypercholesterolemia and hypertension

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Abstract

Pharmacokinetic and pharmacodynamic interactions between simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, and diltiazem, a calcium antagonist, were investigated in 7 male and 4 female patients with hypercholesterolemia and hypertension. The patients were given, for one in a three consecutive 4-week periods, oral simvastatin (5 mg/day), oral simvastatin (5 mg/day) combined with diltiazem (90 mg/day), and then oral diltiazem (90 mg/day), respectively. The area under the plasma concentration versus time curve up to 6 hours post-dose (AUC_{0-6h}) and maximum plasma concentrations (Cmax) of the drugs, serum lipid profiles, blood pressures and liver functions were assessed on the last day of each of the three 4-week periods. After the combined treatment period, Cmax of HMG-CoA reductase inhibitor was elevated from 7.8 ± 2.6 ng/ml to 15.4 ± 7.9 ng/ml ($P < 0.01$) and AUC_{0-6h} from 21.7 ± 4.9 ng·hr/ml to 43.3 ± 23.4 ng·hr/ml ($P < 0.01$), while Cmax of diltiazem was decreased from 74.2 ± 36.4 ng/ml to 58.6 ± 18.9 ng/ml ($P < 0.05$) and its AUC_{0-6h} from 365 ± 153 ng·hr/ml to 287 ± 113 ng·hr/ml ($P < 0.01$). Compared to simvastatin monotherapy, combined treatment further reduced LDL-cholesterol levels by 9%, from 129 ± 16 mg/dl to 119 ± 17 mg/dl ($P < 0.05$). No adverse events were observed throughout the study. These apparent pharmacokinetic interactions, namely the increase of HMG-CoA reductase inhibitor concentration by diltiazem

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and the decrease of diltiazem concentration by simvastatin, enhance the cholesterol-lowering effects of simvastatin during combined treatment.
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Keywords: HMG-CoA reductase inhibitor; Simvastatin; Diltiazem; Pharmacokinetic interaction; Pharmacodynamic interaction

Introduction

Control of hypercholesterolemia is of prime importance for the primary and secondary prevention of coronary artery disease (CAD) (Gould et al., 1995; Tonkin, 1995; Shepherd, 1998). Currently, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors are the first-line therapy for patients with elevated serum low-density lipoprotein (LDL)-cholesterol (Gotto, 1998; Wood, 2001). Among the HMG Co-A reductase inhibitors, simvastatin is widely used and has been shown to reduce morbidity and mortality from CAD (The Scandinavian Simvastatin Survival Study, 1994). Simvastatin is an inactive lactone pro-drug that is hydrolysed by esterases to simvastatin acid, the active competitive inhibitor of HMG-CoA reductase (Vickers et al., 1990, 1990; Prueksaritanont et al., 1997). Since HMG-CoA reductase is responsible for the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in the hepatic cholesterol biosynthesis, the inhibition of HMG-CoA reductase lowers serum cholesterol levels (Goldstein and Brown, 1990). Although cytochrome P450 (CYP) is not involved in the conversion of simvastatin to simvastatin acid, the oxidative metabolism of simvastatin to the metabolites, 3′,5′-dihydrodiol, 3′-hydroxy and 6′-exomethylene, is mainly mediated by CYP3A4 (Vickers et al., 1990, 1990; Prueksaritanont et al., 1997). In a crossover study in healthy volunteers (Neuvonen et al., 1998), the areas under the plasma concentration versus time curves (AUCs) of simvastatin and simvastatin acid after a single oral dose of simvastatin were increased 10-fold and 19-fold, respectively, following 4 days of treatment with 200 mg/day itraconazole, an agent that has been shown to increase the plasma concentrations and half-lives of many drugs metabolized by CYP3A4 by inhibiting the enzyme (Kivistö et al., 1997; Wang et al., 1999).

Hypercholesterolemia is often accompanied by hypertension, an associated risk factor for CAD (Gould et al., 1995; Gotto, 1998; Wood, 2001). The calcium antagonist diltiazem is effective for the management of hypertension, supraventricular arrhythmias and angina pectoris (Chaffman and Brogden, 1985; Hansson et al., 2000; Nakagawa and Ishizaki, 2000), and is often prescribed in association with lipid-lowering agents like simvastatin (The Scandinavian Simvastatin Survival Study, 1994; Gotto, 1998; Wood, 2001). Diltiazem is extensively metabolized in the liver, primarily by deacetylation and demethylation by CYP3A4 into a host metabolite, N-desmethyl-diltiazem, which, together with diltiazem, in turn selectively inhibits CYP3A4, but not CYP1A2, CYP2C9, or CYP2E1 (Sutton et al., 1997; Jones et al., 1999). Accordingly, pharmacokinetic and pharmacodynamic interactions may theoretically happen upon co-administration of diltiazem and a drug metabolized by CYP3A4 like simvastatin.

Indeed, combined treatment of diltiazem and simvastatin has been shown to cause a 5-fold increase in the AUC of simvastatin (Mousa et al., 2000). Lovastatin, which is pharmacokinetically similar to simvastatin, also interacts with diltiazem (Azie et al., 1998). A recent retrospective analysis shows that patients who had taken both simvastatin and diltiazem needed lower doses of simvastatin to achieve

the recommended reduction in serum cholesterol (Yeo et al., 1999), suggesting a pharmacokinetically-driven pharmacodynamic interaction between the two drugs. However, steady state bi-directional pharmacokinetic and pharmacodinamic interactions between simvastatin and diltiazem has not been prospectively evaluated. In this study we prospectively studied the pharmacokinetic and pharmacodynamic interactions between simvastatin and diltiazem in patients with hypercholesterolemia and hypertension.

Methods

Subjects

Enrolled were 7 male and 4 female patients (age: 62.0 ± 7.5 years; body weight: 62.6 ± 5.4 kg, mean ± S.D.) with hypercholesterolemia and hypertension who had taken simvastatin (5 mg/day) and the angiotensin-converting enzyme inhibitor enalapril (5 mg/day) for more than 3 months and had reached the plateau control (Table 1). Inclusion criteria were: age of at least 18 years, basal total cholesterol or LDL-cholesterol levels greater than 220 mg/dl or 140 mg/dl, respectively, and systolic blood pressure (BP) or diastolic BP levels greater than 140 mmHg or 90 mmHg, respectively, without medication. Before the start of any lipid-lowering and antihypertensive therapy, basal total cholesterol levels were 249 ± 28 mg/dl; LDL-cholesterol, 166 ± 23 mg/dl; systolic BP, 151 ± 29 mm Hg; and diastolic BP, 88 ± 11 mm Hg. The subjects had no history of hepatic or renal disease. At the end of the pre-trial phase with simvastatin (5 mg/day) and enalapril (5 mg/day) for more than 3 months, the average total cholesterol level was 207 ± 23 mg/dl; LDL-cholesterol, 129 ± 15 mg/dl; systolic BP, 142 ± 22 mm Hg; and diastolic BP, 84 ± 12 mm Hg.

