Original Article

Effect of Ezetimibe Monotherapy on Lipid Metabolism and Arterial Stiffness Assessed by Cardio-Ankle Vascular Index in Type 2 Diabetic Patients

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Aim: High cholesterol absorption in the small intestine has been proposed to be a risk factor of atherosclerosis. In this study, we evaluated the effect of ezetimibe monotherapy on arterial stiffness in type 2 diabetic patients.

Methods: Forty type 2 diabetes mellitus patients with high serum low-density lipoprotein cholesterol (LDL-C) were enrolled and treated with ezetimibe 10 mg/day for 6 months. HbA1c, serum lipids, remnant-like particle-cholesterol (RLP-C), serum lipoprotein lipase mass (LPL mass) and the cardio-ankle vascular index (CAVI) were measured before and after ezetimibe treatment.

Results: After 6 months of ezetimibe treatment, significant decreases in LDL-C, RLP-C and CAVI were observed. In the group that achieved the LDL-C goal of <120 mg/dL after 6 months of ezetimibe treatment, the pretreatment CAVI was markedly high, and CAVI decreased significantly after ezetimibe treatment.

Conclusions: In type 2 diabetic patients, ezetimibe monotherapy may have the potential to ameliorate arterial stiffness in addition to lowering LDL-C and RLP-C.


Key words; Ezetimibe, Remnant like particle, Cardio-ankle vascular index

Introduction

Diabetic patients complicated with high serum low-density lipoprotein cholesterol (LDL-C) are at increased risk of macroangiopathy1, 2; therefore, in diabetic patients, intensive LDL-C-lowering therapy is needed to prevent the progression of atherosclerosis. Recent reports have suggested that high cholesterol absorption in the small intestine may be one of the risk factors of atherosclerosis3, 4. Type 2 diabetic patients show high cholesterol absorption5; therefore, high cholesterol absorption may be related to the high frequency of atherosclerosis in diabetic patients.

Ezetimibe is a cholesterol absorption inhibitor with demonstrated efficacy and tolerability6. Inhibition of cholesterol absorption is expected to prevent the progression of atherosclerosis in patients showing high cholesterol absorption, such as type 2 diabetes mellitus; however, the effectiveness of ezetimibe monotherapy in preventing cerebro-cardiovascular events has not been reported.

Recently, a novel arterial stiffness parameter, termed the cardio-ankle vascular index (CAVI), was developed, which essentially reflects the stiffness of the aorta, femoral artery and tibial artery7. CAVI is independent of blood pressure, and has adequate reproducibility for clinical use8. Furthermore, no special technique is required for the measurement of CAVI. Several reports have demonstrated the usefulness of CAVI for the detection of atherosclerotic diseases7,10. Furthermore, we have reported that treatment with statin or angiotensin II receptor blocker (ARB) improves CAVI11, 12. These findings indicate that CAVI is use-
ful to assess the anti-atherosclerotic effect of various drugs.

In the present study, we evaluated the effect of ezetimibe monotherapy on arterial stiffness in type 2 diabetic patients with high LDL-C, using CAVI.

**Subjects and Methods**

**Subjects**

A randomized, open study was performed. Forty type 2 diabetes mellitus patients with high LDL-C, who attended Sakura Medical Center of Toho University as outpatients, were enrolled. Patients were excluded if they had received insulin therapy, or had diabetic retinopathy, nephropathy or previous cardiovascular and cerebrovascular diseases. The clinical profile of the subjects is shown in Table 1. The enrolled subjects were treated with ezetimibe 10 mg/day for 6 months. During the study period, all patients maintained the same diet and exercise therapies, and did not change medications. All subjects received nutrition guidance from a dietitian every month. After 6 months of ezetimibe treatment, the subjects were divided into two groups according to whether they had achieved the LDL-C goal of <120 mg/dL, as recommended by the Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases (JAS, 2007). This study was approved by the institutional review board. The purpose of this study was explained to the subjects, and consent was obtained for participation in the study and also for release of the study data.

**Measurements of Body Weight and Blood Pressure**

Body weight and blood pressure were measured in the morning after 12 hours of fasting. Blood pressure was measured at least twice in a sitting position.

