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Effects of fenofibrate on lipoproteins, vasomotor function, and serological markers of inflammation, plaque stabilization, and hemostasis

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Abstract

We investigated the effects of fenofibrate, peroxisome proliferator-activated receptors (PPARs) agonist, on endothelial function in patients with hypertriglyceridemia. We administered placebo or fenofibrate 200 mg daily to 25 patients with hypertriglyceridemia for 8 weeks. This study was randomized, double-blind, placebo-controlled, crossover in design. Compared with placebo, fenofibrate significantly changed lipoprotein levels including non-HDL cholesterol and significantly improved the percent flow-mediated dilator response to hyperemia by 33 ± 6% ($P < 0.001$) and lowered plasma levels of tumor necrosis factor-α by 13 ± 3% ($P = 0.002$). Fenofibrate reduced fibrinogen and plasminogen activator inhibitor type 1 antigen levels by 17 ± 3 and 10 ± 3%, respectively ($P < 0.001$ and $P = 0.014$, respectively). However, fenofibrate did not significantly change plasma levels of nitrate, malondialdehyde, tissue factor activity, and serological markers of plaque stabilization. Fenofibrate significantly changed lipoprotein levels and improved the percent flow-mediated dilator response to hyperemia as well as lowered levels of tumor necrosis factor-α (TNF-α), fibrinogen, and plasminogen activator inhibitor type 1 antigen.

Keywords: Fibrate; Hyperlipidemia; Endothelial function; Atherosclerosis

1. Introduction

Endothelial dysfunction of epicardial coronary arteries precedes development of atherosclerotic disease that is either angiographically apparent or of sufficient obstructive severity to cause myocardial ischemia and angina pectoris [1]. Patients with coronary heart disease or risk factors for coronary heart disease have been associated with impaired functions of the endothelium [2]. The vessel wall in these conditions may promote inflammation, smooth muscle proliferation, extracellular matrix deposition or lysis, and thrombus formation. All of these consequences of endothelial dysfunction contribute to development and clinical expression of atherosclerosis. Nitric oxide (NO) plays a pivotal role in maintaining vascular health and protecting from vascular injury under these pathological conditions.

Plaque disruption and thrombosis remains an important cause of acute coronary syndrome. High-risk lesions are not necessarily the angiographically severe stenosis. Rather, unstable vulnerable lesions have large lipid cores and thin fibrous caps. Plaque instability relates closely to the development of inflammation within the intima. Acute coronary syndromes usually result from rupture of a vulnerable atherosclerotic plaque mechanistically linked to the inflammatory process. Matrix metalloproteinase (MMP), tissue inhibitor of matrix metalloproteinase (TIMP), tissue factor (TF), and plasminogen activator inhibitor type-1 (PAI-1) within the plaque are the major components in determining the plaque instability and thrombogenicity [2,3].

Clinical trials of fibric acid derivatives therapy demonstrate an improvement in cardiovascular end points and

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coronary stenosis [4]. Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors activated by fatty acids and derivatives. PPARα mediates the hypolipidemic action of fibrates and is highly expressed in tissues such as heart, where it stimulates the β-oxidative degradation of fatty acids. PPARα controls plasma levels of cholesterol and triglycerides, which constitute major risk factors for coronary heart disease. Furthermore, experimental studies have shown that PPARα regulates the expression of key proteins involved in all stages of atherogenesis, such as vascular inflammation, plaque stability, and thrombosis, suggesting that PPARα exerts direct anti-atherogenic actions at the level of the vascular wall [5]. These direct vascular effects of fibrates may contribute to the cardiovascular event reduction and explain the clinical benefit in these clinical trials.

Cholesterol level lowering in experimental models was accompanied by a reduction of extracellular matrix and TF within atherosclerotic plaque [6,7]. Accordingly, the mechanism of fibrates therapy on the reduction of cardiovascular risk may be mediated by inhibiting vascular inflammation and thrombosis and stabilizing plaque. Thus, we investigated the vascular effects of fenofibrate such as vasomotor function, inflammation, plaque stability and hemostasis in patients with hypertriglyceridemia and further, the mechanism of regulation suggested by experimental studies [8–13].

2. Methods

2.1. Study population and design

This study was randomized, double-blind, placebo-controlled, crossover in design. None were diabetic, smokers, or had previous angina. We administered placebo or fenofibrate 200 mg daily to 25 patients with hypertriglyceridemia (>200 mg/dl) for 8 weeks, with the second treatment period initiated upon completion of the first treatment period (without washout phase). Any medications were not allowed during the study period to avoid other drugs' effects. The study was approved by the Gil Hospital Institute Review Board and all participants gave written, informed consent.

