Effects of Fluvastatin in Type 2 Diabetic Patients with Hyperlipidemia: Reduction in Cholesterol Oxidation Products and VCAM-1

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The purpose of this study was to investigate the lipid-lowering and anti-oxidative effects of fluvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, in type 2 diabetic patients. Six patients (3 men and 3 women, mean age = 56.2) took 20 mg of fluvastatin once daily (at night) for 12 weeks. Several markers of oxidative stress were then measured in these patients including plasma cholesterol oxidation products, i.e. oxysterols, and the levels of circulating adhesion molecules. Plasma total cholesterol levels were reduced by 12.3% in these individuals after 4 weeks of treatment, with levels remaining below 220 mg/dl for the entire treatment period. LDL levels were significantly reduced at 4 (18.1%) and 12 weeks (16.1%), and triglyceride levels were significantly reduced after 8 (22.5%) and 12 (37.7%) weeks of treatment. HDL-C levels increased from 50.7 ± 15.4 prior to treatment to 63.8 ± 24.3 mg/dl after 12 weeks of treatment, though this increase was not statistically significant. Lipid hydroperoxide, thiobarbituric acid-reactive substance (TBARS), and oxysterol levels were also reduced, suggesting that fluvastatin also had anti-oxidative effects. Finally, VCAM-1 levels were similarly reduced by fluvastatin treatment. We conclude that fluvastatin safely improves the plasma lipid profile in type 2 diabetic patients with hyperlipidemia. We speculate that this drug might be doubly effective in reducing atherosclerosis and cardiac events in these patients as a result of its demonstrated anti-oxidative effects and its ability to reduce VCAM-1 levels. J Atheroscler Thromb, 2004; 11: 56–61.

Key words: Fluvastatin, Type 2 diabetes, Oxysterols, Antioxidant

Introduction

The efficacy of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in lowering serum cholesterol concentrations is well established and has been extensively documented (1–4). Treatment of hyperlipidemia in type 2 diabetic patients is crucial because these patients have an elevated risk of developing atherosclerosis. Results of the Cholesterol and Recurrent Events (CARE) trial (1) and the Scandinavian Simvastatin Survival Study (4S)(2) confirmed the usefulness of statins in reducing cardiovascular events in diabetic patients.

Type 2 diabetics display altered lipid metabolism resulting in increased plasma triglycerides (TG) and reduced plasma high density lipoprotein (HDL) cholesterol (HDL-C) levels, while they demonstrate only a slight increase in their plasma low density lipoprotein (LDL) cholesterol (LDL-C) levels (5). These patients also tend to have a preponderance of atherogenic small-dense (6,7) and glycated LDLs (8).

It has been suggested that statins might reduce the expression of adhesion molecules, inhibit the proliferation and migration of vascular smooth muscle cells (9,10), exert anti-thrombotic effects (11), and suppress inflam-
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Statins have also been reported to possess intrinsic antioxidant activity (13–16). Oxidative stress has been identified as a pathogenic mechanism in the development of diabetic complications, including atherosclerosis. It is now accepted that reactive oxygen species play an important causative role in diseases such as atherosclerosis (17–19) and diabetic micro- and macroangiopathy (20).

Fluvastatin is a synthetic statin that has antioxidant effects similar to those mediated by α-tocopherol. Antioxidants have been suggested to be useful in the treatment of diabetic patients with hyperlipidemia (21, 22). In this study, we examined the anti-oxidant effects of fluvastatin and its effects on the expression of adhesion molecules.

Material and Methods

Subjects

Six (3 men and 3 women) type 2 diabetic patients with hyperlipidemia were enrolled in this study. Their average age was 56.2 ± 6.4 years and their mean BMI was 23.0 ± 1.4 kg/m². Three of the patients were being treated with diet therapy, while the others were taking oral hypoglycemic agents (two were taking 2.5 mg/day of glybenclamide and one was taking 20 mg/day of gliclazide). The patients’ average fasting plasma glucose (FPG) was 119 ± 8 mg/dl (range = 90 to 145), and their average HbA1c was 5.8 ± 0.6 % (range = 4.4 to 7.4 %).

Six type 2 diabetic patients who did not take fluvastatin served as controls. These individuals did not have hyperlipidemia and had a BMI of 24.5 ± 0.24 kg/m², a HbA1c of 6.6 ± 0.7, and the following mean lipid levels: TC = 203 ± 8.35; LDL-C = 122 ± 5; TG = 119 ± 34; HDL-C = 57.3 ± 3.9 mg/dl. Their levels of 7-ketocholesterol and VCAM-1 and ICAM-1 were similarly assessed at the 12 week time point.