**Assays of HbA1c, Serum Lipids and Serum LPL Mass**

Blood samples were collected in the morning after 12 hours of fasting. Serum was separated within 1 hour of blood collection, and samples were used to measure the following chemical parameters. Stable and unstable fractions of glycosylated hemoglobin (HbA1c) were measured by high pressure liquid chromatography using the Hi-Auto A1c kit (Kyoto Daiichi Kagaku, Kyoto, Japan). Data on the stable type were used in the present analysis. Total cholesterol (TC), triglyceride (TG) and LDL-C were measured with an automatic analyzer (Hitachi 7150; Hitachi Tokyo, Japan). High-density lipoprotein cholesterol (HDL-C)

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**Table 1. Clinical parameters of subjects before and after ezetimibe treatment**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>40 (23/17)</td>
<td>23.2 ± 3.2</td>
<td>0.3 ± 2.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 ± 10</td>
<td>128 ± 13</td>
<td>0.8 ± 11.2</td>
</tr>
<tr>
<td>BMI</td>
<td>23.1 ± 3.4</td>
<td>76 ± 8</td>
<td>0.4 ± 12.6</td>
</tr>
<tr>
<td>BP systolic</td>
<td>127 ± 14</td>
<td>6.3 ± 1.2</td>
<td>-3.1 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>76 ± 8</td>
<td>210 ± 24*</td>
<td>-14 ± 11</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.5 ± 1.2</td>
<td>149 ± 78</td>
<td>-4 ± 39</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>246 ± 25</td>
<td>57 ± 16</td>
<td>3.5 ± 9.4</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>155 ± 72</td>
<td>123 ± 24*</td>
<td>-21 ± 14</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>55 ± 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>160 ± 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure-lowering drugs (No)</td>
<td>25 (76%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARBs or ACE inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca channel blockers</td>
<td>12 (36%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sugar-lowering drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonylureas (No)</td>
<td>29 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiazolidinedione (No)</td>
<td>5 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitor (No)</td>
<td>13 (39%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanide (No)</td>
<td>10 (30%)</td>
<td></td>
<td></td>
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</tbody>
</table>

Data are presented as the mean ± SD. BMI: body mass index, BP: blood pressure, HbA1c: glycosylated hemoglobin, TC: total cholesterol, TG: triglycerides, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, ARB: angiotensin II receptor antagonist, ACE inhibitor: angiotensin II converting enzyme inhibitor
*p < 0.01 vs baseline by paired t test.
was measured by the selective inhibition method (Daiichi Pure Chemicals, Tokyo)\(^{13}\). Remnant-like particle-cholesterol (RLP-C) was analyzed by immunoseparation as described previously\(^{14}\). Serum lipoprotein lipase mass (LPL mass) was measured by a sandwich enzyme-linked immunosorbent assay using a specific monoclonal antibody against lipoprotein lipase (Daiichi Pure Chemicals, Tokyo), as described by Kobayashi et al.\(^{15}\).

**Measurement of CAVI**

CAVI was measured using a VaSera CAVI instrument (Fukuda Denshi Co. Ltd., Tokyo) by the methods described previously\(^{7}\). CAVI was measured in the morning after 12 hours of fasting. Briefly, cuffs were applied to the bilateral upper arms and ankles, with the subject supine and the head held in the midline position. After resting for 10 minutes, measurements were performed. To detect the brachial and ankle pulse waves with cuffs, a low cuff pressure of 30 to 50 mmHg was used to ensure the minimal effect of cuff pressure on hemodynamics. Blood pressure was measured thereafter. CAVI was calculated by the following formula:

\[
CAVI = a\left(2\rho/\Delta P\right) \times \ln(Ps/Pd)PWV^2 + b
\]

where \(Ps\) is systolic blood pressure, \(Pd\) is diastolic blood pressure, \(PWV\) is pulse wave velocity, \(\Delta P\) is \(Ps - Pd\), \(\rho\) is blood density, and \(a\) and \(b\) are constants.

Scale conversion was performed to compare CAVI with PWV (Hasegawa’s method). The VaSera was equipped with both measurement and calculation systems, and automatically calculated the CAVI. The average coefficient of variation of CAVI is less than 5%, which is sufficiently small for clinical usage and indicates that CAVI has good reproducibility\(^{7}\).

