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Inhibitory effects of fluvastatin, a new HMG-CoA reductase inhibitor, on the increase in vascular ACE activity in cholesterol-fed rabbits

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1 The effects of fluvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on the vascular angiotensin converting enzyme (ACE) activity in hyperlipidaemic rabbits were compared with those of enalapril, an ACE inhibitor.

2 Rabbits were fed a 1.5% cholesterol containing diet or normal diet for 16 weeks and treated with either fluvastatin or enalapril in the diet at the respective doses of 2 and 10 mg kg^{-1} day^{-1}. The total cholesterol, triglyceride and phospholipid levels in serum were significantly increased in rabbits fed the high cholesterol diet. Treatment with fluvastatin but not enalapril resulted in a decrease in serum lipids.

3 The vascular ACE activities assessed via the cleavage rate from synthetic substrate in the aortic arches and upper thoracic aortae were increased by 8 to 10 times when the rabbits were made hyperlipidaemic. Fluvastatin as well as enalapril significantly lowered the tissue ACE in the aortae.

4 The ACE activities in serum did not alter in hyperlipidaemic rabbits either in the presence or absence of fluvastatin. The serum ACE activity was lowered by enalapril.

5 The lipid peroxide in serum as well as the plaque area in the thoracic aorta was significantly increased in the cholesterol diet-fed rabbits. Treatment with fluvastatin or enalapril reduced both serum lipid peroxide and plaque formation. The relaxant responses to acetylcholine (ACh) were significantly suppressed in the cholesterol-fed rabbits. Treatment with fluvastatin or enalapril significantly reversed the suppression of ACh-induced relaxation.

6 It seems that the reduction of vascular ACE is not coupled to lipids and ACE activity in serum, but rather to lipid peroxidation. Thus, the decrease in vascular ACE activity by fluvastatin as well as the lipid-lowering effect may reduce the risk of atherosclerosis progression in the vasculature.

Keywords: Renin-angiotensin system; vascular angiotensin converting enzyme; HMG-CoA reductase inhibitor; hyperlipidaemic rabbits; atherosclerosis; lipid peroxidation; enalapril; fluvastatin

Introduction

Besides the circulating renin angiotensin system, angiotensin II is formed locally through proteolytic cleavage of angiotensin I by the angiotensin converting enzyme (ACE), which is found predominantly in endothelial cells but also in the mediae and adventitiae of blood vessels (Dzau, 1993a,b). There are several lines of evidence supporting the pathophysiological role of locally formed angiotensin II in blood vessel regulation in relation to hypertension, cardiac hypertrophy, restenosis after angioplasty and atherosclerosis (Dzau, 1993a,b). Increased activity of tissue ACE has been demonstrated in hypertension (Miyazaki *et al.*, 1987), cardiac hypertrophy (Schunkert *et al.*, 1990) and intimal thickening after balloon injury (Shiota *et al.*, 1993).

Angiotensin II formed in the vasculature stimulates growth of vascular smooth muscle cells, associated with an increase in DNA synthesis and the expression of proto-oncogenes and cytokines (Dzau, 1993a,b). Infusion of exogenous angiotensin II enhances the proliferation of smooth muscle cells in the vascular wall after balloon-induced injury (Daemen *et al.*, 1991). It has been demonstrated that ACE inhibitors prevent neointimal proliferation in rats *in vivo* after balloon-induced injury of the vascular wall (Powell *et al.*, 1989). Furthermore, neointimal hyperplasia, which is closely related to tissue ACE activity but not serum ACE (Rakugi *et al.*, 1994), is prevented by a selective angiotensin II receptor antagonist, losartan (Osterrieder *et al.*, 1991). A recent study, using *in vivo* gene transfer has demonstrated that overexpression on the ACE gene in the rat carotid artery increases DNA synthesis and induces vascular structural changes as a result of the increased generation of angiotensin II locally (Morishita *et al.*, 1994). Since ACE is identical to kininase II, an enzyme which inactivates kinins, it is postulated that the inhibition of neointimal formation by ACE inhibitors is accounted for by the consequent increase in kinins, which allows the generation of nitric oxide and prostacyclin which have anti-atherosclerotic effects (Linz *et al.*, 1995).

