The Effects of Statin and Fibrate on Lowering Small Dense LDL-Cholesterol in Hyperlipidemic Patients with Type 2 Diabetes

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Aim: Small dense (sd)-low-density lipoprotein (LDL) is a potent atherogenic lipoprotein. The overall atherogenicity of this lipoprotein can be precisely assessed by quantifying sd-LDL rather than by measuring the LDL size. We studied the effects of representative lipid-lowering agents (statin and fibrate) on sd-LDL-cholesterol (C) in patients with type 2 diabetes.

Methods: Sd-LDL-C was measured by the precipitation method established by Hirano and Ito. Large buoyant (lb)-LDL-C was calculated by subtracting sd-LDL-C from LDL-C. Type 2 diabetes patients (n=72) were administered lipid-lowering agents for three months: patients with hypercholesterolemia received 1 mg of pitavastatin and those with hypertriglyceridemia received 100 mg of micronized fenofibrate.

Results: Pitavastatin reduced LDL-C by 25% and reduced TG by 8%. The statin decreased sd-LDL-C by 26%, and lb-LDL-C by 22%. Fenofibrate reduced TG by 38% and increased HDL-C by 14%. The fibrate decreased sd-LDL-C by 23% without changing LDL-C. The pitavastatin-induced reduction of sd-LDL-C was significantly correlated with the reduction of LDL-C and apo B, whereas the fenofibrate-induced reduction of sd-LDL-C was correlated with the reduction of TG.

Conclusion: Both statin and fibrate reduce the potency of atherogenic sd-LDL particles, but via different mechanisms: the former decreases total-LDL including sd-LDL, while the latter decreases sd-LDL specifically.


Key words; Small dense LDL-cholesterol, Type 2 diabetes, Pitavastatin, Fenofibrate

Introduction

Small dense low-density lipoprotein (sd-LDL) has recently been highlighted as a new risk factor for coronary heart disease (CHD). Sd-LDL is predictive of risk not only in Westerners¹⁻³, but also in Japanese in spite of their somewhat lower levels of LDL-cholesterol (C)⁴⁻⁵. LDL particle size is usually measured by gradient gel electrophoresis (GGE) using non-denaturing polyacrylamide according to the method of Nichols, Krauss, and Musliner⁶. This GGE procedure provides no quantitative determination of sd-LDL, however, and it requires a time-consuming assay consisting of overnight electrophoresis, staining, and destaining. Analytical ultracentrifugation, the standard technique for quantifying sd-LDL⁷, also requires a long running time, and the need for specialized equipment renders it unsuitable for general clinical use. Our group recently established a simple assay for sd-LDL-C using heparin-Mg precipitation followed by direct measurement of LDL-C⁸⁻⁹. We previously reported that the sd-LDL-C level determined by this method was substantially elevated in subjects with CHD and type 2 diabetes¹⁰.

Statin acts potently in reducing LDL-C and is widely used to prevent CHD events. It remains unclear, however, whether statin can reduce sd-LDL-C by mechanisms similar to LDL-C. Sd-LDL particles have weaker affinity to the LDL receptor than large buoyant (lb)-
LDL particles\(^{11}\), and the residence time of sd-LDL particles is much longer than that of lb-LDL particles in the blood circulation\(^{12}\). If the induction of LDL receptors by statin treatment stimulates the uptake of lb-LDL particles more potently than the uptake of sd-LDL particles, as proposed, then statin treatment might not reduce sd-LDL as effectively as lb-LDL. Plasma TG levels exert a strong influence on LDL size\(^{13}\); hence, we know that a potent TG-lowering agent such as fibrate will enlarge the LDL size\(^{13}\). Few studies, however, have examined the quantitative change of LDL subspecies by fibrate treatment. For these reasons, we tried to determine the effects of statin and fibrate on the concentration of LDL subspecies in patients with type 2 diabetes, one of the representative diseases with elevated sd-LDL-C.