2.2. Laboratory assays

Blood samples for laboratory assays were obtained at approximately 8:00 a.m. following overnight fasting at baseline and at the end of each treatment period, and immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, fibrinogen, plasma nitrate (using the Griess reaction), malondialdehyde (MDA), tumor necrosis factor (TNF)-α, MMP-9 activity (Fluorokine® E Active MMP-9 kit), TIMP-1, and serum C-reactive protein (CRP) were performed in duplicate by ELISA (R&D Systems Inc., Minneapolis, MN, USA; BIOXYTECH® LPO-586, Oxis Research, Portland, OR, USA; rate nephelometry; IMMAGE®, Beckman Coulter, Brea, CA, USA) as previously described [14–18]. Assay for PAI-1 antigens and TF activity were measured in duplicates by ELISA (Biopool) and actichrome assays (American Diagnostica, Greenwich, CT) as previously described [16,17]. All samples from the same patient (batch samples) were measured in blinded pairs on the same ELISA kit to minimize run-to-run variability. The inter- and intra-assay coefficients of variation were <6%.

2.3. Vascular studies

Imaging studies of the right brachial artery were performed using a ATL HDI 3000 ultrasound machine equipped with a 10 MHz linear-array transducer, based on a previously published technique [15,16,18]. Measurements were performed by two independent investigators (D.K.J. and H.S.K.) blinded to the subject's identity and medication status. Measurements of maximum diameter and percent flow-mediated dilation were made in 10 studies selected at random. The inter- and intra-observer variability for repeated measurement of maximum diameter were 0.004±0.039 and 0.005±0.089 mm, respectively. The inter- and intra-observer variability for repeated measurement of

Table 1
Effects of Fenofibrate in hypertryglyceridemic patients

Variables	Placebo	Fenofibrate
Lipids (mg/dl)		
Total cholesterol	198 ± 5	191 ± 7
Triglycerides	354 ± 12	137 ± 11***
ApoB	109 ± 2	96 ± 3**
HDL cholesterol	42 ± 2	54 ± 2***
ApoA-I	146 ± 4	166 ± 5***
Non-HDL cholesterol	156 ± 5	137 ± 7**
Vasomotor function (%)		
Flow-mediated dilation	4.99 ± 0.33	6.33 ± 0.34***
Nitroglycerin dilation	13.70 ± 0.58	14.27 ± 0.64
Nitrate (μmol/l)	98 ± 9	98 ± 9
Malondialdehyde (μM)	0.98 ± 0.10	1.20 ± 0.12
Cytokines		
TNF-α (pg/ml)	1.64 ± 0.10	1.43 ± 0.11**
C-reactive protein (mg/dl)	0.17 (0.11–0.28)	0.11 (0.11–0.36)
Plaque stability		
MMP-9 activity (ng/ml)	90 ± 13	72 ± 9
TIMP-1 (ng/ml)	92 ± 4	98 ± 4
MMP-9 activity/TIMP-1	0.92 ± 0.13	0.80 ± 0.11
Hemostasis		
Fibrinogen (mg/dl)	288 ± 9	236 ± 9***
Tissue factor (nM)	0.96 ± 0.16	0.93 ± 0.14
PAI-1 (ng/ml)	94 ± 7	84 ± 7*

Data are expressed as means ± S.E.M. Non-HDL cholesterol = total cholesterol − HDL cholesterol [30].
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ vs. placebo.

percent flow-mediated dilation were 0.07 ± 1.27 and 0.15 ± 1.24%, respectively.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. or median (range:25–75%). After testing data for normality, we used Student's paired t test or Wilcoxon Signed Rank test to compare values after placebo and fenofibrate therapies, as reported in Table 1. We calculated that 25 subjects will provide 80% power for detecting difference of absolute increase 1.3% or greater flow-mediated dilation of the brachial artery on fenofibrate compared with placebo, with $\alpha = 0.05$. The comparison of endothelium-dependent dilation between placebo and fenofibrate therapies was prospectively designated as the primary end-point of the study. For a conservative analysis, a P-value less than the Bonferroni-adjusted α of $0.05/12 = 0.004$ was deemed as statistically significant for each of the 12 parameters that underwent statistical comparison in the study. Pearson or Spearman correlation coefficient analysis was used to assess associations between measured parameters.

3. Results

Baseline total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, apolipoprotein B, and apolipoprotein A-I levels were 201±6, 346 ± 11, 43 ± 2, 161 ± 6, 106 ± 3, and 148 ± 4 mg/dl, respectively. The mean age was 51 ± 2 years and 15 (60%) were male. Mean body mass index was 24.9 ± 0.6.

To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the percent changes of (1) the first treatment placebo and the second treatment placebo (2) the first treatment fenofibrate and the second treatment fenofibrate, relative to baseline values. There were no significant differences in baseline values, vascular function (diameter and flow) and serological markers between each group. No significant differences were found in above two comparisons. (data not shown).

