Anti-oxidative properties of fluvastatin, an HMG-CoA reductase inhibitor, contribute to prevention of atherosclerosis in cholesterol-fed rabbits

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Abstract

Studies in vitro reveal that fluvastatin, an HMG-CoA reductase inhibitor, has a strong DPPH radical scavenging activity and achieves concentration-dependent inhibition of copper- and cell-induced oxidation of low-density lipoprotein (LDL). To further examine the anti-oxidative activity of fluvastatin in vivo, we elucidated the effects of chronic treatment with fluvastatin at a dose insufficient to reduce plasma cholesterol levels (2 mg/kg per day) on vasomotion and vascular oxidative stress in thoracic aortas of 0.5% cholesterol-fed rabbits. After 12 weeks of dietary treatment, aortic segments from rabbits fed cholesterol alone showed impaired endothelium-dependent relaxation responses to acetylcholine and A23187 compared to normal chow-fed rabbits in association with a significant increase in plasma total cholesterol levels. In contrast, although plasma total cholesterol levels were not different from those in control cholesterol-fed rabbits, aortic segments from fluvastatin-treated rabbits showed normal relaxation. Compared with rabbits fed cholesterol alone, fluvastatin treatment decreased susceptibility of LDL to ex vivo copper-induced oxidation, reduced vascular superoxide generation, and atheromatous plaque formation. In conclusion, the potent anti-oxidative properties of fluvastatin in addition to its cholesterol-lowering activity appear to contribute to its anti-atherosclerotic effect in vivo. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Hypercholesterolemia has been shown to be one of the major risk factors of atherosclerosis [1,2]. Recent clinical studies have demonstrated that cholesterol-lowering therapy with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors can achieve a relatively large reduction in plasma cholesterol and they cannot only decrease morbidity and mortality of coronary artery disease in humans[3,4], but also induce regression of atherosclerosis [5]. It has been considered that these anti-atherosclerotic effects of HMG-CoA reductase inhibitors are likely to mainly result from lipid-lowering properties of these drugs.

In arteries in hypercholesterolemic animals and patients, vascular superoxide production and oxidative stress are increased [6]. Oxidation of low-density lipoprotein (LDL) is considered as an important step in the development of atherosclerosis. In vitro studies have demonstrated that oxidized LDL (ox-LDL) activates endothelial cells to increase the expression of chemotactic molecules leading to stimulate transendothelial migration of monocyte and formation of foam cells. Ox-LDL also increases production of growth factors including platelet-derived growth factor that stimulates migration and proliferation of smooth muscle cells.
Furthermore, ox-LDL impairs endothelium-derived nitric oxide (NO) production [7,8]. As reported previously, the antioxidant has the ability to prevent oxidation of LDL in vitro and in vivo. For example, dietary antioxidants such as vitamin E [9] and probucol [10] protect LDL from oxidation. In addition to inhibition of LDL oxidation, antioxidant therapy has been shown to produce beneficial effects on atherosclerosis and to prevent the progression of atherosclerosis in animal models by limiting vascular oxidative stress and superoxide production [11–13].

Endothelial dysfunction including the impaired endothelium-dependent vasodilation is observed in hypercholesterolemia and atherosclerosis. Recent studies have demonstrated that cholesterol-lowering therapy with HMG-CoA reductase inhibitors showed a beneficial effect on endothelium-dependent coronary vasodilation [14,15], whereas others failed to show such an effect [16,17]. Anderson et al. have reported that cholesterol-lowering therapy with Lovastatin, an HMG-CoA reductase inhibitor, and cholestyramine was not able to improve endothelial dysfunction; however, combination therapy with a lipid-lowering drug and an antioxidant effectively improved endothelial function [16]. In contrast to failure of Lovastatin to prevent the progression of atherosclerosis in cholesterol-fed rabbits [17], anti-oxidative drugs like probucol have been shown to have beneficial effects to prevent the development of atherosclerosis and to preserve endothelial function effectively [13].

In recent years, it becomes clear that all the clinical benefits of the HMG-CoA reductase inhibitors therapy cannot be explained solely by their lipid-lowering properties because a variety of experimental data revealed that these drugs have direct anti-atherosclerotic effects that were unrelated to the lipid-lowering effect [18–20]. However, it remains unknown that these pleiotropic effects of HMG-CoA reductase inhibitors serve to improve endothelial dysfunction in present in atherosclerosis. The aim of the present study was to examine anti-oxidative property of fluvastatin in vitro and in vivo. We investigated whether fluvastatin can preserve endothelial function and prevent atherosclerosis in cholesterol-fed rabbit model via its potent anti-oxidative property other than its hypocholesterolemic effect.