**Statistical Analysis**

Two groups were compared using Student’s \(t\)-test or the paired \(t\)-test. The relationship between changes of CAVI and each parameter was analyzed using simple regression analysis. In all comparisons, \(p < 0.05\) was considered significant.

**Results**

**Changes in Clinical Parameters After Ezetimibe Treatment**

The changes in clinical parameters after ezetimibe treatment are shown in Table 1. After ezetimibe administration for 6 months, significant decreases in TC and LDL-C were observed. No significant changes in BMI, blood pressure, HbA1c, TG and HDL-C were detected.

**Changes in RLP-C and LPL Mass After 6 Months of Ezetimibe Treatment**

The changes in RLP-C and LPL mass are shown in Fig. 1. After 6 months of ezetimibe treatment, a significant decrease in RLP-C from 9.44 to 7.41 mg/dL (\(p < 0.05\)) was observed (Fig. 1A), but no significant change in LPL mass was found (Fig. 1B).

**Change in CAVI After 6 Months of Ezetimibe Treatment**

The changes in CAVI are shown in Fig. 2. A sig-
significant decrease in CAVI from 9.17 to 9.00 (p<0.05) was observed after 6 months of ezetimibe treatment; however, the change in CAVI did not correlate with the changes in TC, LDL-C and RLP-C (data not shown).

Comparison of Each Parameter Before and After Ezetimibe Treatment Between Two Groups

Next, the data were compared between the group that achieved the LDL-C goal and the group that did not. The baseline clinical profiles of two groups and the changes in clinical parameters are shown in Table 2. The two groups did not differ significantly in all baseline parameters. After 6 months of ezetimibe treatment, the changes in TC and LDL-C were significantly decreased in two groups while the changes of all other parameters were not significantly different.

Comparison of CAVI Before and After Ezetimibe Treatment Between Two Groups

The changes of CAVI in the two groups are shown in Fig. 2. CAVI before ezetimibe treatment was significantly higher in the group that achieved the LDL-C goal than the group that did not. In the group that achieved the LDL-C goal, a significant decrease in CAVI was observed after 6 months; however, no significant change in CAVI was observed in the group that did not achieve the LDL-C goal.

Discussion

In the present study, LDL-C was significantly decreased by ezetimibe monotherapy, and a mean decrease of 23% was observed. Previous reports demonstrated that ezetimibe monotherapy lowered LDL-C by 18%\(^{10}\). Type 2 diabetic patients have been reported to express a high level of Niemann-Pick C1 like 1 protein, which is an intestinal cholesterol transporter\(^{17,18}\), and to show high cholesterol absorption\(^{19}\). High cholesterol absorption should result in a good response to ezetimibe treatment. This may account for the superior result in the present study compared to previous data. Ezetimibe has also been reported to have an RLP-C-lowering effect\(^{19}\). Fibrate is one of the typical drugs showing high RLP-C-lowering potential. Fibrate

![Fig. 2. Change in CAVI after ezetimibe treatment for 6 months.](image)

Data are presented as the mean ± S.D. *: p<0.05, paired t-test.

Table 2. Comparison of clinical parameters before and after ezetimibe treatment between patients who achieved the LDL-C goal <120 mg/dL and patients who did not achieve the goal

<table>
<thead>
<tr>
<th></th>
<th>LDL-C goal achieved(^a)</th>
<th>LDL-C goal not achieved(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
</tr>
<tr>
<td>n (male/female)</td>
<td>15 (10/5)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65 ±6</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>23.6 ± 2.6</td>
<td>23.5 ± 2.5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.7 ± 1.5</td>
<td>6.5 ± 1.2</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>242 ± 24</td>
<td>195 ± 22**</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>157 ± 79</td>
<td>160 ± 22</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>58.1 ± 14.2</td>
<td>57.3 ± 13.0</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>157 ± 22</td>
<td>100 ± 16**</td>
</tr>
<tr>
<td>RLP-C (mg/dL)</td>
<td>9.43 ± 3.09</td>
<td>7.90 ± 4.82**</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD. \(^a\): Post-treatment LDL-C <120 mg/dL; \(^b\): Post-treatment LDL-C ≥120 mg/dL. BMI: body mass index, HbA1c: glycosylated hemoglobin, TC: total cholesterol, TG: triglycerides, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, RLP-C: remnant-like particle-cholesterol. *: p<0.05, **: p<0.01 vs baseline by paired t test.
increases LPL and promotes the catabolism of TG-rich lipoprotein, such as RLP-C. In the present study, RLP-C was significantly decreased by ezetimibe administration without increasing the serum LPL mass level, which was reported to reflect total LPL production in the whole body.\(^\text{20}\) The effect of ezetimibe on reducing RLP-C was previously reported by Masuda \textit{et al.}\(^\text{21}\). As a possible mechanism, they suggest that the inhibition of chylomicron production by ezetimibe leads to the inhibition of cholesterol inflow into the liver and, as a result, upregulation of remnant receptors may occur. Therefore, one of the mechanisms by which ezetimibe lowers RLP-C may be the inhibition of cholesterol absorption and not the enhancement of LPL expression.

