

Anti-oxidative Effect of Fluvastatin in Hyperlipidemic Type 2 Diabetic Patients

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Abstract. An open-label prospective cross-over trial was performed to evaluate the antioxidative effect of fluvastatin in Japanese type 2 diabetics with hyperlipidemia. The study subjects were 10 patients who were on pravastatin (10 mg/day) or simvastatin (5 mg/day). After at least 12 weeks of continuous pravastatin or simvastatin therapy, the drugs were washed out for 12 weeks and replaced with fluvastatin (30 mg/day), then the treatment was continued for another 12 weeks. Total cholesterol and LDL cholesterol were efficiently and comparably reduced by all three statin agents. There were no differences in serum parameters of oxidative stress such as malondialdehyde-modified low-density lipoprotein, thiobarbituric acid-reactive substances, and 8-iso-prostaglandin F₂ α between pravastatin/simvastatin and fluvastatin. However, fluvastatin, but not pravastatin/simvastatin, significantly reduced 3,5,7-cholestatriene in erythrocyte membrane, representing the extent of membrane cholesterol peroxidation. Our data demonstrated that fluvastatin has a unique anti-oxidative effect in patients with type 2 diabetes and hyperlipidemia, compared with other statins.

Key words: Oxidative stress, HMG-CoA reductase inhibitor, Type 2 diabetes

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ONE of the main goals of treatment of type 2 diabetes mellitus is to prevent its complications. In addition to the well-recognized microvascular complications of diabetes, there is a growing epidemic of macrovascular complications. In particular, type 2 diabetes complicated with hyperlipidemia is associated with increased risk of atherosclerosis. Thus, there is a need for continued search for effective therapies that prevent the progression of atherosclerosis in such patients.

The efficacy of statins in lowering serum cholesterol concentration is well established [1–3]. In addition to their hypocholesterolemic effect, statins are also known to reduce the expression of various adhesion molecules, inhibit the proliferation and migration of vascular smooth muscle cells [4, 5], exert anti-thrombotic

effects [6], and suppress inflammation [7]. These pleiotropic effects are beneficial as they also serve to prevent the development of atherosclerosis. In fact, the results of the Cholesterol and Recurrent Events (CARE) trial [1], Scandinavian Simvastatin Survival Study (4S) [2], and Collaborative Atorvastatin Diabetes Study (CARDS) [8] confirm the usefulness of statins in reducing cardiovascular events in type 2 diabetic patients with hyperlipidemia.

Fluvastatin is one of the frequently prescribed statins worldwide and has a structure similar to α -tocopherol, a natural antioxidant [9]. Increased oxidative stress in vascular wall cells and leukocytes has been identified as an important mechanism for the development of atherosclerosis in type 2 diabetes [10]. To elucidate whether a drug has anti-oxidative effect in clinical setting should provide important information for clinicians to help them select appropriate drugs for their diabetic patients. Recently, we established a method to measure 3,5,7-cholestatriene in erythrocyte membrane, a cholesterol oxidation product in human [11]. Different from other established serum oxidative markers,

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such as malondialdehyde-modified low density lipoprotein (MDA-LDL), thiobarbituric acid-reactive substances (TBARS), and 8-iso-prostaglandin F₂ α (8-epiPGF₂ α), 3,5,7-cholestatriene is estimated by direct measurement of the oxidative product in the cell membrane. Our previous data demonstrated that this marker correlated well with carotid artery intima-media thickness in type 2 diabetics [11]. Using this value as a marker of oxidative stress, in this study we investigated the effects of fluvastatin on oxidative stress in patients with type 2 diabetes and hyperlipidemia, and compared these effects with those of other statins.

Materials and Methods

Subjects and study protocol

To evaluate the effect of vitamin E on the value of 3,5,7-cholestatriene, we measured 3,5,7-cholestatriene in 5 patients with type 2 diabetes before and 12 weeks after treatment with vitamin E (200 mg/day). Their mean age was 57.2 ± 9.8 (\pm SD) and mean duration of diabetes was 11.8 ± 7.9 years. The mean HbA1c level at baseline was $7.2 \pm 1.3\%$. To compare the value of TBARS and 3,5,7-cholestatriene, we measured both values simultaneously in another group of 37 patients with type 2 diabetes. Their average age was 60.2 ± 10.8 years and mean duration of diabetes 10.4 ± 9.5 years.

