Effects of Atorvastatin Therapy on the Low-Density Lipoprotein Subfraction, Remnant-Like Particles Cholesterol, and Oxidized Low-Density Lipoprotein Within 2 Weeks in Hypercholesterolemic Patients

Koichi Sakabe, MD; Nobuo Fukuda, MD; Katsunori Wakayama, MD; Teru Nada, MD; Hisanori Shinohara, MD; Yoshiyuki Tamura, MD

The short- and intermediate-term pleiotropic effects of atorvastatin were investigated in 18 hypercholesterolemic patients, as well as the temporal differences in these pleiotropic effects. Atorvastatin was given for 3 months and fasting lipid concentrations, thiobarbituric acid reactive substances (TBARS), fibrinolytic parameters, and flow-mediated dilation of the brachial artery (FMD) were measured at baseline and after 2 weeks and 3 months of therapy. Atorvastatin reduced the total cholesterol (273±34 vs 188±31 mg/dl, p<0.0001), low-density lipoprotein-cholesterol (LDL-C: 174±28 vs 111±23 mg/dl, p<0.0001), small, dense LDL-C (34±22 vs 18±20%, p<0.01), remnant-like particles cholesterol (RLP-C: 8.8±6.0 vs 5.1±2.6 mg/ml, p<0.01), and TBARS (3.3±1.0 vs 3.1±0.9 nmol/ml, p<0.05) after 2 weeks. Atorvastatin decreased the concentration of small, dense LDL-C again after 3 months (8±13%, p<0.0001). The plasma concentrations of the fibrinolytic parameters did not change significantly after 3 months of atorvastatin therapy, FMD increased significantly after 2 weeks (5.6±2.1 vs 6.3±2.0%, p<0.01) and additionally increased after 3 months of therapy (8.3±1.9%, p<0.0001). There were no correlations between the pleiotropic effects and the improvement in the lipid profile. The results indicate some short-term pleiotropic effects of atorvastatin therapy within 2 weeks, which may be important with respect to the early benefits of statin therapy. (Circ J 2003; 67: 866–870)

Key Words: Atorvastatin; Hypercholesterolemia; Lipid profile; Pleiotropic effects

Hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) decrease the risk of coronary events not only through their lipid-lowering effects, but also through direct effects on the vascular wall.1–5 Statins also have pleiotropic effects, including altering endothelial function, reducing the concentration of C-reactive protein, and other anti-atherogenic effects.6–10 In view of recent data on the effectiveness of statins in acute coronary syndrome,11–13 it is of particular interest to study their short-term effects. It has been reported that short-term atorvastatin therapy can improve endothelial function and decrease the C-reactive protein concentration6–11 but the other rapid pleiotropic effects of atorvastatin and their time course are still unclear. Therefore, we investigated the short- and intermediate-term pleiotropic effects of atorvastatin in patients with primary hypercholesterolemia, and we also evaluated the time course of these effects.

Methods

Patient Population

Eighteen consecutive patients with primary hypercholesterolemia who met the study criteria were prospectively assigned to treatment with atorvastatin (10 mg/day) for 3 months. The drug was administered once daily after the evening meal and concomitant use of any drug that could influence the serum lipid concentrations was prohibited. The enrollment criteria were: low-density lipoprotein-cholesterol (LDL-C) concentration ≥160 mg/dl and triglyceride (TG) concentration ≤400 mg/dl for patients on the ‘American Heart Association Step I diet’ who were not receiving any cholesterol-lowering medications. Patients who smoked, had diabetes, hypertension, an acute vascular event within the previous 3 months, revascularization therapy within the previous 6 months, or active liver disease were excluded.

