Effects of Fluvastatin and Its Major Metabolites on Low-Density Lipoprotein Oxidation and Cholesterol Esterification in Macrophages

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ABSTRACT—We investigated effects of fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, and its major metabolites, M2 and M4, on CuSO₄-induced low-density lipoprotein (LDL) oxidation and cholesteryl ester accumulation in mouse peritoneal macrophages. All the test compounds inhibited LDL oxidation, and M2 had the most potent effect comparable to vitamin E. When LDL was previously incubated with the test compounds in the presence of CuSO₄, the pre-treatment resulted in a marked reduction of facilitated cholesteryl ester accumulation in macrophages. Supplementation of mevalonate did not overcome the inhibitory effects of fluvastatin and its metabolites on both LDL oxidation and facilitated cholesterol esterification. Pravastatin, another HMG-CoA reductase inhibitor, did not show any inhibitory effect. Consequently, these effects of fluvastatin and its metabolites are considered to be derived from their own unique chemical structures. Moreover, fluvastatin and M2 directly inhibited cholesterol esterification induced by oxidized LDL in macrophages, but pravastatin was also found to have a weak effect. As their inhibitory effects were overcome by addition of mevalonate, the direct inhibitory effect on cholesterol esterification would be a common property of HMG-CoA reductase inhibitors. The inhibitory effects of fluvastatin and its metabolites on both LDL oxidation and cholesterol esterification in macrophages may contribute to the antiatherogenic action in vivo.

Keywords: HMG-CoA reductase, Macrophage, Oxidized low-density lipoprotein, Cholesteryl ester, Antioxidant

The oxidative modification of low-density lipoprotein (LDL) and foam cell formation of macrophages through degradation of the oxidized LDL via the scavenger receptors in the arterial wall play important roles in the development and progression of atherosclerotic lesions (1). Impaired removal of plasma LDL via the LDL receptor in the liver results in the sustained presence of LDL in plasma for a long period of time and thus could contribute to its enhanced oxidative modification. Macrophages, which differentiated from monocytes in the subendothelial space, degrade oxidized LDL via the scavenger receptor (2). As the degradation has no negative feedback inhibition mechanism, macrophages accumulate a large amount of cholesteryl ester and turn into foam cells. Therefore, regulation of plasma lipids should be one of the most important therapies to prevent the progression of atherosclerosis (3). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors lower plasma cholesterol levels and various mega studies demonstrate that lowering plasma cholesterol results in prevention of coronary heart disease as a clinical end point (4, 5). Fluvastatin is the first synthetic HMG-CoA reductase inhibitor (6, 7). The Lipoprotein and Coronary Atherosclerosis Study (LCAS) has demonstrated that the lipid-regulating therapy with fluvastatin reduced both the progression of coronary atherosclerosis and new coronary lesion formation in patients with coronary heart disease accompanied by mildly to moderately elevated plasma LDL cholesterol (8). Moreover, Hussein et al. have recently reported the inhibitory effect of fluvastatin on the susceptibility of LDL to oxidation (9). We also have reported the inhibitory effects of fluvastatin and its human metabolites on the susceptibility of LDL to oxidation by measuring conjugated diene formation in vitro (10, 11). On the other hand, mevalonate is not only a precursor of cholesterol, but also of a number of non-steroidal isoprenoid compounds essential for normal cellular activity, so that the inhibition of HMG-CoA reductase might have potential pleiotropic effects (12).

There are some reports that antioxidants demonstrate...
protective effects on LDL oxidation in vitro and show anti-atherogenic effects in animal models (13). However, there are no reports that show clear evidence that the inhibition of LDL oxidation is directly involved in the antiatherogenic effect. In the present study, we investigated the inhibitory effects of fluvastatin and its major human metabolites, M2 and M4 (14) (Fig. 1), on both the oxidation of human LDL in vitro and oxidized LDL-induced cholesteryl ester formation in mouse peritoneal macrophages.

MATERIALS AND METHODS

Chemicals
Fluvastatin sodium, metabolites of fluvastatin, M2 and M4, and pravastatin were donated by Tsukuba Research Institute, Novartis Pharma Co. Vitamin E and mevalonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [1-14C]Oleic acid (51 Ci/mM) and [3H]cholesteryl oleate (71 Ci/mM) were purchased from NEN™ Life Science Products, Inc. (Boston, MA, USA). The bicinechoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA). Other chemicals were the best grade available from commercial sources.