The effect of fluvastatin (30 mg/day) was evaluated in 10 patients with type 2 diabetes and hyperlipidemia (7 males and 3 females). All patients enrolled in this study were on treatment with pravastatin (10 mg/day) ($n = 7$), or simvastatin (5 mg/day) ($n = 3$). Their clinical characteristics are shown in Table 1. With regard to the treatment of diabetes, 3 patients were treated with diet therapy only, 6 patients were treated with oral hypoglycemic agents, and 1 patient was treated with insulin. The dose of antidiabetic drug was not changed during this study. None of the patients was taking antioxidants such as vitamin C, vitamin E, or probucol. Subjects already being treated with simvastatin or pravastatin continued to take the respective drug for more than 12 weeks. At the end of that period, a fasting blood sample was collected from each patient to evaluate the effect of pravastatin or simvastatin. This was followed by withdrawal of statins for 12 weeks. Then, a fasting blood sample was collected from each patient as a control. After that, the patients received

fluvastatin for 12 weeks, after which another fasting blood sample was collected to evaluate the effect of fluvastatin. Each patient was reviewed once a month including evaluation of general health, compliance with medication, and body weight.

Assays

Blood was collected from each subject and glycated albumin (Gly-Alb), 1,5-AG (1,5-anhydroglucitol), total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglycerides, TBARS, 8-epiPGF₂ α , MDA-LDL, were measured by SRL Co. (Tachikawa, Japan). TBARS was measured using a test kit (Wako, Osaka, Japan) according to the method described previously [12]. To measure 8-epiPGF₂ α , enzyme immunoassay (EIA) was used as described previously [13]. MDA-LDL was measured by an enzyme-linked immunosorbent assay (ELISA) method based on the principle previously reported by Kotani *et al.* [14]. To estimate the extent of cholesterol peroxidation, the level of 3,5,7-cholestatriene in erythrocyte membranes was measured using gas chromatography-mass spectrometry, as described previously [11, 15].

Data analysis and ethical considerations

The effect of vitamin E on 3,5,7-cholestatriene was evaluated by the Student's paired *t*-test. The relation between TBARS and 3,5,7-cholestatriene was evaluated by linear regression analysis. The effects of fluvastatin were evaluated by comparison of the laboratory data using repeated measures ANOVA. When significant differences were observed, Student-Newman-Keuls analysis was applied. All data are expressed as mean \pm SD. A *P* value less than 0.05 denoted the presence of a statistically significant difference. The study protocol was approved by the Human Ethics Review Committee of Juntendo University School of Medicine and a signed informed consent form was obtained from each participating subject.

Results

To confirm that 3,5,7-cholestatriene reflects oxidative stress clinically, we treated type 2 diabetics with vitamin E, an anti-oxidative agent, and examined the

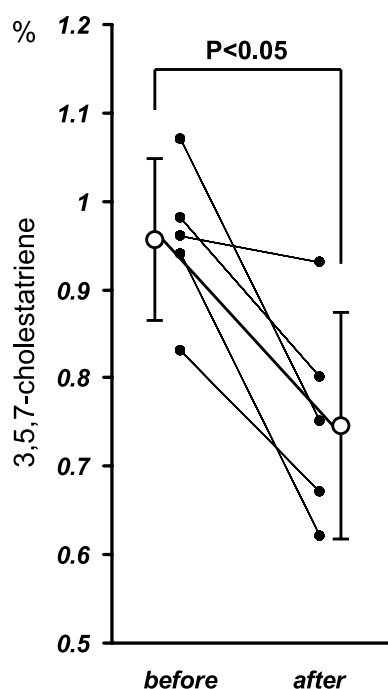


Fig. 1. Vitamin E reduced 3,5,7-cholestatriene in type 2 diabetics. 3,5,7-cholestatriene levels were measured in patients with type 2 diabetes ($n = 5$) before and after treatment with vitamin E for 12 weeks. Solid circles: individual subjects, Open circles: mean \pm SD of each group. Student's paired t -test was used to compare laboratory data between the groups.

