Atorvastatin 10 mg plus ezetimibe 10 mg compared with atorvastatin 20 mg: Impact on the lipid profile in Japanese patients with abnormal glucose tolerance and coronary artery disease

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Received 3 August 2011; received in revised form 23 August 2011; accepted 5 September 2011
Available online 17 November 2011

Summary

\textit{Background:} Oxidized low-density lipoprotein (LDL) cholesterol is a sensitive lipid marker for predicting atherosclerosis. Ezetimibe and statins are reported to decrease both LDL cholesterol and oxidized LDL cholesterol. This prospective randomized open-label crossover study compared combination therapy with atorvastatin plus ezetimibe versus high-dose atorvastatin monotherapy. Changes in serum lipids, including malondialdehyde-modified LDL (MDA-LDL) as a representative form of oxidized LDL cholesterol, and glucose metabolism were assessed.

\textit{Methods and results:} The subjects were 39 Japanese patients with coronary artery disease and type 2 diabetes or impaired glucose tolerance who were taking 10 mg/day of atorvastatin (30 men and 9 women with a mean age of 67.8 years). They were randomized to a group that first received add-on ezetimibe (10 mg/day) or a group that first received atorvastatin monotherapy at a higher dose of 20 mg/day. Both treatments were given for 12 weeks each in a crossover fashion. Add-on ezetimibe significantly decreased MDA-LDL (109.0 ± 31.9 mg/dl to 87.7 ± 29.4 mg/dl, \( p = 0.0009 \)), while up-titration of atorvastatin did not. The decrease with add-on ezetimibe was significantly greater than with up-titration of atorvastatin (\( p = 0.0006 \)). Total cholesterol and LDL cholesterol were significantly decreased by both treatments,
Introduction

It is well known that the serum level of low-density lipoprotein (LDL) cholesterol is the most significant risk factor for coronary artery disease (CAD) [1]. In addition, there is a close relationship between a decrease in LDL cholesterol and an anti-inflammatory effect [2,3]. High-dose statin therapy has these beneficial effects, resulting in the prevention of cardiovascular events [4].

Ezetimibe inhibits the uptake of dietary and biliary cholesterol [5]. Intensive lipid lowering can be achieved by the combination of a statin with ezetimibe [6], but it remains controversial whether such combined therapy significantly prevents atherosclerosis compared with statin monotherapy. It was reported that combined therapy with simvastatin (80 mg/day) and ezetimibe (10 mg/day) does not significantly reduce intima—media thickness compared with simvastatin monotherapy [7]. In contrast, other studies have shown that combination therapy with a statin and ezetimibe significantly preserves endothelial function after a fat load and significantly prevents inflammation compared with high-dose statin monotherapy [8,9]. Accordingly, we hypothesized that the combination of atorvastatin (10 mg/day) and ezetimibe (10 mg/day) could have a beneficial effect (due to inhibition of cholesterol absorption) on the lipid profile of Japanese patients with type 2 diabetes or impaired glucose tolerance (IGT) and CAD compared with high-dose atorvastatin monotherapy (20 mg/day).

Methods

Study population

This was a prospective, randomized, open-label, clinical trial in Japanese subjects at the Department of Cardiology of Anjo Kosei Hospital (Anjo, Japan), in which atorvastatin plus ezetimibe therapy was switched to high-dose atorvastatin alone or vice versa. There was no washout period between the two treatments because of ethical considerations. The first subject was enrolled in October 2008 and the last subject completed the study in July 2009. Patients eligible for inclusion had IGT or type 2 diabetes, were on treatment with atorvastatin (10 mg/day) for dyslipidemia, and had CAD with angiographic stenosis (>50% diameter stenosis on quantitative coronary angiography) or a history of coronary revascularization for stable angina. Exclusion criteria were acute coronary syndrome within 6 months, inflammatory disease, infectious disease, malignancy, a history of hypersensitivity to ezetimibe or atorvastatin, severe liver dysfunction or renal dysfunction, and insulin treatment for diabetes. Pregnant women, women who might be pregnant, and breast-feeding women were also excluded. The dosage of anti-angina agents, antihypertensive agents, and lipid-lowering agents was not changed throughout the study period.

A diagnosis of IGT was based on a fasting plasma glucose level <126 mg/dl and a 2-h plasma glucose level of 140—199 mg/dl in the 75 g oral glucose tolerance test (OGTT), while diabetes was defined by casual plasma glucose ≥200 mg/dl, fasting plasma glucose ≥126 mg/dl, or 2-h plasma glucose ≥200 mg/dl.