We have recently demonstrated that vascular ACE activity was significantly increased in the aortae of rabbits fed a high cholesterol diet, and there was a significant correlation between plaque area and vascular ACE activity (Mitani *et al.*, 1996). In addition, ACE inhibitors slow down plaque formation in the progression of atherosclerosis in hyperlipidaemia in Watanabe heritable hyperlipidaemic (WHHL) rabbits (Chobanian *et al.*, 1990), cynomolgus monkeys (Aberg & Ferrer, 1990), and high cholesterol-fed hamsters (Kowala *et al.*, 1994) and rabbits (Fennessy *et al.*, 1994). However, the anti-atherosclerotic effects of ACE inhibitors are not necessarily consistent, as ramipril has no significant effect on plaque formation in high cholesterol-fed rabbits (Finta *et al.*, 1993).

Fluvastatin sodium is a potent inhibitor of 3-hydroxy-3-methylgluytaryl coenzyme A (HMG-CoA) reductase inhibitor, the rate limiting enzyme in cholesterol biosynthesis. Previous studies showed marked reduction in serum lipids or lipoprotein-cholesterol in WHHL rabbits (Shiomi *et al.*, 1994) and serum low density lipoprotein (LDL)-cholesterol in hypercholesterolaemia patients (Davidson, 1994). Fluvastatin, furthermore, inhibits the proliferation of human smooth muscle

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cells and the migration of rat aortic myocytes, and prevents the neointimal formation following the insertion of a flexible collar around one carotid artery in rabbits without changing the plasma cholesterol level (Corsini *et al.*, 1995b). These results suggest that fluvastatin has pharmacological profiles of both lipid lowering and vascular actions.

The present study with long-term treatment with drugs was designed to address the questions of whether fluvastatin or an ACE inhibitor, enalapril, modulates the vascular ACE activity and plaque formation, and to characterize better two mechanistically-different inhibitors concerning lipids, ACE, lipid peroxide and vascular function.

Methods

Animals and tissue preparation

Thirty two male Japanese white rabbits (2.5–3.0 kg) were obtained from Kitayama Labes Co. Ltd. (Japan) and divided into four groups; normal diet group, 1.5% cholesterol diet group, 1.5% cholesterol diet group containing fluvastatin or enalapril. Each diet was set at 100 g day^{-1} but water was available *ad libitum*.

After 16 weeks, the aortae were isolated and blood samples were taken. The high fat diets resulted in a high incidence of death; e.g., 3 out of 8 animals in the 1.5% cholesterol group.

Serum lipids and lipid peroxides

Blood samples were taken to determine serum lipid and lipid peroxide levels. Total cholesterol, triglycerides and phospholipids in serum were measured by cholesterol oxidase-3, 5-dimethoxy-N-ethyl-(2-hydroxy-3-sulphoproryl)-aniline sodium (DAOS), glycerol-3 phosphate oxidase-DAOS, and choline oxidase-DAOS, respectively, using commercially available kits (Wako Pure Chemical Industries, Japan). The lipid peroxides in serum were measured as thiobarbituric acid-reactive substances (TBARS) using commercial kits (Wako Pure Chemical Industries, Japan) and the results are given in nmol malondialdehyde (MDA)-equivalents per ml.

ACE activities

Aortic arches and upper thoracic aortae were isolated for the assessment of the vascular ACE activity. The tissue ACE activity of isolated aortae was determined by the production rate of hippuric acid from the synthetic tripeptide substrate, hippuryl-L-histidyl-L-leucine (HHL), by previously described methods (Miyazaki *et al*, 1984). All samples were cut into small pieces and immediately placed into ice-cold Tris-HCl buffer solution (1 ml per 100 mg sample wet weight). The buffer solution consisted of 20 mM Tris-HCl (pH 8.3), 5 mM $Mg(CH_3COO)_2$, 30 mM KCl, 250 mM sucrose and 0.5% Nonidet P-40. The sample suspension was homogenized on ice in a Polytron homogenizer (Microtec Co., Japan), and stored overnight at 4°C. The homogenized sample was centrifuged for 30 min at 15,000 r.p.m. at 4°C. The supernatant was then incubated with the substrate.

Two hundred μl of assay medium composed of 100 mM potassium phosphate buffer (pH 8.3), 300 mM NaCl and 5 mM HHL, was mixed with 50 μl of sample supernatant and incubated for 30 min at 37°C. When the *in vitro* assay was performed, the drugs were added just before incubation with the substrate. The enzyme reaction was terminated by adding 3% metaphosphoric acid, and the mixture was placed in iced water for 10 min. After centrifugation of the reaction mixture for 10 min at 3,000 r.p.m., 50 μl of the supernatant was applied to a reverse-phase column (TSK-GEL, ODS-80TM, Tosoh Co., Japan) and eluted with 10 mM KH_2PO_4-methanol (1:1, pH 3.0) at a rate of 0.7 ml min^{-1} at 38°C. The hippuric acid concentration was assessed by ultra violet absorbance at 228 nm. The protein concentration was measured according to the method of Bradford (1976) with a commercial kit (Bio-Rad Laboratories, U.S.A.). ACE activity was expressed as nmol min^{-1} per mg protein or per ml of serum.

