Original Article

Effects of Low-Dose Simvastatin on the Distribution of Plasma Cholesterol and Oxidized Low-Density Lipoprotein in Three Ultracentrifugally Separated Low-Density Lipoprotein Subfractions: 12-Month, Open-Label Trial

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Aim: The effects of statins on the distribution of oxidized LDL in plasma LDL subfractions have not been well defined. Effects of 12-month treatment with low-dose simvastatin on the distribution of cholesterol and oxidized LDL in 3 ultracentrifugally separated plasma LDL subfractions were compared in patients with hypercholesterolemia.

Methods: Simvastatin was administered to 30 hypercholesterolemic subjects for 12 months at an initial dose of 5 mg/day, which was increased to 20 mg/day via 10mg/day to decrease plasma LDL-cholesterol (C) lower than 130 mg/dL. Simvastatin dose was fixed after 3 months of treatment. The amounts of cholesterol and oxidized LDL in 3 ultracentrifugally separated plasma LDL subfractions were compared between 0 and 12 months of treatment.

Results: The distribution of ox-LDL skewed to denser LDL fractions, compared with cholesterol in plasma LDL subfractions. Plasma cholesterol in low-density LDL, medium-density LDL and high-density LDL decreased significantly by 31%, 30%, and 25%, respectively ($p < 0.0001$) after 12 months of simvastatin treatment. Plasma oxidized LDL was decreased from 70 U/L to 56 U/L in medium-density LDL ($p = 0.042$). Oxidized LDL in low-density LDL and high-density LDL did not change significantly after 12 months of treatment.

Conclusion: Treatment with low-dose simvastatin decreased plasma cholesterol in 3 LDL subfractions and oxidized LDL in medium-density LDL. The decrease of oxidized LDL seemed to be not due to the decrease of cholesterol in plasma LDL subfractions because the decreasing patterns of cholesterol and ox-LDL were different in 3 LDL subfractions.

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Key words; Simvastatin, Plasma lipids, ox-LDL, hs-CRP, LDL subfractions, Plasma lipoprotein subfractions

Introduction

A statin, simvastatin, has been used for the treatment of hypercholesterolemia and has been proved

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effective for the prevention of coronary artery diseases (CAD)¹⁻³. Low-density lipoprotein (LDL) is a heterogeneous lipoprotein fraction and the atherogenicity of each subfraction is not the same⁴⁻⁶. Plasma oxidized LDL (ox-LDL) is atherogenic⁷⁻¹², and statins decrease plasma ox-LDL as well as LDL-cholesterol^{13, 14}. Small, dense LDL is easily oxidizable and believed to be very atherogenic^{15, 16}, and simvastatin was reported to reduce plasma small, dense LDL¹⁷. We reported the decrease of plasma cholesterol in 2 ultracentrifugally

separated LDL subfractions after 3 months of treatment with simvastatin¹⁸). The decrease in cholesterol in less-dense LDL was more marked than that in dense LDL in that study. The effect of simvastatin on the distribution of plasma ox-LDL levels in LDL subfractions has not been well defined. We investigated the effect of simvastatin on cholesterol and ox-LDL levels in 3 ultracentrifugally separated plasma LDL subfractions after 12 months of treatment with low-dose simvastatin in order to know whether the decreasing patterns of cholesterol and ox-LDL in 3 plasma LDL subfractions are same or not.

Materials and Methods

Protocol

The study protocol was approved by the ethics committees of each hospitals and the trial was started after obtaining written informed consent.

Simvastatin was administered to 30 patients with hypercholesterolemia at an initial dose of 5 mg/day after dinner. The duration of the trial was 12 months. The daily dose was increased to 20 mg via 10 mg to reduce plasma LDL-cholesterol lower than 130 mg/dL, and the daily dose was fixed at 3 months of treatment and was not changed until the end of treatment. Plasma lipids, apo(lipo)proteins, ox-LDL, high sensitivity (hs)-CRP, and cholesterol and ox-LDL in 3 LDL subfractions were compared between the start and 12 months of treatment with simvastatin. Blood sampling was made in a 12-hour fasting state.

Laboratory Procedures

Cholesterol (C) and triglyceride (TG) were measured with an autanalyzer. Apoproteins were estimated with a turbidimetric immunoassay¹⁹. Remnant-like particle (RLP)-cholesterol (C) was measured with a precipitation method using monoclonal antibodies against apoprotein (apo) AI and apo B-100²⁰. Ox-LDL was estimated with an ELISA method using monoclonal antibodies against malondialdehyde (MDA)-LDL and apo B²¹. One U/L of plasma ox-LDL was equivalent to 1mg/dL MDA in medium *in vitro*. Hs-CRP was measured with particle-enhanced immuno- nephelometry²².

