Original Article

The Effects of Ezetimibe on Surrogate Markers of Cholesterol Absorption and Synthesis in Japanese Patients with Dyslipidemia

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Aim: To demonstrate the clinical benefit of inhibiting intestinal cholesterol absorption, we evaluated the effects of ezetimibe on surrogate markers of cholesterol absorption and synthesis, lipid and glucose metabolism, and markers of obesity and inflammation.

Methods: A total of 120 patients with dyslipidemia (46 men; mean age 66.5 years), who had not achieved the low density lipoprotein cholesterol (LDL-C) goal recommended by the Japan Atherosclerosis Society Guideline despite diet and exercise or any statin therapy, were enrolled and additionally treated with ezetimibe (10 mg/day) for 12 weeks.

Results: Compared to the baseline, LDL-C was reduced by 19.2% (p < 0.001) after ezetimibe monotherapy and by 24.7% (p < 0.001) after co-administration with ezetimibe and any statin. Ezetimibe therapy decreased cholesterol absorption markers and increased a cholesterol synthesis marker. Treatment with ezetimibe reduced the fasting serum insulin level (p < 0.05) and HbA1c (p < 0.05), increased serum adiponectin (p < 0.01), and showed a significant decrease of high-sensitive C-reactive protein (hsCRP, p < 0.01). No adverse events occurred during the study.

Conclusion: Thus, cholesterol absorption inhibition by ezetimibe is an important therapeutic strategy since LDL-C and cholesterol absorption markers had a positive correlation. Ezetimibe not only reduced the serum LDL-C level but also improved glucose metabolism as well as obesity and inflammation markers. These findings support the benefit of ezetimibe as a new option for the treatment of dyslipidemia.


Key words: Cholesterol, Absorption, Synthesis, Ezetimibe

Introduction

Elevated low density lipoprotein cholesterol (LDL-C) is a major risk factor for cardiovascular diseases and its two sources, i.e., de novo synthesis in the liver and the absorption of dietary and biliary cholesterol in the intestine, have offered important targets for the treatment of hypercholesterolemia. HMG-CoA reductase inhibitors (statins), which target cholesterol synthesis in the liver, are the mainstay of the currently available LDL-C-lowering therapy. Statins inhibit cholesterol synthesis from mevalonate by inhibiting HMG-CoA reductase, a rate-limiting enzyme in the mevalonic pathway of cholesterol de novo synthesis. This effect in turn upregulates the expression of LDL receptors in the liver, thereby reducing the serum LDL-C level. Recent studies have elucidated the molecular mechanisms underlying intestinal cholesterol absorption, another potential therapeutic target for hypercholesterolemia. In 2000, Niemann-Pick C1 like 1 (NPC1L1), a transporter protein important for intestinal cholesterol absorption, was cloned. NPC1L1 is composed of 1,359 amino acids, possesses 13 transmembrane spanning
Effects of Ezetimibe on Dyslipidemia

segments, and is highly expressed on the surface of jejunal enterocytes. NPC1L1 has sterol-sensing domains and is known to have about 50% amino acid homology to Niemann-Pick C1 protein, which is affected in the cholesterol storage disease Niemann-Pick type C2.

Ezetimibe is a selective intestinal cholesterol transporter inhibitor which selectively inhibits cholesterol absorption by inhibiting NPC1L1 protein. The recent introduction of ezetimibe into clinical use3, together with a growing body of evidence that relates cholesterol absorption to the risk of cardiovascular events4, 5, has made investigators more aware of the importance of controlling cholesterol absorption for the prevention of these cardiovascular events. Some investigators have reported increased intestinal cholesterol absorption in US and/or European individuals with a history of coronary artery disease6, 7, or type 2 diabetes mellitus8, although the relevance of this alteration to these and other pathologies remains to be determined.

Based on these considerations, we evaluated the clinical benefit of ezetimibe and its effect on surrogate markers of cholesterol absorption and synthesis in Japanese patients with hypercholesterolemia in order to establish more efficient lipid-lowering therapy.