Table 1
Patient demographics and basic medical data (mean ± S.D.)

Age (y)	62.0 ± 7.5
Sex (M/F)	7/4
Body weight (kg)	62.6 ± 5.4
Serum creatinine (mg/dl)	0.72 ± 0.19
AST (IU/l)	21.4 ± 3.8
ALT (IU/l)	20.0 ± 9.3
Creatine kinase (IU/l)	109 ± 48
Total cholesterol (mg/dl)	249 ± 28
LDL-cholesterol (mg/dl)	166 ± 23
HDL-cholesterol (mg/dl)	50 ± 10
Triglyceride (mg/dl)	168 ± 82
Systolic BP (mmHg)	151 ± 29
Diastolic BP (mmHg)	88 ± 11
Heart rate (beats/min)	72 ± 10

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure.

Study design

This was a three-phase fixed-order design study: (1) administration of oral simvastatin (5 mg/day) for 4 weeks, (2) co-administration of oral diltiazem (30 mg three times a day) and simvastatin (5 mg/day) for 4 weeks, and (3) administration of oral diltiazem (90 mg/day) alone for another 4 weeks. The AUC up to 6 hours post-dose (AUC_{0-6h}) and Cmax of the drugs, serum lipid profiles and liver function were evaluated, as specified below. No drug other than simvastatin and/or diltiazem was taken during the study period. Patients who developed symptoms due to withdrawal of lipid-lowering medication or whose systolic BP or diastolic BP respectively exceeded 180 mmHg or 110 mmHg following discontinuation of antihypertensive therapy were withdrawn from the study and appropriate therapy re-established. The study protocol, consent forms, and volunteer information documents were approved by Hamamatsu University School of Medicine Independent Review Board. All subjects provided written informed consent before participating in the trial.

Blood sampling

Blood samples were obtained on the last day of each of the three 4-week periods. After an overnight fast, a pre-dosing venous blood sample was taken, and then simvastatin (5 mg) and/or diltiazem (30 mg) was/were given. All patients drank a glass of water after swallowing the tablets. Blood samples were then taken 2, 3, 4 and 6 hours later. Standardized breakfast and lunch were served 2 and 4 hours after drug intake. Plasma was separated within 30 minutes and stored at −70 °C until analysis.

Blood pressure measurement

On the last day of each trial periods, systolic BP and diastolic BP were measured twice each using an automatic electronic sphygmomanometer (BP-103i II, Nippon Colin, Komaki, Japan) at the sitting position before and 2, 3, 4 and 6 hours after the administration of the drug(s).

Determination of diltiazem concentration

Diltiazem concentrations were measured by an HPLC assay with an ultraviolet detection, as described by Abernethy et al. (1985). Diltiazem was resolved from the internal standard desipramine with a mobile phase of 0.06 mol/l acetate buffer/acetonitrile/methanol (58:37:5) that contained 5 mmol/l heptane sulfonic acid and glacial acetic acid to adjust pH to 6.4. A reversed-phase C_{18}μBondapak column (30 cm×3.9 mm, Waters Chromatography, Milford, MA) was eluted at 1.8 ml/min and detection was performed by ultraviolet absorbance at 254 nm. The calibration range was 5–300 ng/ml. The intra-day and inter-day coefficients of variation were less than 9%.

Determination of simvastatin HMG-CoA reductase inhibitor concentrations

HMG-CoA reductase inhibitor concentrations were determined as previously described (Arnadottir et al., 1993). An equal volume of methanol was added to the plasma samples and the mixtures were vortexed thoroughly, kept on ice for 10 minutes and centrifuged. Fifty microliters of the supernatants were dried in an evaporator (SpeedVac, Savant Instr. Farmingdale, NY). The reaction mixture (96 μl) was added

directly to the dried residues to make a final volume of 100 μl containing 0.1 M KPO_4 (pH 7.4), 10 mM 1, 4-dithiothreitol (DTT), 0.2 mM $NADH^+$ (made fresh daily), 5 mM glucose-6-phosphate, 1.4 U/ml glucose-6-phosphate dehydrogenase and 1 mg/ml bovine serum albumin. The reaction mixture was incubated for 5 minutes at 37 °C and soluble rat liver HMG-CoA reductase was added to 2 μl buffer A: 0.04 M KPO_4 (pH 7.4), 0.05 M KCl, 0.1 M sucrose, 0.03 M ethylenediaminetetraacetic acid (EDTA) and 0.01 M DTT (added immediately before use). The mixture was incubated at 37 °C for 5 minutes in the presence of the inhibitor-containing plasma sample. The reaction was then started with 2 μl of 1.25 mg/ml HMG-CoA containing 17.5 μCi/ml glutaryl-3-[^{14}C]-HMG-CoA. After an additional 6-minute incubation at 37 °C, 20 μl of 5 N HCl was added to lactonize the mevalonic acid formed. After 15 minutes, 3.5 ml of a 1:1 suspension of BioRad AG 1 × 8 resin (200–400 mesh) was added and the tubes (13 × 100) were thoroughly vortexed. [^{14}C]-mevalonolactone was filtered from the resin suspension through polystyrene filters (pore size 70 μm, EverGreen, Los Angeles, CA) into scintillation vials containing 15 ml of Aquasol-2 (New England Nuclear, Newton, MA) and counted on a scintillation counter. Percent inhibition was converted to the inhibitor concentration using a standard curve constructed by extracting from the control plasma containing known amounts of L-654, 969, the free acid form of simvastatin. The results were expressed as nanograms of inhibitor per milliliter of plasma. The intra-day and inter-day coefficients of variation for the HMG-CoA reductase activity assay were less than 6%.

Statistical analysis

Data were analyzed by 2-way ANOVA, a paired Student's *t* test, or Wilcoxon signed-rank test where appropriate. Differences with P values < 0.05 were considered statistically significant. All values are given as means ± S.D.

Results

Pharmacokinetic interactions between simvastatin and diltiazem

HMG-CoA reductase inhibitor concentrations after simvastatin administration with or without diltiazem are shown in Fig. 1A. HMG-CoA reductase inhibitor values for Cmax, time to Cmax (Tmax) and AUC_{0-6h} after simvastatin administration without diltiazem were 7.8 ± 2.6 ng/ml, 2.3 ± 0.5 h and 21.7 ± 4.9 ng·h/ml, respectively. Co-administration of diltiazem with simvastatin increased Cmax and AUC_{0-6h} of HMG-CoA reductase inhibitor concentrations to 15.4 ± 7.9 ng/ml ($P < 0.01$) and 43.3 ± 23.4 ng·h/ml ($P < 0.01$), respectively (Fig. 1B), but did not affect Tmax of HMG-CoA reductase inhibitor (2.3 ± 0.5 h). There was a considerable inter-individual variability in the effect of diltiazem on the levels of HMG-CoA reductase inhibitor (Fig. 1B): the AUC_{0-6h} of HMG-CoA reductase inhibitor concentration was increased by 422% in a patient and 7% in another.

