Fluvastatin Increases LDL Particle Size and Reduces Oxidative Stress in Patients with Hyperlipidemia

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The effects of fluvastatin on levels of urinary 8-iso-prostaglandin F_{2α} (iPF_{2α}III), a marker of oxidative stress, and low-density lipoprotein (LDL) particle size in serum were investigated in patients with hypercholesterolemia. After 6 months of fluvastatin therapy, levels of urinary iPF_{2α}III decreased from 1,720.1 ± 392.0 to 539.6 ± 75.5 pg/mg ($p < 0.01$), and LDL particle size increased from 24.3 ± 0.3 to 26.5 ± 0.2 nm ($p < 0.001$). These changes from the treatment of fluvastatin were not correlated with those of the serum LDL cholesterol (LDL-C) levels. The results imply that fluvastatin, with its unique antioxidant property among statins, reduces oxidative stress and increases LDL particle size simultaneously in hyperlipidemic patients. *J Atheroscler Thromb*, 2003; 10: 343–347.

Key words: 8-iso-prostaglandin F_{2α}, Isoprostane, Low-density lipoprotein, Statins

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are used worldwide for the treatment of hypercholesterolemia due to their potent blood cholesterol-lowering action and excellent safety profile. Numerous studies have shown that statins suppress both the onset and recurrence of coronary heart disease (CHD) as a result of their lowering blood cholesterol levels (1, 2).

Atherosclerotic plaques arise from the scavenger re-

ceptor-mediated uptake of oxidatively modified low-density lipoproteins (LDL) by macrophages in the subintimal space. This uptake promotes the formation of foam cells loaded with cholesterol esters (3), accompanied by proliferation of smooth muscle cells and elaboration of an extracellular lipid core and fibrous cap (4). Possible antiatherosclerotic mechanisms of statins include prevention of LDL modification, inhibition of cholesterol esterification and accumulation, and attenuation of smooth muscle cell proliferation (5).

Oxidation of LDL particles can be estimated by measuring levels of a novel marker of lipid peroxidation, 8-iso-prostaglandin F_{2α} (iPF_{2α}III) (6–8). iPF_{2α}III is a chemically stable prostaglandin directly (nonenzymatically) produced by free radicals from arachidonic acid in the phospholipids of cell membranes (6–8). iPF_{2α}III is cleaved, presumably by phospholipases, and excreted in urine, the rate of excretion providing an index of lipid peroxidation *in vivo*. To determine the effects of fluvastatin, which has antioxidant properties (9–14), on the prevention of modification of LDL, levels of urinary

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iPF2 α III were measured in patients with hypercholesterolemia following 6 months of fluvastatin therapy.

In addition, the effects of fluvastatin on LDL particle size were monitored.

Subjects

Twenty hypercholesterolemic patients (12 female, 8 male; mean age 63.0 ± 2.6 years) were enrolled in the study after informed consent was given. They were treated on an outpatient basis. Patients with diabetes mellitus and/or essential hypertension were included. Patients who might have been pregnant or lactating, had a history of hypersensitivity to HMG-CoA reductase inhibitors, severe hepatic damage or nephropathy, or were receiving other antihyperlipidemic medications were excluded from the study.

Methods

Patients were placed on a diet based on the National Cholesterol Education Program (NCEP) guideline (15) to confirm that their serum total cholesterol (TC) levels at baseline were > 220 mg/dl. Twenty mg fluvastatin was administered once daily after dinner for 6 consecutive months. Blood and urine samples were taken following overnight fasting prior to and 1, 3, and 6 months after the start of fluvastatin administration. Urine samples were placed in containers containing 5-hydroxy-TEMPO/EDTA and quickly stored at -80°C until analysis. TC, triglycerides (TG), and high-density lipoprotein (HDL)-cholesterol levels were measured by the enzymatic method, and LDL-C was calculated using Friedewald's formula (16). Samples with TG levels > 400 mg/dl were not used. LDL particle size was determined using a LipoprintTM LDL

System (Quantimetrix Corporation, Redondo Beach, CA). The reliability of this system was confirmed in our previous study (17), and the results obtained using this system correlated well with those obtained by the method of Krauss and Burke (18).

Hepatic function (aspartate transaminase (AST), alanine transaminase (ALT), γ -glutamyl transpeptidase (γ -GTP), alkaline phosphatase (ALP), and lactic acid dehydrogenase (LDH)), renal function (blood urea nitrogen (BUN) and serum creatinine), and CPK were assessed. Urinary iPF2 α III (6–8) levels were measured using high-performance liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis (19) using a modified method of FitzGerald *et al* (6–8). The levels were corrected by measuring the concentration of urinary creatinine.

Statistical analyses were carried out using the paired *t*-test. Correlative analysis was assessed by the Spearman rank correlation test. Significance level was established at 5% (bilateral). All results were expressed as mean $\pm$ standard error (SE).

The study was conducted in accordance with the Declaration of Helsinki; all patients gave informed consent to participate prior to entering the study.

Results

Mean baseline levels of TC, HDL-cholesterol, TG, and LDL-cholesterol were 270.7 ± 5.9 mg/dl, 51.2 ± 2.6 mg/dl, 177.0 ± 11.3 mg/dl, and 181.7 ± 5.5 mg/dl, respectively. Changes in the levels of serum lipids following fluvastatin administration are shown in Fig. 1. After 1 month, TC and LDL-cholesterol levels decreased significantly, to 228.2 ± 5.9 mg/dl and 140.9 ± 5.0 mg/dl, respectively, and remained low after 3 months (225.8 ± 5.4

Fig. 1. Effect of fluvastatin on levels of serum lipids (mean $\pm$ standard error) in patients with hypercholesterolemia. TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. **p* < 0.05; ***p* < 0.001.