### Methods

Seventy-two patients (30 men and 42 women) with type 2 diabetes, aged 60.2±9.6, were enrolled in this study. Dietary therapy was supervised by a diettian and exercise therapy was prescribed by a physician according to the recommendations of the Japanese Diabetes Association. Patients were treated with diet therapy alone \((n=11)\), sulfonylureas \((n=12)\), alpha-glucosidase inhibitors \((n=21)\), metformin \((n=11)\), insulin \((n=2)\), and combinations of oral hypoglycemic agents \((n=15)\). All hypoglycemic agents were continued during the study without any changes in dose. Hypercholesterolemic subjects \((LDL-C > 140 \text{mg/dL at baseline})\) received pitavastatin \((1 \text{mg})\) once daily for three months. Hypertriglyceridemic subjects \((TG > 150 \text{mg/dL at baseline})\) received micronized fenofibrate \((100 \text{mg})\) once daily for three months. Patients with combined hyperlipidemia were given either pitavastatin or fenofibrate, based on the severity of hypercholesterolemia or hypertriglyceridemia. There was no significant difference between statin and fibrate groups in the method used for glycemic control. Fasting serum samples were collected before and three months after the commencement of lipid-lowering therapy. Informed consent was obtained from all subjects, and this study was approved by the local ethics committee.

Sd-LDL-C was measured using a commercially available test kit \((sd-LDL-C \text{ “Seiken”, Denka Seiken, Tokyo, Japan})\). The principle of this method has been described in detail elsewhere\(^\text{a, b}\). When serum \((0.2 \text{mL})\) was incubated with heparin-magnesium precipitation agents, all of the apoB-containing lipoproteins but sd-LDL were aggregated, and the aggregate was trapped in a filter by centrifugation \((5000 \text{rpm for 1 min})\). sd-LDL-C was obtained by subsequently measuring the remainder by direct homogenous LDL-C assay \((LDL-EX, \text{Denka Seiken})\). The sd-LDL-C level was identical to cholesterol in the denser LDL fraction with a density \((d) = 1.044 – 1.063 \text{g/mL}\). Large buoyant \((lb)\)-LDL-C was calculated by subtracting sd-LDL-C from LDL-C. We demonstrated an excellent relationship between estimated lb-LDL-C and cholesterol in the lighter LDL fraction with a density \((d) = 1.019 – 1.044 \text{g/mL}\). Apolipoprotein (apo) A1 and B were measured by immunoturbidometry \((\text{Daiichi Pure Chemical Co})\). LDL-C and HDL-C were measured by direct assay using commercially available kits \((LDL-EX \text{ and HDL-EX; Denka Seiken Co.)})\).

The paired Student’s t-test was used to assess the significance of differences between before and after lipid-lowering treatments. Correlations between two variables were calculated by Pearson’s simple linear regression analysis. Multiple linear regression analysis was performed to evaluate the independent influence of changes of LDL-C, apoB, TG or HDL-C on the changes of sd-LDL-C by lipid-lowering treatments. Statistical significance was accepted at \(p < 0.05\).

### Results

**Table 1** (left panel) shows the serum levels of various parameters before and three months after treatments with pitavastatin (left panel) and fenofibrate (right panel). Pitavastatin treatment brought about no changes in glycemic control determined by fasting glucose and HbA1c. LDL-C was significantly reduced from 171 to 125 mg/dL in response to treatment, apo B was significantly reduced while TG was only slightly reduced, HDL-C was unchanged, whereas apo AI was slightly elevated. Sd-LDL-C was significantly reduced from 37 to 25 mg/dL and Lb-LDL-C was significantly decreased from 134 to 99 mg/dL. The treatment brought about no change in the percentage of sd-LDL-C in total LDL-C (22 to 20%).

Fenofibrate treatment induced no changes in FPG or HbA1c; the levels of both were comparable to those measured in the statin group. Plasma TG was markedly reduced from 352 to 179 mg/dL by fibrate treatment, whereas LDL-C was essentially unchanged, HDL-C was significantly increased from 49 to 55 mg/dL, whereas apo AI was unchanged, apo B was significantly reduced, Sd-LDL-C was significantly reduced from 37 to 25 mg/dL and Lb-LDL-C was significantly decreased from 134 to 99 mg/dL. The treatment brought about no change in the percentage of sd-LDL-C in total LDL-C (22 to 20%).