Diltiazem concentrations after diltiazem administration with and without simvastatin are shown in Fig. 2A. After the last oral intake of diltiazem without simvastatin, Cmax, Tmax and AUC_{0-6h} of diltiazem were 74.2 ± 36.4 ng/ml, 3.4 ± 1.2 h and 365 ± 153 ng·h/ml, respectively. In contrast to the effects of the combined treatment on the pharmacokinetics of HMG-CoA reductase inhibitor concentrations, co-administration of simvastatin with diltiazem decreased Cmax and AUC_{0-6h} of diltiazem to 58.6 ± 18.9 ng/ml ($P < 0.05$) and 287 ± 113 ng·h/ml ($P < 0.01$), respectively, while the

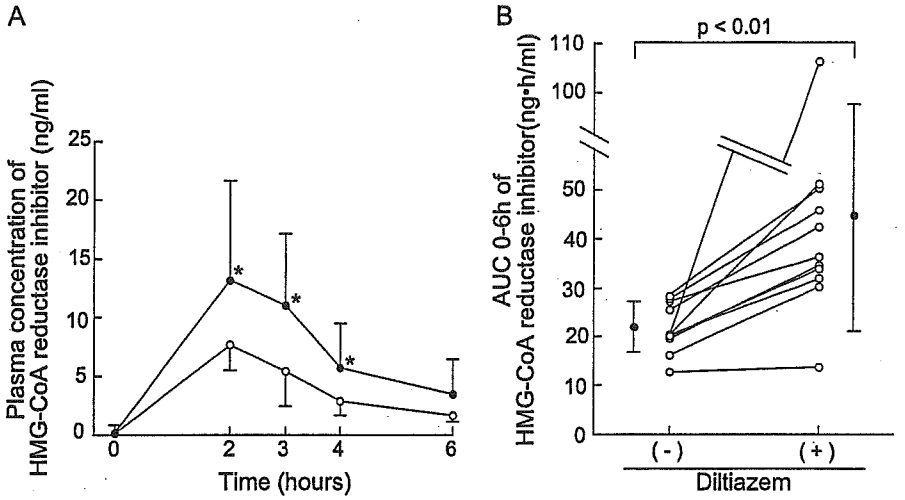

Fig. 1. Effect of diltiazem on plasma concentration and AUC_{0-6h} of HMG-CoA reductase inhibitor. (A) Plasma concentrations of HMG-CoA reductase inhibitor observed on the last day of 4 weeks of treatment with simvastatin (5mg/day) (open circles) or combined treatment with simvastatin (5mg/day) and diltiazem (90mg/day) (closed circles). Error bars represent S.D. *Significant difference from simvastatin monotherapy ($P < 0.05$). (B) Individual AUC_{0-6h} values for HMG-CoA reductase inhibitor (open circles) with (right) and without diltiazem (left) in the 11 patients. Closed circles with the bars indicate means ± S.D.

Tmax of diltiazem was not affected (3.1 ± 0.9 h) by simvastatin. Plasma diltiazem AUC_{0-6h} values were decreased by simvastatin in 9 of the 11 patients (Fig. 2B).

Pharmacodynamic interactions between simvastatin and diltiazem

Following 4 weeks of simvastatin monotherapy, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride levels were 206 ± 26 mg/dl, 129 ± 16 mg/dl, 50 ± 10 mg/dl, and 135 ± 73 mg/dl,

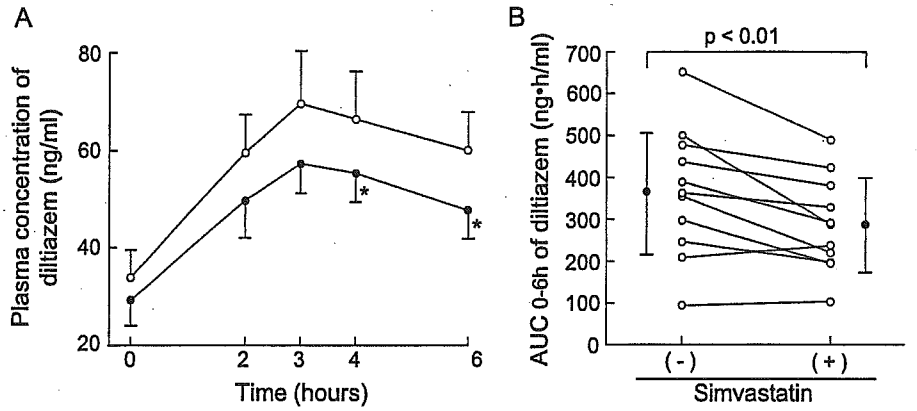

Fig. 2. Effect of simvastatin on plasma concentration and AUC_{0-6h} of diltiazem. (A) Plasma concentrations of diltiazem observed on the last day of 4 weeks of treatment with diltiazem (open circles) or combined treatment with simvastatin and diltiazem (closed circles). *Significant difference from diltiazem monotherapy ($P < 0.05$). (B) Individual AUC_{0-6h} values of diltiazem (open circles) with (right) and without simvastatin (left). Closed circles with the bars indicate means ± S.D.

respectively (Fig. 3A). These values were not different with those at the end of pretrial phase with simvastatin (5 mg/day) and enalapril (5 mg/day) (total cholesterol, 207 ± 23 mg/dl; LDL-cholesterol, 129 ± 15 mg/dl; HDL-cholesterol, 50 ± 10 mg/dl; triglyceride, 137 ± 68 mg/dl), suggesting that the treatment with simvastatin reached the plateau control during the pretrial phase. Co-administration of diltiazem and simvastatin further reduced the mean total and LDL-cholesterol levels to 196 ± 32 mg/dl ($P < 0.05$) (Fig. 3B) and 119 ± 17 mg/dl ($P < 0.05$), respectively, but did not influence HDL-cholesterol and triglyceride levels, which were 49 ± 11 mg/dl and 140 ± 72 mg/dl, respectively. On the other hand, after simvastatin was withdrawn during the last 4 weeks of diltiazem monotherapy, total cholesterol and LDL-cholesterol levels increased to 245 ± 33 mg/dl and 163 ± 21 mg/dl ($P < 0.01$), respectively, while HDL-cholesterol and triglyceride levels were not affected (51 ± 12 mg/dl and 157 ± 77 mg/dl, respectively).