Plaque area analysis

Photographs of thoracic aortae were computer-analysed (NIH Image 1.44, U.S.A.) according to the method of Schuh *et al.* (1993). The computer-analysed images of plaque area were assessed, so that plaque coverage was then expressed as a percentage of the lesioned area to the total area in each morphological preparation.

Vascular responsiveness

The right femoral artery was isolated and placed into ice-cold modified Krebs-Henseleit buffer solution composed of the following composition (mM): NaCl 119.2, KCl 4.7, $NaHCO_3$ 23, NaH_2PO_4 1.2, $CaCl_2$ 1.8, $MgCl_2$ 1.2 and glucose 7.9. The vessel was cleaned of excess fat and connective tissue, and cut into rings 3 mm in longitudinal length. The ring preparations were suspended between stainless steel triangular holders and transferred to muscle baths containing bathing medium at 37°C and bubbled with 95% O_2 and 5% CO_2 gas mixture as previously described (Kimura *et al.*, 1992). The changes in isometric force under a resting force of 1 g were monitored through a force-displacement transducer (Nihon Kohden, Japan). Before measurements were taken, the preparation was allowed to equilibrate for 90 min in the bathing medium, during which time the medium was replaced every 30 min.

First, KCl 40 mM (submaximal concentration) was added to the bath to induce contraction. Then, before acetylcholine (ACh) at concentrations of 0.01 to 10 μM was added to examine the relaxant effects, the preparations were contracted with 0.1 μM phenylephrine. Relaxant responses to ACh were expressed as a percentage of the phenylephrine pre-induced contraction.

Materials

The drugs used were fluvastatin ((±)-(3**RS**, 5**SR**, 6E)-sodium-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoate) (Sandoz Research Institute, U.S.A.), and enalapril maleate (Sigma Chemical Co., U.S.A.). Fluvastatin and enalapril were added separately to a 1.5% cholesterol-containing diet (LRC-4 of diet base, Oriental Yeast Co., Japan) at concentrations of 0.03% and 0.15%, respectively. The drug concentration in the diet was adjusted with the diet containing 1.5% cholesterol, according to the dose level. The dosage of fluvastatin and enalapril was 2 and 10 mg kg^{-1} day^{-1}, respectively. Enalapril at a dose not sufficient to elicit a hypotensive effect was used in high cholesterol-fed rabbits (Schuh *et al.*, 1993).

Statistical analysis

Data are expressed as the mean ± s.e. Statistical analysis of the data was performed using one-way analysis of variance, followed by Tukey's multiple comparison test. Statistical significance was accepted at $P<0.05$.

Results

Serum lipids

After 16 weeks, the total cholesterol, triglyceride and phospholipid levels in serum were significantly increased in rabbits fed the 1.5% cholesterol diet, compared with those of the normal diet group (Figure 1). Fluvastatin significantly lowered the total cholesterol, triglyceride and phospholipid levels, compared with those of the 1.5% cholesterol diet group. No effects were observed for enalapril-treated group.