Patients received 20 mg of fluvastatin once daily in the evening for 12 weeks and were assessed at 4-week intervals. During that period, their average fasting glucose levels and BMI remained constant.

The patients’ clinical laboratory data at the start of, and at key points during, the study appear in Table 1. Their starting average total cholesterol (TC) level was 238 ± 15 mg/dl (range = 208 to 316), while their average LDL level was 155 ± 15 mg/dl (range = 125 to 225). These values, coupled with an average plasma TG level of 162 ± 23 mg/dl (range = 105 to 249) satisfy the criteria necessary for a diagnosis of hyperlipidemia (23). Our patients’ average HDL-C level was 50.7 ± 6 (range = 30.1 to 76.5). Exclusion criteria included poorly controlled hypertension, liver disease, chronic renal failure, lipid-lowering therapy before the study, and use of insulin at the start of the study. Before the study, the protocol was explained to all of our subjects and their consent was obtained.

Assay methods

Blood samples were obtained after a 12 hour overnight fast. HbA1c was measured using a high performance liquid chromatography system (Kyoto Daichikagaku, Kptyo, Japan) and TC, TG, and HDL-C levels were measured using enzymatic colorimetric commercial kits (Wako, Osaka, Japan). LDL-C was calculated by the Friedewald method. The plasma LDL fraction was separated by ultracentrifugation on a density gradient of between 1.019 to 1.063, as previously described (24), after which it was dialyzed for 24 hours against phosphate buffered saline (PBS) at 4°C. Lipoproteins were subjected to polyacrylamide gel electrophoresis (LipoPhor system, Yokoh) (25) and LDL particle size was determined by measuring the migration distance of LDL relative to the HDL fraction (Rf value) (26). Lipoperoxidation was determined by measuring the amount of lipid hydroperoxide (LHPO) using the methylene blue-hemoglobin method (Kowa Medics, Tokyo, Japan); values were corrected using LDL protein and were expressed as nmol of LHPO/mg LDL protein. α-Tocopherol in the LDL fraction was quantified as previously described (27). The degree of lipid peroxidation was determined by malondialdehyde (MDA) analysis using the thiobarbituric acid-reactive substance (TBARS) assay (28). TBARS levels in the LDL fraction were expressed as the concentration of MDA (nmol of MDA/mg LDL protein). The concentration of LDL protein was determined using the Lowry method (29). Plasma oxysterols were measured by gas chromatography and mass spectrometry as previously described (30). Plasma levels of

| Table 1. Plasma lipid, glucose, and glycated hemoglobin levels as well as BMI in our six diabetic patients. |
|-----------------|-------|------|------|------|-------|
|                 | 0W    | 4W   | 8W   | 12W  |
| **BMI (kg/m²)** | 23.5 ± 1.7 | 23.5 ± 1.8 | 23.4 ± 1.7 | 23.4 ± 1.8 |
| **FPG (mg/dl)** | 119 ± 8.6 | 121 ± 5.7 | 129 ± 7.7 | 126 ± 8.2 |
| **HbA1c (%)**   | 5.8 ± 0.6 | 6.4 ± 0.2 | 6.3 ± 0.3 | 6.3 ± 0.4 |
| **TC (mg/dl)**  | 239 ± 16 | 209 ± 8* | 212 ± 16 | 214 ± 12 |
| **LDL (mg/dl)** | 155 ± 14 | 127 ± 7* | 132 ± 15 | 130 ± 12* |
| **TG (mg/dl)**  | 162 ± 23 | 141 ± 27 | 126 ± 27* | 101 ± 15* |
| **HDL (mg/dl)** | 50.7 ± 6.3 | 53.8 ± 4.6 | 55.2 ± 6.3 | 63.8 ± 9.9 |

* p < 0.05 compared to baseline (0W).
VCAM-1, ICAM-1 (Biosource International, CA, USA), and PAI-1 (Biopool International, CA, USA) were determined using specific ELISA kits.

Data analysis

All data were expressed as the mean ± SE. Differences between baseline (wk 0) and later time points were compared using paired and unpaired t-tests. A p value of less than 0.05 was considered statistically significant.