2. Methods

2.1. DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois [21]. In brief, HMG-CoA reductase inhibitors (final 160 μM) were incubated with 16 μM DPPH in acetate buffer (0.1 M, pH 5.5)/methanol, 2:3 (v/v) solution at room temperature. The absorbance at 523 nm of the tested sample solution was measured with a spectrophotometer (U-3300, Hitachi Ltd.).

2.2. Preparation of LDL

Human LDL (density, 1.019–1.063 g/ml) was isolated from the plasma of healthy volunteers by sequential ultracentrifugation as described previously [22]. The isolated LDL was dialyzed against 150 mM NaCl and 1 mM EDTA at 4°C, stored at 4°C and used within 2 weeks. Before used in the experiments, LDL was freshly dialyzed against phosphate-buffered saline (PBS) at 4°C and was sterilized by passage through a 0.22 μm filter (Acrogel). LDL protein concentrations were determined by the method of Lowry et al. [23].

2.3. Copper ion-mediated oxidation of LDL

LDL (50 μg/ml) in PBS was incubated with freshly prepared CuSO₄ (1 μM) in the presence of the indicated concentrations of fluvastatin or vehicle. After incubation at 37°C for 2 h, the extent of LDL oxidation was assessed by measuring of thiobarbituric acid-reactive substances (TBARS) formation [22].

2.4. Measurement of diene formation

LDL (50 μg/ml) in PBS was incubated with freshly prepared CuSO₄ (1 μM) at 37°C in the presence of the indicated concentrations of fluvastatin or vehicle. Diene formation was measured as the increase in absorbance at 234 nm every 5 min, monitored by a spectrophotometer (UV-1600, Shimadzu) [23]. The lag time and T_max were determined as described previously [24].

2.5. VSMC-mediated oxidation of LDL

LDL modification by cultured rat vascular smooth muscle cells (VSMC) was done as described previously [22]. Briefly, confluent VSMC at passages 9–12th were cultured with serum-free Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL) for 24 h and the medium was changed to serum-free Ham’s F-10 medium (Gibco BRL) containing 100 μg/ml LDL for 24 h at 37°C with the indicated concentrations of fluvastatin or vehicle. At the end of incubation (in a humidified incubator; 37°C, 5% CO₂), the culture medium was collected and then centrifuged (1500 × g for 10 min) to remove detached cells. The extent of LDL oxidation was assessed by measuring of TBARS formation and electrophoretic mobility of LDL [22].
2.6. Animal studies

Male Japanese White rabbits (n = 50) were divided into three groups: received normal standard chow (n = 14); normal standard chow supplemented with 0.5% cholesterol alone (n = 18); and 0.5% cholesterol with oral gavage of fluvastatin (2 mg/kg per day) (n = 18). Each treatment was continued for 12 weeks. At the end of the experimental period, all rabbits were canulated into cervical artery and blood was collected. Plasma total cholesterol and triglycerides were quantified by enzymatic methods. LDL (density, 1.019–1.063 g/ml) was isolated from the plasma by sequential ultracentrifugation [22] and diene formation was examined. LDL (50 μg/ml) in PBS was incubated with freshly prepared CuSO4 (5 μM) at 37°C and absorbance at 234 nm was monitored.

2.7. Endothelial function

Strip rings were prepared from rabbits' aortas and isometric tensions were measured as described previously [25]. In brief, aortic strips were suspended in 30-ml organ baths containing Krebs' bicarbonate buffer of following composition (mM): NaCl 118, KCl 4.0, CaCl2 1.5, MgSO4 1.2, NaH2PO4 1.2, NaHCO3 25 and glucose 5, and equilibrated at 37°C with a 95% O2–5% CO2 gas mixture. The strips were constricted with 0.3 μM phenylephrine and subsequently relaxed by cumulative additions of acetylcholine (1 nM–10 μM), sodium nitroprusside (1 nM–3 μM), or the calcium ionophore A23187 (1 nM–1 μM). Relaxation values were expressed as percent decreases of the phenylephrine-induced constrictor tone.

