Effects of Fluvastatin on Plasma Levels of Low-Density Lipoprotein Subfractions, Oxidized Low-Density Lipoprotein, and Soluble Adhesion Molecules: A Twenty-Four-Week, Open-Label, Dose-Increasing Study

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ABSTRACT

Background: Statins not only lower low-density lipoprotein (LDL) levels, but also have several antiarteriosclerotic effects (eg, decreasing arterial inflammation and arterial smooth muscle cell proliferation, as well as antioxidant effects). The relationship between the dose of statin and its effects on plasma LDL levels and other arteriosclerosis-related effects remains to be clarified.

Objective: We investigated the effect of a statin, fluvastatin, on plasma levels of lipoprotein subfractions, oxidized LDL (Ox-LDL), Ox-LDL immunoglobulin G (IgG), soluble adhesion molecules, reverse cholesterol transport (ie, transport of esterified high-density lipoprotein cholesterol [HDL-C] to triglyceride [TG]-rich lipoproteins by cholesteryl ester transfer protein [CETP] and reduction of plasma HDL-C levels), and on the intima-medial thickness (IMT) of the common carotid arteries.

Methods: Patients with nonfamilial type 2 hyperlipoproteinemia were eligible for this open-label, dose-increasing study. Fluvastatin 20 mg/d was administered for the first 12 weeks, and the daily dose was increased to 40 mg for the subsequent 12 weeks. Patients were examined at baseline and after 12 and 24 weeks of treatment. Plasma lipoprotein subfractions were determined using sequential ultracentrifugation at 100,000g. The plasma levels of Ox-LDL, Ox-LDL-IgG, CETP, and soluble adhesion molecules were measured using sandwich enzyme-linked immunosorbent assay. The maximum IMT of the common carotid arteries was measured using sonography.

Results: The plasma levels of LDL cholesterol (LDL-C) and apolipoprotein (apo) B were reduced by 25% and 17%, respectively ($P < 0.001$ for both), after 12 weeks of treatment with fluvastatin 20 mg/d; no further significant reductions

in LDL were observed after increasing the daily dose to 40 mg. Fluvastatin 20 mg/d for 12 weeks decreased plasma levels of intermediate-density lipoprotein cholesterol, LDL-I-C, LDL-II-C, and LDL-III-C by 25% ($P < 0.01$), 30% ($P < 0.001$), 23% ($P < 0.01$), and 20% ($P = 0.02$), respectively. No further significant reductions in these levels were observed after increasing the daily dose to 40 mg. The plasma levels of Ox-LDL decreased in a similar fashion to the plasma levels of LDL-C ($P < 0.001$). However, plasma levels of Ox-LDL-IgG and soluble P-selectin did not decrease after 12 weeks of fluvastatin 20 mg/d, but did decrease significantly (both 22%) after the next 12 weeks of treatment with fluvastatin 40 mg/d ($P < 0.05$). Plasma levels of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 and CETP mass were not altered by fluvastatin treatment. Significant changes in maximum IMT of the common carotid arteries were not seen throughout 24 weeks of fluvastatin treatment.

Conclusions: In this patient population, fluvastatin 20 mg/d was sufficient to significantly reduce plasma levels of LDL, the 3 LDL subfractions, and Ox-LDL, but was not sufficient to reduce plasma levels of Ox-LDL-IgG and soluble P-selectin. It is important to check not only plasma lipoprotein levels but also other factors relating to arteriosclerosis during treatment with statins for the prevention and treatment of arteriosclerosis. (*Curr Ther Res Clin Exp.* 2003;64: 236–247) Copyright © 2003 Excerpta Medica, Inc.

Key words: fluvastatin, lipoprotein subfractions, oxidized LDL, adhesion molecules, reverse cholesterol transport, carotid arteriosclerosis.

INTRODUCTION

Statins decrease plasma levels of low-density lipoprotein (LDL), mainly through the suppression of hepatic LDL uptake via LDL-receptors,^{1,2} and have been shown to be effective for the primary and secondary prevention of coronary heart disease (CHD).^{3–7} Statins mainly decrease the plasma levels of large, less dense LDL.^{8,9} However, atorvastatin was reported to decrease plasma small, dense LDL.¹⁰ The effect of fluvastatin on plasma LDL subfractions remains to be determined.

Statins also have been reported to suppress the oxidation of LDL^{11–14} and the proliferation of arterial smooth muscle cells,^{15–17} while preventing arterial inflammation and endothelial injury.^{18–21} The relationship between the dose of statin and its effectiveness in lowering LDL and on arteriosclerosis-related factors remains to be clarified.

