To clarify whether 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statin) increases lipoprotein lipase mass in preheparin plasma (preheparin LPL mass), we observed the change in preheparin LPL mass during administration of atorvastatin and pravastatin to type 2 diabetes mellitus patients with hypercholesterolemia. The subjects were randomly divided into two groups. One group was 24 patients given atorvastatin (10 mg/day), and the other was 23 patients given pravastatin (20 mg/day) for 4 months. After 4 months of administration, no significant change of HbA1c was observed. TC significantly decreased in the atorvastatin group compared to the pravastatin group. TG significantly decreased in the atorvastatin group. Low density lipoprotein cholesterol level significantly decreased in both groups (– 36.3%, p < 0.01 in atorvastatin, – 24.3%, p < 0.01 in pravastatin). Preheparin LPL mass slightly increased in both groups after 4 months of administration. Especially in patients who showed low preheparin LPL mass (less than 50 ng/ml) before statin administration, preheparin LPL mass significantly increased in both groups (+ 25.8% in the atorvastatin group, + 24.39% in the pravastatin group). These results suggested that administration of atorvastatin and pravastatin to type 2 diabetic patients with hypercholesterolemia increased serum preheparin LPL mass concentration. Especially, its effect was remarkable in patients who showed low preheparin LPL mass. J Atheroscler Thromb, 2004; 11: 341–347.

Key words: HMG-CoA reductase inhibitor, Pleiotropic effect, Type 2 diabetes mellitus, Hypercholesterolemia, Preheparin LPL mass

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) is known to have a lowering effect of low-density lipoprotein cholesterol (LDL-C). Various studies demonstrated that the frequency of coronary heart disease was significantly decreased by cholesterol lowering therapy with statin administration (1–3). For example, in the West of Scotland Coronary Prevention Study (WOSCOPS), pravastatin administration for 5 years reduced by 33% the number of definite nonfatal cases of myocardial infarction, as compared with a placebo (1). Furthermore, Atorvastatin versus Revascularization Treatment Study (AVERT) recognized that treatment by atorvastatin in stable coronary artery disease was equal to coronary angioplasty in incidence of acute coronary syndrome (2). However, Gaw fored that statins showed anti-atherogenic effects potentially in addition to their lipid-lowering action (4). So various pleiotropic effects of statin were demonstrated except LDL-C lowering effect (5–7).

Lipoprotein lipase (LPL) hydrolyzes triglycerides (TG) in
circulating chylomicrons and very low-density lipoproteins (VLDL) on the surface of endothelial cells (8). LPL is produced mainly in adipocytes and skeletal muscle cells (8, 9), and is transported to the surface of endothelial cells (10, 11). In clinical studies, LPL analysis has been conducted by using postheparin plasma, because LPL is detached from the endothelial cells by heparin injection and is released into the blood stream (12). Tornvall et al report that LPL mass exists in preheparin serum (preheparin LPL mass), even though lipase activity is scarcely recognized (13). We reported that administration of bezafibrate and insulin sensitizer, troglitazone, increases preheparin LPL mass, indicating that preheparin LPL mass could reflect the amount of LPL produced in a whole body, and might be related to insulin sensitivity (14, 15).

Diabetes mellitus is a very important risk factor for acute coronary syndrome (16, 17). Miyashita et al reported that preheparin LPL mass in type 2 diabetes mellitus patients was significantly low compared to that in healthy subjects (18). Hitsumoto et al reported that patients with recognized low serum preheparin LPL mass concentration had high risk of acute coronary syndrome (19, 20). From these findings, serum low concentration of preheparin LPL mass might be related to be a risk factor for coronary arterial disease.

In the present study, we examined the possibility that atorvastatin and pravastatin increase serum concentration of preheparin LPL mass in type 2 diabetes mellitus patients with hypercholesterolemia as one of the pleiotropic effects of statins.