Fig. 2. Effect of fluvastatin on low-density lipoprotein particle size (mean $\pm$ standard error) in patients with hypercholesterolemia. **p* < 0.001.

mg/dl and 140.7 ± 5.7 mg/dl, respectively) and 6 months (228.4 ± 6.7 mg/dl and 143.6 ± 6.7 mg/dl, respectively) (all $p < 0.001$ vs baseline). TG levels at 1, 3, and 6 months after the start of administration were 171.7 ± 18.3 , 152.2 ± 11.5 , and 149.6 ± 12.8 mg/dl, respectively. The reductions were significant at 3 and 6 months ($p < 0.05$). Significant changes in HDL-cholesterol levels were also observed at 3 and 6 months; the levels increased to 52.9 ± 2.5 , 54.7 ± 3.0 , and 55.6 ± 3.3 mg/dl at 1, 3, and 6 months, respectively.

Mean LDL particle size was < 25.5 nm (*i.e.*, small, dense LDL) prior to study entry. After fluvastatin administration, mean particle size increased significantly from 24.3 ± 0.3 to 26.5 ± 0.2 nm ($p < 0.001$; Fig. 2). Urinary iPF2 α III levels decreased significantly from $1,720.1 \pm 392.0$ pg/mg to 539.6 ± 75.5 ($p < 0.01$, Fig. 3). Although the reductions of urinary iPF2 α III levels were manifest in the patients with iPF2 α III > 2000 pg/mg creatinine, there were no baseline characteristics compared with other patients except for the age (73.3 vs 62.5 years old).

There were no correlations between the respective changes of iPF2 α III, LDL size, and LDL-C by the treatment of fluvastatin (data not shown).

No significant changes in clinical laboratory test results were observed during the study.

Discussion

Numerous large-scale epidemiological studies have shown that elevated levels of serum LDL-cholesterol have a causative effect in the onset and progression of CHD (1, 2). Nevertheless, more than half of patients with CHD have normal levels of LDL-cholesterol (20), and it has been hypothesized that the particle size and composition of LDL have clinical significance in the progression

of CHD. Small, dense LDL particles (21, 22) and oxidized LDL (3, 4) are independently associated with increased risk of atherosclerosis. Austin *et al.* (21) reported that the prevalence of CHD was 3-fold higher in patients with the small, dense LDL phenotype than in those whose LDL profile was normal. In a previous study we found that more than 70% of Japanese patients with CHD exhibited the small, dense LDL subclass pattern, compared with 30% of healthy individuals (23). It is well established that oxidized LDL is taken up by scavenger receptors on macrophages and smooth muscle cells in the intima of blood vessels, leading to foam cell formation (3, 4). Small, dense LDL is easily oxidized (24, 25) and displays high affinity to cell surface binding sites independent of LDL receptors (26). Thus, monitoring of LDL particle size and oxidation is invaluable in the management of CHD.

Currently, there are 5 commercially available statins (27), but to the authors' knowledge there have been no studies carried out on the effects of statins on both the oxidative modification and particle size of LDL. Fluvastatin is known to be unique among statins in having antioxidant action on serum lipids (9–14). In addition, fluvastatin has been reported to change the distribution of LDL sub-species in hypercholesterolemic patients, although the change was not statistically significant (28). Thus, the present study was conducted to assess the effects of fluvastatin on whole body oxidative stress as well as particle size in hypercholesterolemic patients. The whole body oxidative stress was assessed by measuring the level of the novel parameter iPF2 α III. Since LDL particles contain iPF2 α III, the concentration of this molecule in plasma and urine is elevated in patients with high blood cholesterol (29, 30). iPF2 α III levels have also been shown to increase in smokers (31, 32) and have been detected in atherosclerotic plaque layers (33). Consequently, iPF2 α III levels have been used as a marker of oxidative stress (6–8).

In the present study, iPF2 α III levels decreased in 18 of 20 patients following fluvastatin therapy. Fluvastatin is known to have antioxidant properties attributable to its structure (13), and has been shown to suppress the oxidation of LDL (9–12). Furthermore, fluvastatin was reported to impair the elevation of plasma iPF2 α III levels in vitamin E-deficient hamsters (14). When vitamin E was administered to diabetic patients, urinary levels of iPF2 α III decreased (34); the results of the present study suggest that fluvastatin also inhibits *in vivo* oxidation.

However, the mechanisms through which fluvastatin increases LDL particle size are not clear. Previously, we observed that fluvastatin suppresses plasma TG in heterozygotes of Watanabe heritable hyperlipidemic (WHHL) rabbits (35). Enhancement of TG metabolism induced by fluvastatin can be explained by a dual mechanism, suppression of hepatic production of very low-density lipoprotein (VLDL)-TG, a precursor of LDL particles, and in-

Fig. 3. Effect of fluvastatin on 8-iso-prostaglandin F2 (iPF2 α III) levels (mean $\pm$ standard error) in patients with hypercholesterolemia. * $p < 0.001$.

creased VLDL turnover by stimulation of LDL-receptor activity (35). It is known that TG-rich (large sized) VLDL is a better substrate for small, dense LDL. Thus, the effects of fluvastatin on LDL size can be partially explained by suppressed hepatic TG production by fluvastatin.

Fluvastatin, with its unique antioxidant property among statins, reduces oxidative stress and increases LDL size simultaneously in hyperlipidemic patients. Since the changes of urinary iPF2 α III levels and LDL size by treatment of fluvastatin were not correlated with those of serum LDL-C levels, it is likely that the antioxidant property and the increasing effect on LDL size of fluvastatin were independent of its cholesterol-lowering effect. These results warrant further large-scale clinical trials.

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