Materials

Between June and December 2007, patients who presented to the outpatient clinic of Fujita Ezetimibe Study Assembly were enrolled if they had not achieved the LDL-C goal levels recommended in the 2007 Guideline of the Japan Atherosclerosis Society (JAS)9 despite diet and exercise without anti-dyslipidemic agents for at least 4 weeks (ezetimibe monotherapy group) or had not achieved the LDL-C management goal levels despite at least 4 weeks of statin therapy (co-administration with ezetimibe and any statin). The study protocol was approved by the Fujita Health University Ethics Committee and all patients gave written informed consent.

Methods

At enrollment, all patients were examined for coronary risk factors, including advanced age (≥45 years for men and ≥55 years for women), hypertension, diabetes mellitus (including impaired glucose tolerance), smoking, hypo-high-density-lipoprotein cholesterolemia (<40 mg/dL), and a family history of coronary artery disease. Information was also obtained about their body mass index (BMI), waist circumference at the umbilical level, and use of antihypertensive, anti-diabetic, and anti-platelet agents.

The patients enrolled were given diet and exercise guidance and then treated orally with ezetimibe 10 mg once a day for 12 weeks. During treatment, the following parameters were monitored: serum lipid profile, glucose metabolism, markers of obesity and inflammation, markers of cholesterol absorption and synthesis, BMI, waist circumference, blood pressure, and heart rate. During the study period, the use of non-study anti-dyslipidemic agents was prohibited and anti-hypertensive, anti-diabetic, and anti-platelet therapy used at enrollment was to be continued without dosage modifications.

The serum lipid profile was assessed in terms of total cholesterol (TC), LDL-C (measured directly), triglycerides (TG), and high-density-lipoprotein cholesterol (HDL-C). Glucose metabolism was assessed by measuring fasting blood glucose, fasting insulin, and HbA1c levels. Serum levels of adiponectin and high-sensitive C-reactive protein (hsCRP) were measured as markers of obesity and inflammation, respectively. Two plant sterols not synthesized de novo, sitosterol and campesterol, were measured as markers of cholesterol absorption. Cholestanol (a bile acid metabolite) was measured as a surrogate marker of cholesterol re-sorption, and lathosterol (a cholesterol synthesis intermediate) respectively. The serum levels of these markers were determined by gas chromatography as described by Miettinen et al.10 TC and TG levels were determined using enzymatic methods HDL-C was determined by the detergent selective-inhibition method (Daiichi Pure Chemical Industries, Tokyo, Japan), and LDL-C was determined by the N-geneous assay (Daiichi Pure Chemical). Serum glucose concentrations were determined by the glucose oxidase method. Serum insulin levels were determined by an enzyme-linked immunosorbent assay, and HbA1c was determined by high-performance liquid chromatography. Serum high-sensitivity C-reactive protein levels were determined by the nephelometry assay used by N latex CRP II (Siemens Healthcare Diagnostics, Tokyo, Japan), and serum high molecular adiponectin levels were determined by an enzyme-linked immunosorbent assay (FUJIREBIO Inc., Tokyo, Japan). All laboratory analyses were exclusively performed at SRL Inc., an external laboratory (Hachioji, Tokyo, Japan).

The primary endpoints of this study were to clarify the patient’s lipid profile prescribing the surrogate marker of cholesterol absorption and synthesis and the effects of ezetimibe treatment on cholesterol absorption and synthesis markers. Secondary endpoints included changes from the baseline after treatment...
with ezetimibe in LDL-C, TG, and HDL-C levels, glucose metabolism, and biomarkers of obesity and inflammation.

Pearson correlation coefficients were used to assess the correlations between cholesterol absorption markers and individual baseline characteristic variables. Baseline clinical and laboratory characteristics were compared between those with and without enhanced cholesterol absorption markers using ANOVA, the chi-square test or the paired and non-paired t-test. Laboratory data obtained before and after treatment with ezetimibe were compared using the paired t-test. A value of $p < 0.05$ was considered significant. Statistical analyses were performed with statistical software JMP (version 5.1; SAS Institute, Inc., Cary, NC).

The planned sample size was set at 100 based on the feasibility of recruitment in view of the exploratory nature of this study and the lack of previous publications which permitted us to make appropriate hypotheses about the primary endpoints.