Next, we examined whether ezetimibe administration improves vascular stiffness, assessed by CAVI, which decreased significantly after 6 months of ezetimibe treatment; however, the change in CAVI did not correlate with the change in LDL-C or RLP-C (data not shown). These results suggest that ezetimibe potentially improves vascular stiffness and this potential is not necessarily due to the LDL-C- or RLP-C-lowering effect.

Cholesterol oxidation products, commonly known as oxysterols, are known to contribute to the development of atherosclerosis.\(^\text{22-24}\) Furthermore, a high intake of atherosclerosis is reported to deteriorate atherosclerosis.\(^\text{25}\) Our previous data demonstrated that the serum oxysterol concentration in type 2 diabetic patients, who are reported to have high absorption of cholesterol, was significantly higher than in healthy subjects.\(^\text{26}\) Ezetimibe was reported to inhibit oxysterol absorption.\(^\text{27}\) From these findings, we speculate that type 2 diabetic patients may have high absorption of oxysterols along with high absorption of cholesterol, and the inhibition of oxysterol absorption by ezetimibe may be associated with the improvement of CAVI.

To characterize patients who achieved the LDL-C goal of \(<120\ mg/dL\), as recommended by the Japanese guideline, we divided the subjects into two groups by the cutoff LDL-C level of \(120\ mg/dL\). Baseline CAVI in patients who achieved the LDL-C goal was significantly lower than that in patients not achieving the goal, in spite of similar LDL-C levels at baseline in the two groups. The CAVI difference between the two groups at baseline could not be explained by differences in age, BMI, HbA1c and serum lipid levels at baseline. Recently, it has been reported that high cholesterol absorption in the small intestine may be one of the risk factors for atherosclerosis.\(^\text{3, 4}\) In this study, the LDL-C-lowering response to ezetimibe treatment was higher in patients who achieved the LDL-C goal than in patients not achieving the goal. From these findings, we speculate that patients who achieved the LDL-C goal may correspond to high cholesterol absorbers, and high CAVI in these patients may be due to high cholesterol absorption; however, the change in RLP-C, which is atherogenic, was not significant between the two groups. Different mechanisms may exist between the conversion of LDL from RLP-C and cholesterol absorption. Further studies are required to clarify the detailed effects of ezetimibe on CAVI.

Our study had some limitations. First, the sample size was relatively small. Second, whether our findings are specific to type 2 diabetes mellitus is unknown, because a nondiabetic control group was not included. Third, since serum oxysterol levels were not measured before and after ezetimibe administration, whether oxysterol is related to the improvement of CAVI was not confirmed. In addition, our patients were mostly elderly. Thus, our findings may not be generalized to younger patients. To clarify the mechanism of the CAVI-ameliorating effect of ezetimibe, further studies are required in the future.

In summary, six months of ezetimibe monotherapy improved CAVI in addition to lowering LDL-C.
and RLP-C in type 2 diabetic patients. Furthermore, patients with a high response to ezetimibe showed significant improvement of CAVI. From these findings, we conclude that high cholesterol absorption may be one of the factors that aggravate vascular stiffness, and ezetimibe monotherapy may have the potential to ameliorate vascular stiffness through the inhibition of cholesterol absorption.

References
23) Miyashita Y, Shirai K, Ito Y, Watanabe J, Urano Y, Murano T, Tomioka H: Cytotoxicity of some oxysterols on human vascular smooth muscle cells was mediated by