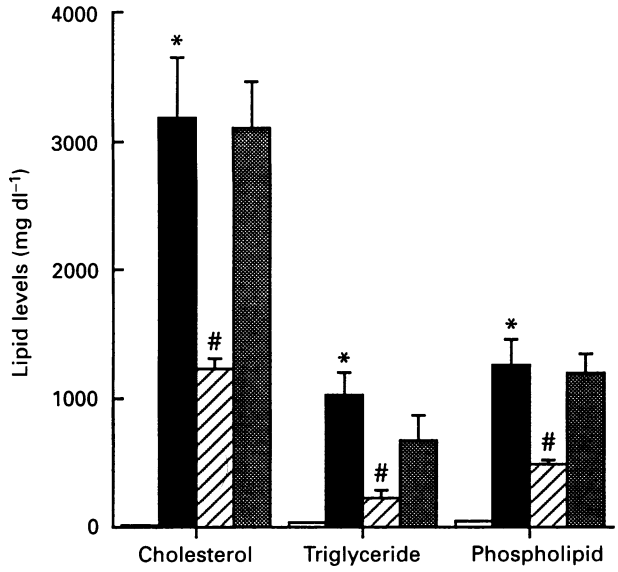

Figure 1 Effects of fluvastatin on lipids in rabbit serum. Rabbits were fed a normal diet (open columns), 1.5% cholesterol diet (solid columns) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\,day^{-1}$; hatched columns) or enalapril ($10\,mg\,kg^{-1}\,day^{-1}$; stippled columns) for 16 weeks. After long term treatment with the drug, the total cholesterol, triglyceride and phospholipid levels in serum were measured by enzymatic assays (see Methods). The values are the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the normal diet group. $\#P<0.05$, significantly different from the 1.5% cholesterol diet group.

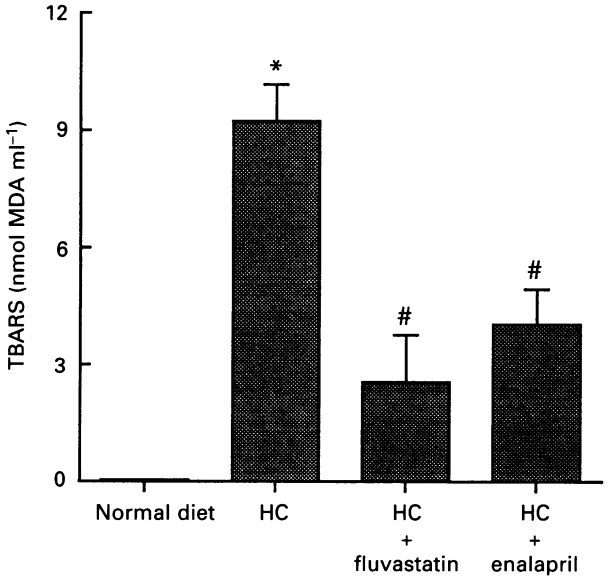

Figure 2 Effect of fluvastatin on lipid peroxide in rabbit serum. Rabbits were fed a normal diet, 1.5% cholesterol diet (HC) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\,day^{-1}$) or enalapril ($10\,mg\,kg^{-1}\,day^{-1}$) for 16 weeks. Lipid peroxides in serum were assessed by the formation of thiobarbituric acid-reactive substances (TBARS) and the figures are given in nmol malondialdehyde (MDA) equivalents per ml. The values presented the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the normal diet group. $\#P<0.05$, significantly different from the 1.5% cholesterol diet group.

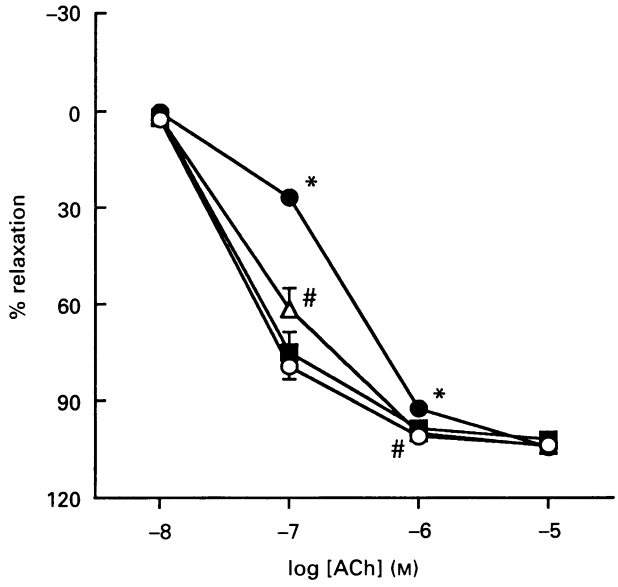

Figure 3 Effects of fluvastatin on the acetylcholine (ACh)-induced relaxation in the right femoral arteries of rabbits. Rabbits were fed a normal diet (○), 1.5% cholesterol diet (●) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\,day^{-1}$; △) or enalapril ($10\,mg\,kg^{-1}\,day^{-1}$; ■) for 16 weeks. Changes in the isometric force of the ring preparation were monitored through a force-displacement transducer. Before ACh at concentrations of 0.01 to 10 μM was added to examine the relaxant effects, preparations were contracted with 0.1 μM phenylephrine. Relaxant responses to ACh were expressed as a percentage of the phenylephrine-induced contraction. The values presented the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the normal diet group. $\#P<0.05$, significantly different from the 1.5% cholesterol diet group.