Fluvastatin has been reported to suppress LDL oxidation and to reduce plasma soluble P-selectin.^{14,22,23} However, it is not known whether fluvastatin reduces the plasma levels of very atherogenic LDL, oxidized LDL (Ox-LDL), Ox-LDL-immunoglobulin G (IgG), and other adhesion molecules (eg, intercellular adhesion molecule [ICAM]-1 and vascular cell adhesion molecule [VCAM]-1).

Adhesion molecules mediate the adhesion of leukocytes to the endothelial surface of the arteries.^{24,25} The mechanism of the transport of esterified cholesterol (C) produced by lecithin:cholesterol acyltransferase (LCAT) from high-density lipoprotein (HDL) to triglyceride (TG)-rich lipoproteins by cholesteryl ester transfer protein (CETP) is called *reverse cholesterol transport*.^{26,27} Plasma HDL-C is low when reverse cholesterol transport is active.

The aim of our study was to investigate the effects of fluvastatin treatment on the plasma levels of 3 LDL subfractions (which were separated by ultracentrifugation), Ox-LDL, Ox-LDL-IgG, reverse cholesterol transport, and soluble adhesion molecules (ie, ICAM-1, VCAM-1, and P-selectin). We also studied the effects of fluvastatin treatment on the intima-medial thickness (IMT) of the common carotid arteries as an indicator of the proliferation of arterial smooth muscle cells.

PATIENTS AND METHODS

Patients

Patients with nonfamilial type 2 hyperlipoproteinemia who were seen at the lipid clinic at Tokai University School of Medicine (Boseidai, Isehara, Japan) were eligible for the study. Patients with familial hypercholesterolemia and familial combined hyperlipoproteinemia were excluded from the study, as were patients with plasma TG levels >350 mg/dL and those treated with probucol. In addition, patients with diabetes mellitus, CHD, or cerebrovascular disease were excluded.

The institutional review board of the Tokai University Hospital approved the study. Written informed consent was obtained from all patients before the start of the trial.

Methods

Patients were observed during a washout period of at least 8 weeks, during which they received no hypolipoproteinemic drugs. Patients with >10% changes in plasma total cholesterol (TC) level during the washout period were excluded. The patients had been treated with statin drugs and given dietary restrictions before the washout period. Hypertensive medications were not altered during the washout and treatment periods. The patients were requested not to change their dietary habits.

Fluvastatin 20 mg was administered daily after dinner for 12 weeks, and then the daily dose was increased to 40 mg for the subsequent 12 weeks. Patients were examined at baseline and after 12 and 24 weeks of treatment.

Twelve-hour fasting blood samples were collected to determine the plasma levels of lipids; apolipoproteins (apos); remnant-like particles (RLPs), which are equivalent to TG-rich lipoprotein remnants; lipoprotein subfractions; Ox-LDL; Ox-LDL-IgG; and soluble adhesion molecules at baseline and after 12 and 24 weeks of fluvastatin treatment. Ox-LDL-IgG was measured only in 21 of 30

patients because the commercial kits were no longer manufactured. The cholesterol esterification rate (LCAT activity) and plasma level of CETP also were estimated to assess the effect of fluvastatin on plasma reverse cholesterol transport at baseline and at weeks 12 and 24. The apo E phenotyping and assaying of lymphocyte LDL-receptor activity were done during the washout period. Sonography of the carotid arteries also was done at baseline and weeks 12 and 24.

Laboratory Procedures

The lipids were measured using a Hitachi autoanalyzer (Tokyo, Japan). Apo levels were measured with an automated turbidimetric immunoassay.²⁸ The plasma levels of HDL-C were obtained by dextran sulfate-Ca²⁺ precipitation.²⁹ The plasma levels of LDL-C were estimated using the following equation by Friedewald et al³⁰:

$$\text{Est. LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$$

Cholesterol of remnant-like particles equivalent to remnants of TG-rich lipoproteins (RLP-C) was estimated using a precipitation method with monoclonal antibodies against apo AI and apo B-100.³¹ The plasma lipoproteins were subfractionated into very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL-I (1.019 < density < 1.035), LDL-II (1.035 < density < 1.045), LDL-III (1.045 < density < 1.063), HDL2, and HDL3 by sequential ultracentrifugation at 100,000g.³² Plasma LCAT activity was estimated using the method by Nagasaki and Akanuma.³³ Plasma levels of CETP were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies against human CETP (REAADS Bio-Medical Products, Inc., Westminster, Colorado).³⁴ Plasma Ox-LDL levels were measured using sandwich ELISA with monoclonal antibodies against malondialdehyde-modified LDL (MDA-LDL) and apo B-100.³⁵ Ox-LDL-IgG was measured using sandwich ELISA by first incubating the plasma with microplate-coated MDA-LDL, followed by the incubation of microplate-adherent Ox-LDL-IgG with alkaline phosphatase-labeled goat IgG against human IgG.³⁶ The plasma levels of soluble ICAM-1, VCAM-1, and P-selectin were estimated with the sandwich ELISA method using corresponding monoclonal antibodies and Bender MedSystem kits (Vienna, Austria).³⁷ The LDL-receptor activity of lymphocytes cultured in a lipoprotein-deficient medium for 72 hours was assayed using flow cytometry.³⁸ Apo E phenotyping was done by isoelectric focusing immunoblot.³⁹

Sonography of the Carotid Arteries

Sonography of the carotid arteries was conducted by the same examiner, using Logic 700 MR® (GE, Milwaukee, Wisconsin) and a linear probe (739L®; GE). The maximum IMT of the common carotid arteries was measured, whereas the plaque portion was excluded.

Statistical Analysis

Significant differences between baseline and 12 and 24 weeks of treatment were analyzed using analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

Thirty patients (26 women, 4 men; mean [SD] age, 67 [7] years; mean [SD] body mass index, 24 [3] kg) were included in the study. Baseline characteristics of the study patients are shown in Table I.

Mean change in body weight during the 24 weeks of treatment was <1 kg.

The effects of fluvastatin on plasma levels of lipids and apos are shown in Table II. The mean plasma levels of TC, LDL-C, and apo B were significantly reduced (by ~16%, 25%, and 17%, respectively [$P < 0.001$ for all]) at 12 weeks of treatment with fluvastatin 20 mg/d. No further significant reductions were observed after the next 12 weeks of treatment, when the fluvastatin dose was increased to 40 mg/d. The mean plasma levels of apo E were significantly reduced from baseline after 24 weeks of fluvastatin treatment ($P = 0.03$). The

Table I. Demographic characteristics and laboratory values at baseline (N = 30). (Values are expressed as mean [SD], unless otherwise indicated.)

Characteristic	Normal Range	Value
Sex, no. (%) of patients		
Female	–	26 (87)
Male	–	4 (13)
Age, y	–	67 (7)
BMI, kg/m ²	<26	24 (3)
TC, mg/dL	120–200	300 (45)
TG, mg/dL	<150	168 (90)
LDL-C, mg/dL	50–140	203 (42)
HDL-C, mg/dL	40–80	64 (16)
Apo A-I, mg/dL	120–160	152 (23)
Apo B, mg/dL	70–110	158 (27)
Apo E, mg/dL	2–5	6 (2)
Apo E phenotype (E 3/3; E 4/3)	–	27; 3
LDL-receptor activity, %*	80–120	108 (22)
Concomitant diseases, no. (%) of patients		
None	–	16 (53)
Hypertension	–	14 (47)
Diabetes mellitus	–	0
Coronary heart disease	–	0

BMI = body mass index; TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; Apo = apolipoprotein.

*Estimated with flow cytometry in lymphocytes cultured in lipoprotein-deficient medium for 72 hours.

Table II. Effects of fluvastatin treatment on plasma levels (mg/dL) of lipids and apolipoproteins (apos). (Values are expressed as mean [SD].)

Level	Normal Range	Baseline	12 Weeks	24 Weeks
TC	120–200	300 (45)	251 (39)*	237 (36)*
TG	<150	168 (90)	162 (100)	159 (117)
LDL-C [†]	50–140	203 (42)	152 (36)*	141 (31)*
HDL-C	40–80	64 (16)	69 (16)	66 (17)
Apo A-I	120–160	152 (23)	156 (24)	160 (32)
Apo A-II	25–35	31 (5)	30 (5)	31 (5)
Apo B	70–110	158 (27)	131 (23)*	125 (23)*
Apo C-II	2–5	6 (3)	6 (3)	6 (3)
Apo C-III	5–10	13 (4)	13 (4)	13 (5)
Apo E	2–5	6 (2)	6 (2)	5 (2) [‡]

TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

* $P < 0.001$ versus baseline.

[†]Obtained using the equation by Friedewald et al³⁰: Est. LDL-C = TC – HDL-C – TG/5.

[‡] $P = 0.03$ versus baseline.

plasma levels of TG, HDL-C, apo A-I, apo A-II, apo C-II, and apo C-III all remained unchanged throughout the 24 weeks of treatment.