3.1. Effects of therapies on lipids and vasomotor function

Compared with placebo, fenofibrate significantly changed lipoprotein levels. As expected, fenofibrate decreased total cholesterol, non-HDL cholesterol, apolipoprotein B, and triglyceride and increased HDL-C and apolipoprotein A-I. Fenofibrate significantly improved the percent flow-mediated dilator response to hyperemia by 33 ± 6% ($P < 0.001$, Fig. 1), however, the brachial artery dilator response to nitroglycerin was not significantly changed ($P = 0.200$). Fenofibrate did not change plasma levels of nitrate and MDA.

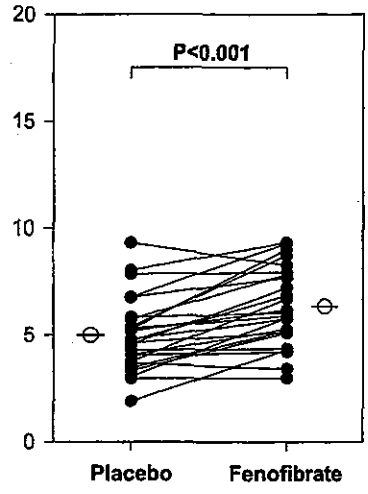

Fig. 1. Flow-mediated dilation on fenofibrate. Compared with placebo, fenofibrate significantly improved the percent flow-mediated dilator response to hyperemia. Mean values are identified by open circles.

3.2. Effects of therapies on TNF-α and markers of plaque stability

Compared with placebo, fenofibrate significantly lowered plasma levels of TNF-α by 13 ± 3% ($P = 0.002$). Fenofibrate lowered MMP-9 activity by 6 ± 10% ($P = 0.152$) and changed TIMP-1 by 9 ± 4% ($P = 0.180$) and lowered the ratio of MMP-9 activity over TIMP-1 (MMP-9 activity/TIMP-1) by 0 ± 14% ($P = 0.494$).

There were no significant inverse correlations between the degree of changes in flow-mediated dilation or HDL cholesterol and the degree of changes in MMP-9 activity ($r = -0.180$ and -0.144, respectively). However, a weak correlation between TNF-α levels and MMP-9 activity levels was determined ($r = 0.317$, $P = 0.123$).

3.3. Effects of therapies on CRP and markers of thrombosis

Compared with placebo, fenofibrate lowered serum levels of CRP from 0.17 to 0.11 mg/dl ($P = 0.424$). Fenofibrate reduced fibrinogen and PAI-I antigen levels relative to placebo by 17 ± 3 and by 10 ± 3%, respectively ($P < 0.001$ and $P = 0.014$, respectively). However, fenofibrate did not lower plasma levels of TF activity relative to baseline measurements ($P = 0.903$).

There were significant inverse correlation between the degree of changes in TNF-α levels and the degree of changes in flow-mediated dilation ($r = -0.409$, $P = 0.042$). However, no significant correlations between the degree of changes in lipoproteins or CRP levels and the degree of changes in TF activity on fenofibrate were determined ($-0.237 \leq r \leq 0.154$).

4. Discussion

In the current study, we observed that compared with placebo, fenofibrate significantly decreased total cholesterol, non-HDL cholesterol, apolipoprotein B, and triglyceride and increased HDL-C and apolipoprotein A-I. Several studies have examined the effect of fibrates on vasomotor function, but results were controversial. Improved flow-mediated dilation after oral fat loading has been shown in type 2 diabetes after 12 weeks of ciprofibrate therapy [19], but similar benefits were not confirmed in healthy volunteers after 3 weeks of gemfibrozil [20]. In patients after coronary angioplasty, exercise-induced coronary artery dilation measured by quantitative coronary angiogram increased after bezafibrate therapy compared with placebo [21]. However, in patients with coroanry artery disease, Andrews et al. [22] reported that gemfibrozil alone or in combination with niacin did not significantly improve flow-mediated dilation. In the current study, we observed that fenofibrate significantly improved the percent flow-mediated dilator response to hyperemia. However, fenofibrate did not change plasma levels of nitrate and MDA. Fenofibrate may not have anti-oxidant effect in humans. We did not see any correlations between lipoprotein changes and flow-mediated dilation percent changes.

In order to gain insight as to mechanisms of potential vasculoprotective effects of fenofibrate, we measured vasomotor function, plasma TNF-α, and markers of plaque stability. Lowering blood LDL cholesterol levels may facilitate plaque stability either through a reduction in size or by an alteration of the physiochemical properties of lipid cores [2]. However, changes in plaque size by lipid lowering tend to occur over an extended period of time and are quite minimal, as assessed by angiography. Rather, the clinical benefits from lipid lowering are probably due to decreases in macrophage accumulation in atherosclerotic lesions and inhibition of MMP production by activated macrophages. In this regard, Aikawa et al. [6] demonstrated that intimal smooth muscle cells in the low cholesterol group displayed reduced expression of MMP-9 compared with the high cholesterol groups. Lipid-lowering therapies diminished accumulation of macrophages as well as macrophage expression of MMP-9 in animal studies [7,23]. Indeed, Xu et al. [9] demonstrated that oxidized LDL upregulated MMP-9 expression while reducing TIMP-1 in monocyte-derived macrophages. Furthermore, HDL abrogated oxidized LDL-induced MMP-9 expression. However, we did not observe significant correlations between lipoprotein levels and MMP-9 activity or TIMP-1 levels on fenofibrate. On the other hand, PPAR activator inhibited the expression of MMP-9 [11]. This PPAR-dependent inhibition may prevent the rupture of the atherosclerotic plaque and subsequent thrombosis. Despite the experimental observations, we observed that fenofibrate did not significantly change serological markers of plaque stability in the current study.