After 4 weeks of simvastatin monotherapy, baseline systolic and diastolic BP increased from 142 ± 22 mm Hg to 152 ± 28 mm Hg ($P < 0.05$) and from 84 ± 12 mm Hg to 89 ± 10 mm Hg ($P < 0.05$), respectively, compared to baseline BP during the pre-trial phase with simvastatin and enalapril. Simvastatin did not exert any BP-lowering effect. Diltiazem decreased systolic BP from 146 ± 26 mm Hg to 124 ± 9 mm Hg and diastolic BP from 84 ± 11 mm Hg to 75 ± 6 mm Hg at 2 hours post-dose. This effect was not influenced by the combined treatment with simvastatin (baseline systolic BP, 138 ± 18 mm Hg; baseline diastolic BP, 83 ± 13 mm Hg; systolic BP at 2 hours post-dose, 129 ± 19; diastolic BP at 2 hours post-dose, 76 ± 12 mm Hg) (Fig. 4).

Serum aspartate aminotransferase (AST; normal range, 11–30 IU/l), alanine aminotransferase (ALT; normal range, 5–42 IU/l), lactate dehydrogenase (LDH; normal range, 115–208 IU/l) and creatine kinase (CK; normal range, 55–204 IU/l) levels appeared to increase, albeit without statistical significance, during the combined therapy period compared with those observed during the simvastatin monotherapy

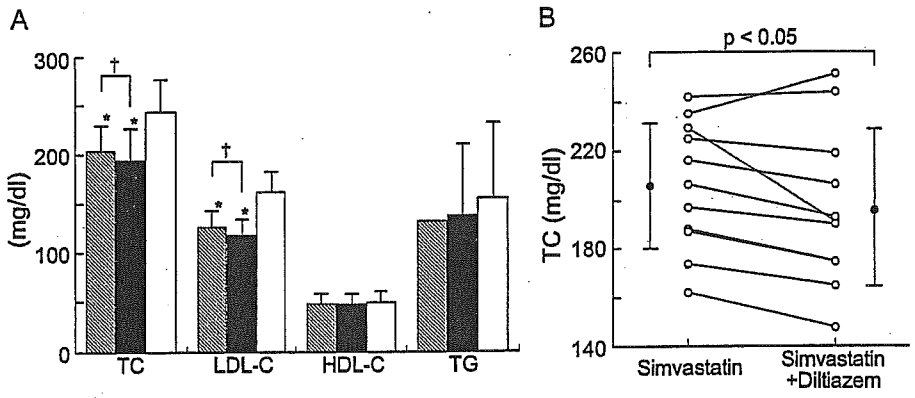

Fig. 3. Lipid profiles during simvastatin monotherapy, combined therapy with diltiazem and simvastatin, and diltiazem monotherapy. (A) Lipid profiles after 4 weeks of simvastatin monotherapy (5mg/day, hatched columns), combined treatment with simvastatin (5mg/day) and diltiazem (90mg/day) (closed columns) or diltiazem monotherapy (90mg/day, open columns). TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol and TG, triglyceride. * Significant difference from diltiazem monotherapy ($P < 0.05$). †Significant difference between simvastatin monotherapy and combined treatment with simvastatin and diltiazem ($P < 0.05$). (B) Total cholesterol levels in the 11 patients observed after 4 weeks of treatment with simvastatin (90mg/day) (left) or combined treatment with simvastatin (5mg/day) and diltiazem (90mg/day) (right). Closed circles with the bars indicate means ± S.D.

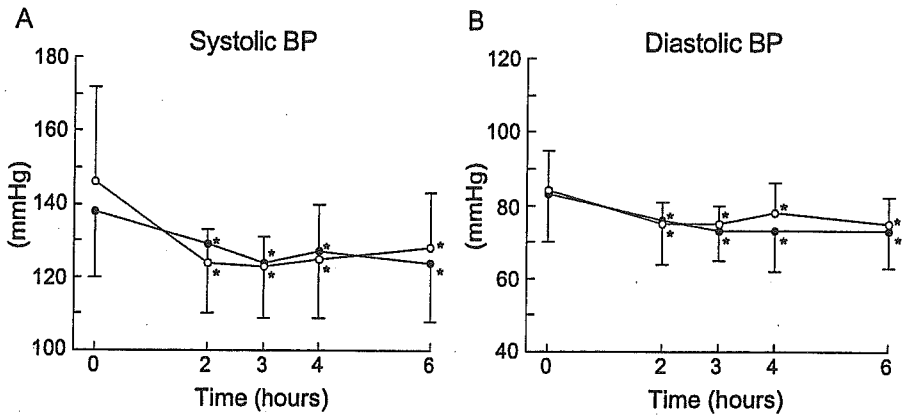

Fig. 4. Blood pressures during combined therapy with diltiazem and simvastatin, and diltiazem monotherapy. Systolic (A) and diastolic (B) BP before and 2, 3, 4 and 6 hours after an oral 30 mg dose of diltiazem with (closed circles) or without (open circles) simvastatin following 4 weeks of treatment with diltiazem alone (90mg/day) (open circles) or combined treatment with simvastatin (5mg/day) and diltiazem (90mg/day) (closed circles). * Significant difference from BP at 0 h ($P < 0.05$). Data are expressed as means ± S.D.

period: AST, 23.4 ± 4.3 IU/l vs. 21.3 ± 5.1 IU/l, ALT, 22.1 ± 5.6 IU/l vs. 18.9 ± 5.6 IU/l, LDH, 196 ± 42 IU/l vs. 187 ± 32 IU/l, and CK 142 ± 111 IU/l vs. 107 ± 45 IU/l, respectively.

Discussion

Simvastatin and diltiazem are often prescribed together for the treatment of hypercholesterolemia in patients with hypertension and/or angina pectoris (Gould et al., 1995; Gotto, 1998; Wood, 2001). In the Scandinavian Simvastatin Survival Study (4S) (1994), which demonstrated a reduction in nonfatal myocardial infarction, cardiovascular death, and total mortality by simvastatin treatment in patients with angina pectoris or previous myocardial infarction, more than 30% of the study population were treated with calcium antagonists including diltiazem. The efficacy and safety profiles of simvastatin and diltiazem are widely accepted (Chaffman and Brogden, 1985; The Scandinavian Simvastatin Survival Study, 1994; Hansson et al., 2000). The effect of diltiazem on the pharmacokinetics of simvastatin has been previously described, such that the Cmax and AUC of simvastatin after a single 20 mg oral dose of simvastatin increased by 3.6-fold and 5-fold, respectively, after 2 weeks of treatment with 120 mg diltiazem twice a day (Mousa et al., 2000). However, bi-directional pharmacokinetic interactions and the potential pharmacodynamic impact have not been prospectively studied.