Blood Sample Analysis

We measured the plasma concentrations of total cholesterol (TC), LDL-C, high-density lipoprotein-cholesterol (HDL-C), TG, the subfractions of LDL-C, and remnant-like particles cholesterol (RLP-C). In addition, thiobarbituric acid reactive substances (TBARS) were measured as a marker of lipid peroxide, and fibrinolytic parameters (tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1)) were also measured. Measurements were performed at baseline and after 2 weeks and 3 months of therapy. Blood samples were drawn after more than 12 h of fasting.

The TC and TG concentrations were measured by enzymatic methods17 and HDL-C was quantified by the heparin-Ca2+ precipitation method18. Low-density lipoprotein-cholesterol (LDL-C) concentration ≥160 mg/dl and triglyceride (TG) concentration ≤400 mg/dl for patients on the ‘American Heart Association Step I diet’ who were not receiving any cholesterol-lowering medications. Patients who smoked, had diabetes, hypertension, an acute vascular event within the previous 3 months, revascularization therapy within the previous 6 months, or active liver disease were excluded.

Key Words: Atorvastatin; Hypercholesterolemia; Lipid profile; Pleiotropic effects

(Received April 21, 2003; revised manuscript received July 24, 2003; accepted July 31, 2003)

Department of Cardiology and Clinical Research, National Zentsuji Hospital, Kagawa, Japan

Mailing address: Koichi Sakabe, MD, Department of Cardiology and Clinical Research, National Zentsuji Hospital, 2-1-1 Senyu-cho, Zentsuji, Kagawa 765-8507, Japan. E-mail: ksakabe@jun.ncvc.go.jp
teins were fractionated from freshly drawn plasma samples by ultracentrifugation according to the method of Havel et al\textsuperscript{19} and the concentrations of LDL-C were assayed enzymatically.\textsuperscript{19}

The LDL subfractions were identified by electrophoresis using the polyacrylamide gel method.\textsuperscript{20} In brief, we used the LipoPrint LDL system (Quantimetri Co, USA). The test is performed by adding 25 μl of patient serum and 200 μl of loading gel into a precast gel tube. After photopolymerization of the loading gel, electrophoresis is carried out at a constant current of 3 mA per tube for approximately 60 min or until the HDL fraction migrates approximately 40 mm. The various preeminent lipoproteins and subfractions are separated on the basis of molecular size. The electrophoresed gels are scanned at 610 nm on a densitometer and the lipoprotein subfractions are identified by their migration distance (D) relative to the HDL fraction (D=1.00). In this study, the LDL subfractions were divided into small, dense LDL (D>0.58) and large, buoyant LDL (D>0.28).

RPL-cholesterol was determined by an immunoadsorption method using an immunoaffinity gel mixture of anti-apolipoprotein A-I and anti-apolipoprotein B monoclonal antibodies.\textsuperscript{21} For the RPL-cholesterol assay, 300 μl of the immunoaffinity gel was pipetted into separation cups containing steel balls and placed in a magnetic mixer. Next, 5 μl of patient serum was pipetted onto the surface of the gel and incubated with continuous mixing for 2 h. After incubation, 200 μl of the supernatant was placed into sample cups, and the RPL-cholesterol concentrations were measured with the Hitachi 7170 autoanalyzer. TBARS were determined by the reaction of malondialdehyde, a secondary breakdown product of lipid hydroperoxides, with thiobarbituric acid.\textsuperscript{22} Plasma t-PA and PAI-1 concentrations were measured by enzyme-linked immunosorbent assay with goat anti-human t-PA immunoglobulin and rabbit anti-human PAI-1 immunoglobulin, respectively.\textsuperscript{23,24}