Moreover, endothelial NO synthase gene transfer significantly decreased MMP-2 and MMP-9 activities simultaneously with increase of TIMP-2 levels in the conditioned medium [10]. Furthermore, TNF-α, a proinflammatory cytokine, stimulated the synthesis and secretion of MMP-9 [8]. In the current study, we observed a weak correlation between the degree of changes in TNF-α and the degree of changes in MMP-9 activity on fenofibrate.

Two experimental studies demonstrated that native or oxidized LDL enhanced lipopolysaccharide-induced TF expression [24,25]. Furthermore, CRP, a proinflammatory cytokine, stimulated the synthesis of TF [26]. Meanwhile, PPARα also inhibits the expression of TF in human monocytes and macrophages [12]. However, we observed no effects of fenofibrate on TF activity and no correlations between the degree of changes in lipoproteins or CRP levels and the degree of changes in TF activity on fenofibrate.

Impaired fibrinolysis as measured by an elevation in PAI-1 and fibrinogen is predictive of ischemic heart disease [27,28]. Several lipid-lowering agents may potentiate fibrinolysis independent of alterations in plasma lipoproteins. In this regard, the gemfibrozil and fenofibrate reduced PAI-1 and fibrinogen plasma levels in hypercholesterolemic subjects [5,13,29]. Fibrates also modulate the secretion of the thrombosis inducer PAI-1 [13]. These actions of fenofibrate on fibrinogen and PAI-1 antigen levels may result in a decreased thrombogenic response.

Our current observations are consistent with the Adult Treatment Panel III Guidelines reported in 2001 [30]. Since metabolic syndrome is one of the most important clinical features underlying atherosclerosis, and since the Adult Treatment Panel III Guidelines use non-HDL cholesterol as one of the indexes to evaluate the dyslipidemic status including hypertriglyceridemia, our current observations showing the effects of fenofibrate on lipoproteins and endothelial function in patients with hypertriglyceridemia, may have important clinical implications to reduce cardiovascular events in these patients.

In conclusion, fenofibrate significantly changed lipoprotein levels including non-HDL cholesterol and improved the percent flow-mediated dilator response to hyperemia as well as lowered levels of TNF-α, fibrinogen, and PAI-1 antigen.

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CLINICAL PHARMACOLOGY GRAND ROUNDS

Altered pharmacokinetics and excessive hypotensive effect of candesartan in a patient with the *CYP2C9*1/*3* genotype

An 89-year-old man with severe hypertension (190/82 mm Hg) and chronic heart failure (New York Heart Association class II) despite treatment with benidipine, doxazosin mesylate (INN, doxazosin), and furosemide was given oral candesartan cilexetil (4 mg/d), an angiotensin II type 1 receptor blocker metabolized via cytochrome P450 (CYP) 2C9. Two days later, he started to have severe dizziness and returned to the hospital on the fourth day without taking any of his medications. The blood pressure 30 hours after the last dose of candesartan was 126/64 mm Hg. Polymorphism analysis revealed the heterozygous poor metabolizer genotype *CYP2C9*1/*3*. The area under the concentration-time curve and the mean residence time of candesartan were both increased 2.5-fold, and the oral clearance of candesartan was 48% lower than that of the average elderly Japanese patient with hypertension. These results suggest that the *CYP2C9*1/*3* genotype could be associated with decreased clearance and increased plasma concentration of candesartan, potentially enhancing its hypotensive effect. (Clin Pharmacol Ther 2003;74:505-8.)