The effects of fluvastatin treatment on the plasma levels of the lipoprotein subfractions are shown in Table III. The plasma levels of IDL-C, LDL-I-C, and LDL-III-C all significantly decreased (by 25% [$P < 0.01$], 30% [$P < 0.001$], 23% [$P < 0.01$], and 20% [$P = 0.02$], respectively) after 12 weeks of treatment with

Table III. Effects of fluvastatin treatment on plasma lipoprotein subfraction levels (mg/dL). (Values are expressed as mean [SD].)

Level	Normal Range	Baseline	12 Weeks	24 Weeks
VLDL-C	<20	18 (14)	17 (13)	16 (14)
IDL-C	2–14	17 (8)	12 (5)*	11 (5) [†]
LDL-I-C [‡]	25–45	48 (16)	34 (13) [†]	29 (10) [†]
LDL-II-C [§]	40–70	64 (24)	49 (18)*	45 (15)*
LDL-III-C	15–40	33 (12)	26 (10) [¶]	25 (11)*
HDL2-C	15–25	25 (12)	26 (10)	25 (10)
HDL3-C	20–25	27 (4)	26 (10)	25 (11)

VLDL = very low-density lipoprotein; C = cholesterol; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

* $P < 0.01$ versus baseline.

[†] $P < 0.001$ versus baseline.

[‡]1.019 < density < 1.035.

[§]1.035 < density < 1.045.

^{||}1.045 < density < 1.063.

[¶] $P = 0.02$ versus baseline.

fluvastatin 20 mg/d. No further significant reductions in LDL were observed after the dose was increased to 40 mg/d. The plasma levels of VLDL-C, HDL2-C, and HDL3-C did not change throughout 24 weeks of fluvastatin treatment.

The effects of fluvastatin treatment on the plasma levels of RLP-C, Ox-LDL, Ox-LDL-IgG, plasma LCAT activity, CETP mass, soluble adhesion molecules, and IMT of the common carotid arteries are shown in Table IV. The plasma levels of Ox-LDL significantly decreased by 28% after 12 weeks of treatment with fluvastatin 20 mg/d ($P < 0.001$), but did not decrease further after 12 additional weeks of treatment with fluvastatin 40 mg/d. The plasma levels of Ox-LDL-IgG did not decrease after 12 weeks of treatment but significantly decreased (by 22%) after 24 weeks of fluvastatin treatment ($P < 0.05$ vs baseline and week 12). The plasma levels of RLP-C did not decrease throughout the 24 weeks of fluvastatin treatment. The LCAT activities decreased in a dose-dependent manner during fluvastatin treatment ($P < 0.001$), but the plasma CETP mass did not change throughout the study. The plasma levels of soluble P-selectin did not decrease after 12 weeks of fluvastatin 20 mg/d but did significantly decrease (by 22%) after the subsequent 12 weeks at 40 mg/d ($P < 0.05$ vs baseline and week 12). The plasma levels of soluble ICAM-1 and VCAM-1 did not change throughout 24 weeks of fluvastatin treatment.

The maximum IMT of the common carotid arteries did not change throughout 24 weeks of fluvastatin treatment.

No treatment-related adverse events were observed throughout the study.

Table IV. Effects of fluvastatin treatment on plasma remnants of triglyceride (TG)-rich lipoproteins, oxidized low-density lipoprotein (Ox-LDL), immunoglobulin G Ox-LDL (Ox-LDL-IgG), reverse cholesterol transport, soluble adhesion molecules, and on intima-medial thickness (IMT) of the common carotid arteries. (Values are given as mean [SD].)

Level	Normal Range	Baseline	12 Weeks	24 Weeks
RLP-C, mg/dL	<7	8 (4)	6 (4)	6 (5)
Ox-LDL, U/L	40–140	182 (57)	131 (48)*	122 (36)*
Ox-LDL-IgG, U/mL	10–20	16 (4)	16 (5)	13 (4) ^{†‡}
LCAT activity, $\mu\text{mol/mL} \cdot \text{h}^{-1\S}$	60–100	146 (29)	117 (28)*	100 (22)* [‡]
CETP mass, $\mu\text{g/mL}$	1–4	5 (2)	4 (2)	4 (2)
sICAM-1, ng/mL	90–250	239 (92)	238 (106)	219 (90)
sVCAM-1, ng/mL	450–800	738 (213)	776 (270)	746 (226)
sP-selectin, ng/mL	100–280	250 (103)	258 (117)	196 (81) ^{†‡}
Carotid artery IMT, mm	<1	0.92 (0.26)	0.93 (0.24)	0.94 (0.26)

RLP-C = cholesterol of remnant-like particles equivalent to remnants of TG-rich lipoproteins; LCAT = lecithin:cholesterol acyltransferase; CETP = cholesteryl ester transfer protein; sICAM-1 = soluble intercellular adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; sP-selectin = soluble P-selectin.