Lipoproteins and their oxidative modification
Human LDL (d = 1.019 – 1.063 g/ml) was isolated by sequential ultracentrifugation (15) of fresh EDTA-treated plasma obtained from healthy male volunteers. Before use, LDL was dialyzed overnight at 4°C against phosphate-buffered saline (PBS) (pH 7.4) to remove EDTA and then filtered through a 0.22 μm filter (Millipore Corporation, Bedford, MA, USA). The protein concentration of LDL was determined with the BCA protein assay kit by using bovine serum albumin as a standard and was adjusted to 100 μg/ml with PBS (pH 7.4). Oxidized LDL was prepared by incubation of LDL with 5 μM CuSO4 at 37°C for 100 min.

Cell culture
The experimental protocol was approved by the Animal Experimentation Ethics Committee of our company. Peritoneal macrophages were obtained by peritoneal lavage from mice (BALB/c; SLC, Shizuoka) 4 days after intraperitoneal injection of thioglycollate (Eiken Chemical Co., Tokyo). Cells (1 × 10⁶/22 mm dish) were plated in each well of 12-well tissue culture plates (22-mm diameter; Ibaki Glass, Chiba) in Dulbecco’s modified Eagle’s medium (DMEM) (Nissui Seiyaku, Tokyo) containing 10% heat-inactivated fetal bovine serum (JRH Bioscience, Woodland, CA, USA), 100 μg/ml streptomycin, 100 units/ml penicillin and 589 μg/ml glutamine (Gibco BRL, Grand Island, NY, USA). After 3 h, the dishes were washed with PBS to eliminate unattached cells, the medium was changed to DMEM containing 10% lipoprotein-deficient serum (Sigma Chemical Co.). Macrophages were confirmed by morphological features of mononuclear cells on Giemsa staining, and the cells prepared contained more than 95% macrophages. The cultured cells were used for the experiments described below, and all cellular experiments were performed at 37°C in a humidified atmosphere of 5% CO₂ in air.

Determination of cholesterol esterification
Cholesterol esterification was evaluated by measuring the incorporation of [1-14C]oleic acid into cholesteryl ester in the whole cells by the method of Tabas et al. (16). Briefly, cells were incubated for 24 h in a medium containing 10% lipoprotein-deficient serum and the test compound under investigation and further incubated for 18 h in the same fresh medium containing 50 μg of oxidized LDL protein/ml and the test compound. We confirmed that the concentration of oxidized LDL used was not toxic to macrophages. The rate of cholesterol esterification was measured by determining incorporation of radioactivity into cellular cholesteryl esters after the addition of [1-14C]oleic acid-albumin complex during the last 2 h of incubation. To evaluate the cellular esterified cholesterol, the incubation medium was discarded, the cells were washed with PBS and the lipids were extracted with hexane : isopropanol (3:2). Esterified cholesterol were partitioned by thin-layer chromatography (hexane/ethyl acetate/acetic acid, 85:15:4 by volume). Radioactivity of the spot was determined by
liquid scintillation counting. $[^{14}{}H]$Cholesteryl oleate was added as an internal standard during the extraction procedure. Analysis of samples was run in duplicate.

*CuSO$_4$-induced LDL oxidation and cholesteryl ester formation in macrophages*

LDL (100 $\mu$g/ml) in PBS (pH 7.4) was incubated with CuSO$_4$ at 37°C for 100 min in the absence (control group) or presence of the indicated concentrations of test compounds. In every experiment, the exact terminal point of oxidation reaction was determined as follows: The kinetics of LDL oxidation was continuously monitored by measuring the increase in the absorbance at 234 nm as the index of conjugated diene formation (17). At the point where the absorbance of the control group reached the maximum, the oxidative reaction of LDL was stopped by the addition of 1 mM EDTA and cooling. Then, the LDL solution was concentrated about tenfold using Centricon (Millipore Corporation), and the concentration of LDL was determined. The migration of LDL was assessed by agarose gel electrophoresis using an electrophoresis system (Helena Laboratories, Saitama). The gel was stained with Fat Red 7B to develop the lipoprotein bands, and the mobility of LDL was determined by measuring the distance of migration from the point of application (origin) toward the anode. The extent of lipid peroxidation was measured as thiobarbituric acid-reactive substances (TBARS) by the method of Uchiyama and Mihara (18) with some modification, and the results were given in malondialdehyde equivalents per mg of LDL protein.