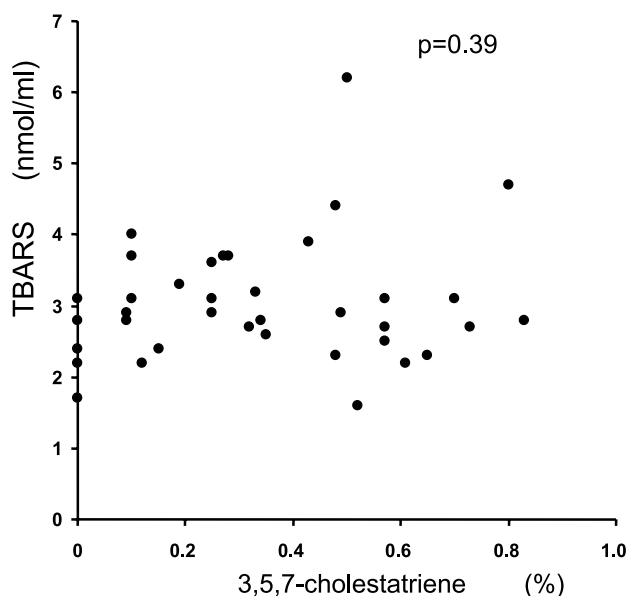


Fig. 2. No relation between 3,5,7-cholestatriene and TBARS in type 2 diabetes. TBARS and 3,5,7-cholestatriene were measured simultaneously in patients with type 2 diabetes. Linear regression analysis was used to compare the relationship.

effect of 12-week treatment. The mean HbA_{1c} value before and after the treatment in the subjects was comparable (before, $7.2 \pm 1.3\%$; after, $7.1 \pm 0.9\%$). As shown in Fig. 1, vitamin E efficiently reduced 3,5,7-cholestatriene levels in all patients. The result clearly supports the notion that 3,5,7-cholestatriene is a clinically useful marker of oxidative stress. Next we investigated the relationship between 3,5,7-cholestatriene and TBARS, an established serum oxidative marker, by measuring both values in another group of patients with type 2 diabetes. As shown in Fig. 2, 3,5,7-cholestatriene level did not correlate with that of TBARS, suggesting that the clinical value of 3,5,7-cholestatriene reflects different aspects of oxidative stress from TBARS in diabetic patients. Furthermore, the results suggest it is worth measuring both values in the same patient, and to compare the features and usefulness of each value in clinical setting. Accordingly, we measured both markers to evaluate oxidative stress.

Table 1 summarizes the demographic characteristics and baseline data of the patients who participated in the statin study. All the subjects took their drug almost exactly as specified. Table 2 compares the laboratory data at each stage of the study protocol. Gly-Alb and 1,5-AG were similar in all stages. Total cholesterol and LDL-cholesterol levels were significantly and comparably reduced with the treatment of both types of statins. Accompanying the reduction of LDL-cholesterol, MDA-LDL was decreased after treatment with both types of statins. However, after correction using the level of LDL-cholesterol, no differences were noted among the groups. Other serum oxidative markers, TBARS and 8-epiPGF₂ α were not different among the groups. However, the level of 3,5,7-cholestatriene was reduced relative to no treatment following treatment with fluvastatin but not with the simvastatin or pravastatin. These results demonstrate that fluvastatin has antioxidative effect, when using 3,5,7-cholestatriene level in erythrocyte cell membrane as an index of oxidative stress.

Discussion

Fluvastatin is the first totally synthesized statin. Its chemical structure differs from that of pravastatin and simvastatin, which were produced by chemical modification of fungal metabolites. Previous data showed that fluvastatin has a potent hydroxyl radical scaveng-

Table 1. Clinical characteristics of the study subjects

	Pravastatin	Simvastatin	Total
N	7	3	10
Age (years)	58.0 ± 7.2	45.0 ± 18.4	54.1 ± 12.2
Male/Female	1/6	2/1	3/7
Body mass index (kg/m ²)	21.8 ± 2.2	24.7 ± 1.3	22.7 ± 2.4
Duration of diabetes (years)	5.7 ± 2.8	4.0 ± 2.6	5.2 ± 2.7

Data are expressed as n or mean ± SD.