Lipid peroxides

After 16 weeks, the lipid peroxide levels in serum were significantly increased in rabbits fed the cholesterol diet, compared with the normal diet group (Figure 2). Fluvastatin and enalapril reduced the lipid peroxide levels markedly, compared with those of the cholesterol diet group.

Vascular responsiveness

The contractile response to 40 mM KCl was not affected by 1.5% cholesterol or drug treatment for 16 weeks (data not shown). The relaxant responses to ACh at concentrations of 0.1 and 1 μM were significantly suppressed by the 1.5% cholesterol diet, compared with those of the normal diet group (Figure 3). Both fluvastatin and enalapril reversed the high cholesterol diet-induced suppression of the relaxation significantly.

Plaque areas

The plaque area in the thoracic aortae increased by more than 70% in the 1.5% cholesterol diet group after 16 weeks with the cholesterol diet (Figure 4). Both fluvastatin and enalapril significantly reduced the formation of plaque area.

ACE activities

After 16 weeks, the ACE activities in serum did not alter in rabbits fed the 1.5% cholesterol diet nor the 1.5% cholesterol diet containing fluvastatin (Figure 5). On the other hand, enalapril treatment significantly reduced the serum ACE activities.

Vascular ACE activities in aortic arches and upper thoracic aortae were increased by 10 and 8 times, respectively, in rabbits fed the 1.5% cholesterol diet, compared with the normal diet group (Figure 6). Both fluvastatin and enalapril reduced the ACE activities in the aortic arches, compared with the 1.5% cholesterol diet group. In the upper thoracic aorta, both fluvastatin and enalapril also reduced the tissue ACE activity significantly.

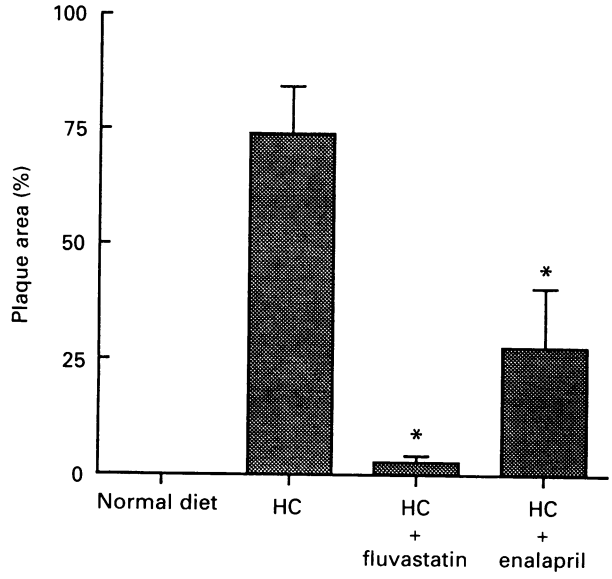

Figure 4 Effects of fluvastatin on the plaque area in the aortae of rabbits. Rabbits were fed a normal diet, 1.5% cholesterol diet (HC) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\ day^{-1}$) or enalapril ($10\,mg\,kg^{-1}\ day^{-1}$) for 16 weeks. Plaque area is expressed as a percentage of the lesioned area to the total area in each sample. The values presented the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the 1.5% cholesterol diet group.

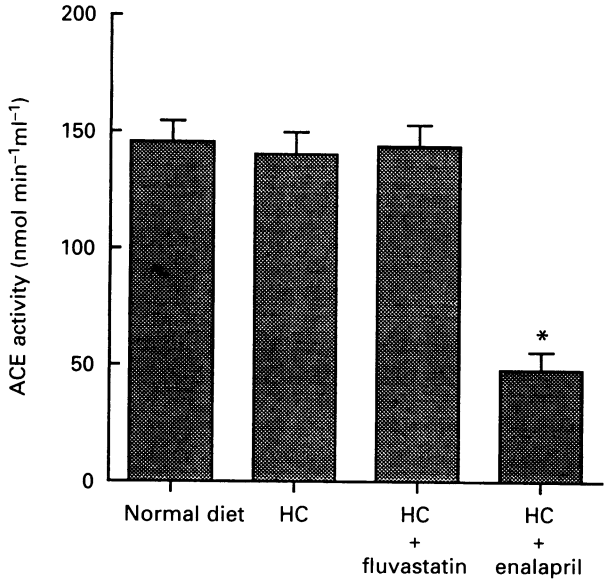

Figure 5 Effects of fluvastatin on the serum ACE activity in atherogenic diet fed rabbits. Rabbits were fed a normal diet, 1.5% cholesterol diet (HC) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\ day^{-1}$) or enalapril ($10\,mg\,kg^{-1}\ day^{-1}$) for 16 weeks. After drug treatments, the ACE activity of serum was determined by the production rate of hippuric acid from the synthetic tripeptide substrate. ACE activity is expressed as nmol min^{-1} ml^{-1}. The values presented the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the 1.5% cholesterol diet group.