2.8. Vascular superoxide generation

Superoxide production was measured using lucigenin chemiluminescence as described previously [13]. Briefly, ring segments of thoracic aorta were incubated with 10 mM diethylthiocarbamate (Sigma), an inhibitor of copper zinc superoxide dismutase, for 30 min in Hepes-buffered physiologic saline solution and then gently transferred to vials containing 0.25 mM lucigenin (Sigma). Chemiluminescence values were obtained at 1-min intervals over 30 min using a luminescence reader (BLR-201, Aloka) and the counts of the last 5 min in the plateau phase were averaged. Background counts were determined from identically processed vessel-free incubations and subtracted from the readings obtained with vessels. Chemiluminescence was completely inhibited by pretreatment of rings with 10 mM Tiron (Sigma), a superoxide scavenger.

2.9. Pathological examination and histological analysis

To assess the lipid accumulation area, the aortas from cholesterol-fed and fluvastatin-treated rabbits were randomly chosen and dissected from its origin at the aortic valve to the bifurcation of the internal iliac arteries. The aortas were opened longitudinally, fixed in 10% formalin, and stained with Sudan IV.

Segments of descending thoracic aortas taken 10 mm below the bifurcation of subclavian arteries were fixed
Fig. 2. Conjugated diene formation and TBARS formation of LDL.

**A**

Absorbance at 234 nm

- 0 μM
- 1 μM
- 3 μM
- 10 μM
- 30 μM

Time (min)

**B**

TBARS (nmol MDA/mg LDL protein)

- 0
- 1
- 3
- 10
- 30

Fluvastatin (μmol/L)

* P < 0.05, ** P < 0.01 vs. control.

in 3.7% parahormaldehyde and histologic sections were prepared, followed by being stained with hematoxylin-eosin. At least three slices for each rabbit aorta were examined with a light microscope and photographed. Percent of aortic surface areas covered by atheromatous plaques was evaluated. The mean value of each aorta was used for statistics.

2.10. Statistical analysis

Data are expressed as mean ± S.E. The significance of the difference between group means was analyzed by one-way ANOVA followed by post hoc tests or two-way ANOVA (for Endothelial Function). Values of P < 0.05 were considered statistically significant.

3. Results

3.1. DPPH radical scavenging activity

First, we investigated the free radical scavenging activities of various HMG-CoA reductase inhibitors. DPPH is a very stable free radical and widely used for evaluation of anti-oxidative activities [21]. Probucol [10] and four HMG-CoA reductase inhibitors, fluvastatin, lovastatin, pravastatin, and simvastatin were tested. As shown in Fig. 1, probucol showed a very rapid and large decrease in the absorbance of DPPH solution, indicating that probucol has a potent anti-oxidative activity. Similarly, fluvastatin showed the strong free radical scavenging activity, whereas other tested HMG-CoA reductase inhibitors yielded only very weak effects. Similar results that fluvastatin has an anti-oxidative activity were confirmed in electron spin resonance experiments using DPPH or active oxygen species (H. Nishi., unpublished observations).

3.2. Copper ion-mediated oxidation of LDL

The effects of HMG-CoA reductase inhibitors, fluvastatin, pravastatin, and simvastatin on copper ion-mediated oxidation of LDL were studied. The formation of conjugated dienes during copper ion-mediated oxidation, which represents the early peroxidative changes in the lipids of LDL, were determined by measuring changes in absorbance at 234 nm. At the concentration of 10 μM, fluvastatin decreased the rate of increases in absorbance at 234 nm, while neither pravastatin nor simvastatin did (data not shown). Fluvastatin significantly increased $T_{\text{max}}$ by 48 min (P < 0.05 vs. control), whereas pravastatin and simvastatin did not show such an effect (14 and 8 min, respectively, $P = \text{NS}$). Fluvastatin-induced increases in the lag time and $T_{\text{max}}$ were concentration-dependent (Fig. 2A). We also estimated the effect of fluvastatin on the formation of TBARS of LDL incubated with copper ion. Fluvastatin inhibited the formation of TBARS in a concentration-dependent manner (Fig. 2B).

3.3. VSMC-mediated oxidation of LDL

Since intimal cells are known to cause LDL oxidation, we investigated the ability of fluvastatin to affect
Fig. 3. Effects of fluvastatin on VSMC-mediated oxidation of LDL as measured by TBARS formation (A) and electrophoretic mobilities (B). LDL (100 μg/ml) was incubated with cultured rat VSMC in the presence of the indicated concentrations of fluvastatin or vehicle. After incubation for 24 h, TBARS formation and electrophoretic mobilities of LDLs were measured. Data represent means ± S.E. of four independent experiments. * P<0.05, ** P<0.01 vs. control.