**Fig. 1** shows the percent changes in TG, total-LDL-C, sd-LDL-C and lb-LDL-C after pitavastatin...
Table 1. Serum levels of various parameters before and 3 months after treatment with 1 mg of pitavastatin or 100 mg of micronized fenofibrate in patients with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Pitavastatin</th>
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<th>Fenofibrate</th>
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<tr>
<td></td>
<td>pre</td>
<td>post</td>
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<td></td>
<td>pre</td>
<td>post</td>
<td>p</td>
<td></td>
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<tr>
<td>Glucose</td>
<td>143 ± 52</td>
<td>155 ± 66</td>
<td>NS</td>
<td></td>
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<td>159 ± 82</td>
<td>140 ± 62</td>
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<td>HbA1c</td>
<td>6.8 ± 1.5</td>
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<td>HbA1c</td>
<td>6.7 ± 1.5</td>
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<td>LDL-C</td>
<td>171 ± 40</td>
<td>125 ± 34</td>
<td>&lt;0.0001</td>
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<td>LDL-C</td>
<td>135 ± 36</td>
<td>132 ± 35</td>
<td>NS</td>
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<tr>
<td>TG</td>
<td>206 ± 159</td>
<td>160 ± 91</td>
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<td>TG</td>
<td>352 ± 230</td>
<td>179 ± 84</td>
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<td>HDL-C</td>
<td>58 ± 13</td>
<td>59 ± 15</td>
<td>NS</td>
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<td>HDL-C</td>
<td>49 ± 12</td>
<td>55 ± 13</td>
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<tr>
<td>ApoA1</td>
<td>142 ± 21</td>
<td>149 ± 26</td>
<td>&lt;0.01</td>
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<td>ApoA1</td>
<td>140 ± 26</td>
<td>147 ± 28</td>
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<tr>
<td>ApoB</td>
<td>132 ± 25</td>
<td>101 ± 23</td>
<td>&lt;0.0001</td>
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<td>118 ± 22</td>
<td>103 ± 24</td>
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<td>sd-LDL-C</td>
<td>37 ± 20</td>
<td>25 ± 15</td>
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<td>sd-LDL-C</td>
<td>45 ± 21</td>
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<tr>
<td>lb-LDL-C</td>
<td>134 ± 39</td>
<td>99 ± 29</td>
<td>&lt;0.0001</td>
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<td>lb-LDL-C</td>
<td>90 ± 34</td>
<td>99 ± 31</td>
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Data represent the mean ± SD. NS = not significant (p > 0.05), Pre = pre-treatment, Post = post-treatment.

Fig. 1

Percent changes in TG, total-LDL-C, small dense (sd)-LDL-C and large buoyant (lb)-LDL-C after pitavastatin treatment (1 mg/d). *p < 0.01 vs pre-treatment.

Fig. 2

Percent changes in TG, total-LDL-C, sd-LDL-C and lb-LDL-C after micronized fenofibrate treatment (100 mg/d). *p < 0.01 vs pre-treatment.

Table 2. Correlations between changes of sd-LDL-C and changes of various parameters before and 3 months after treatment with 1 mg of pitavastatin or 100 mg of micronized fenofibrate in patients with type 2 diabetes

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<tr>
<th></th>
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<td>NS</td>
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<tr>
<td>HbA1c</td>
<td>0.07</td>
<td>NS</td>
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<td>HbA1c</td>
<td>0.15</td>
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<tr>
<td>TG</td>
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<td>NS</td>
<td></td>
<td></td>
<td>LDL-C</td>
<td>0.04</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
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<td>NS</td>
<td></td>
<td></td>
<td>TG</td>
<td>0.60</td>
<td>&lt;0.002</td>
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<td>LDL-C</td>
<td>0.36</td>
<td>&lt;0.02</td>
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<td>HDL-C</td>
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<td>lb-LDL-C</td>
<td>0.02</td>
<td>NS</td>
<td></td>
<td></td>
<td>lb-LDL-C</td>
<td>0.15</td>
<td>NS</td>
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<tr>
<td>ApoA1</td>
<td>0.04</td>
<td>NS</td>
<td></td>
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<td>ApoA1</td>
<td>0.38</td>
<td>NS</td>
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<tr>
<td>ApoB</td>
<td>0.48</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>ApoB</td>
<td>0.26</td>
<td>NS</td>
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with the reduction of LDL-C and apo B, respectively.