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Candesartan is a long-acting angiotensin II type 1 receptor blocker (ARB) that is widely used to treat patients with hypertension. The oral form is candesartan cilexetil, which is completely converted during enteric absorption to the active form, candesartan. Candesartan is metabolized in the liver by cytochrome P450 (CYP) 2C9 to the inactive metabolite CV15959 and is excreted as such through renal and biliary routes.[1] The existence of genetic polymorphisms in CYP2C9 has been recognized to influence the enzyme's activity. The most common variants of CYP2C9 alleles are *CYP2C9*2*, a 430C<T polymorphism that results in the amino acid exchange Arg144Cys, and *CYP2C9*3*, a 1075A<C polymorphism that causes an Ile359Leu exchange.[2-4] In vitro studies have suggested that the *CYP2C9*3* variant markedly reduces catalytic activity and binding affinity of the enzyme.[2,5] In addition, studies in humans have demonstrated that this variant is associated with poor metabolism of classic CYP2C9 substrates such as tolbutamide,[2] phenytoin,[6] and warfarin.[7] Nonetheless, it is not clear whether the *CYP2C9*3* allele affects pharmacokinetics and therapeutic effects of candesartan. Here we describe a clinical case in which the heterozygous poor metabolizer genotype of CYP2C9 (*CYP2C9*1/*3*) is associated with decreased clearance and increased plasma concentration of candesartan, manifesting clinically by severe dizziness and hypotension.

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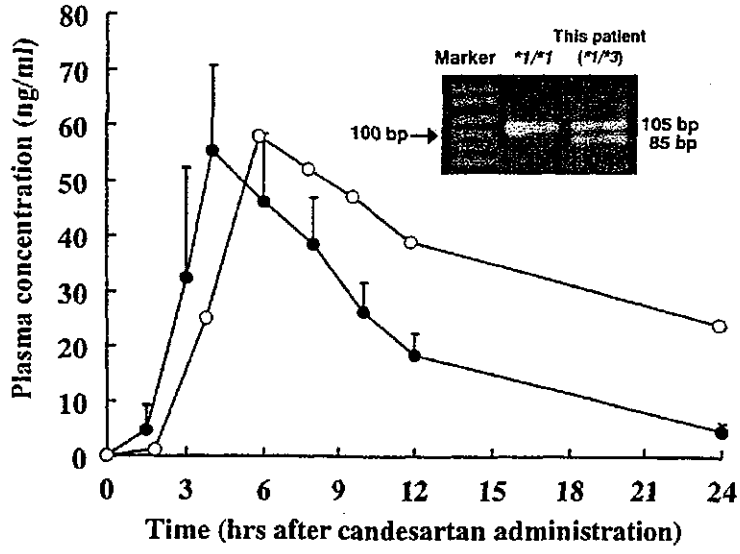

Fig 1. Plasma concentration–time curves of candesartan after oral administration of candesartan cilexetil in our *CYP2C9*1/*3* patient (*open symbols*) and in hypertensive elderly patients (from reference 9) (*solid symbols*, n = 6). *Bars* represent mean ± SD. *Inset*, Polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis of the CYP2C9 gene in a *CYP2C9*1/*1* patient (*lane 1*) and our *CYP2C9*1/*3* patient (*lane 2*). PCR products were digested with *Kpn*I before electrophoresis. The PCR product containing the **1* allele remained uncut, whereas the other produced 85- and 20-base pair (bp) fragments. Only the 85-bp fragment was visible.

CASE REPORT

An 89-year-old man was seen at the outpatient clinic of Hamamatsu University Hospital, Hamamatsu, Japan, with severe hypertension and chronic heart failure (New York Heart Association functional class II). The blood pressure was uncontrollable (190/82 mm Hg) despite treatment with benidipine (4 mg/d), doxazosin mesylate (INN, doxazosin) (2 mg/d), and furosemide (40 mg/d). The ARB candesartan cilexetil, 4 mg/d orally, was thus added for better control of blood pressure. The first day passed without the patient having any symptoms of severe hypotension such as dizziness and syncope. From the second day, he started to have severe dizziness and had to return to the hospital on the fourth day without taking any of his medications. The blood pressure 30 hours after the last intake of candesartan was found to be only 124/64 mm Hg. Candesartan was withdrawn and the symptoms were rapidly alleviated. Serum aspartate aminotransferase and alkaline aminotransferase levels were 17 IU/L and 13 IU/L, respectively. Serum electrolyte levels were as follows: sodium, 142 mEq/mL; potassium, 3.9 mEq/mL; and chloride, 103 mEq/mL. The ratio of blood urea nitrogen to serum creatinine was 14.8, and creatinine clearance was 88.7 mL/min. There was no evidence of significant hepatic or renal dysfunction or dehydration. These results suggest that the metabolism of candesartan was significantly reduced in this patient. Polymerase chain reaction–restriction fragment length polymorphism analysis[8] then revealed that the CYP2C9 genotype of the patient was heterozygous of the wild-type (**1*) and Leu359 (**3*) allele (*CYP2C9*1/*3*) (Fig 1).