Mouse peritoneal macrophages were obtained by peritoneal lavage from mice 4 days after intraperitoneal injection of thioglycollate. Cells were plated in each well of 12-well tissue culture plates in DMEM containing 10% heat-inactivated fetal bovine serum, 100 $\mu$g/ml streptomycin, 100 units/ml penicillin and 589 $\mu$g/ml glutamine. After 3 h, the dishes were washed with PBS to eliminate unattached cells, and the medium was changed to DMEM containing 10% lipoprotein-deficient serum. Then, each LDL solution was added to the mouse peritoneal macrophages (final concentration: 50 $\mu$g LDL protein/ml) and incubated for 18 h. The rate of cholesterol esterification was measured by determining incorporation of radioactivity into cellular cholesteryl esters after the addition of [1-$^{14}$C]oleic acid-albumin complex during the last 2 h of incubation. To evaluate the cellular esterified cholesterol, the incubation medium was discarded, the cells were washed with PBS, and the lipids were extracted with hexane:isopropanol (3:2). Esterified cholesterol was partitioned by thin-layer chromatography (hexane/ethyl acetate/acetic acid, 85:15:4 by volume). Radioactivity of the spot was determined by liquid scintillation counting. $[^{14}{}H]$Cholesteryl oleate was added as an internal standard during the extraction procedure. Analysis of samples was run in duplicate.

**Statistical analyses**

Results were expressed as means ± S.E.M. of three to six independent experiments. Statistical comparisons among the groups were carried out by Dunnett’s multiple comparison following a complete randomized block design. A probability level of <0.05 was considered to be significant. Inhibitory concentration 50% (IC$_{50}$) value was calculated using the GraphPad Prism 2.0 software computer program (GraphPad Software).

**RESULTS**

**Effects of fluvastatin, M2 and M4 on LDL oxidation**

The inhibitory effects of fluvastatin, M2 and M4 on CuSO$_4$-induced LDL oxidation were first evaluated by determining the change in mobility of LDL on agarose gel electrophoresis. The data of electrophoresis of LDL after incubation with CuSO$_4$ in the presence or absence of the tested compounds are shown in Table 1. Upon incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.M. (cm)</th>
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<tbody>
<tr>
<td>Native LDL</td>
<td>0.89 ± 0.10</td>
</tr>
<tr>
<td>Control</td>
<td>1.48 ± 0.21**</td>
</tr>
<tr>
<td>FV (1 $\mu$M)</td>
<td>1.47 ± 0.20</td>
</tr>
<tr>
<td>FV (3 $\mu$M)</td>
<td>1.47 ± 0.20</td>
</tr>
<tr>
<td>FV (10 $\mu$M)</td>
<td>1.38 ± 0.19</td>
</tr>
<tr>
<td>FV (30 $\mu$M)</td>
<td>1.22 ± 0.16**</td>
</tr>
<tr>
<td>V.E (3 $\mu$M)</td>
<td>1.12 ± 0.15**</td>
</tr>
</tbody>
</table>

| Native LDL | 0.93 ± 0.15 |
| Control | 1.59 ± 0.26** |
| M2 (0.3 $\mu$M) | 1.60 ± 0.27 |
| M2 (1 $\mu$M) | 1.57 ± 0.27 |
| M2 (3 $\mu$M) | 1.17 ± 0.21** |
| V.E (3 $\mu$M) | 1.26 ± 0.21** |

| Native LDL | 0.87 ± 0.13 |
| Control | 1.46 ± 0.20** |
| M4 (1 $\mu$M) | 1.43 ± 0.19 |
| M4 (3 $\mu$M) | 1.34 ± 0.18 |
| M4 (10 $\mu$M) | 1.21 ± 0.17** |
| M4 (30 $\mu$M) | 1.06 ± 0.14** |
| V.E (3 $\mu$M) | 1.07 ± 0.12** |