The right panel of Table 2 shows the correlations between the change of sd-LDL-C and those of serum parameters during fenofibrate treatment. The reduction of sd-LDL-C by fenofibrate treatment was substantially associated with the reduction of TG and the increase of HDL-C, but was not associated with the reduction of LDL-C. Multiple regression analysis revealed that the reduction of sd-LDL-C was significantly correlated with the reduction of TG, but not with the increase of HDL-C.

Creatinine kinase and laboratory examinations related to liver function were essentially unchanged by pitavastatin and fenofibrate treatments (data not shown).

Discussion

Pitavastatin is a newly developed statin now widely used in Japan and many other countries. The usual dose is 2 mg/day, and the maximal dose is 4 mg/day. Preliminary studies have revealed that 2 mg of pitavastatin confers about the same cholesterol-lowering effect as 10 mg of atorvastatin. In the present study, we examined the effect of a relatively low dose of pitavastatin (1 mg) on LDL subspecies. Even at the low dose administered, pitavastatin decreased LDL-C by 25% (significant). The low dose also reduced sd-LDL-C and lb-LDL by comparable levels, and the former reduction was significantly correlated with the pitavastatin-induced reduction of LDL-C. These results suggest that the induction of LDL receptors by statin stimulates the uptake of all LDL particles, irrespective of their size. Another study found that 2 mg of pitavastatin significantly increased the LDL size. It may be that the potent induction of LDL receptors by a high-dose of pitavastatin preferentially facilitates the removal of sd-LDL particles. Pitavastatin also appears to reduce the TG level with a 2 mg dose, and its hypotriglyceridemic action was confirmed even at the lower dose of 1 mg administered in the present study. The ratio of sd-LDL in LDL-C was unchanged by pitavastatin treatment, implying no change in LDL size. While a lower TG is generally expected to increase the size of LDL particles, the mild reduction in TG by low-dose pitavastatin might be insufficient to bring about this effect.

There have been a number of reports that fibrate, a potent TG-lowering agent, enlarges LDL in patients with type 2 diabetes. Few reports, however, have demonstrated the effects of fibrate on the concentrations of LDL subspecies. In the present study we used a low dose of fenofibrate (100 mg) as a suitable comparison with the low dose of pitavastatin (1 mg). As a result, fenofibrate substantially lowered TG and elevated HDL-C even at the low dose administered. Lb-LDL was unchanged by fibrate treatment, whereas sd-LDL-C was markedly decreased. When TG-rich lipoproteins are increased, LDL particles become TG-enriched and cholesterol in the blood is depleted by the action of cholesterol ester transfer protein. Sd-LDL particles are generated from TG-rich and cholesterol-poor LDL particles by the action of hepatic TG lipase. Fibrates likely suppress lipid exchange between TG-rich lipoprotein and LDL via the marked reduction in TG-rich lipoproteins. TG-rich large VLDL (VLDL1) is proposed to be a precursor of sd-LDL, while smaller VLDL (VLDL2) is proposed to be a precursor of lb-LDL. Fibrates may reduce sd-LDL particles by suppressing the production of VLDL1 and stimulating the transfer from VLDL1 to VLDL2 via the activation of lipoprotein lipase. In an earlier study, we reported the enlargement of LDL by another fibrate, bezafibrate, and proposed that the change was wholly attributable to the TG-lowering effect of the agent. Similarly, the present study confirmed an association between the decrease of sd-LDL-C and the decrease of TG during fenofibrate treatment. Though we did not measure the actual changes of the LDL size in this study, the specific decrease in sd-LDL-C without affecting lb-LDL-C implied that the average LDL size was increased.

We finally determined that low doses of statin and fibrate were both effective in decreasing sd-LDL-C concentration, and by comparable rates of reduction. We should bear in mind, however, that the baseline sd-LDL-C level and the phenotype of hyperlipidemia differed significantly between the statin- and fibrate-treated groups. Though we cannot readily conclude which drug reduces sd-LDL more potently, we can expect substantial reductions in sd-LDL-C when statin is administered to hypercholesterolemic subjects and when fibrate is administered to hypertriglyceridemic subjects. Large-scale clinical trials will help to elucidate whether the effects of statin and fibrate in reducing sd-LDL particles are associated with the prevention of CHD events.

References

3) Gardner CD, Fortmann SP, and Krauss RM: Association
of small dense low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA, 1996; 276:875-881