To determine whether the pharmacokinetic properties of candesartan were altered in this patient, we obtained written informed consent for an in-hospital readministration of 4 mg candesartan cilexetil. This study was approved by the Ethical Committee of Hamamatsu University School of Medicine. The pharmacokinetic parameters of candesartan in this patient were compared with published values from 6 Japanese patients with hypertension (mean age, 67.2 years).[9] Blood samples were taken at 2, 4, 6, 8, 10, 12, and 24 hours after the administration of candesartan, and plasma concentrations of the drug were determined by use of HPLC.[10] Fig 1 shows the plasma concentration–time curves of candesartan after oral administration of candesartan cilexetil (4 mg) in our patient and the 6 elderly patients with hypertension previously de-

Table I. Pharmacokinetics of candesartan after oral administration of candesartan cilexetil in our *CYP2C9*1/*3* patient and published data from hypertensive elderly patients (from reference 9)

Pharmacokinetic parameter	*CYP2C9*1/*3 patient*	*Other hypertensive elderly patients*
CL_{oral} ($mL \cdot kg^{-1} \cdot h^{-1}$)	52.6	109
MRT (h)	27.8	11.2
AUC(0-∞) (h · ng/mL)	1382	549

The area under the concentration-time curve [AUC(0-∞)] and the area under the first moment (plasma concentration multiplied by time)–time curve (AUMC) were calculated by use of the trapezoidal rule for the observed values and with subsequent extrapolation to infinity. The mean residence time (MRT) was determined by dividing AUMC by AUC(0-∞). The oral clearance (CL_{oral}) was calculated as dose divided by AUC(0-∞).

scribed.[9] Peak plasma concentrations (C_{max}) were similar in our patient and the other 6 patients (58.0 ng/mL versus 55.4 ng/mL), whereas the time to reach C_{max} was 4 hours in our patient and 2 hours in the 6 elderly patients. Candesartan in our patient was more slowly eliminated than in the other patients. The area under the concentration-time curve extrapolated to infinity [AUC(0-∞)] and the mean resident time (MRT) of candesartan in our patient were both increased 2.5-fold, and the oral clearance (CL_{oral}) was 48.3% lower than that of the 6 elderly patients (Table I). After a single dose of the drug, the blood pressure of our patient was slightly decreased (from 139/63 mm Hg before dosing to 123/58 mm Hg at 6 hours, 118/66 mm Hg at 10 hours, and 110/62 mm Hg at 24 hours), but he had no significant symptoms of severe hypotension.

DISCUSSION

The patient described in this case report, who carried the *CYP2C9*1/*3* genotype, was found to have a markedly higher plasma drug concentration and significantly lower clearance in comparison with values in other elderly patients. Although renal impairment could alter the pharmacokinetics of candesartan,[11] both renal and hepatic functions were normal in our patient. Our data are consistent with previous reports that the *CYP2C9*3* allele, even when heterozygous with the wild-type allele, alters the pharmacokinetics of its substrates. Lee et al[12] have recently reported that the oral clearance of tolbutamide is reduced by approximately 50% in individuals carrying the *CYP2C9*1/*3* genotype in comparison with those carrying the *CYP2C9*1/*1* genotype. In a group of Japanese patients with the *CYP2C9*1/*3* genotype, the maximal elimination rate of phenytoin was 33% lower than in a *CYP2C9*1/*1* group.[6] There appears to be no significant difference between *CYP2C9*1/*1* and *CYP2C9*1/*3* in human liver microsomes with regard to the kinetics of candesartan. However, expression of the *CYP2C9*3* variant in yeast has been shown to change the metabolism of the drug.[13] It is, therefore, very likely that the *CYP2C9*1/*3* genotype is an important factor that disturbed candesartan pharmacokinetics in our patient. Given that the patient's plasma concentration of candesartan remained high even at 24 hours after candesartan administration, it is likely that the concentration was very high on later days of treatment, which would explain the patient's severe dizziness from the second day and the markedly reduced blood pressure (126/64 mm Hg) when he returned to the hospital on the fourth day. These results suggest that the *CYP2C9*1/*3* genotype could be associated with decreased clearance and increased AUC(0-∞) and MRT of candesartan in humans, resulting in an excessive hypotensive effect. In this context, additive effects of other factors affecting drug disposition in geriatric patients, such as changes in plasma protein binding, hepatic blood flow, and enzyme activity, cannot be ruled out.[14]

This report is the first to suggest an association between CYP2C9 genomic polymorphism and altered pharmacokinetics of candesartan. Previous reports have shown a link between the *CYP2C9*3* allele and deficient conversion of losartan, another ARB, to its metabolite E-3174.[15,16] Because E-3174 is more potent than losartan, the lowered concentrations of the metabolite in association with the *CYP2C9*3* allele may somewhat reduce the blood pressure–lowering effect of losartan. However, in a recent study, healthy subjects with the *CYP2C9*1/*1* and *CYP2C9*1/*3* genotypes did not show any difference in blood pressure after a single dose of losartan.[17] Consistent with this finding, our patient was treated with losartan after the episode of severe dizziness that occurred with candesartan and he did not have any symptoms of hypotension. In the case of candesartan, however, the metabolite is inactive and the decreased metabolism of the drug associated with the *CYP2C9*3* allele may thus be responsible for the observed excessive hypotensive effect. Therefore this report is also the first to demonstrate the potential pharmacodynamic consequences of the *CYP2C9*1/*3* genotype with an ARB, candesartan.