Table 1
Body weights and plasma lipid profiles in rabbits after 12 weeks of experimental diets

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Body weight (kg)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
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<tr>
<td>Normal</td>
<td>14</td>
<td>3.43 ± 0.06</td>
<td>13 ± 2</td>
<td>68 ± 8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>18</td>
<td>3.15 ± 0.05</td>
<td>1130 ± 114*</td>
<td>98 ± 13</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>18</td>
<td>3.10 ± 0.06**</td>
<td>970 ± 120*</td>
<td>81 ± 15</td>
</tr>
</tbody>
</table>

* Body weights and plasma lipid profiles in rabbits received normal diet (normal), 0.5% cholesterol (cholesterol), and 0.5% cholesterol with fluvastatin (fluvastatin) for 12 weeks are shown. Values represent the mean ± S.E.
* * P<0.001,
** P<0.005 vs. normal.

cell-mediated oxidation of LDL. For this purpose, LDL was incubated with cultured rat VSMC with increasing amounts of fluvastatin. In this model, oxidation of LDL is mainly mediated by superoxide from VSMC [22,26]. Fluvastatin inhibited the VSMC-mediated formation of TBARS (Fig. 3A) and the increases in electrophoretic mobilities of LDLs (Fig. 3B), which reflected increases in their negative charges.

3.4. Plasma lipid level and body weight

To determine whether the anti-oxidative effect of fluvastatin is observed in vivo and whether the anti-oxidative property of the drug can prevent the atherosclerosis, we examined the effect of administration of fluvastatin to cholesterol-fed rabbits. In this study, to exclude the possibility that lipid-lowering property of fluvastatin may prevent the susceptibility of LDL to oxidation through the reduction of vascular oxidative stress [27], we employed fluvastatin at a dose insufficient to reduce plasma cholesterol levels. Total cholesterol and triglyceride levels in plasma and the body weights are shown in Table 1. Plasma cholesterol levels in 0.5% cholesterol-fed rabbits were significantly increased compared with those in normal-diet rabbits. Similarly, fluvastatin-treated rabbits showed a significant increase in plasma cholesterol that was not statistically different from cholesterol-fed rabbits. Body weights of rabbits at the start of the experiment were similar among three groups and there were no significant differences in weight gains between cholesterol-fed and fluvastatin-treated rabbits.

Table 2
LDL susceptibility to ex vivo oxidation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Lag time (min)</th>
<th>T_{max} (min)</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>18</td>
<td>70.7 ± 3.5</td>
<td>178.7 ± 8.2</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>18</td>
<td>84.7 ± 5.3*</td>
<td>201.1 ± 8.7</td>
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</tbody>
</table>

* LDL was isolated from the plasma of rabbits received 0.5% cholesterol (cholesterol) and 0.5% cholesterol with fluvastatin (fluvastatin). LDL (100 μg/ml) in PBS was incubated with CuSO₄ (5 μM) and both lag time and \( T_{max} \) were measured. Values represent the mean ± S.E.
* * P<0.05 vs cholesterol.
3.5. Lag time

As shown in Table 2, LDL isolated from fluvastatin-treated rabbits showed to be resistant to susceptibility to ex vivo copper ion-induced oxidation as assessed by a lag phase ($P < 0.05$ vs. cholesterol-fed rabbits). $T_{\text{max}}$ in fluvastatin-treated rabbits showed a tendency to be increased, but not statistically different from that in cholesterol-fed rabbits ($P = 0.069$). Since plasma cholesterol levels were not different between cholesterol-fed and fluvastatin-treated rabbits (Table 1), this result suggests that fluvastatin has an anti-oxidative property in vivo.

3.6. Endothelial function

The contractile responses in aortic rings to 40 mM KCl and 0.3 μM phenylephrine were similar between three groups (Table 3). As shown in Fig. 4A and B, aortic rings from cholesterol-fed rabbits showed significantly impaired endothelium-dependent relaxation responses to acetylcholine ($P < 0.01$ vs. normal-diet...
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Fig. 5. Effects of fluvastatin administration on vascular superoxide generation in thoracic aortas of cholesterol-fed rabbits. Aortic rings from rabbits fed normal diet (normal, n = 14), 0.5% cholesterol-diet (cholesterol, n = 14), or 0.5% cholesterol-diet and fluvastatin (cholesterol, n = 14) were examined. Vascular superoxide generation was estimated by lucigenin chemiluminescence as described in Section 2. Data are expressed as means ± S.E. * P < 0.05 vs. normal.

rabbits) and A2387 (P < 0.01 vs. normal-diet rabbits). In contrast, aortic rings from fluvastatin-treated group showed normal responses to both acetylcholine and A23187 (P = NS vs normal-diet rabbits). Endothelium-independent relaxations in response to sodium nitroprusside were not different among three groups (Fig. 4C).