Our prospective study demonstrates that long-term and low-dose co-administration of diltiazem and simvastatin results in two-fold increase of Cmax and AUC of HMG-CoA reductase inhibitor, which is accompanied by enhanced cholesterol-lowering effect of simvastatin in patients with hypercholesterolemia and hypertension. Interestingly, in contrast to the effect on the pharmacokinetics of simvastatin, the co-administration of simvastatin with diltiazem decreased the Cmax and AUC of diltiazem without affecting its BP-lowering effects.

These results are consistent with a retrospective study demonstrating that simvastatin caused a 33.3% cholesterol reduction in patients using diltiazem compared with 24.7% in those not using diltiazem (Yeo

et al., 1999). It has also been reported that doubling the dose of simvastatin further reduces serum cholesterol by an average of 5% (Roberts, 1997). This is compatible with our finding that a two-fold increase in the Cmax and AUC of HMG-CoA reductase inhibitor by co-administration of diltiazem with simvastatin was accompanied by a further 5% reduction in total cholesterol level. The results of our study suggest that patients who require both simvastatin and diltiazem may need a lower dose of simvastatin than when simvastatin is prescribed alone to achieve the desired reduction in total and LDL-cholesterol levels.

The mechanism underlying the decrease in the AUC of diltiazem by the combined therapy with simvastatin remains unknown. Diltiazem is extensively metabolized in the liver into its host metabolites, primarily by deacetylation and demethylation by CYP3A4 in vitro and in vivo (Chaffman and Brogden, 1985; Pichard et al., 1990; Sutton et al., 1997; Jones et al., 1999; Nakagawa and Ishizaki, 2000; Yeo and Yeo, 2001; Kosuge et al., 2001), and probably in part by CYP2C8/9 (Sutton et al., 1997). In addition, diltiazem has been shown to increase the metabolic ratio of debrisoquine (Sakai et al., 1991), suggesting a possible interference with CYP2D6 (Molden et al., 2002). It is possible that the relevant enzyme activity to metabolize diltiazem or its metabolite(s) might be induced by themselves. Alternatively, simvastatin and/or its metabolite(s) might enhance the activity of enzyme(s) involved in the metabolism of diltiazem after the long term coadministration. Although the Cmax and AUC of diltiazem were decreased by simvastatin, blood pressure-lowering effect of diltiazem was not influenced by simvastatin. Heart rate of the patients during combined treatment with simvastatin did not differ from that during the diltiazem monotherapy period: 70 ± 10 beats/min vs. 68 ± 7 beats/min, respectively. It is likely that the pharmacokinetic interaction such as the 21% reduction in both the Cmax and AUC of diltiazem was not sufficient to alter pharmacodynamic response. However, we cannot exclude the possibility that the power was not enough to detect the pharmacodynamic differences. Further investigation is required to clarify the pharmacodynamic impact on blood pressure and the mechanism responsible for the changes in the pharmacokinetic behavior of diltiazem by the combined treatment with simvastatin.

The combined therapy increased the AUC of HMG-CoA reductase inhibitor by as much as 422% in one patient and as little as 7 % in another, suggesting a considerable inter-individual variability in the effect of diltiazem on the levels of HMG-CoA reductase inhibitor (Fig. 1B). However, this pharmacokinetic variation did not account for the differences in the pharmacodynamic responses to simvastatin (correlation coefficient: $r = 0.106$, not significant) (Fig. 5A). On the other hand, there was a significant correlation between the AUC of diltiazem and the AUC of HMG-CoA reductase inhibitor ($r = 0.73$, $P < 0.05$) (Fig. 5B). For example, one patient showing the lowest value of the AUC of diltiazem showed the lowest value for the AUC of HMG-CoA reductase inhibitor, suggesting that this patient might be an individual with a high CYP3A4 activity. These findings taken together strongly suggest that simvastatin and diltiazem could be metabolized, at least in part, through a common or shared pathway.

Simvastatin is generally well tolerated and causes few subjective side-effects during chronic treatment, however, rhabdomyolysis is a rare side effect of this HMG-CoA reductase inhibitor that appears to be dose-related. The doses of simvastatin (5 mg/day) and diltiazem (90 mg/day) used in this study are lower than those recommended in Western countries, because these doses are common and approved in the Japanese formulary and have been shown to be sufficient to treat Japanese patients at the clinical practice (Matsuzaki et al., 2002). It is noteworthy that the pharmacokinetic and pharmacodynamic interactions take place even at the lower doses. Furthermore, the levels of AST, ALT, LDH and CK appeared to increase during the combined therapy with simvastatin and diltiazem compared to the

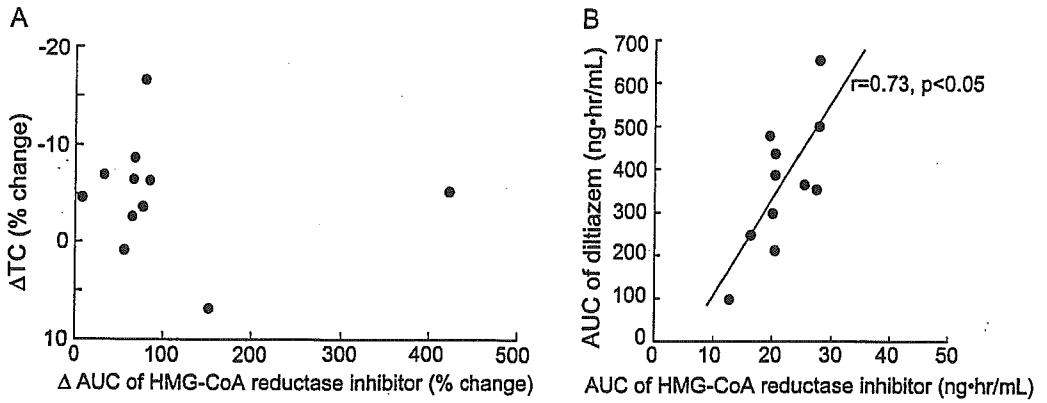

Fig. 5. (A) Percent changes in plasma concentration of HMG-CoA reductase inhibitor versus plasma total cholesterol (TC) concentration after the combined treatment with simvastatin and diltiazem in the 11 patients. Correlation coefficient was 0.106 (not significant). (B) Relationship between the AUCs of HMG-CoA reductase inhibitor and diltiazem in the 11 patients during monotherapy ($r = 0.73$, $P < 0.05$).

simvastatin mono-therapy. The findings strongly suggest that careful monitoring should be carried out for patients under combined treatment with simvastatin and diltiazem at higher doses to avoid any increase in risk of serious adverse effects.