Table 2. Comparison of the effects of fluvastatin and pravastatin/simvastatin

	Statin (–)	Pravastatin or Simvastatin	Fluvastatin
Gly-Alb (%)	18.1 ± 3.5	17.9 ± 3.3	17.8 ± 3.5
1,5-AG (mg/ml)	9.4 ± 4.0	9.9 ± 3.3	9.2 ± 4.5
Total cholesterol (mg/dl)	282.3 ± 43.8	231.0 ± 27.2*	233.8 ± 49.6*
HDL (mg/dl)	58.7 ± 11.4	60.5 ± 11.1	62.5 ± 13.0
LDL (mg/dl)	193.2 ± 47.9	145.4 ± 29.7*	146.8 ± 44.9*
Triglyceride (mg/dl)	138.9 ± 70.7	126.5 ± 40.4	123.2 ± 61.1
TBA (nmol/ml)	2.8 ± 0.7	2.8 ± 0.7	3.1 ± 1.2
8-epiPGF ₂ α	239.6 ± 112.0	204.2 ± 113.8	255.4 ± 79.5
MDA-LDL (U/l)	215.5 ± 79.6	140.5 ± 44.8*	143.4 ± 61.3*
MDA-LDL (U/g-LDL)	118.2 ± 63.3	96.7 ± 28.1	98.6 ± 24.8
3,5,7-cholestatriene (%)	0.38 ± 0.21	0.25 ± 0.23	0.16 ± 0.16*

Data are expressed as mean ± SD.

*p<0.05 vs. Statin (–) group, by repeated measures ANOVA.

ing activity that was not observed in pravastatin and simvastatin *in vitro* [16]. Thus, the hydroxyl radical scavenging activity of fluvastatin is not considered a common property of statins, but is likely to be derived from the unique chemical structure of this compound. With regard to the anti-oxidant effect, to date anti-oxidant activity has been reported for pravastatin [17], simvastatin [18] and atorvastatin [19]. Thus, to elucidate the unique feature of fluvastatin, it is essential to compare its anti-oxidative potency with other statins. In this study, we reported that fluvastatin but not simvastatin or pravastatin reduced the level of 3,5,7-cholestatriene in erythrocyte membrane in type 2 diabetics with hyperlipidemia, while there were no differences in other serum markers of oxidative stress between the different types of statins. Considering the importance of oxidative stress in the pathophysiology of diabetic complication, our data provide useful information for physicians when choosing the appropriate drug for patients with type 2 diabetes.

3,5,7-cholestatriene is produced from cholesterol by radical reaction. It is suggested that the generation of reactive oxygen species in excess of the detoxifying capacity of erythrocytes could cause oxidative damage to

phospholipids in the inner layer of the cell membrane. Therefore, the value of 3,5,7-cholestatriene reflects the extent of oxidative stress in the cell membrane. In fact, our data indicated that vitamin E efficiently reduced the value of 3,5,7-cholestatriene *in vivo* (Fig. 1). While there are several serum markers of oxidative stress available clinically, such as MDA-LDL, TBARS and 8-epiPGF₂α, these markers do not necessarily correlate well with each other in clinical setting. In fact, we could not find significant association between TBARS and 3,5,7-cholestatriene in type 2 diabetes (Fig. 2). This is most likely due to the difference of the substrate used to measure. 3,5,7-cholestatriene is measured using cell membrane as a sample. The turnover rate of the substrate should be longer than TBARS and MDA-LDL. Thus, theoretically, 3,5,7-cholestatriene might reflect the accumulation of oxidative stress better than other markers. This feature may explain why the anti-oxidative effect of fluvastatin was only detected by measuring 3,5,7-cholestatriene, not by other serum markers in this study. However, the exact property of 3,5,7-cholestatriene has not been revealed, thus further studies are needed to elucidate this feature of 3,5,7-cholestatriene.

Although no direct *in vivo* and *in vitro* comparison has been made, anti-oxidant effect has been reported for fluvastatin [16], pravastatin [17], and simvastatin [18]. However, in this study, we could not detect anti-oxidative effect of pravastatin and simvastatin as assessed by the oxidative markers used in this study. Two major differences between our data and previous data were 1) the dose of statins used in this study was relatively small. 2) The subjects of this study were patients with diabetes. Accordingly, it is likely that the dosage we employed was too low to detect anti-

oxidative activity of pravastatin and simvastatin for diabetic patients. Our data indicated that fluvastatin has stronger anti-oxidative effect than other statins when comparing the effect with the doses which similarly reduce cholesterol level.

In conclusion, the unique feature of fluvastatin as an anti-oxidant was confirmed in type 2 diabetic patients with hyperlipidemia, in addition to its hypocholesterolemic effect. Our results should assist physicians in finding more suitable drugs for their patients.

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