### Results

A total of 120 patients (80 for ezetimibe monotherapy and 40 for co-administration with ezetimibe and any statin) were enrolled during the planned enrollment period (from June to December 2007). According to the risk category recommended by the JAS Guideline, the study population consisted of 1 low-risk, 55 moderate-risk, and 33 high-risk patients treated for primary prevention as well as 31 patients treated for secondary prevention. Table 1 shows the baseline characteristics of the patients.

### Correlations between Cholesterol Absorption Markers and Baseline Characteristics

The serum LDL-C level had a significant positive correlation with cholesterol absorption markers as
Correlation between a surrogate marker of cholesterol absorption and serum lipid profile in patients without any statin

The relationships between cholesterol absorption markers and baseline characteristics were further assessed in 68 patients enrolled for ezetimibe monotherapy who had evaluable baseline data on cholesterol absorption and synthesis markers. These patients were stratified by quartiles of the serum level of cholestanol, which is not affected by dietary factors. The serum LDL-C level was significantly lower in patients in the lower quartile of the cholestanol level (estimated ‘low cholesterol absorbers’) than in those in the highest quartile of the cholestanol level (estimated ‘high cholesterol absorbers’) (146.6 ± 28.3 vs. 166.3 ± 19.8 mg/dL; p < 0.05), and a constant tendency was shown for LDL-C and markers of cholesterol absorption level, indicating that estimated higher absorbers have a higher LDL-C level (not significant by ANOVA). The serum HDL-C level was significantly lower in estimated low cholesterol absorbers than in estimated high cholesterol absorbers (50.3 ± 10.8 vs. 62.5 ± 13.7 mg/dL; p < 0.01), despite a constant tendency in the quartiles of HDL-C and cholesterol absorption (Table 2).

None of the other risk factors or laboratory parameters at baseline were significantly correlated with cholesterol absorption markers or differed significantly between estimated low and high cholesterol absorbers.

Levels of cholesterol absorption and synthesis markers at baseline were compared between patients previously untreated (enrolled for ezetimibe monotherapy) and treated with statins (enrolled for coad-

<table>
<thead>
<tr>
<th>Quartile of cholestanol level</th>
<th>1st (Lower level)</th>
<th>2nd</th>
<th>3rd</th>
<th>4th (Higher level)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>219.0 ± 35.7</td>
<td>239.6 ± 33.8</td>
<td>244.2 ± 47.1</td>
<td>253.1 ± 28.8**</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>146.6 ± 28.3</td>
<td>156.7 ± 26.1</td>
<td>164.8 ± 37.0</td>
<td>166.3 ± 19.8*</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>113.5 ± 53.5</td>
<td>128.4 ± 49.3</td>
<td>132.6 ± 40.7</td>
<td>137.2 ± 51.1</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>50.3 ± 10.8</td>
<td>58.3 ± 14.5</td>
<td>53.9 ± 11.4</td>
<td>62.5 ± 13.7*</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

For each serum lipid parameter, the mean (±SD) values were calculated in patients stratified by quartiles of the serum cholestanol level, a cholesterol absorption marker. The number of patients in each quartile was 17 cases. Compared to the lowest quartile of serum cholestanol level (low cholesterol absorbers), the highest quartile (high cholesterol absorbers) was associated with significantly higher serum levels of total cholesterol, LDL-C, and HDL-C. Statistical analysis was used by ANOVA and non-paired t-test (*p < 0.05, **p < 0.01, versus low cholesterol absorbers).

Values presented are the means (± SD) observed at baseline. Levels of cholesterol absorption and synthesis markers were compared between patients previously treated and untreated with statins. For comparison, serum sterol levels were adjusted for the serum total cholesterol level (expressed in terms of μg sterol/mg total cholesterol), which differed significantly between the two groups at baseline. Previous treatment with statins was associated with a significantly lower level of the cholesterol synthesis marker lathosterol (p < 0.001) and significantly higher levels of the cholesterol absorption markers sitosterol and campesterol (p < 0.001). Statistical analysis was performed using the non-paired t-test. NS: not significant.