In order to rule out the possibility that fluvastatin might be a potent ACE inhibitor, the effect on the vascular ACE activity *in vitro* was examined. Fluvastatin was found to have no inhibitory activity on the tissue ACE *in vitro* (Figure 7).

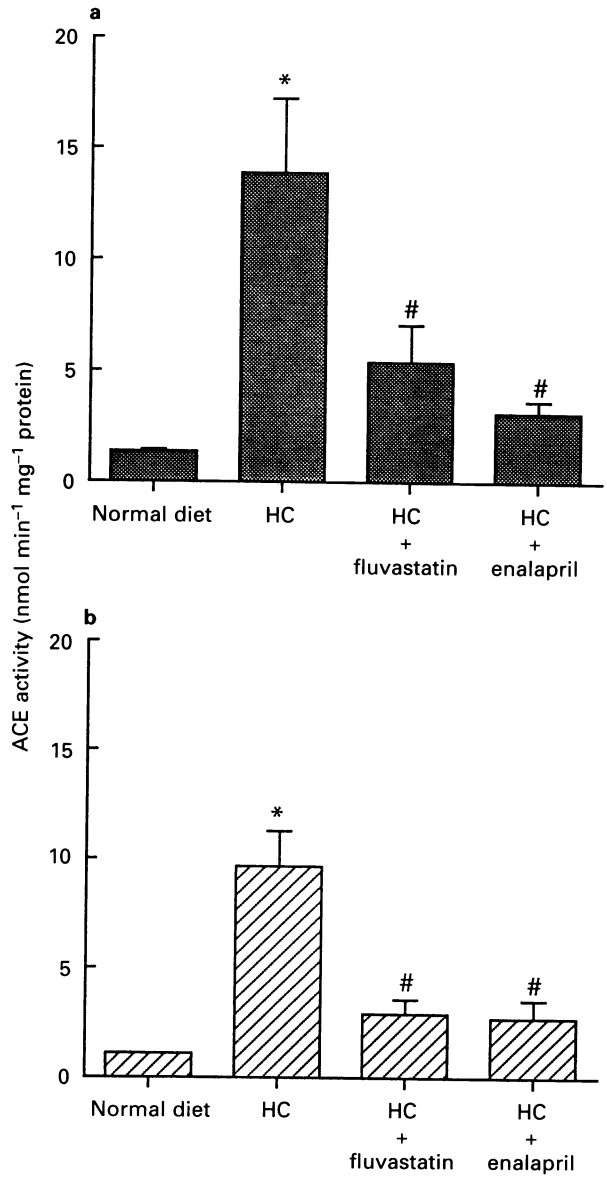

Figure 6 Effects of fluvastatin on the vascular ACE activity in rabbits. Rabbits were fed a normal diet, 1.5% cholesterol diet (HC) or cholesterol diet containing fluvastatin ($2\,mg\,kg^{-1}\ day^{-1}$) or enalapril ($10\,mg\,kg^{-1}\ day^{-1}$) for 16 weeks. ACE activity was assessed in the aortic arches (a) and upper thoracic aortae (b). After drug treatments, the ACE activity of isolated aortae was determined by the production rate of hippuric acid from the synthetic tripeptide substrate. ACE activity was expressed as nmol min^{-1} mg^{-1} protein. The values presented the mean ± s.e. from 5 to 8 animals. $*P<0.05$, significantly different from the normal diet group. $\#P<0.05$, significantly different from the 1.5% cholesterol diet group.

Discussion

Fluvastatin reduced the tissue ACE activity but not the serum ACE activity in cholesterol diet-fed rabbits. This was associated with the anti-atherosclerotic findings as shown with decrease in plaque formation. By contrast, both serum and tissue ACE activities were attenuated by enalapril with a resultant decrease in plaque formation. In a previous study, we demonstrated that hyperlipidaemia, an atherogenic factor, does not increase serum ACE in contrast to tissue ACE which is directly correlated to plaque formation (Mitani *et al.*, 1996). These results suggest that the reduction of tissue ACE activity is more closely related to the anti-atherosclerotic effect than the reduction of the serum ACE.