Results

BMI, FPG, and HbA1c did not change significantly during the 12 week study (Table 1). Blood pressure, renal function, and urinary albumin levels were within the normal range and did not deteriorate during the study. Fluvastatin treatment lowered plasma TC, TG, and LDL-C concentrations compared to baseline values. Specifically, TC levels were significantly reduced 4-weeks after initiation of treatment and remained below 220 mg/dl (10.5 to 12.3% reduction relative to baseline). TG levels were significantly less than baseline at 8 (22.5%) and 12 weeks (37.7%). Especially noteworthy was the fact that LDL levels were significantly reduced at 4 (18.1%) and 12 weeks (16.1%). HDL-C levels increased from 50.7 ± 6.3 at the before drug treatment to 63.8 ± 9.9 mg/dl at 12-weeks, representing a 25.9% increase, though this increase did not reach statistical significance.

The values of some other lipid and oxidative stress parameters are summarized in Table 2. LDL size as measured by the LipoPhor system did not demonstrate any alteration in its Rf value. LHPO diminished gradually with treatment and levels were significantly reduced at 8 (55.2%) and 12 (66.6%) weeks. TBARS were also significantly reduced at 8 (39.6%) and 12 (51.7%) weeks.

Patients also showed reductions in their plasma oxysterols, such as 7β-hydroxy (46.9%), epoxy (25.6%), 7keto- (47.6%), 25-hydroxy (27.0%), and 27-hydroxy (33.7%) cholesterol at 12 weeks. Overall, total plasma oxysterols decreased significantly from 61.25 at the beginning of the study to 38.95 ng/ml after 12 weeks of treatment.

VCAM-1 levels diminished gradually, with levels reaching statistical significance at 8 (28.2%) and 12 (18.0%) weeks; on the other hand, ICAM-1 and PAI-1 levels did not change appreciably during the study period.

The levels of 7-ketocholesterol (16.71 ± 4.20), VCAM-1 (2.29 ± 0.29) and ICAM-1 (10.15 ± 1.59) in the fluvastatin-treated group at 12 weeks tended to be lower than in control diabetics that didn’t have hyperlipidemia (7-ketocholesterol; 25.48 ± 4.02, VCAM-1; 2.84 ± 0.13, ICAM-1; 11.73 ± 1.00).

None of our patients experienced any drug-related adverse side effects and their creatine phosphokinase levels remained steady throughout the study period.

### Table 2. Patients’ plasma lipids and parameters of oxidative stress.

<table>
<thead>
<tr>
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<th>0W</th>
<th>4W</th>
<th>8W</th>
<th>12W</th>
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</thead>
<tbody>
<tr>
<td>LDL size (Rf)</td>
<td>0.39 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>LHPO (nmol/mgLDL pro)</td>
<td>43.3 ± 13.1</td>
<td>43.4 ± 11.4</td>
<td>19.4 ± 8.1*</td>
<td>14.4 ± 5.1*</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>8.83 ± 1.11</td>
<td>10.75 ± 1.25</td>
<td>5.33 ± 0.97*</td>
<td>4.27 ± 0.65*</td>
</tr>
<tr>
<td>Oxysterols (ng/ml each)</td>
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<tr>
<td>7α-hydroxycholesterol</td>
<td>21.50 ± 3.17</td>
<td>25.31 ± 7.56</td>
<td>25.13 ± 8.04</td>
<td>17.24 ± 2.67</td>
</tr>
<tr>
<td>7β-hydroxycholesterol</td>
<td>3.46 ± 0.68</td>
<td>3.75 ± 1.28</td>
<td>3.61 ± 1.44</td>
<td>1.84 ± 0.38*</td>
</tr>
<tr>
<td>Epoxy-cholesterol</td>
<td>2.93 ± 0.17</td>
<td>3.08 ± 0.70</td>
<td>3.26 ± 0.50</td>
<td>2.18 ± 0.22*</td>
</tr>
<tr>
<td>7-ketocholesterol</td>
<td>31.89 ± 5.61</td>
<td>22.08 ± 7.43</td>
<td>29.61 ± 11.2</td>
<td>16.71 ± 4.20*</td>
</tr>
<tr>
<td>25-hydroxycholesterol</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td>27-hydroxycholesterol</td>
<td>1.39 ± 0.02</td>
<td>1.28 ± 0.17</td>
<td>1.07 ± 0.15</td>
<td>0.92 ± 0.24*</td>
</tr>
<tr>
<td>Total oxysterols (ng/ml)</td>
<td>61.25 ± 8.32</td>
<td>55.58 ± 16.8</td>
<td>62.78 ± 18.8</td>
<td>38.95 ± 6.57*</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>2.79 ± 0.37</td>
<td>2.31 ± 0.033</td>
<td>2.00 ± 0.28*</td>
<td>2.29 ± 0.29*</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>11.16 ± 1.66</td>
<td>11.25 ± 1.55</td>
<td>10.50 ± 1.47</td>
<td>10.15 ± 1.59</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>19.8 ± 5.1</td>
<td>23.1 ± 4.6</td>
<td>16.9 ± 3.1</td>
<td>19.3 ± 6.48</td>
</tr>
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</table>