Although the incidence of the *CYP2C9*3* allele is relatively low among white subjects (0.06)[3] and Japanese populations (0.02),[8] the pharmacokinetic disturbance of candesartan in *CYP2C9*1/*3* patients may be important because approximately 4% of Japanese sub-

jects compared with 10% of white subjects are known to express this heterozygous genotype.

In conclusion, this case report suggests that reduced CYP2C9 activity associated with the *CYP2C9*1/*3* genotype may be clinically meaningful in patients receiving candesartan cilexetil. Larger studies on the pharmacokinetic and pharmacodynamic characteristics of candesartan are necessary to confirm the increased drug exposure and hypotensive effect of the drug in *CYP2C9*3* carriers. However, from a practical point of view, determination of the patient's genotype for CYP2C9 should be considered at the first sign of severe hypotension after candesartan administration so that the treatment regimen can be adjusted in a timely manner and further deleterious effects avoided.

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All authors of this manuscript disclose any significant relationship with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of this study. None of the authors have any conflicts of interest.

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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.

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 IIMG-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

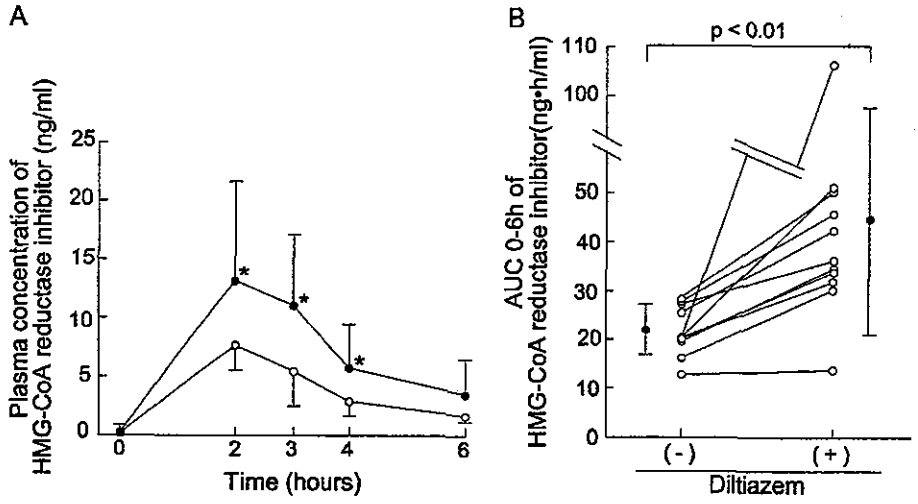

Fig. 1. Effect of diltiazem on plasma concentration and $AUC_{0\ 6h}$ of HMG-CoA reductase inhibitor. (A) Plasma concentrations of HMG-CoA reductase inhibitor observed on the last day of 4 weeks of treatment with simvastatin (5mg/day) (open circles) or combined treatment with simvastatin (5mg/day) and 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,

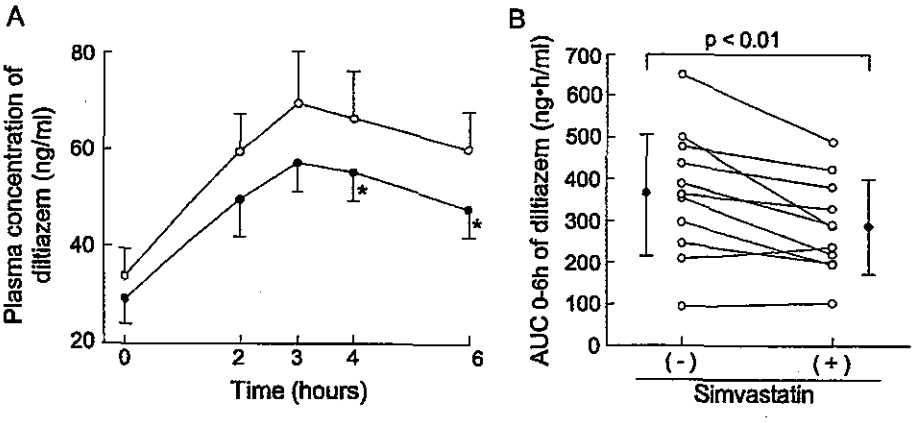

Fig. 2. Effect of simvastatin on plasma concentration and $AUC_{0\ 6h}$ of diltiazem. (A) Plasma concentrations of diltiazem observed on the last day of 4 weeks of treatment with diltiazem (open circles) or combined treatment with simvastatin and diltiazem (closed circles). *Significant difference from 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