Fig. 1. Cholesterol absorption and synthesis levels at baseline with or without any statin therapy.
Table 3. Serum lipid profile before and after ezetimibe therapy

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline (mg/dL)</th>
<th>After treatment (mg/dL)</th>
<th>Change (%)</th>
</tr>
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<tbody>
<tr>
<td>Monotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>79</td>
<td>239.7 ± 38.2</td>
<td>204.3 ± 29.3</td>
<td>−13.7 ± 13.5***</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>80</td>
<td>158.4 ± 28.5</td>
<td>125.8 ± 23.1</td>
<td>−19.2 ± 16.5***</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>80</td>
<td>131.4 ± 60.9</td>
<td>120.0 ± 52.2</td>
<td>3.7 ± 29.5*</td>
</tr>
<tr>
<td>Hyper triglyceridemia</td>
<td>24</td>
<td>201.7 ± 60.0</td>
<td>159.2 ± 64.8</td>
<td>−20.2 ± 23.9***</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>80</td>
<td>56.7 ± 13.9</td>
<td>56.8 ± 12.7</td>
<td>1.8 ± 13.4</td>
</tr>
<tr>
<td>Hypo HDL cholesterol</td>
<td>9</td>
<td>35.1 ± 1.8</td>
<td>39.7 ± 4.0</td>
<td>13.0 ± 8.8**</td>
</tr>
<tr>
<td>Co-administration with any statin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>40</td>
<td>225.7 ± 38.3</td>
<td>192.2 ± 46.9</td>
<td>−15.0 ± 10.9***</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>40</td>
<td>142.4 ± 27.0</td>
<td>108.2 ± 37.7</td>
<td>−24.7 ± 14.0***</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>40</td>
<td>170.9 ± 108.0</td>
<td>157.9 ± 113.3</td>
<td>−4.4 ± 33.5</td>
</tr>
<tr>
<td>Hyper triglyceridemia</td>
<td>19</td>
<td>250.8 ± 108.5</td>
<td>225.7 ± 130.6</td>
<td>−9.6 ± 28.9</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>40</td>
<td>54.9 ± 18.3</td>
<td>58.0 ± 18.3</td>
<td>6.1 ± 14.8*</td>
</tr>
<tr>
<td>Hypo HDL cholesterol</td>
<td>3</td>
<td>34.0 ± 6.1</td>
<td>41.6 ± 2.3</td>
<td>24.4 ± 17.4</td>
</tr>
</tbody>
</table>

Values presented are the means (±SD) observed at baseline and after 12 weeks of ezetimibe monotherapy or co-administration with ezetimibe and any statin as well as mean (±SD) percent changes from baseline to the end of treatment. Hyper triglyceridemia was defined as serum triglycerides level over 150 mg/dL and hypo HDL cholesterolemia was defined as serum HDL cholesterol level less than 40 mg/dL. Statistical analysis was performed using the paired t-test (*p < 0.05, **p < 0.01, ***p < 0.001, versus baseline).

11.7 ± 5.7 μg/chol·mg; p < 0.001), and cholestanol levels (8.4 ± 1.9 vs. 7.9 ± 1.8 μg/chol·mg). These data indicate that previous treatment with any statin decreased the cholesterol synthesis marker and increased cholesterol absorption markers (Fig. 1). There were no critical differences in patient characteristics between groups (data not shown).

Effects of Ezetimibe on Serum Lipid Profile

In the 80 patients receiving ezetimibe monotherapy for 12 weeks, the serum LDL-C level was reduced by 19.2 ± 16.5% from 158.4 ± 28.5 mg/dL at baseline to 125.8 ± 23.1 mg/dL at the end of treatment (p < 0.001), while the serum total cholesterol level was reduced by 13.7 ± 13.5% from 239.7 ± 38.2 mg/dL at baseline to 204.3 ± 29.3 mg/dL at the end of treatment (p < 0.001). Ezetimibe monotherapy caused a 20.2 ± 23.9% decrease in the serum TG level (p < 0.001) in patients with hypertriglyceridemia (TG ≥ 150 mg/dL; n = 24) and a 13.0 ± 8.8% increase in the serum HDL-C level (p < 0.001) in patients with hypo-HDL-C (HDL-C < 40 mg/dL; n = 9) (Table 3).