3.7. Vascular superoxide generation

As reported previously, vascular superoxide production is increased in arteries in hypercholesterolemic rabbits [5,27]. As shown in Fig. 5, chemiluminescence in aortic rings showed a 1.6-fold increase in cholesterol-fed rabbits compared with normal-diet rabbits (P < 0.05). In contrast, treatment with fluvastatin prevented increases in superoxide production. In some experiments, superoxide generation was measured in aortic segments whose endothelium was removed. In cholesterol-fed rabbits, superoxide production was decreased by removal of the endothelium, while in both normal and fluvastatin-treated rabbits superoxide production was increased by removal of the endothelium (data not shown), which is consistent with the previous report [27].

3.8. Histology

To assess the lipid accumulation area, randomly chosen aortas from cholesterol-fed (n = 4) and fluvastatin-treated rabbits (n = 4) were dissected from its origin at the aortic valve to the bifurcation of the internal iliac arteries and stained with Sudan IV. Representative results were shown in Fig. 6A. Fluvastatin prevented the plaque area formation particularly in thoracic descending aortas and abdominal aortas around the origins of abdominal branches. We also examined the quantitative analysis of plaque area using the cross sectional area of aortic segment taken 10 mm below the bifurcation of subclavian artery in cholesterol-fed rabbits (n = 14) and fluvastatin-treated rabbits (n = 14) including those used for vascular reactivity. In cholesterol-fed rabbits, intimal plaque area was seen in 38.3 ± 4.4% in surface area of aortas (Fig. 6B). In contrast, fluvastatin prevented the intimal plaque formation to 14.5 ± 5.2% (P < 0.005 vs. cholesterol-fed rabbits).

4. Discussion

The present in vitro study shows that fluvastatin has anti-oxidative activities as demonstrated that this drug has a radical scavenging activity and is capable of inhibiting copper ion- and VSMC-mediated oxidation of LDL. These anti-oxidative effects of fluvastatin were observed at therapeutic concentrations [28]. Although previous studies have shown that HMG-CoA reductase inhibitors, lovastatin [29], pravastatin [30], or simvastatin [31], also have inhibitory effects on LDL oxidation, these drugs were not demonstrated to have radical scavenging activities and direct anti-oxidative effects on LDL oxidation in vitro at therapeutic concentrations. Inhibitory effects of these drugs on LDL oxidation were suggested to be caused mainly by increased removal of aged plasma LDL as a result of up-regulation of LDL receptor. Aviram and colleagues have demonstrated that fluvastatin exerted its anti-oxidative effect as a result of binding to LDL [32]. Fluvastatin bound mainly to the LDL surface phospholipids, but not to the LDL apoB-100, and resulted in the prevention of diffusion of free radicals into the lipoprotein core. They also described that inhibitory effects of fluvastatin on LDL oxidation were not due to binding of copper ions or free radical scavenging because of its inability to reduce linoleic acid peroxidation. However, we have confirmed that fluvastatin has a radical scavenging activity (Fig. 1). We cannot clearly explain this discrepancy at present.

In the present animal experiments, we demonstrated that administration of fluvastatin to cholesterol-fed rabbits prevented progression of atherosclerosis assessed by plaque formation. Fluvastatin prevented impairment of endothelium-dependent relaxation, as well as an increase in superoxide production in thoracic aortas from cholesterol-fed rabbits in association with an increase in resistance to ex vivo oxidation of LDL. Anti-atherosclerotic effects of fluvastatin demonstrated here
appear not to be mediated by the lipid-lowering property of this drug. Fluvastatin at the dose in this protocol (2 mg/kg per day) did not reduce plasma lipids, although fluvastatin at the concentration of 12.5–50 mg/kg per day has been shown to effectively reduce plasma lipids in Watanabe heritable hyperlipidemic rabbits [33]. Fluvastatin treatment increased resistance to susceptibility of ex vivo oxidation of LDL in cholesterol-fed rabbits, which indicates that fluvastatin acts as an antioxidant in vivo. The reduced endothelium-dependent relaxation in hypercholesterolemia is in part due to the increased production of superoxide, which can inactivate NO [6,27]. NO has been considered as an anti-atherosclerotic molecule to inhibit LDL oxidation [22,34], chemotaxis of VSMC [35], and platelet aggregation [36], leading to prevention of atheromatous plaque. Therefore, reduced NO activity may enhance progression of atherosclerosis in hypercholesterolemia.