Conclusion

This study is the first to show the bi-directional pharmacokinetic and pharmacodynamic interactions between diltiazem and simvastatin after long-term treatment with both drugs. Combined treatment with diltiazem and simvastatin increases the Cmax and AUC of HMG-CoA reductase inhibitor and further reduces total and LDL-cholesterol levels. On the other hand, the combination decreases the Cmax and AUC of diltiazem without affecting its blood pressure-lowering effect. These interactions should therefore be taken into consideration, and pharmacokinetic and pharmacodynamic monitoring may be necessary when these drugs are used concomitantly.

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References

Abernethy, D.R., Schwartz, J.B., Todd, E.L., 1985. Diltiazem and desacetyldiltiazem analysis in human plasma using high-performance liquid chromatography: Improved sensitivity without derivation. Journal of Chromatography 342, 216–220.

Arnadottir, M., Eriksson, L.O., Thysell, H., Karkas, J.D., 1993. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron 65, 410–413.

Azie, N.E., Brater, D.C., Becker, P.A., Jones, D.R., Hall, S.D., 1998. The interaction of diltiazem with lovastatin and pravastatin. Clinical Pharmacology and Therapeutics 64, 369–377.

Chaffman, M., Brogden, R.N., 1985. Diltiazem. A review of its pharmacological properties and therapeutic efficacy. Drugs 29, 387–454.

Goldstein, J.L., Brown, M.S., 1990. Regulation of the mevalonate pathway. Nature 343, 425–430.

Gotto Jr., A.M., 1998. Risk factor modification: rationale for management of dyslipidemia. American Journal of Medicine 104, 6S–8S.

Gould, K.L., Casscells, S.W., Buja, L.M., Goff, D.C., 1995. Non-invasive management of coronary artery disease. Report of a meeting at the University of Texas Medical School at Houston. Lancet 346, 750–753.

Hansson, L., Hedner, T., Lund-Johansen, P., Kjeldsen, S.E., Lindholm, L.H., Syvertsen, J.O., Lanke, J., de Faire, U., Dahlof, B., Karlberg, B.E., 2000. Randomised trial of effects of calcium antagonists compared with diuretics and beta-blockers on cardiovascular morbidity and mortality in hypertension: the Nordic Diltiazem (NORDIL) study. Lancet 356, 359–365.

Jones, D.R., Gorski, J.C., Hamman, M.A., Mayhew, B.S., Rider, S., Hall, S.D., 1999. Diltiazem inhibition of cytochrome P-450 3A activity is due to metabolite intermediate complex formation. Journal of Pharmacology and Experimental Therapeutics 290, 1116–1125.

Kivistö, K.T., Lamberg, T.S., Kantola, T., Neuvonen, P.J., 1997. Plasma buspirone concentrations are greatly increased by erythromycin and itraconazole. Clinical Pharmacology and Therapeutics 62, 348–354.

Kosuge, K., Jun, Y., Watanabe, H., Kimura, M., Nishimoto, M., Ishizaki, T., Ohashi, K., 2001. Effects of CYP3A4 inhibition by diltiazem on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. Drug Metabolism and Disposition 29, 1284–1289.

Matsuzaki, M., Kita, T., Mabuchi, H., Matsuzawa, Y., Nakaya, N., Oikawa, S., Saito, Y., Sasaki, J., Shimamoto, K., Itakura, H., 2002. Large scale cohort study of the relationship between serum cholesterol concentration and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia. Circulation Journal 66, 1087–1095.

Molden, E., Johansen, P.W., Bøe, G.H., Bergan, S., Christensen, H., Rugstad, H.E., Rootwelt, H., Reubsaet, L., Lehne, G., 2002. Pharmacokinetics of diltiazem and its metabolites in relation to CYP2D6 genotype. Clinical Pharmacology and Therapeutics 72, 333–342.

Mousa, O., Brater, D.C., Sunblad, K.J., Hall, S.D., 2000. The interaction of diltiazem with simvastatin. Clinical Pharmacology and Therapeutics 67, 267–274.

Nakagawa, K., Ishizaki, T., 2000. Therapeutic relevance of pharmacogenetic factors in cardiovascular medicine. Pharmacology and Therapeutics 86, 1–28.

Neuvonen, P.J., Kantola, T., Kivisto, K.T., 1998. Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. Clinical Pharmacology and Therapeutics 63, 332–341.

Pichard, L., Gillet, G., Fabre, I., Dalet-Beluche, I., Bonfils, C., Thenot, J.P., Maurel, P., 1990. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metabolism and Disposition 18, 711–719.

Prueksaritanont, T., Gorham, L.M., Ma, B., Liu, L., Yu, X., Zhao, J.J., Slaughter, D.E., Arison, B.H., Vyas, K.P., 1997. In vitro metabolism of simvastatin in humans: identification of metabolizing enzymes and effect of the drug on hepatic P450s. Drug Metabolism and Disposition 25, 1191–1199.

Roberts, W.C., 1997. The rule of 5 and the rule of 7 in lipidlowering by statin drugs. American Journal of Cardiology 80, 106–107.

Sakai, H., Kobayashi, S., Hamada, K., Iida, S., Akita, H., Tanaka, E., Uchida, E., Yasuhara, H., 1991. The effects of diltiazem on hepatic drug metabolizing enzymes in man using antipyrine, trimethadione and debrisoquine as model substrates. British Journal of Clinical Pharmacology 31, 353–355.

Shepherd, J., 1998. Preventing coronary artery disease in the West of Scotland: implications for primary prevention. American Journal of Cardiology 82, 57T–59T.

Sutton, D., Butler, A.M., Nadin, L., Murray, M., 1997. Role of CYP3A4 in human hepatic diltiazem N-demethylation: inhibition of CYP3A4 activity by oxidized diltiazem metabolites. Journal of Pharmacology and Experimental Therapeutics 282, 294–300.

The Scandinavian Simvastatin Survival Study (4S), 1994. Randomised trial of cholesterol lowering in 4444 patients with coronary artery disease. Lancet 344, 1383–1389.

Tonkin, A.M., 1995. Management of the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) study after the Scandinavian Simvastatin Survival Study (4S). American Journal of Cardiology 76, 107C–112C.

Vickers, S., Duncan, C.A., Chen, I.W., Rosegay, A., Duggan, D.E., 1990. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metabolism and Disposition 18, 138–145.

Vickers, S., Duncan, C.A., Vyas, K.P., Kari, P.H., Arison, B., Prakash, S.R., Ramjit, H.G., Pitzenberger, S.M., Stokker, G., Duggan, D.E., 1990. In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase. Drug Metabolism and Disposition 18, 476–483.

Wang, J.S., Wen, X., Backman, J.T., Taavitsainen, P., Neuvonen, P.J., Kivisto, K.T., 1999. Midazolam alpha-hydroxylation by human liver microsomes in vitro: inhibition by calcium channel blockers, itraconazole and ketoconazole. Pharmacology and Toxicology 85, 157–161.