* $P < 0.001$ versus baseline.

[†] $P < 0.05$ versus baseline.

[‡] $P < 0.05$ versus week 12.

[§]Cholesterol esterification rate.

DISCUSSION

The effects of fluvastatin on the plasma levels of lipoprotein subfractions, Ox-LDL, and Ox-LDL-IgG, soluble adhesion molecules, reverse cholesterol transport, and IMT of the common carotid arteries were investigated in this study.

Treatment with fluvastatin 20 mg/d was equally effective in reducing the plasma levels of LDL-C at a daily dose of 40 or 80 mg in Western studies.⁴⁰⁻⁴² In our study, the increase in the daily dose from 20 to 40 mg did not significantly enhance the decrease in the plasma levels of LDL-C and apo B. Fluvastatin may be more effective for Japanese patients as opposed to people living in the West.^{3-9,43,44} The typical Japanese diet is very similar to the treatment diet for hyperlipoproteinemia in Western patients.

Plasma lipid levels usually are stable during observation without medication if body weight is stable. Twelve weeks is a sufficient period of time to examine the effects of a hypolipoproteinemic drug. Therefore, the significant changes that occurred after increasing the daily dose of fluvastatin from 20 to 40 mg were due to increasing the dose and not to the additional 12 weeks of treatment.

The effects of statins on the plasma levels of lipoprotein subfractions are controversial.⁸⁻¹⁰ Fluvastatin decreased all 3 subfractions of plasma LDL in this study. Thus, the reduction in plasma Ox-LDL with fluvastatin treatment was not due to the preferential decrease in the plasma levels of small, dense LDL, which is more susceptible to oxidation than less dense LDL fractions.^{45,46} The plasma level of IDL, which is equivalent to the remnants of VLDL, a part of TG-rich lipoproteins, also decreased by fluvastatin treatment.

Plasma cholesterol esterification was inhibited by the fluvastatin treatment. However, reverse cholesterol transport did not appear to be altered because the plasma levels of TG-rich lipoproteins, which accept esterified cholesterol and HDL, which in turn donates esterified cholesterol of reverse cholesterol transport by CETP, were not altered by fluvastatin treatment.^{26,27}

Plasma Ox-LDL decreased after 12 weeks of treatment with fluvastatin 20 mg/d in parallel with plasma LDL-C. However, the plasma levels of Ox-LDL-IgG did not decrease after 12 weeks of treatment with 20 mg/d, but did decrease after an additional 12 weeks of treatment at 40 mg/d. The plasma Ox-LDL-IgG levels have been reported to be elevated in atherosclerotic diseases.⁴⁷⁻⁵⁰ Thus, in Japanese patients, treatment with fluvastatin 20 mg/d may be considered sufficient to reduce plasma LDL levels, but not sufficient for reducing plasma Ox-LDL-IgG levels.

The plasma levels of soluble P-selectin significantly decreased after fluvastatin treatment, as previously reported.^{22,23} Again, a dose of 40 mg/d was necessary to achieve such a decrease. Fluvastatin treatment did not affect plasma levels of ICAM-1 and VCAM-1 in this study. Thus, fluvastatin appeared to suppress only the first step of the adhesion of leukocytes to the endothelial surface of arteries (ie, rolling).^{24,25}

The reduction in plasma levels of Ox-LDL-IgG and soluble P-selectin was not parallel with the reduction in plasma LDL-C, apo B, and Ox-LDL levels. Therefore, the reduction in plasma levels of Ox-LDL-IgG and soluble P-selectin did not appear to be due to the decrease in plasma LDL; instead, it seemed to be due to the direct pharmacologic action of fluvastatin.

As previously reported,⁴² we found that fluvastatin treatment had no significant effects on carotid arteriosclerosis.

CONCLUSIONS

In this patient population, fluvastatin treatment at a daily dose of 20 mg was sufficient to significantly decrease the plasma levels of LDL, the 3 LDL subfractions, and Ox-LDL, but was not sufficient to decrease the plasma levels of Ox-LDL-IgG and soluble P-selectin in Japanese patients. It is important to check not only plasma lipoprotein levels but also other factors relating to arteriosclerosis during treatment with statins for the prevention and treatment of arteriosclerosis.

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