Plasma lipoprotein subfractionation with ultracentrifugation into very low-density lipoprotein (VLDL, density; $d < 1.006$), intermediate-density lipoprotein (IDL, $1.006 < d < 1.019$), low-density LDL (ld-LDL, $1.019 < d < 1.035$), medium-density LDL (md-LDL, $1.035 < d < 1.045$), high-density LDL (hd-LDL, $1.045 < d < 1.063$), high-density lipoprotein (HDL)₂ ($1.063 < d < 1.125$), and HDL₃ ($d > 1.125$)

Table 1. Demographics of subjects

N (M/F)	30 (11/19)
Age (y/o)	64.8 ± 8.4 ¹
BMI (kg/m ²)	23.5 ± 2.7
Total cholesterol (TC) (mg/dL)	264 ± 34
Triglyceride (TG) (mg/dL)	137 ± 60
LDL-C (mg/dL)	182 ± 27
HDL-C (mg/dL)	55 ± 14
RLP-C (mg/dL)	5.4 ± 2.5
Ox-LDL (U/L)	162 ± 56
Hs-CRP (ng/mL)	1,050 ± 805
FPG (mg/dL)	103 ± 16

1. Mean ± S.D.

was performed sequentially with the method of Havel *et al.*²³. The duration of ultracentrifugation for lipoprotein subfractionation was 16 h for VLDL, 18 h for IDL, 20 h for LDL, and 24 h for HDL₂ at 100,000 g.

Statistical Analysis

Statistical comparison of serial changes of measured items was performed by ANOVA to identify trends of changes, and further comparison of values between the start and end of the trial was performed with the Dunnett's repeated measurement analysis.

Results

The demographics of subjects are shown in **Table 1**. The daily dose of simvastatin at 12 months of treatment was 7.7 ± 5.2 mg (mean ± S.D.) (5 mg for 22 subjects, 10 mg for 4 subjects, and 20 mg for 4 subjects).

Changes of plasma lipids, apoproteins, ox-LDL, and hs-CRP after 12 months of treatment with simvastatin are shown in **Table 2**. TC, TG, LDL-C, RLP-C, apo B, apo E, and ox-LDL were significantly decreased by 12 months of treatment with simvastatin. Plasma levels of HDL-C, apo AI, and hs-CRP were not changed significantly by treatment with simvastatin.

Distributions of cholesterol and ox-LDL in plasma LDL subfractions at the start of treatment are demonstrated in **Fig. 1**. The distribution of ox-LDL skewed to higher density fractions, compared with that of cholesterol. A small amount of ox-LDL was recovered in IDL and HDL₂ fractions.

Changes in plasma cholesterol and ox-LDL levels in lipoprotein subfractions are shown in **Table 3**. Plasma levels of VLDL-C, IDL-C, ld-LDL-C, md-LDL-C, hd-LDL-C were significantly decreased after 12 months of treatment with simvastatin. Decreases in

Table 2. Changes in plasma levels of lipids, apolipoproteins, ox-LDL, and hs-CRP after 12-month treatment with low-dose simvastatin

Treatment duration (m)	0	12	<i>p</i> ¹
Total cholesterol (TC) (mg/dL)	264 ± 33 ²	200 ± 32 ^c	<0.0001
Triglyceride (TG) (md/dL)	137 ± 60	114 ± 58 ^a	0.0415
LDL-cholesterol (LDL-C) (mg/dL)	182 ± 26	117 ± 25 ^c	<0.0001
HDL-cholesterol (HDL-C) (mg/dL)	55 ± 14	60 ± 17	0.3825
RLP-cholesterol (RLP-C) (mg/dL)	6.4 ± 2.5	3.8 ± 1.2 ^c	<0.0001
Apoprotein AI (apo AI) (mg/dL)	140 ± 26	154 ± 27	0.2566
Apo B (mg/dL)	143 ± 19	102 ± 19 ^c	<0.0001
Apo E (mg/dL)	5.3 ± 1.4	4.2 ± 1.2 ^c	0.0001
Oxidized LDL (ox-LDL) (U/L)	161 ± 133	133 ± 41 ^c	<0.0001
Hs-CRP (ng/mL)	1,040 ± 818	844 ± 1,096	0.4502

1. Statistical analysis by ANOVA

2. Mean ± S.D.

3. Statistical significance by repeated measurement analysis: a; *p* < 0.05, b; *p* < 0.01, c; *p* < 0.001 **Fig. 1.** Distributions of cholesterol and ox-LDL in plasma LDL subfractions at the start of the trial. Absolute amounts are demonstrated at the bottom (mean ± S.D.).Small amount of ox-LDL was recovered in IDL and HDL₂

the absolute amounts of Id-LDL-C and md-LDL-C were significantly greater than the decrease of hd-LDL-C., but percent decreases of cholesterol in 3 LDL subfractions were not significantly different among them (percent decreases of cholesterol: 31% in Id-LDL, 30% in md-LDL, and 25% in hd-LDL). Plasma levels of HDL₂-C and HDL₃-C were not changed by simv-

astatin treatment. Ox-LDL was significantly decreased only in plasma md-LDL.