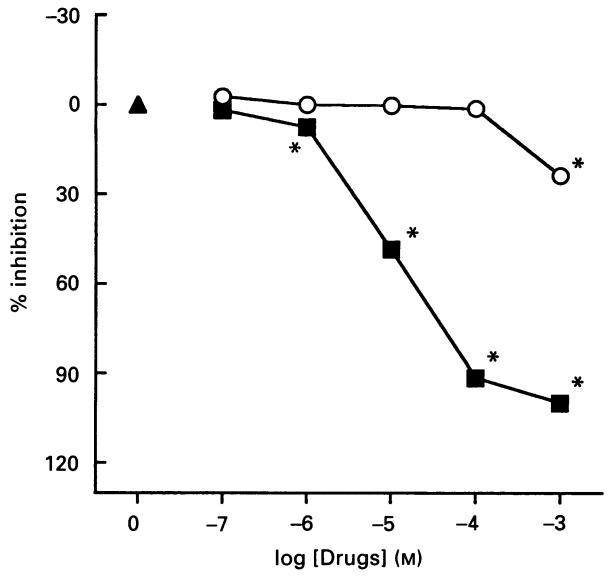

Figure 7 *In vitro* effects of fluvastatin on the ACE activity in the rabbit aortae. The ACE activity of isolated aortae was determined by the production rate of hippuric acid from the synthetic tripeptide substrate. Fluvastatin (○) and enalapril (■) were added just before the assay. Inhibitory activity was expressed as a percentage of the control activity (▲). The values presented the mean ± s.e. of 3 experiments. $*P<0.05$, significantly different from the control.

Serum lipid levels were markedly lowered by chronic treatment with fluvastatin, while no reduction was induced by enalapril as expected. Either treatment could result in less formation of plaque in the aortic surface. The reduction of vascular lesions is generally accounted for by the lipid lowering effects of fluvastatin via HMG-CoA reductase inhibition in the liver and up-regulation of hepatic LDL receptor (Corsini *et al.*, 1995a). On the other hand, vascular mechanism(s) other than mediation of systemic lipid lowering may be attributed to reduction of vascular lesions. Reduced ACE activity in the vasculature is likely to account for the suppression of plaque formation in common with two inhibitors of HMG-CoA reductase and ACE. Does fluvastatin exert a covert action directly on vascular ACE? As shown in isolated aortic tissues as well as serum ACE, there was no inhibitory effect of fluvastatin at the concentration range found in human plasma, around 0.1 to 1 μM (Plosker & Wagstaff, 1996). Enalapril did not show any lipid lowering effect. Thus, modulation of ACE in the vasculature may be another target for the anti-atherosclerotic, as presumed with findings that ACE inhibitors reduce the incidence of myocardial infarction in patients with asymptomatic left ventricular dysfunction (The SOLVD Investigators, 1992) and presumably the progression of atherosclerosis (Texter *et al.*, 1993). The key questions that remain to be addressed are what pathway for increasing vascular ACE activity is targeted by the two different inhibitors against ACE and lipids, or how is the HMG-CoA pathway linked with modulation of tissue ACE activity.

Lipid peroxides in serum were markedly reduced by fluvastatin and enalapril. Because of the lipid lowering effects, fluvastatin-induced inhibition of lipid peroxidation may be accounted for partly by the lipid lowering effects through reduced oxidized LDL. Giroux *et al.* (1993) have recently demonstrated that simvastatin, an HMG-CoA reductase inhibitor, inhibits the production of the superoxide anion in human monocyte-derived macrophages. The inhibition was presumed to be due to reduced production of isoprenoids in the mevalonate pathway and the subsequent inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity which is activated by isoprenylated guanosine triphosphate (GTP)-binding protein. In a similar fashion, fluvastatin may interrupt the production of reactive oxygen radicals in the monocyte/macrophage, or suppress their injurious actions. The inhibition of lipid peroxidation by fluvastatin may be due to the reduced accumulation of cholesterol caused by the inhibition of lipoprotein endocytosis seen in mouse peritoneal macrophages *in vitro* (Bernini *et al.*, 1995). The mechanism for enalapril-induced attenuation of lipid peroxidation remains to be determined with respect to the common characteristics of ACE or kininase II inhibitors and to the linkage between the converting enzyme and activation of NADPH oxidase. Enalapril is not capable of inhibiting free radical-induced lipid peroxidation (Mak *et al.*, 1990) or scavenging oxygen-derived free radicals (Westlin & Mullane, 1988). However, a role of angiotensin II in macrophages and LDL receptors has been proposed in which exogenously applied angiotensin II binds to LDL, enhances the uptake via scavenger receptors (Keidar *et al.*, 1996) and stimulates macrophage-mediated oxidation of LDL (Keidar *et al.*, 1995). Thus, it is speculated that tissue ACE may be attributable to oxidation in macrophages via angiotensin II.