Subjects and Methods

Subjects
The subjects were 47 type 2 diabetes mellitus patients with hypercholesterolemia (TC > 220 mg/dl). This trial was carried by non-blind. They were randomly assigned to one of the following two groups. One group of subjects was treated with atorvastatin (10 mg/day, n = 24), and the other was treated with pravastatin (20 mg/day, n = 23) for 4 months. The clinical profile of the subjects at the baseline is shown in Table 1. During this study, all patients kept the same diet and exercise therapy, and did not change medications. The purpose, nature and potential risk of this study were explained to all patients and their voluntary consent was obtained before they were enrolled.

Blood sampling
Blood samples were taken in the morning after 12 hours of fasting. Serum was obtained within 1 hour, and samples were used for measuring of HbA1c, serum total cholesterol (TC), triglyceride (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C) and preheparin LPL mass.

Table 1. The profile of all patients at baseline.

<table>
<thead>
<tr>
<th>Case</th>
<th>Atorvastatin</th>
<th>Pravastatin</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>57.8 ± 2.2</td>
<td>59.9 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.06 ± 0.20</td>
<td>6.15 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>275.5 ± 13.1</td>
<td>268.5 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>196.0 ± 21.3</td>
<td>186.3 ± 17.0</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>54.6 ± 2.2</td>
<td>56.0 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>180.9 ± 10.1</td>
<td>179.0 ± 5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Preheparin LPL mass (ng/ml)</td>
<td>48.0 ± 4.1</td>
<td>49.5 ± 4.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SE

Assay of preheparin LPL mass
Preheparin LPL mass was measured by the sandwich enzyme-linked immunosorbent assay using a specific monoclonal antibody against lipoprotein lipase (Daiichi Pure Chemicals, Tokyo), as described by Kobayashi et al. (21). The linearity of this assay system was observed from 5 to 400 ng/ml. Within-run coefficient variation was 2.8%. Between-day coefficient variation was 4.3%.

HbA1c analysis
HbA1c, including stable and unstable fractions was measured with high pressure liquid chromatography method using Hi-Auto A1c (Kyoto Daiichi Kagaku, Kyoto, Japan). As data of this study, stable type was used.

Serum lipids analysis
TC, TG and LDL-C concentrations were measured enzymatically using a kit available from Nippon Shoji (Osaka, Japan) and an automatic analyzer (Hitachi 7150 available from Hitachi Tokyo, Japan). HDL-C was measured by the selective inhibition method (Daiichi Pure Chemicals, Tokyo).

Statistical analysis
Data was expressed as the mean ± SE. A t-test was used for group comparisons. p values less than 0.05 were considered significant.

Results

Changes of HbA1c
During this study, no significant change of HbA1c was observed in both two groups, and also no significant difference of HbA1c between the two groups was observed (Fig. 1).
Effect of Statins on Preheparin LPL Mass

Changes of serum lipids

In the atorvastatin and pravastatin groups, TC decreased from 275 mg/dl to 193 mg/dl (−29.7%, \( p < 0.01 \) versus before administration), and from 268 mg/dl to 217 mg/dl (−18.9%, \( p < 0.01 \) versus before administration) respectively 4 months later (Fig. 2A). Furthermore, TC showed a larger decrease in the atorvastatin group compared to the pravastatin group after 4 months of administration (Fig. 2A). TG significantly decreased in the atorvastatin group (−21.0%, \( p < 0.05 \)). Although TG decreased in the pravastatin group, no significant change was observed (Fig. 2B). LDL-C significantly decreased in the atorvastatin group compared to the pravastatin group (−36.3%, \( p < 0.01 \) in atorvastatin, −24.3%, \( p < 0.01 \) in pravastatin) (Fig. 2C). There was no significant change in HDL-C in both groups (Fig. 2D).

Changes of preheparin LPL mass

Preheparin LPL mass was slightly increased by about 5% in the atorvastatin group, and 10% in the pravastatin group during statin administration, but no significant difference was observed between the two groups (Fig. 3).