Discussion

Effects of treatment with low-dose simvastatin on the distribution of cholesterol and ox-LDL in

Table 3. Changes in plasma cholesterol and ox-LDL in lipoprotein subfractions after 12-month treatment with low-dose simvastatin

Treatment duration (m)	0	12	<i>p</i> ¹
VLDL-cholesterol (C) (mg/dL)	15.2 ± 11.1 ²	9.9 ± 8.4 ^a	0.0235
IDL-C (mg/dL)	14.0 ± 5.8	10.1 ± 4.7 ^c	< 0.0001
Low-density LDL-C (ld-LDL-C) (mg/dL)	60.4 ± 18.3	41.2 ± 11.6 ^c	< 0.0001
ld-LDL ox-LDL (U/L)	41.8 ± 20.6	35.2 ± 11.3	0.1795
Medium-density LDL-C (md-LDL-C) (mg/dL)	70.7 ± 12.6	49.3 ± 16.4 ^c	< 0.0001
md-LDL ox-LDL (U/L)	70.3 ± 29.4	56.2 ± 20.6 ^a	0.0421
High-density LDL-C (hd-LDL-C) (mg/dL)	20.3 ± 7.7	15.3 ± 5.4 ^c	0.0002
hd-LDL ox-LDL (U/L)	32.1 ± 16.0	31.1 ± 15.5	0.6572
HDL2-C (mg/dL)	24.2 ± 9.0	25.5 ± 8.4	0.4175
HDL3-C (mg/dL)	22.6 ± 3.9	23.5 ± 3.3	0.6426

1. Statistical analysis by ANOVA

2. Mean ± S.D.

3. Statistical significance by repeated measurement analysis: a; *p* < 0.05, b; *p* < 0.01, c; *p* < 0.001

plasma LDL subfractions were compared.

Low-dose simvastatin was very effective for the treatment of Japanese subjects with hypercholesterolemia.

Plasma levels of TC, TG, LDL-C, apo B, apo E, and ox-LDL were decreased by treatment with simvastatin, as previously reported^{1, 2, 18, 24, 25}; however, plasma levels of HDL-C, apo AI, hs-CRP did not change significantly in this study.

The metabolism of plasma LDL subfractions is complicated. Plasma levels of LDL subfractions are mainly determined by cascade-like catabolism from VLDL to small, dense LDL, direct secretion of LDL subfractions, and their removal via LDL receptors, especially in the liver²⁶⁻²⁸. The production of VLDL and LDL subfractions is inhibited and their removal via LDL receptors is stimulated by statin treatment. Plasma levels of VLDL, IDL, ld-LDL, md-LDL, hd-LDL were significantly decreased after-12 month treatment with low-dose simvastatin in this study probably due to these mechanisms. The metabolism of ox-LDL and the effect of simvastatin treatment have not been well defined¹². The selective decrease of ox-LDL in md-LDL might be due to the stimulation of minimally oxidized LDL removal through LDL receptors because md-LDL had the highest affinity to LDL receptors among 3 LDL fractions²⁹⁻³²; however, it is impossible to know the mechanisms of changes of cholesterol and ox-LDL in LDL subfractions in this kind of clinical study. The decrease of ox-LDL was speculated to be due to the decrease of cholesterol¹². This study suggested that the decrease of ox-LDL was not due to the decrease of cholesterol because the decreasing patterns of cholesterol and ox-LDL in LDL

subfractions were different.

We concluded that 12 months of treatment with low-dose simvastatin reduced plasma levels of cholesterol in all 3 LDL subfractions and ox-LDL in md-LDL.

Appendix

Members of Kanagawa Lipid Research Group: Yasuhiko Homma*, Isehara; Ichiro Michishita, Yokohama; Hiroshi Hayashi, Atami; Hiroshi Shigematsu, Yokohama; Yuichi Fusegawa, Sagami-hara; Makoto Ujihara, Yokohama; Toshimasa Fujihara, Narashino; Osamu Mizuno, Ohuna; Hiroshi Yoshida, Kashiwa; Tadashi Yamakawa, Yokohama; Akira Tanaka, Sakato; and Koichiro Homma, Shinjuku

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