Eligible patients who gave written informed consent were randomly allocated to two groups on the day after coronary angiography, if coronary stenosis was confirmed. Patients allocated to the group receiving combination therapy first were initially given 10 mg/day of atorvastatin combined with 10 mg/day of ezetimibe, while patients allocated to the group receiving up-titration of atorvastatin first were given 20 mg/day of atorvastatin. After 12 weeks, each regimen was switched to the other one and treatment was continued for another 12 weeks (Fig. 1). Subjects attended the hospital monthly for assessment of their general condition, adherence to treatment, and adverse events. At the start of treatment and at the end of the first and second treatment periods, the subjects also underwent laboratory tests.

The study protocol was approved by the hospital ethics committee and written informed consent was obtained from every patient.

Laboratory tests

Venous blood samples for determination of serum lipids and other laboratory parameters [malondialdehyde-modified LDL (MDA-LDL), total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoproteins A-I and B, and remnant-like particle (RLP) cholesterol] were obtained between 7:00 and 8:00 AM on the day of the 75 g OGTT (baseline data). Serum lipids were measured by enzymatic methods using an autoanalyzer (Hitachi Co., Tokyo, Japan) in the hospital laboratory immediately after blood sampling. Levels of MDA-LDL were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) (SRL Inc., Tokyo, Japan). In the ELISA kit, test wells are coated with anti-MDL-LDL monoclonal antibody, which binds with MDA-LDL in the sample. After removing all of the unbound material, horseradish peroxidase-labeled anti-apolipoprotein B monoclonal antibody is added, which binds to the MDA-LDL captured on the plate. After that, substrate solution and stop reagent are added. The intensity of color that develops is read by a microplate reader. The
Absorbance is proportional to the concentration of MDA-LDL in the sample. Apolipoproteins were determined by fixed-rate immunonephelometry using a JEOL JCA-BM8000 (JEOL Inc., Tokyo, Japan). Plasma RLP cholesterol was determined with a JIMRO-II assay kit (Otsuka Inc., Tokyo, Japan) and a Hitachi autoanalyzer 7070 (Hitachi Co.), using an immunoseparation method.

Statistical analysis

Statistical analysis was performed using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA). Continuous variables are presented as the mean±standard deviation and were compared by using Student’s t-test. Comparison of various parameters among the baseline values after both treatments was done by Wilcoxon’s signed rank test. Data on the lipid profiles were evaluated by analysis of variance (ANOVA) with respect to treatment sequence, treatment period, treatment regimen, and patients. Differences between the two treatment regimens were determined by the paired t-test. In all analyses, p < 0.05 was considered statistically significant.

Results

Thirty-nine patients were enrolled in the study. Twenty patients initially received 10 mg of atorvastatin combined with 10 mg of ezetimibe, while nineteen patients initially received 20 mg of atorvastatin. No patient in either group withdrew from treatment during the follow-up period, although 2 patients experienced abdominal discomfort without any evidence of liver dysfunction or diarrhea. No musculoskeletal adverse events were observed and no patient in either group had an increase in serum aminotransferases above the normal range. Follow-up data were collected from all enrolled patients. The baseline characteristics of the subjects are shown in Table 1.

Table 2 shows the levels of MDA-LDL, LDL cholesterol, and other parameters before and after treatment with either combination therapy or high-dose atorvastatin monotherapy. According to ANOVA, there was no influence of the sequence and treatment period. Both treatments produced significant reduction in the serum levels of LDL cholesterol and total cholesterol, along with a significant increase in HDL cholesterol. Ezetimibe plus atorvastatin achieved a significantly greater reduction in LDL cholesterol and total cholesterol than up-titration of atorvastatin to 20 mg/day (p < 0.0001). The increase in HDL cholesterol with combination therapy was similar to that observed with up-titration. Reduction in the serum triglyceride level was not significant in either group.