In the 40 patients co-administered with ezetimibe and any statin for 12 weeks, the serum LDL-C level was reduced by 24.7 ± 14.0% from 142.4 ± 27.0 mg/dL at baseline to 108.2 ± 37.7 mg/dL at the end of treatment (p < 0.001), while the serum total cholesterol level was reduced by 15.0 ± 10.9% from 225.7 ± 38.3 mg/dL at baseline to 192.2 ± 46.9 mg/dL at the end of treatment (p < 0.001). Co-administration with ezetimibe and any statin caused no significant changes in the serum TG or HDL-C level (Table 3).

The target LDL-C level recommended in the JAS Guideline was achieved in 45/80 patients (56.3%) receiving ezetimibe monotherapy and 29/40 patients (72.5%) receiving co-administration with ezetimibe and any statin.

Effects of Ezetimibe on Cholesterol Absorption and Synthesis Markers

Ezetimibe administered alone and co-administered with any statin significantly decreased cholesterol absorption markers; the respective percent reductions were 27.2 ± 24.2% and 33.4 ± 18.8% for sitosterol (both p < 0.001), 46.4 ± 21.0% and 50.0 ± 18.5% for campesterol (both p < 0.001), and 15.0 ± 17.1% and 15.8 ± 13.1% for cholestanol (both p < 0.001). On the other hand, the serum lathosterol level, a surrogate marker of cholesterol synthesis, increased by 45.3 ± 76.4% (p < 0.001) after ezetimibe monotherapy and by 25.4 ± 66.7% (not significant) after co-administration with ezetimibe and any statin (Table 4).

Effects of Ezetimibe on Glucose Metabolism as well as Biomarkers of Inflammation and Obesity

After 12 weeks of treatment with ezetimibe, the fasting blood glucose level did not change significantly, while the fasting insulin level and HbA1c decreased significantly from 12.7 ± 17.5 μIU/mL and 6.2 ± 1.0% at baseline to 9.4 ± 8.2 μIU/mL (p < 0.05) and 5.9 ±
After treatment levels of glucose metabolism, obesity, and inflammation markers before and after ezetimibe therapy

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After treatment</th>
<th>% change</th>
<th>Baseline</th>
<th>After treatment</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lathosterol (μg/mL)</td>
<td>2.2 ± 1.0</td>
<td>2.9 ± 1.4</td>
<td>45.3 ± 76.4***</td>
<td>1.4 ± 0.6</td>
<td>1.8 ± 1.2</td>
<td>25.4 ± 66.7***</td>
</tr>
<tr>
<td>Sitosterol (μg/mL)</td>
<td>1.8 ± 0.7</td>
<td>1.2 ± 0.4</td>
<td>-27.2 ± 24.2***</td>
<td>2.1 ± 0.9</td>
<td>1.3 ± 0.4</td>
<td>-33.4 ± 18.8***</td>
</tr>
<tr>
<td>Campesterol (μg/mL)</td>
<td>2.8 ± 1.4</td>
<td>1.4 ± 0.8</td>
<td>-46.4 ± 21.0***</td>
<td>3.8 ± 2.1</td>
<td>1.7 ± 0.8</td>
<td>-50.0 ± 18.5***</td>
</tr>
<tr>
<td>Cholesterol (μg/mL)</td>
<td>1.9 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>-15.0 ± 17.1***</td>
<td>1.8 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>-15.8 ± 13.1***</td>
</tr>
</tbody>
</table>

Values presented are the means (± SD) observed at baseline and after 12 weeks of ezetimibe therapy as well as the mean (± SD) percent changes from baseline to the end of treatment. The levels of cholesterol absorption markers were significantly reduced by ezetimibe therapy (p < 0.001), while the level of the cholesterol synthesis marker significantly increased only after ezetimibe monotherapy (p < 0.001). Statistical analysis was performed using the paired t-test (**p < 0.001, versus baseline).