Wood, D., 2001. Asymptomatic individuals–risk stratification in the prevention of coronary heart disease. British Medical Bulletin 59, 3–16.

Yeo, K.R., Yeo, W.W., Wallis, E.J., Ramsay, L.E., 1999. Enhanced cholesterol reduction by simvastatin in diltiazem-treated patients. British Journal of Clinical Pharmacology 48, 610–615.

Yeo, K.R., Yeo, W.W., 2001. Inhibitory effects of verapamil and diltiazem on simvastatin metabolism in human liver microsomes. British Journal of Clinical Pharmacology 51, 461–470.

Original Article

Interaction between Amlodipine and Simvastatin in Patients with Hypercholesterolemia and Hypertension

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3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors are often prescribed in association with antihypertensive agents, including calcium antagonists. Simvastatin is an HMG-CoA reductase inhibitor that is metabolized by the cytochrome P450 (CYP) 3A4. The calcium antagonist amlodipine is also metabolized by CYP3A4. The purpose of this study was to investigate drug interactions between amlodipine and simvastatin. Eight patients with hypercholesterolemia and hypertension were enrolled. They were given 4 weeks of oral simvastatin (5 mg/day), followed by 4 weeks of oral amlodipine (5 mg/day) co-administered with simvastatin (5 mg/day). Combined treatment with simvastatin and amlodipine increased the peak concentration (C_{max}) of HMG-CoA reductase inhibitors from 9.6±3.7 ng/ml to 13.7±4.7 ng/ml ($p<0.05$) and the area under the concentration-time curve (AUC) from 34.3±16.5 ng h/ml to 43.9±16.6 ng h/ml ($p<0.05$) without affecting the cholesterol-lowering effect of simvastatin. This study is the first to determine prospectively the pharmacokinetic and pharmacodynamic interaction between amlodipine and simvastatin. (*Hypertens Res* 2005; 28: 223–227)

Key Words: **drug interaction, simvastatin, amlodipine, hypercholesterolemia**

Introduction

Control of hypercholesterolemia is important for the prevention of coronary artery disease (CAD) (*1–5*). Currently, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors are the first-choice therapeutic agents for patients with hypercholesterolemia (*6–8*). The HMG-CoA reductase inhibitor simvastatin is widely used and has been shown to reduce morbidity and mortality from CAD (*9*). Simvastatin is an inactive lactone pro-drug that is hydrolyzed by esterases to simvastatin acid, the active competitive inhibitor of HMG-CoA reductase (*10–12*). Simvastatin and simvastatin acid are mainly metabolized by the cytochrome P450 (CYP) 3A4 to 3′,5′-dihydrodiol, 3′-hydroxy and 6′-exomethylene (*10–12*). The pharmacokinetics of simvastatin has been reported to be affected by potent CYP3A4 inhibitors such as itraconazole (*13*), erythromycin (*14*), verapamil (*14*) and nelfinavir (*15*). Moreover, we have previously reported that diltiazem, which is a selective inhibitor of CYP3A4 (*16, 17*), caused a 2-fold increase of the area under the concentration-time curve (AUC) of HMG-CoA reductase inhibitors (*18*).

Hypercholesterolemia is often accompanied by hypertension, an associated risk factor for CAD (*19–21*). Calcium antagonists have been widely used in the treatment of hypertension and/or angina pectoris (*22–26*), and are often prescribed in association with a lipid-lowering agent such as simvastatin. Amlodipine is one of the 1,4-dihydropyridine calcium antagonists with a long elimination half-life (*27–29*). Amlodipine undergoes the oxidative metabolism of dihydro-

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Table 1. Patient Demographics and Basic Medical Data

Age (years old)	64.1±6.0
Sex (male/female)	5/3
Body weight (kg)	61.5±5.9
Total cholesterol (mg/dl)	253±31
LDL-cholesterol (mg/dl)	164±26
HDL-cholesterol (mg/dl)	54±9
Triglyceride (mg/dl)	179±95

Values are mean±SD. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

pyridine to a pyridine analogue by CYP3A4 (*30*). In an *in-vitro* study, amlodipine was shown to have strong inhibitory effects on CYP1A1, CYP2B6 and CYP2C9, and a weak inhibitory effect on CYP3A4 when using microsomes from human B-lymphoblast cells expressing CYP (*31*). Although amlodipine is one of the most frequently used calcium antagonists, the drug interaction between amlodipine and substrate drugs for CYP3A4 has not been clinically investigated. In this study we prospectively studied the pharmacokinetic and pharmacodynamic drug interaction between amlodipine and simvastatin in patients with hypercholesterolemia and hypertension.

Methods

Subjects

Eight patients with mild hypertension and hypercholesterolemia who had been treated with simvastatin (5 mg/day) and the angiotensin-converting enzyme inhibitor enalapril (5 mg/day) for more than 3 months were enrolled. Before the start of any antihypertensive therapy, the mean systolic and diastolic blood pressure levels (SBP/DBP) were 151±29 mmHg and 88±11 mmHg, respectively. The patient demographics and basic medical data are shown in Table 1. Patients had no history of hepatic or renal disease. The study protocol was approved by the Ethical Committee of Hamamatsu University School of Medicine. All subjects gave written informed consent before participating in the study.

Study Design

This was a two-phase fixed-order design study. In the first period, patients were administered oral simvastatin (5 mg/day) alone for 4 weeks. In the second period, patients were co-administered amlodipine (5 mg/day) and simvastatin (5 mg/day) for 4 weeks. No drug other than simvastatin and amlodipine was taken during the study period.

Blood Sampling

Blood samples were obtained on the last day of each of the trial periods. After an overnight fast, a pre-dosing venous blood sample was taken, which was used to measure serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) enzymatically, and the low-density lipoprotein cholesterol (LDL-C) concentration was calculated according to the Friedewald formula method (*32*). All patients drank a glass of water after swallowing the tablets. Blood samples were then taken 2, 3, 4 and 6 h after simvastatin administration. A standardized breakfast and lunch were served 2 and 4 h after drug intake. Plasma was separated within 30 min and stored at −70°C until analysis.