Ezetimibe plus atorvastatin significantly reduced the serum concentration of MDA-LDL from 109.0 ± 31.9 IU/l to 87.7 ± 29.4 IU/l (p = 0.0009). On the other hand, MDA-LDL was not significantly decreased in the high-dose atorvastatin group [109.0 ± 31.9 IU/l to 106.0 ± 34.9 IU/l (p = NS)]. The MDA-LDL level was significantly lower after combination therapy than after up-titration monotherapy (p = 0.0006) (Table 2 and Fig. 2). An increase in apolipoprotein A-I levels was only seen in the subjects receiving combination therapy (p = 0.0005 versus baseline). A significant reduction in apolipoprotein B was seen in both groups, but it was much greater in the combination therapy group (p = 0.0063) (Table 2 and Fig. 3). Only combination therapy significantly reduced the apolipoprotein B/apolipoprotein A-I ratio (p < 0.0001 versus baseline) and the level of RLP cholesterol (Table 2 and Fig. 4).

Discussion

In the present crossover study, both combination therapy with atorvastatin (10 mg/day) plus ezetimibe (10 mg/day) and high-dose atorvastatin monotherapy (20 mg/day) significantly decreased the levels of total cholesterol, LDL cholesterol, and apolipoprotein B, while increasing HDL cholesterol, compared to treatment with 10 mg/day of
Table 2  MDA-LDL, LDL cholesterol, other lipid parameters, and hemoglobin A1c at baseline and after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After combination therapy</th>
<th>After monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-LDL, IU/l</td>
<td>109.0 ± 31.9</td>
<td>87.7 ± 29.4(^*)</td>
<td>106.0 ± 34.9</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>111.6 ± 16.6</td>
<td>82.9 ± 20.5(^*)</td>
<td>98.4 ± 22.7(^*)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>46.7 ± 10.2</td>
<td>52.8 ± 12.6(^*)</td>
<td>50.0 ± 11.6(^*)</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>124.3 ± 55.9</td>
<td>113.7 ± 58.6</td>
<td>125.7 ± 77.9</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>178.1 ± 23.8</td>
<td>147.8 ± 21.3(^*)</td>
<td>164.3 ± 25.8(^*)</td>
</tr>
<tr>
<td>Apolipoprotein A-I, mg/dl</td>
<td>126.3 ± 19.6</td>
<td>133.5 ± 22.5(^*)</td>
<td>126.4 ± 21.1</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dl</td>
<td>90.1 ± 12.4</td>
<td>73.9 ± 18.0(^*)</td>
<td>83.7 ± 17.2(^*)</td>
</tr>
<tr>
<td>Apolipoprotein B/apolipoprotein A-I ratio</td>
<td>0.73 ± 0.15</td>
<td>0.57 ± 0.18(^*)</td>
<td>0.68 ± 0.19</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>111.1 ± 19.0</td>
<td>112.5 ± 20.9</td>
<td>115.4 ± 30.3</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>5.70 ± 0.61</td>
<td>6.03 ± 0.64(^*)</td>
<td>5.97 ± 0.67(^*)</td>
</tr>
<tr>
<td>RLP-cholesterol, mg/dl</td>
<td>4.40 ± 2.93</td>
<td>3.85 ± 2.68(^*)</td>
<td>4.85 ± 4.90</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD. MDA, malondialdehyde-modified; LDL, low-density lipoprotein; HDL, high-density lipoprotein; RLP, remnant-like particle.
\(^*\) p < 0.05 versus baseline.
\(\dagger\) p < 0.05 versus monotherapy group.

Atorvastatin alone. However, only combination therapy with atorvastatin plus ezetimibe had the effect of reducing MDA-LDL levels and increasing apolipoprotein A-I levels. Previous reports have shown that both ezetimibe and statin in monotherapy decrease the plasma level of MDA-LDL [10,11]. The present study demonstrated for the first time that addition of ezetimibe therapy is more effective in the decrease of MDA-LDL level than up-titration statin therapy.