LDL was incubated with CuSO$_4$ (5 $\mu$M) at 37°C for 100 min in the presence or absence of the indicated concentrations of tested compounds. Values are the mean ± S.E.M. of 3 – 6 separate experiments. **P<0.01: statistically significant compared with native LDL group (Student’s t-test); ††P<0.01: statistically significant compared with the control group (Dunnett’s multiple comparisons test). FV: fluvastatin, PV: pravastatin, V.E: vitamin E.
with CuSO₄, LDL became more negatively charged and migrated faster toward the anode from the origin compared to native LDL. Fluvastatin, M2 and M4 prevented the CuSO₄-induced increase in electrophoretic mobility of LDL in a concentration-dependent manner. The potency of M2 was greatest among the three compounds, and the magnitude of its effect was almost equal to that of vitamin E (3 mg/ml), a well-known antioxidant. M4 was more potent than fluvastatin in this respect.

Table 2 shows the comparative effects of fluvastatin and pravastatin on the mean migration distance of LDL from the origin on agarose gel electrophoresis. Whereas fluvastatin (30 mg/ml) apparently prevented the CuSO₄-induced increase in electrophoretic mobility of LDL, the same concentration of pravastatin did not show any preventive effect. The presence of excess mevalonate did not affect the inhibitory effect of fluvastatin.

Next, we measured the extent of lipid peroxidation by the thiobarbituric acid method. The data presented in Fig. 2 show the inhibitory effects of the test compounds on CuSO₄-induced LDL oxidation. The value of TBARS markedly increased after incubation with CuSO₄ (Fig. 2B). Fluvastatin (30 μM) significantly inhibited the increase of TBARS and the effect of fluvastatin was as potent as that of 3 μM vitamin E. Fluvastatin, M2 and M4 significantly inhibited the increase of TBARS in a concentration-dependent manner (Fig. 2A). The inhibitory effect of M2 was the most potent and its potency was almost equal to that of vitamin E (the value of the vitamin E (3 μM)-treated group was 46.61 ± 3.31% of the control). The presence of excess mevalonate did not affect the inhibitory effect of fluvastatin (Fig. 2B).

No inhibitory effect by pravastatin was observed even at the concentration of 30 μM. These results are also consistent with the electrophoretic mobility assay of LDL (Tables 1 and 2).

![Image](image-url)

**Table 2.** Effects of test compounds on agarose gel electrophoretic mobility of LDL incubated with CuSO₄

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± S.E.M. (cm)</th>
</tr>
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<tbody>
<tr>
<td>Native LDL</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>Control</td>
<td>1.72 ± 0.14**</td>
</tr>
<tr>
<td>FV (30 μM)</td>
<td>1.47 ± 0.08†</td>
</tr>
<tr>
<td>PV (30 μM)</td>
<td>1.75 ± 0.13</td>
</tr>
<tr>
<td>FV (30 μM) + Mevalonate (100 μM)</td>
<td>1.43 ± 0.09</td>
</tr>
<tr>
<td>V.E (3 μM)</td>
<td>1.30 ± 0.09††</td>
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</table>

LDL was incubated with CuSO₄ (5 μM) at 37°C for 100 min in the presence or absence of the indicated concentrations of tested compounds. Values are the mean ± S.E.M. of 3 separate experiments. **P<0.01: statistically significant compared with native LDL group (Student’s t-test); †P<0.05; ††P<0.01: statistically significant compared with the control group (Dunnett’s multiple comparisons test). FV: fluvastatin, PV: pravastatin, V.E: vitamin E.

![Image](image-url)

Fig. 2. Effects of test compounds on the production of thiobarbituric acid-reactive substances (TBARS) in LDL during incubation with 5 μM CuSO₄. A) TBARS value of control was 15.43 ± 1.13 nmol malondialdehyde/mg LDL protein. Data are expressed as the mean ± S.E.M. of 5–6 separate experiments. B) Data are expressed as the mean ± S.E.M. of 3 separate experiments. *P<0.05, **P<0.01: statistically different compared with the control group (Dunnett’s multiple comparisons test); ††P<0.01: statistically different compared with the native LDL group (Student’s t-test). C: Control, FV: fluvastatin, PV: pravastatin, V.E: vitamin E.