W = weeks; values are expressed as the mean ± SE. * p < 0.05 compared to baseline (0W).
Discussion

The Lipoprotein and Coronary Atherosclerosis Study (LCAS) showed that fluvastatin therapy in patients with coronary artery disease and mild to moderate hypercholesterolemia reduced the rate of progression of coronary atherosclerotic lesions in patients with mildly to moderately elevated LDL cholesterol (31). The data presented above showed that fluvastatin effectively reduced plasma TC, LDL, and TG levels as well; while patients’ HDL-C levels also improved, their values did not reach statistical significance.

Tilly-Kiesi reported reductions not only in TG but also in dense LDL in response to lovastatin treatment (32). Though Winkler et al. reported beneficial effects of fluvastatin on LDL particle size in patients with type 2 diabetes, the issue as to whether statins are effective in lowering the concentration of dense LDLs is still controversial (33). In our study, we did not detect any significant changes in LDL size even though our patients showed reduced plasma TG levels. Other factors influence the structure of LDL in diabetic patients including their degree of glycation (34). Small, dense LDL particles are known to be atherogenic probably because they are more susceptible to oxidation.

With regard to its anti-oxidant effects, fluvastatin treatment was shown to have significant antioxidant effects against both peroxyl and hydroxyl radicals (35). To date, antioxidant activity has been reported for probucol (36), fluvastatin (13), pravastatin (15), simvastatin (16) and atorvastatin (14), though no direct in vitro or in vivo comparisons of their relative potencies have been made. The mechanism(s) by which statins exert their anti-oxidant effects and the clinical significance of this effect remain to be elucidated.

We previously reported that plasma lipids in patients with diabetes were very susceptible to copper ion-mediated oxidation and that, in vitro, these lipids contained high amounts of autoxidative products of cholesterol i.e., oxysterols (30). Thus, we speculate that plasma oxysterol levels might represent a useful marker of oxidative stress in patients with diabetes and hyperlipidemia (30). Kummerow FA et al. suggested that plasma oxysterol levels may be a more sensitive marker of heart disease than cholesterol levels (37, 38).

We demonstrated that 7-ketocholesterol (major oxysterol) was particularly effective in increasing solubleVCAM-1 due to the suppression of the inhibitor of the proteolytic process (tissue inhibitor of membrane metalloproteinase-2; TIMP-2) on the cell surface (39). Then we examined the effect of fluvastatin, as an antioxidant statin, on the plasma levels of VCAM-1. In the present study, plasma levels of oxysterols, except 7β-hydroxycholesterol, decreased significantly after 12 weeks of fluvastatin treatment. Thus, autooxidation products of cholesterol such as 7β-hydroxy-, 7-keto-, epoxy, 25-hydroxy-cholesterol could be potential markers of oxidative stress. Furthermore, Murakami et al. demonstrated that plasma levels of oxysterols including 7-ketocholesterol, and VCAM-1 increased in parallel with the progression of diabetic nephropathy (40).

We hypothesized the possibility that fluvastatin reduce plasma VCAM-1 levels mediated by the reduction of oxysterols (39). However, other mechanisms may also be considered, because the reduction of the plasma VCAM-1 levels preceded those of oxysterols (Fig. 1). For instance, the influence of various statins in vitro has been reported to reduce the expression of adhesion molecules through their anti-inflammation effect (41). In our study, plasma VCAM-1, but not ICAM-1, was similarly reduced in response to fluvastatin treatment, though the relationship between this reduction and the reduction in autooxidation products is unclear (Fig. 1). The levels of 7-ketocholesterol and VCAM-1 after 12 weeks of fluvastatin treatment fell to levels that approached those seen in our nonlipidemic type 2 diabetic patients.

We conclude that fluvastatin safely improves the plasma lipid profile in type 2 diabetic patients with hyperlipidemia. We speculate that this drug might be doubly effective in reducing atherosclerosis and cardiac events in these patients as a result of its demonstrated anti-oxidative effects and its ability to reduce VCAM-1 levels.
References