Changes of preheparin LPL mass and serum lipids in patients showed low preheparin LPL mass before statin administration

In patients with recognized low preheparin LPL mass before statin administration (less than 50 ng/ml), changes of preheparin LPL mass and serum lipids were studied. The clinical profiles of these patients are shown in Table 2. Preheparin LPL mass significantly increased from 39.7 ng/ml to 47.2 ng/ml (+25.3%, \( p < 0.05 \)) in the atorvastatin group, and from 38.7 ng/ml to 47.9 ng/ml (+24.4%, \( p < 0.05 \)) in the pravastatin group (Fig. 4). The increasing rate of preheparin LPL mass was not significant between the atorvastatin group and the pravastatin group. HbA1c slightly decreased in both groups (Table 3). In serum lipids, TC showed a larger decrease in the atorvastatin group compared to the pravastatin group after 4 months of administration (Table 3). HDL-C was significantly increased both groups, but no significant difference was observed.
between two groups (Table 3). ∆TG negatively correlated with ∆preheparin LPL mass ($r = -0.26$, $p = 0.27$, Fig. 5). ∆HDL positively correlated with ∆preheparin LPL mass ($r = 0.52$, $p = 0.02$, Fig. 6).

Discussion

In this study, atorvastatin (10 mg/day) and pravastatin (20 mg/day) were administered to type 2 diabetes mellitus patients with hypercholesterolemia, and these pa-

Table 2. The profile of patients at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin</th>
<th>Pravastatin</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (years old)</td>
<td>59.8 ± 2.2</td>
<td>58.1 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.14 ± 0.27</td>
<td>6.18 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>268.8 ± 14.2</td>
<td>270.0 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>177.6 ± 24.3</td>
<td>169.6 ± 22.8</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51.4 ± 2.6</td>
<td>53.6 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>188.9 ± 11.2</td>
<td>191.4 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Preheparin LPL mass (ng/ml)</td>
<td>39.7 ± 2.8</td>
<td>38.7 ± 4.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Patients with their preheparin LPL mass < 50 ng/dl before administration.

$\text{Mean} \pm \text{SE}$

Fig. 3. Changes in preheparin LPL mass during statin administration. Closed circle shows atorvastatin group, and open circle shows pravastatin group. Data are shown as mean ± SE.

Fig. 4. Changes of preheparin LPL mass in low preheparin LPL mass patients. Low preheparin LPL mass patients mean patients whose concentration of preheparin LPL mass before statin administration showed less than 50 ng/ml. Closed circle shows atorvastatin group ($n = 12$), and open circle shows pravastatin group ($n = 12$). Data are shown as mean ± SE. * $p < 0.05$ versus before administration.

Miyashita et al. reported that preheparin LPL mass was significantly low in diabetes mellitus patients compared to non-diabetic healthy subjects (18). He reported that preheparin LPL mass in non-diabetic healthy subjects and type 2 diabetes mellitus patients were 57 ng/ml and 36 ng/ml respectively. In this study, in the atorvastatin and pravastatin groups, preheparin LPL mass before statin administration was higher than that of subjects reported by Miyashita et al. For this reason, subjects selected in this study kept good or fair control of diabetic condition. Preheparin LPL mass was also reported to be affected by diabetic condition, such as increasing according to improvement of HbA1c (15). In this study, there was no significant change in HbA1c. Accordingly, it is indicated that diabetic condition did not influence preheparin LPL mass during this study.