Inhibitory effects of fluvastatin, M2 and M4 on the formation of cholesteryl esters in macrophages through inhibition of LDL oxidation

We investigated the cholesteryl ester formation in mouse peritoneal macrophages induced by native LDL or LDL, which was previously incubated with CuSO₄ in the pre-
Fluvastatin Inhibits Foam Cell Formation

As shown in Fig. 3B, a marked increase of cholesteryl ester formation was observed by the addition of LDL, which was previously incubated with CuSO₄. Fluvastatin, M2 and M4 inhibited the rates of cholesteryl ester formation in mouse peritoneal macrophages induced by LDL treated with these compounds in concentration-dependent manners (Fig. 3A). The inhibitory effect of M2 was the most potent and its potency was equal to that of vitamin E (the value of the vitamin E (3 μM)-treated group was 29.21 ± 2.91% of the control). The inhibitory effect was not observed in LDL treated with pravastatin (Fig. 3B). The inhibitory effect of fluvastatin was not affected by the presence of mevalonate. These findings were consistent with the inhibitory effects of the test compounds on the production of TBARS in LDL during incubation with CuSO₄.

Effects of fluvastatin, M2 and M4 on the oxidized LDL-induced accumulation of cholesteryl esters in mouse peritoneal macrophages

The direct effect of fluvastatin on the accumulation of cholesteryl esters in mouse peritoneal macrophages induced by oxidized LDL is shown in Fig. 4. Fluvastatin reduced the oxidized LDL-induced cholesteryl ester formation in a concentration-dependent manner. The inhibitory effect of fluvastatin was statistically significant even at the lowest concentration, 0.03 μM (P<0.05), and its IC₅₀ value was 0.09 μM. M2 also inhibited the oxidized LDL-induced cholesteryl ester formation in macrophages, but its potency was lower than that of fluvastatin, and its IC₅₀ value was 2.57 μM. M4 did not inhibit the oxidized LDL-induced cholesteryl ester formation in macrophages even at 30 μM.

In order to evaluate whether the direct inhibitory effect of fluvastatin on cholesteryl ester formation depends on its unique property or a property common to HMG-CoA reductase inhibitors, we examined the inhibitory effects of fluvastatin and pravastatin and their reversal by mevalonate. As shown in Fig. 4B, under the conditions in which fluvastatin evidently reduced the cholesteryl ester formation induced by oxidized LDL at a concentration of 1 μM, pravastatin only slightly, and not significantly, reduced the cholesteryl ester formation even at a concentration of 10 μM. Furthermore, the inhibitory effects of fluvastatin and pravastatin were both overcome by simultaneous addition of exogenous mevalonate. The inhibitory effect of M2 on the oxidized LDL-induced cholesteryl ester formation was also overcome by the presence of mevalonate (data not shown). Vitamin E did not show any inhibitory effect on the oxidized LDL-induced cholesteryl ester formation.