In our preliminary studies (Niwa *et al.*, unpublished data), fluvastatin reduced the expression of lymphocyte-function associated antigen-1 (LFA-1), an adhesion molecular, in a human monocyte cell line, associated with the inhibition of leukocyte binding to human umbilical vein endothelial cells. The inhibition of leukocyte adhesion to the endothelium was also demonstrated *in vivo* with repeated administration of 6 mg kg^{-1} fluvastatin to the hypercholesterolaemic rats for 10 consecutive days, while there was no change in serum lipids (Kimura *et al.*, 1996). Thus, inhibition by fluvastatin of leukocyte adhesion, which seems independent of serum lipid modulation, may contribute to the reduction of atherogenic factors particularly in the early stage of plaque formation. The present findings for tissue ACE are derived from a long-term treatment study in which it was not clear whether reduced ACE was a consequence of attenuated expression of the adhesion molecule. Alternatively, the involvement of growth and stimulatory factors, including angiotensin II, for reduced adhesion activity cannot be ruled out.

Fluvastatin inhibits overproliferation of vascular smooth muscle cells *in vitro* and *in vivo* (Corsini *et al.*, 1995b). In our study on balloon catheterization-induced thickening of the intima in rabbits at the normal cholesterol level, fluvastatin actually elicited an inhibitory effect on the intimal thickening, which was reversed by treatment with mevalonate, without lipid lowering effects (Bandoh *et al.*, 1996). Thus, it is likely that fluvastatin reduces the excessive growth of smooth muscle cells in the intima, presumably by acting on smooth muscle cells but not indirectly via a serum lipid-lowering action. Angiotensin II formed locally in the intima stimulates the growth of the smooth muscle cells (Dzau, 1993a,b). Reduced formation of angiotensin II would account for the reduced accumulation of the smooth muscle cells in the intimal layer, although whether other cytokine or growth factors, including e.g. platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and transforming growth factor (TGF), are mediated was not determined in the present study. Alternatively, increased bradykinin by kininase II inhibition associated with ACE inhibition may suppress the cell growth via the stimulated production of nitric oxide and prostacyclin, both of which inhibit the proliferation of the smooth muscle cells (Linz *et al.*, 1995). Besides ACE or kininase II, reduced isoprenoid production may be involved in the anti-proliferative action of fluvastatin (Corsini *et al.*, 1995b), but the exact process is not yet known.

Our findings concerning the lipid and ACE profiles suggest that fluvastatin has certain advantages over enalapril in producing anti-atherosclerotic effects: first, fluvastatin has a lipid lowering effect (Davidson, 1994) which is definitely required in the treatment of coronary heart disease (Lipid Research Clinics Program, 1984). Secondly, the animal effective dose of fluvastatin is much closer to the human dose; a dose of fluvastatin (2 mg kg^{-1} day^{-1}) used was 2 to 4 times higher than

the expected clinical dose (0.5 to 1 mg kg^{-1}). No significant effects on atherosclerosis have been observed at low dosage ($\leqslant$0.1 mg kg^{-1} day^{-1}) of enalapril (Overturf *et al.*, 1986), and the effective dose of enalapril (10 mg kg^{-1} day^{-1}) required to establish an anti-atherosclerotic effect was about 60 times higher than that for clinical use (Schuh *et al.*, 1993). Recently, a large placebo-controlled study demonstrated a reduction in overall mortality in patients with both hypercholesterolaemia and established coronary heart diseases who received long term treatment with simvastatin, another HMG-CoA reductase inhibitor (Scandinavian Simvastatin Survival Study Group, 1994). These findings further implicate the potential advantage of fluvastatin in atherogenesis-related coronary heart diseases.

In conclusion, vascular ACE activity which was augmented in hyperlipidaemia was lowered by fluvastatin and enalapril. This was associated with reduced formation of atherosclerotic plaque. It seems that the reduction of vascular ACE is not coupled to lipids and ACE activity in serum, but rather to lipid peroxidation. Thus, the decrease in vascular ACE activity by fluvastatin as well as the lipid lowering effect may reduce the risk of atherosclerosis progression in the vasculature.

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