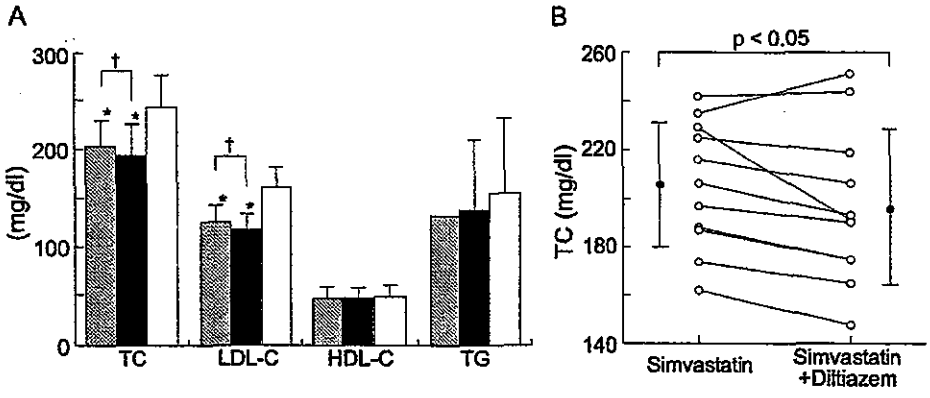

Fig. 3. Lipid profiles during simvastatin monotherapy, combined therapy with diltiazem and simvastatin, and diltiazem monotherapy. (A) Lipid profiles after 4 weeks of simvastatin monotherapy (5mg/day, hatched columns), combined treatment with simvastatin (5mg/day) and diltiazem (90mg/day) (closed columns) 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.

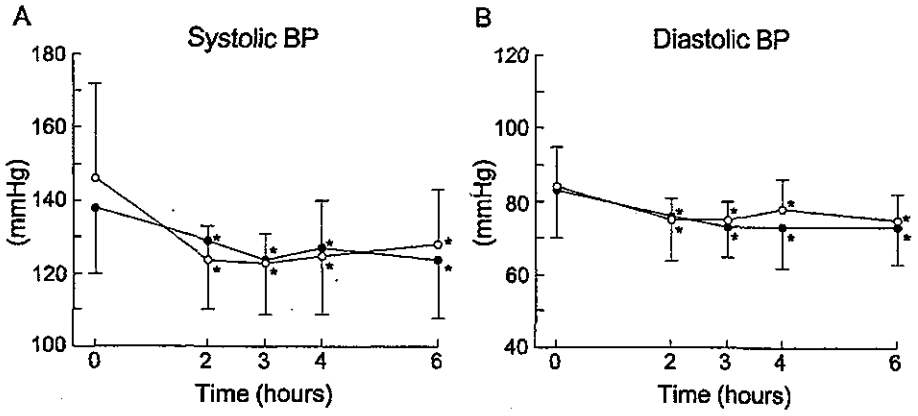

Fig. 4. Blood pressures during combined therapy with diltiazem and simvastatin, and diltiazem monotherapy. Systolic (A) and diastolic (B) BP before and 2, 3, 4 and 6 hours after an oral 30 mg dose of diltiazem with (closed circles) or without (open circles) simvastatin following 4 weeks of treatment 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

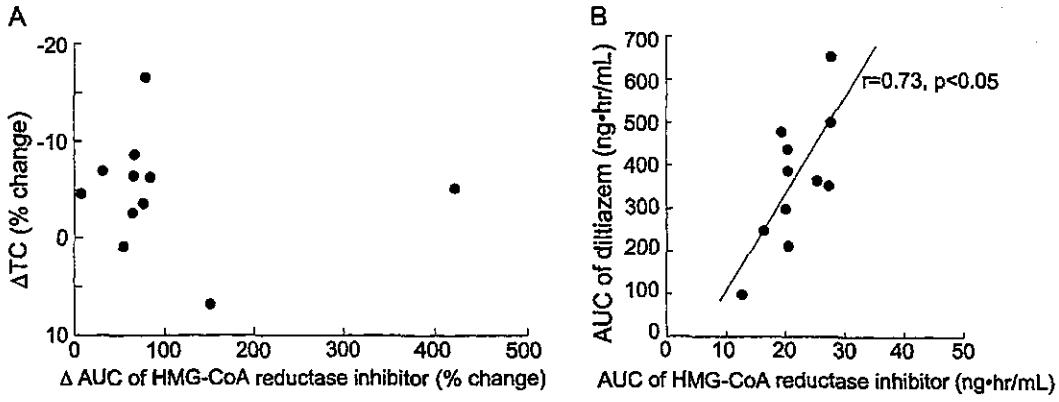

Fig. 5. (A) Percent changes in plasma concentration of HMG-CoA reductase inhibitor versus plasma total cholesterol (TC) concentration after the combined treatment with simvastatin and diltiazem in the 11 patients. Correlation coefficient was 0.106 (not significant). (B) Relationship between the AUCs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