DISCUSSION

We investigated the inhibitory effects of fluvastatin and its metabolites, M2 and M4, on CuSO₄-induced LDL oxidation. We showed that these compounds prevented the CuSO₄-induced increase in electrophoretic mobility of LDL and inhibited also the increase of TBARS values both in a concentration-dependent manner. Although it was an acellular condition, we confirmed that the inhibitory
effects of fluvastatin were not affected by the presence of mevalonate. Pravastatin did not show any inhibitory effect, and M4, which is not an HMG-CoA reductase inhibitor, demonstrated the preventive effect on CuSO₄-induced LDL oxidation. The above result indicates that the inhibitory effects of fluvastatin, M2 and M4 should not depend on HMG-CoA reductase inhibition, but might result from its own antioxidative character. We have previously reported that fluvastatin and its major metabolites prolonged the lag time for initiation of CuSO₄-induced LDL oxidation in vitro (10, 11), and that fluvastatin has a direct scavenging activity on hydroxyl radicals (19) and superoxide anions (20). Moreover, Nakamura et al. demonstrated that the double bond conjugated with the indole ring plays an important role in the high antioxidative activity of fluvastatin (21). Thus, the mechanism of the inhibitory effects of fluvastatin, M2 and M4 on CuSO₄-induced LDL oxidation is supposed to be derived from their scavenging activities on oxygen radicals. In the present study, we demonstrated that LDL oxidation caused cholesteryl ester accumulation in macrophages and that inhibition of LDL oxidation by fluvastatin, M2 and M4 resulted in a marked reduction of facilitated cholesteryl ester accumulation with a parallel magnitude of inhibition of LDL oxidation. Although further investigation is needed for evaluation of the antioxidative effect of fluvastatin in vivo as well as the reducing effect on the susceptibility of LDL to oxidation which comes from the common property of HMG-CoA reductase inhibitors, fluvastatin is clinically expected to prevent LDL oxidation by the direct antioxidative effect in cooperation with M2 and M4. There are few reports demonstrating the inhibition of foam cell formation in macrophages by preventing LDL oxidation. In this paper, we clearly showed that prevention of LDL oxidation caused a marked reduction of facilitated cholesteryl ester accumulation in macrophages. Vitamin E protects LDL from oxidation in vivo or in vitro (22–24). It has been reported that vitamin E supplementation appears to prevent coronary artery disease (25, 26). There are also some reports demonstrating that the antioxidative effect prevents the progression of atherosclerosis in animal models (27–30). These results may suggest that the protection of LDL from oxidation by antioxidants could be important in preventing atherosclerosis. It might be suggested that fluvastatin, which possesses both HMG-CoA reductase inhibitory and antioxidative actions, would be singly effective to prevent the progression of atherosclerosis. Furthermore, our results clearly indicate that fluvastatin has an inhibitory potential against cholesteryl ester formation induced by oxidized LDL in macrophages. This effect was overcome by mevalonate, a precursor of isoprenoids. Pravastatin also tended to show the same inhibitory effect, but its effect was weaker than that of fluvastatin and was also overcome by mevalonate. Considering these results, the inhibitory effects of fluvastatin and pravastatin on the cholesteryl ester formation induced by oxidized LDL in macrophages seem to be a common property of HMG-CoA reductase inhibitors. Our findings correspond to the obser-

**Fig. 4.** Effects of test compounds on [¹⁴C]oleate incorporation into cholesteryl esters in mouse peritoneal macrophages incubated with oxidized LDL. A) The [¹⁴C]cholesterol oleate formation rate of the control was 174.98 ± 16.93 pmol/mg cell protein per hour. Data are expressed as the mean ± S.E.M. of 3–4 separate experiments. *P<0.05, **P<0.01: statistically different compared with the control group (Dunnett’s multiple comparisons test). B) Data are expressed as the mean ± S.E.M. of 3 separate experiments. **P<0.01: statistically different compared with the control (mevalonate (−)) group (Dunnett’s multiple comparisons test), †P<0.05: statistically different compared with fluvastatin (FV) (mevalonate (−)) group (Student’s t-test), ‡P<0.05: statistically different compared with pravastatin (PV) (mevalonate (−)) group (Student’s t-test). V.E: vitamin E.
Fluvastatin Inhibits Foam Cell Formation

It has been reported that, after the final oral dose of 40 mg of fluvastatin once daily for 6 days, the plasma concentration of fluvastatin reaches 1 \( \mu \text{M} \) (38). In this study, as we used a high concentration (5 \( \mu \text{M} \)) of CuSO\(_4\) to oxidize LDL within a short time, a 10 – 30 \( \mu \text{M} \) concentration of fluvastatin was needed to prevent the LDL oxidation. However, it has previously been reported that fluvastatin significantly prevented LDL oxidation at 1 \( \mu \text{M} \) under mild conditions (11). We demonstrated that the inhibitory effects of M2 and M4, which were major metabolites in humans, on CuSO\(_4\)-induced LDL oxidation are more potent than fluvastatin. The peak of plasma concentration of M4 was one-fourth that of fluvastatin, but M4 was present in plasma for longer period of time than fluvastatin (14). M2 was the major component present in feces and existed in a relatively low level in plasma; it is considered to show antioxidative activity in liver and to have a chance to reduce the oxidizability of very low-density lipoprotein which is secreted from the liver into the circulation (10). Although the plasma concentrations of M2 and M4 are less than that of fluvastatin (14), these metabolites should contribute to the inhibition of LDL oxidation in vivo. In conclusion, our data clearly indicate that fluvastatin and its metabolites inhibit both LDL oxidation and foam cell formation by macrophages in vitro. Considering the in vivo circumstances mentioned above, fluvastatin is expected to be a new type of anti-atherosclerotic drug that exerts both HMG-CoA reductase inhibition and antioxidative action.

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