Table 5. Levels of glucose metabolism, obesity, and inflammation markers before and after ezetimibe therapy

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline</th>
<th>After treatment</th>
<th>% change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood Glucose (mg/dL)</td>
<td>116</td>
<td>94.0 ± 23.8</td>
<td>94.3 ± 28.4</td>
<td>0.5 ± 14.7</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting Insulin (μIU/mL)</td>
<td>102</td>
<td>12.7 ± 17.5</td>
<td>9.4 ± 8.2</td>
<td>-12.8 ± 9.8</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>35</td>
<td>6.2 ± 1.0</td>
<td>5.9 ± 1.0</td>
<td>-3.4 ± 8.6</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>High sensitive C reactive protein (ng/mL)</td>
<td>76</td>
<td>601.8 ± 461.6</td>
<td>485.1 ± 366.9</td>
<td>-10.8 ± 36.8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>High molecular weight adiponectin (μg/mL)</td>
<td></td>
<td>Total 102</td>
<td>11.8 ± 6.8</td>
<td>13.4 ± 47.5</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male 42</td>
<td>8.9 ± 4.8</td>
<td>5.4 ± 26.2</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female 60</td>
<td>13.8 ± 7.2</td>
<td>19.2 ± 57.6</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Values presented are the means (± SD) observed at baseline and after 12 weeks of ezetimibe therapy as well as the mean (± SD) percent changes from baseline to the end of treatment. Treatment with ezetimibe for 12 weeks caused significant improvements of fasting insulin (p < 0.05), HbA1c (p < 0.05), high sensitive C reactive protein (p < 0.001) and High molecular weight adiponectin levels (p < 0.01). Statistical analysis was performed using the paired t-test (versus baseline).

1.0% (p < 0.05), respectively.

Treatment with ezetimibe resulted in a significant increase in the serum adiponectin level from 10.8 ± 5.9 μg/mL at baseline to 11.8 ± 6.8 μg/mL overall (p < 0.01). Ezetimibe increased the serum adiponectin level in female from 12.4 ± 6.2 μg/mL at baseline to 13.8 ± 7.2 μg/mL (p < 0.01), otherwise, there was no difference between baseline and after treatment in male patients. Ezetimibe also improved the serum hsCRP level from 601.8 ± 461.6 ng/mL at baseline to 485.1 ± 366.9 ng/mL (p < 0.01) (Table 5).

There was no significant change in metabolic syndrome markers including BMI, waist circumference and blood pressure before and after ezetimibe treatment.

Safety of Ezetimibe

No clinical adverse events potentially related to ezetimibe occurred during the study period and were no significant changes in blood pressure or heart rate after treatment with ezetimibe. Laboratory data showed no clinically significant alterations of hepatic enzymes (e.g., AST and ALT) or creatine phosphokinase.

Discussion

Statins that inhibit cholesterol synthesis in the liver are the mainstay of current therapy for hypercholesterolemia. Evidence from many large clinical trials shows the effectiveness of statins in reducing the incidence of cardiovascular events; however, the mean cardiovascular risk reductions achieved by statins were only 32% in the study by the West of Scotland Coronary Prevention Study Group (WOS-COPS)11, 34% in the Scandinavian Simvastatin Survival Study (‘4S’ Study)12, and 33% in the Japanese MEGA Study13. This means that currently available statins fail to prevent cardiovascular events in about 70% of patients with dyslipidemia.

Recent epidemiological studies have suggested the importance of inhibiting intestinal cholesterol absorption for the primary and secondary prevention of cardiovascular events. A subanalysis of serum samples collected from patients treated with statins for second-
ary prevention of cardiovascular events in the '4S' Study\(^1\)\(^2\) showed that the prevention of clinical events by statins was adequate in patients with a lower cholestanol/total cholesterol ratio, estimated low cholesterol absorbers, but suboptimal in patients with a higher cholestanol/total cholesterol ratio, estimated high cholesterol absorbers\(^4\). The Drugs and Evidence-Based Medicine in the Elderly (DEBATE) study, which was designed to prospectively investigate the relationship between the cholestanol/total cholesterol ratio, a surrogate marker of cholesterol absorption and cardiovascular events, showed that patients with increased cholesterol absorption markers were at higher risk of all-cause death and cardiovascular events compared with those without increased cholesterol absorption markers who had equivalent serum LDL-C levels\(^5\).