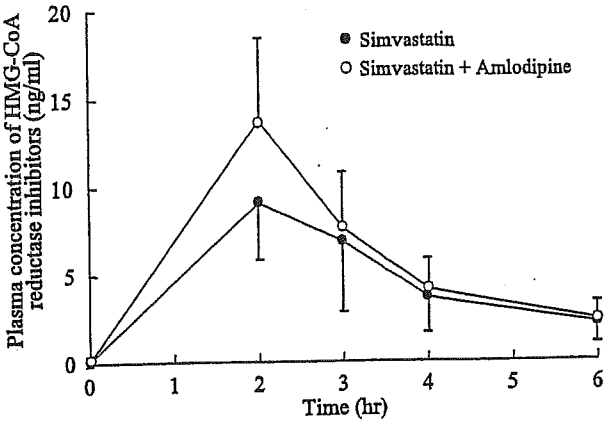

Fig. 1. *Time profiles of the mean plasma concentrations of HMG-CoA reductase inhibitors on the last day of 4 weeks of treatment with simvastatin (5 mg/day) or combined treatment with simvastatin (5 mg/day) and amlodipine (5 mg/day). Each point represents the mean±SD.*

Determination of Simvastatin HMG-CoA Reductase Inhibitor Concentrations

Plasma concentrations of HMG-CoA reductase inhibitors were determined as previously described (*33*). An equal volume of methanol was added to the plasma samples and the mixtures were vortexed thoroughly, kept on ice for 10 min, and centrifuged. Fifty microliters of the supernatants were dried in an evaporator (SpeedVac; Savant Instruments, Farmingdale, USA). The reaction mixture (96 µl) was added directly to the dried residues to make a final volume of 100 µl containing 0.1 mol/l KPO_4 (pH 7.4), 10 mmol/l 1,4-dithiothreitol (DTT), 0.2 mmol/l $NADH^+$ (made fresh daily), 5 mmol/l glucose-6-phosphate, 1.4 U/ml glucose-6-phosphate dehydrogenase and 1 mg/ml bovine serum albumin. The reaction mixture was incubated for 5 min at 37°C, and soluble rat liver HMG-CoA reductase was added to 2 µl buffer A: 0.04 mol/l KPO_4 (pH 7.4), 0.05 mol/l KCl, 0.1 mol/l sucrose, 0.03 mol/l ethylenediaminetetraacetic acid (EDTA) and 0.01 mol/l DTT (added immediately before use). The mixture was incubated at 37°C for 5 min in the presence of the inhibitor-con-

Table 2. Pharmacokinetic Parameters of Simvastatin HMG-CoA Reductase Inhibitor Concentrations

	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC(0–∞) (ng h/ml)
Simvastatin	9.6±3.7	2.08±0.59	34.3±16.5
Simvastatin+amlodipine	13.7±4.7*	1.97±0.61	43.9±16.6*

Values are mean±SD. C_{max}, maximal measured concentration; $t_{1/2}$, the elimination half-life; AUC(0–∞), area under the concentration-time curve. *$p<0.05$ *vs.* simvastatin monotherapy.

taining plasma sample. The reaction was started with 2 µl of 1.25 mg/ml HMG-CoA containing 17.5 µCi/ml glutaryl-3-[^{14}C]HMG-CoA. After an additional 6-min incubation at 37°C, 20 µl of 5 mol/l HCl was added to lactonize the mevalonic acid formed. After 15 min, 3.5 ml of a 1:1 suspension of BioRad AG 1 × 8 resin (200–400 mesh) was added and the tubes (13 × 100) were thoroughly vortexed. [^{14}C]Mevalonolactone was filtered from the resin suspension through polystyrene filters (pore size 70 µm; EverGreen, Los Angeles, USA) into scintillation vials containing 15 ml of Aquasol-2 (New England Nuclear, Newton, USA) and counted on a scintillation counter. The percentage of inhibition was converted to the inhibitor concentration using a standard curve constructed by extracting from the control plasma containing known amounts of L-654, 969, the free acid form of simvastatin. The results were expressed as nanograms of inhibitor per milliliter of plasma. The intra- and inter-day coefficients of variation for the HMG-CoA reductase activity assay were less than 6%.

Data Analysis

The pharmacokinetics of simvastatin was characterized by the peak concentration (C_{max}), the time to C_{max} (T_{max}), the elimination half-life ($t_{1/2}$) and the area under the plasma concentration-time curve from 0 to infinity [AUC(0–∞)]. The C_{max} and T_{max} were obtained directly from the original data. The terminal rate constant (k_e) used for the extrapolation was determined by regression analysis of the log-linear part of the concentration-time curve for each subject. The $t_{1/2}$ was determined by $0.693/k_e$. The AUC(0–∞) was calculated by the trapezoidal rule for the observed values and subsequent extrapolation to infinity. Data are represented as the mean±SD. Data were analyzed by a paired *t*-test or Wilcoxon signed-rank test where appropriate. Differences with p values <0.05 were considered statistically significant.

Results

No subjects reported a serious clinical, laboratory or other adverse effect, and no subjects were discontinued.

Pharmacokinetics of Simvastatin HMG-CoA Reductase Inhibitor Concentrations

Plasma concentrations of HMG-CoA reductase inhibitors after oral simvastatin dosing with or without amlodipine are shown in Fig. 1, and pharmacokinetic parameters of simvastatin are shown in Table 2. Co-administration of amlodipine with simvastatin significantly increased the C_{max} and AUC(0–∞) of HMG-CoA reductase inhibitors to 1.4- and 1.3-fold, respectively, in simvastatin monotherapy, but did not affect the $t_{1/2}$ and T_{max} of HMG-CoA reductase inhibitors.

Pharmacodynamics

Lipid profile, including TC, LDL-C, HDL-C, and TG during simvastatin monotherapy and combined treatment with simvastatin and amlodipine, are shown in Fig. 2. There were no significant differences in lipid profiles between the two periods.

The SBP and DBP values are shown in Table 3. Both measures were significantly higher during simvastatin monotherapy than during the pretrial control period with enalapril. After administration of amlodipine, both SBP and DBP tended to decline ($p=0.06$ and $p=0.08$, respectively). The blood pressure values during combined treatment with simvastatin and amlodipine did not differ from those during the pretrial control period with enalapril.

Discussion

Calcium antagonists and HMG-CoA reductase inhibitors are often prescribed together for the treatment of hypertension and/or angina pectoris in patients with hypercholesterolemia (*1*, *6*, *7*). Amlodipine is used with many drugs, such as oral hypoglycemic drugs, β-blockers, angiotensin-converting enzyme inhibitors, and so on. However, there have been no reports on the interaction between amlodipine and any other drug, with the exception that the interaction of amlodipine with grapefruit juice was shown to increase the AUC of amlodipine (*34*). This study is the first to report that amlodipine affected the plasma concentrations of HMG-CoA reductase inhibitors.

Simvastatin is hydrolyzed by esterases to simvastatin acid, which is an active inhibitor of HMG-CoA reductase (*10–12*). Simvastatin is extensively metabolized to several oxidative products by CYP3A4 (*10–12*). Some of the hydroxyl acid forms of these products also inhibit HMG-CoA reductase (*10*, *11*). In this study, we measured the total HMG-CoA reductase inhibitory activity resulting from simvastatin acid and all other active acid metabolites of simvastatin, since this level is