The prognosis is often worse for patients who have IGT compared with subjects who have normal glucose tolerance or impaired fasting glucose [12,13]. In patients with impaired glucose metabolism, cholesterol absorption is often enhanced and postprandial hypertriglyceridemia is common [14]. Postprandial hyperglycemia induces oxidative stress, decreases flow-mediated vasodilation, and impairs endothelial nitric oxide release [15,16]. In such a situation, it may be useful to investigate oxidative stress. Oxidised LDL cholesterol (including MDA-LDL) is a relatively new marker of oxidative stress, which has been reported to be a stronger predictor of cardiovascular risk than standard lipid parameters [17,18]. In particular, the plasma level of MDA-LDL is related to atherogenic processes such as foam cell formation, endothelial dysfunction, and vascular inflammation [17–19]. Although combination therapy with ezetimibe and a statin has been shown to reduce atherogenic oxidized LDL cholesterol in addition to lowering LDL cholesterol [20], a clinical trial has not been performed to investigate the differential effects of these drugs. In the present study, we investigated changes of apolipoprotein A-I and apolipoprotein B. Apolipoprotein A-I has a protective effect against premature atherosclerosis [21,22], while apolipoprotein B and apolipoprotein B/apolipoprotein A-I ratio are considered to be a better indicator of atherogenic lipoprotein particles than the LDL cholesterol level or non-HDL cholesterol level [22,23]. Also, the remnant-like particle cholesterol level can predict future cardiovascular events [24,25]. A previous report has suggested that the association between high levels of remnant lipoprotein cholesterol and high MDA-LDL levels might be linked to atherogenesis in patients with coronary artery disease [26]. Accordingly, our findings might be of clinical significance.

In the current study, only combination therapy with atorvastatin plus ezetimibe had the effect of reducing MDA-LDL levels. Some mechanisms are possible to explain this. First, ezetimibe effectively decreased the uptake of oxidized LDL from dietary intake. Previous reports have suggested that cholesterol absorption increases in patients with diabetes, with long administration of statins, and a history of coronary artery disease [14,27–29]. We enrolled these patients in this study. Second, ezetimibe inhibited the postprandial dyslipidemia, and this effect resulted in the decrease of atherogenic small, dense LDL and oxidative LDL. Insulin resistance causes postprandial dysmetabolism, which causes the increased oxidative stress and atherogenesis [30].

It has been reported that combined therapy with ezetimibe plus simvastatin significantly decreases both serum LDL cholesterol and C-reactive protein compared with simvastatin alone [7]. There is an association between inflammation as indicated by the C-reactive protein level.
and atherosclerosis [31,32]. It was reported that combination therapy with simvastatin (10 mg/day) plus ezetimibe (10 mg/day) for 6 weeks preserved postprandial endothelial function unlike treatment with simvastatin (80 mg/day) alone in men with metabolic syndrome [9]. Thus, combination therapy may be a good choice in subjects with abnormal glucose tolerance.

Simvastatin was the statin combined with ezetimibe in previous large-scale trials [7,33]. Unfortunately, combination therapy with simvastatin (80 mg/day) plus ezetimibe (10 mg/day) was not shown to have a superior effect on intima–media thickness compared with simvastatin (80 mg/day) alone [7]. In addition, combination therapy with simvastatin (40 mg/day) plus ezetimibe (10 mg/day) did not reduce the incidence of major adverse cardiac events in patients with aortic stenosis [33]. On the other hand, strong statins such as atorvastatin significantly increase the dietary absorption of cholesterol compared with moderate statins due to the significantly greater decrease in cholesterol synthesis [27]. It has been reported that decreased cholesterol absorption is associated with fewer recurrent cardiovascular events in elderly patients [34]. Conversely, increasing cholesterol absorption was related to recurrence of major coronary events during simvastatin treatment in a
Finnish subgroup of the Scandinavian study [35]. Therefore, it may be possible to obtain a better outcome by combination therapy with a statin plus ezetimibe if other statins including atorvastatin are tested, so further investigations are needed.

**Study limitations**

Several limitations of this study should be discussed. First, this was a single-centre crossover study with only 39 patients, resulting in a low statistical power that should be considered. In addition, the findings of this study cannot be extrapolated to the general population. Third, the effects noted were related to specific drugs and drug combinations. Fourth, a carry-over effect might exist because there were no drug-free periods when switching arms. Fifth, data on changes in cardiovascular events should be collected in the future by a long-term study because such findings might have great clinical importance.

**Conclusion**

In Japanese patients with coronary artery disease and abnormal glucose tolerance, both combination therapy with atorvastatin (10 mg/day) plus ezetimibe (10 mg/day) and high-dose atorvastatin monotherapy (20 mg/day) significantly decreased total cholesterol and LDL cholesterol, while raising HDL cholesterol, compared with low-dose atorvastatin monotherapy (10 mg/day). Combination therapy with atorvastatin plus ezetimibe achieved superior reduction of MDA-LDL and apolipoprotein B levels, as well as an increase in apolipoprotein A-I levels, compared to high-dose atorvastatin monotherapy.

**Acknowledgment**

This study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (Identifier: UMIN000005078).

**References**


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