These findings suggest that the two treatment options, statins targeting cholesterol synthesis and ezetimibe targeting cholesterol absorption, should be effectively combined more efficient LDL-C-lowering therapy that can further reduce the risk of vascular events. To achieve this it is important to clearly define the clinical characteristics of patients likely to benefit from the respective therapies. No sufficient data have become available for characterizing patients with enhanced cholesterol absorption who need therapeutic interventions and no previous studies have addressed this issue in Japanese patients.

This was the first study to correlate the serum LDL-C level with surrogate markers of cholesterol absorption and synthesis in Japanese patients with hypercholesterolemia. The study showed that the serum LDL-C level was not correlated with a cholesterol synthesis marker but had a significant positive correlation with cholesterol absorption markers. Quartile analysis by a cholesterol absorption marker also showed a significantly higher serum LDL-C level in patients with a higher cholestanol/total cholesterol ratio, estimated high cholesterol absorbers, than in patients with a lower cholestanol/total cholesterol ratio, estimated low cholesterol absorbers. This finding was consistent with the results of a Finnish study reported by Kesaniemi et al.\(^1\)\(^4\). The mean serum cholesterol level in the Japanese population has steadily been increasing since the 1970s and is now comparable to Western countries\(^1\)\(^5\),\(^1\)\(^6\). An increase of dietary cholesterol intake in an era of food abundance might be one of the greatest causes of this increase in Japanese people\(^1\)\(^7\). Inhibiting cholesterol absorption by ezetimibe, which is expected to offset the influence of excessive dietary cholesterol, seems to be an attractive option for the treatment of hypercholesterolemia in the Japanese. In the present study, ezetimibe monotherapy caused a 19.2% reduction of serum LDL-C level and improved hyper-triglycerides and hypo-HDL-C in patients with hypercholesterolemia who had failed with diet and exercise. A similar lipid-lowering effect of ezetimibe was previously reported in non-Japanese patients\(^1\)\(^8\). Treatment with ezetimibe also caused a significant reduction of the serum hsCRP level, which has been associated with the risk of atherosclerotic diseases.

Ezetimibe has been shown to improve insulin resistance in Zucker fatty rats, a model of obesity\(^1\)\(^9\). Consistent with this nonclinical finding, ezetimibe significantly reduced the fasting insulin level and HbA1c and significantly increased the serum level of adiponectin, an obesity marker involved in the lipid and glucose metabolism\(^2\)\(^0\). These findings suggest that inhibition of cholesterol absorption by ezetimibe produces not only a reduction of the serum LDL-C level but also beneficial secondary effects on the general aspects of metabolism.

Insulin resistance, hyper-triglycerides, and hypo-HDL-C are important pathologic components of metabolic syndrome\(^2\)\(^1\). Since ezetimibe caused overall improvements of these abnormalities and significantly increased adiponectin in the circulation, candidate patients suitable for ezetimibe monotherapy may have multiple metabolic abnormalities, such as metabolic syndrome and obesity, with underlying hyperinsulinemia. Ezetimibe may be considered as a first-line therapy for this population after failure with diet and exercise.

In the present study, screening examinations performed at enrollment showed a suppressed cholesterol synthesis marker and increased cholesterol absorption markers in patients previously treated with any statin. This finding confirms that inhibition of cholesterol synthesis by statins results in compensatory upregulation of cholesterol absorption in Japanese patients, who are known to generally more sensitive to statins, as in patients in Western countries, as reported by Miettinen et al.\(^2\)\(^2\).