It is known that statins decrease TG in the addition of LDL-C lowering effect (22), and TG lowering effect of atorvastatin is stronger than that of pravastatin (22). The mechanisms of TG decreasing and HDL-C increasing effect by statin have been unclear. Totsuka et al reported
Effect of Statins on Preheparin LPL Mass

**Table 3. The changes in HbA₁c and serum lipids of patients during administration.**

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin</th>
<th>Pravastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 0 month</td>
<td>4 months</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>6.14 ± 0.27</td>
<td>5.98 ± 0.36</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>268.8 ± 14.2</td>
<td>188.0 ± 8.7*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>177.6 ± 24.3</td>
<td>121.6 ± 23.1*</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51.4 ± 2.6</td>
<td>57.1 ± 3.6*</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>188.9 ± 11.2</td>
<td>103.4 ± 8.8**</td>
</tr>
</tbody>
</table>

* p < 0.05 versus 0 M, * p < 0.05 versus pravastatin.

Patients with their preheparin LPL mass < 50 ng/dl before administration.

![Fig. 5](image1.png) Correlation between ∆TG and ∆preheparin LPL mass, in low preheparin LPL mass patients. Closed circle shows atorvastatin group (n = 12), and open circle shows pravastatin group (n = 12). Low preheparin LPL mass patients means patients whose concentration of preheparin LPL mass before statin administration showed less than 50 ng/ml. ∆TG and ∆preheparin LPL mass mean difference between before administration and after 4 months.

![Fig. 6](image2.png) Correlation between ∆HDL-C and ∆preheparin LPL mass in low preheparin LPL mass patients. Closed circle shows atorvastatin group (n = 12), and open circle shows pravastatin group (n = 12). Low preheparin LPL mass patients means patients whose concentration of preheparin LPL mass before statin administration showed less than 50 ng/ml. ∆HDL-C and ∆preheparin LPL mass mean difference between before administration and after 4 months.

that preheparin LPL mass negatively correlated with TG and positively correlated with HDL-C, indicating that preheparin LPL mass reflect the amount of working LPL in a whole body (14). In this study, preheparin LPL mass slightly increased in the atorvastatin group and the pravastatin group accompanying decrease of TG and increase of HDL-C. Further, in the low preheparin LPL mass group, ∆preheparin LPL mass had negative correlation with ∆TG and positive correlation with ∆HDL-C. These results suggested that atorvastatin and pravastatin might increase LPL production, resulting in decrease of TG and increase of HDL-C.

In the low preheparin LPL mass group, preheparin LPL mass was significantly increased by atorvastatin and pravastatin administration, and there was no significant difference in increasing rate between two groups. Hitsumoto et al. reported that patients with recognized low serum preheparin LPL mass concentration had high risk of acute coronary syndrome (19, 20). On the other hand, diabetes mellitus is known to be a very important risk factor for acute coronary syndrome, and the risk was accelerated by the addition of hypercholesterolemia (16).
So, it was considered that the risk of the patients who showed low preheparin LPL mass in this study was higher than the risk of the other patients. Accordingly, increase of preheparin LPL mass in diabetic patients with both hypercholesterolemia and low preheparin LPL mass could be a favorable effect to prevent acute coronary syndrome. TC levels were significantly decreased in the atorvastatin group compared to the pravastatin group (Fig. 2A). ∆LDL-C during administration did not correlate with ∆preheparin LPL mass (data not shown). These results suggested that increasing effect of preheparin LPL mass by statins did not depend on the difference of LDL-C lowering potential of each statins.

As to the mechanisms by which statin increased preheparin LPL mass, there are at least two possible explanations. The first is that statin might act directly to adipose cell or muscle cell, and enhance appearance of PPAR-α. Another possibility is that statin might enhance appearance of PPAR-γ. If statin might stimulate PPAR-γ, HbA1C might be decreased. But no significant change of HbA1C was observed. Accordingly, we considered that statins stimulate appearance of PPAR-α. But the precise mechanism should be studied more.

In this study, administration of atorvastatin and pravastatin to type 2 diabetic patients with hypercholesterolemia increased serum preheparin LPL mass concentration. Especially, its effect was remarkable in patients who showed low preheparin LPL mass. These effects might be considered to be one of pleiotropic effects in statin.

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