Besides their lipid-lowering properties, HMG-CoA reductase inhibitors have the ability to modulate other cellular functions. For example, fluvastatin inhibits VSMC proliferation [18] and pravastatin reduces monocyte chemotaxis [37]. Lovastatin, simvastatin, and fluvastatin can prevent in vivo carotid intimal thickening in rabbits [20]. Recently, Liao and colleagues have shown that simvastatin and lovastatin increase expressions of mRNA and protein of endothelial NO synthase in cultured endothelial cells and prevent hypoxia- or ox-LDL-induced decrease in these expression levels [38,39]. Other report has shown that atrovastatin and simvastatin inhibit pre-pro endothelin-1 mRNA expression and endothelin-1 synthesis in cultured endothelial cells [40]. These investigations have prompted us to assume that the anti-atherosclerotic effect of fluvastatin observed in the present study was mediated by up-regulation of endothelial NO synthase and down-regulation of endothelin-1. Interestingly, a recent study showed that fluvastatin up-regulated endothelial NO synthase mRNA in aortas of high fat diet-treated rabbits. Therefore it is possible that the anti-atherosclerotic effects of fluvastatin was partly mediated by its effect on those gene expressions.

Fig. 6. Effects of fluvastatin administration on atheromatous plaque formation in thoracic aortas of cholesterol-fed rabbits. (A) Representative aortas from rabbits fed 0.5% cholesterol-diet (right, two) or 0.5% cholesterol-diet and fluvastatin (left, two) were dissected from its origin at the aortic valve to the bifurcation of the internal iliac arteries and stained with Sudan IV. (B) Aortic rings from rabbits fed 0.5% cholesterol-diet (cholesterol, n = 14), or 0.5% cholesterol-diet and fluvastatin (cholesterol, n = 14) were stained with hematoxylin-eosin. Percent of aortic surface areas covered by plaque in the cross sectional aortic segments taken 10 mm below the bifurcation of subclavian artery was shown. At least three slices were taken in each animals and mean value was used for statistics. Values are expressed as means ± S.E. * P < 0.005 vs. cholesterol.
In the present study, fluvastatin prevented the impairment of endothelial-dependent relaxation in cholesterol-fed rabbits. It is likely that the inhibition of superoxide generation by fluvastatin prevented NO inactivation by superoxide and resulted in prevention of the impaired endothelial-dependent relaxation. Another possible mechanism is the inhibition of ox-LDL and lysophosphatidylcholine formation in the arterial wall. We [41,42] and others [43,44] have demonstrated that ox-LDL and lysophosphatidylcholine inhibit endothelial-dependent relaxation by interfering with receptor-mediated signaling to activation of endothelial NO synthase. We did not measure ox-LDL formation directly, however, the hypothesis that fluvastatin prevented ox-LDL formation in the aortas in cholesterol-fed rabbits is strongly implicated in the following findings: (1) fluvastatin inhibited both copper- and cell-mediated LDL oxidation in vitro, (2) fluvastatin also prevented susceptibility of LDL oxidation ex vivo. These actions of fluvastatin are consistent with those of probucol on atherosclerotic arteries as reported previously [13]. Therefore fluvastatin can inhibit progression of atherosclerosis and improves endothelial dysfunction by the effects independent of its lipid-lowering effect. We demonstrated here the unique anti-oxidative property of fluvastatin. The present results suggest that anti-oxidative properties of fluvastatin are likely involved in the mechanisms of its beneficial effect on atherosclerosis, although other properties common to HMG CoA reductase inhibitors such as up-regulation of endothelial NO synthase and down-regulation of endothelin-1 may play some roles. The participation of these effects on anti-atherosclerotic property of fluvastatin will be clarified in the future studies.

The Lipoprotein and Coronary Atherosclerosis Study (LCAS) [45], that investigated the progression and regression of coronary stenosis by fluvastatin therapy and assessed by quantitative coronary angiography, showed greater angiographic improvement compared with other previously published angiographic trials that achieved a greater reduction in LDL cholesterol than LCAS. This result suggests that there is a possibility that other mechanisms besides lipid-lowering effect of fluvastatin contributes to the greater angiographic benefit in LCAS. Our findings provide the clue that an anti-oxidative effect of fluvastatin may be related to the greater angiographic benefit in LCAS.

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