In our study, co-administration of ezetimibe plus statins resulted in a 24.7% reduction of the serum LDL-C level in patients with dyslipidemia who had failed to achieve the LDL-C management goal levels recommended in the JAS Guideline. As for ezetimibe monotherapy, ezetimibe co-administered with any statin thus caused a favorable LDL-C-lowering effect, consistent with a previous report\(^2\)\(^3\). In our study, inhibition of cholesterol absorption by ezetimibe monotherapy was associated with an increased surrogate marker of cholesterol synthesis. This may also be a
compensatory response to inhibited cholesterol absorption by ezetimibe but no such responses have been reported overseas. Compared to ezetimibe monotherapy, co-administration of ezetimibe plus statin therapy caused a greater inhibition of cholesterol absorption markers without inducing a significant increase of the cholesterol synthesis marker. It has been reported that in patients who fail to obtain an adequate reduction of the serum LDL-C level with any statin, doubling the statin dose has gained an additional LDL-C reduction of only about 6%. Additionally, Pisciotta et al. reported that the efficacy of ezetimibe coadministered with any statin on LDL-C showed a negative correlation with the efficacy of statin alone on LDL-C. This finding supports the choice of ezetimibe coadministration therapy with any statin when the LDL-C level could not achieve the target level by any statin treatment. In an overseas clinical trial, the combination of ezetimibe with atorvastatin 10 mg/day reduced the serum LDL-C level by about 53%, a much greater decrease than that achieved by doubling the atorvastatin dose to 20 mg/day. It has been shown that coadministration of ezetimibe with any statin can cause an additional about 25% reduction of the serum LDL-C level. These findings support the use of ezetimibe as a first-line therapy of hypercholesterolemia after failure with diet and exercise and as an adjunct to any statin after failure to achieve the LDL-C management goal levels with statin therapy.

Based on overall consideration of these findings, the addition of ezetimibe is more recommendable than titration of the statin dose in high-risk patients who need more aggressive reduction of LDL-C or in patients who cannot achieve the target LDL-C level despite statin therapy. This study also confirms that statins increase cholesterol absorption markers, while ezetimibe increases cholesterol synthesis markers. Therefore, combination with ezetimibe, which inhibits cholesterol absorption, will offer a more effective treatment of hypercholesterolemia for patients receiving statin therapy.

The Fujita Ezetimibe Study Assembly involved in this study is composed of certified cardiologists, and many of the patients enrolled were also hypertensive. Treatment with ezetimibe caused no significant changes in blood pressure or heart rate. None of the patients treated with ezetimibe, including those who had previously suffered from statin-related myopathy, developed any treatment-related clinical adverse events or significant changes in hepatic enzymes (e.g., ALT and AST). A US placebo-controlled study of ezetimibe administered alone or in co-administration with statins showed statistically similar rates of adverse events between patients receiving ezetimibe monotherapy and those receiving a placebo and between those receiving statin monotherapy and those receiving co-administration with ezetimibe and any statin therapy. Our findings confirm that ezetimibe has an excellent safety profile when administered alone or in co-administration with any statin.

It has not yet been reliably shown that correction of hypercholesterolemia can lead to a significant prevention of cardiovascular events, which is the hard endpoint of lipid-lowering therapy. In conclusion, the results of the present study suggest that ezetimibe-produced inhibition of cholesterol absorption may provide an important method of lowering LDL-C for the ultimate goal of preventing cardiovascular events.

**Study Limitations**

Since we measured the levels of plant sterols and a precursor of cholesterol synthesis as surrogate markers of cholesterol absorption and synthesis, the meanings of “absorption” and “synthesis” were limited in our previous study; therefore, further studies, such as studies to measure isotope-labelled cholesterol turnover or to measure oral intake of cholesterol and fecal output of sterols are needed. The number of patients examined was still small. Since levels of cholesterol absorption and synthesis markers could only be determined in a limited number of patients, this study might be underpowered for assessing the correlations between cholesterol absorption/synthesis markers and baseline characteristics. Larger studies should be performed to confirm the findings from this study. Further clinical as well as laboratory investigations are also needed to establish the beneficial effects of ezetimibe observed on the levels of hsCRP and adiponectin.

**Conclusions**

The inhibition of cholesterol absorption by ezetimibe not only reduced the serum LDL-C level but also improved glucose metabolism as well as obesity and inflammation markers. These findings support the benefit of ezetimibe as a new option for the treatment of dyslipidemia.

**Acknowledgements**

We are very grateful to Nagoya Clinical Center, Falco Biosystems Ltd., Nagoya Medical Academic Center, and Medical Information Center Group for their technical assistance in collecting, separating and storing serum samples for this study.
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