**Vascular Function**

We assessed endothelial function from the flow-mediated dilation of the brachial artery (FMD) at the time of blood sampling. Endothelium-dependent and -independent vasodilation were measured by ultrasound, as described previously.\textsuperscript{25} In brief, the brachial artery was imaged using a 13.0 MHz linear array transducer and an Acuson Sequoia 512 ultrasound unit by an investigator unaware of the other clinical data. The brachial artery was imaged just above the antecubital fossa, and its distance from an anatomical marker by 2 independent observers unaware of the other clinical data. The ultrasound images were recorded by the Acuson Sequoia 512 ultrasound unit, and the arterial diameter was measured with ultrasonic calipers at a fixed distance from an anatomical marker by 2 independent observers unaware of the other clinical data. The brachial artery was imaged just above the antecubital fossa, and its diameter was calculated for 3 cardiac cycles synchronized to the R-wave peaks on the electrocardiogram. Reactive hyperemia was induced by inflating a blood pressure cuff around the forearm to an occlusive pressure of 200 mmHg for 5 min, and then rapidly deflating the cuff. To assess endothelium-dependent vasodilation, the brachial artery diameter was measured before and then 50–60 s after cuff deflation, and FMD was calculated as the % change in diameter. Fifteen minutes later, the second resting scan was recorded, and then 300 μg of sublingual nitroglycerin spray (Toa Eiyou Co) was administered to assess endothelium-independent vasodilation (nitroglycerin-induced dilation). The brachial artery diameter was measured 5 min after nitroglycerin administration. There was close correlation between the 2 observers of the measurements of vasoactivity (γ=0.95×–0.4, r=0.80, SD=4.7%), and the means of the individual observer determinations were calculated.

As the control group, 20 healthy individuals (age 61±9 years), including 11 females, were examined for comparison with the baseline parameters of the patients. Written informed consent was obtained from all individuals before the study.

**Statistical Analysis**

Data are presented as mean±SD. Comparisons of data obtained at different time intervals were performed using repeated measures ANOVA followed by post-hoc analysis. An unpaired Student’s t test was used to compare the results between different groups. Comparisons of categorical variables were evaluated by chi-square analysis. Correlation coefficients for the relationship between 2 variables were determined using standard linear regression methods. A value of p<0.05 was considered statistically significant.

**Results**

Eighteen patients were prospectively treated with atorvastatin (10 mg/day) for 3 months while keeping constant their lifestyle measures such as diet, weight, and smoking. The mean age of the patients was 62±11 years (range: 40–76 years), and 12 patients were female. Table 1 summarizes the plasma lipid profile, TBARS, fibrinolytic parameters, FMD, and other characteristics of the control group and the patients at baseline, and the patients after 2 weeks and 3 months of lipid-lowering therapy. Total cholesterol, LDL-C, RLP-C, TBARS and PAI-1 concentrations were significantly higher at baseline in the hyperlipidemic patients than in the control group. Baseline FMD was significantly lower in the patients than in the control group, although the baseline brachial artery diameter was similar.

Atorvastatin reduced the TC (273±34 vs 188±31 mg/dl, p<0.0001) and LDL-C (174±28 vs 111±23 mg/dl, p<0.0001) concentrations between the baseline and 2 week measurements. Atorvastatin maintained lower TC and LDL-C concentrations from 2 weeks to 3 months. The percent changes in TC and LDL-C concentrations were, respectively, −31±10% and −36±10% after 2 weeks, and −34±10 and −44±12% after 3 months of therapy. Atorvastatin decreased the small, dense LDL-C subfraction concentration after 2 weeks (34±22% vs 18±20%, p<0.01) with a further decrease after 3 months of therapy (8±13%, p<0.0001). The absolute small, dense LDL-C concentration also decreased after 2 weeks (62±42 vs 22±25 mg/dl, p<0.0001) and additionally decreased after 3 months (8±14 mg/dl, p<0.0001). In contrast, the large, buoyant LDL-C subfraction concentration increased after 2 weeks (65±21 vs 82±19 mg/dl, p<0.01) and additionally increased after 3 months (91±13 mg/dl, p<0.0001). TBARS (3.3±1.0 vs 3.1±0.9 nmol/ml, p<0.05), RLP-C (8.8±6.0 vs 5.1±2.6 mg/ml, p<0.01) and TG (156±83 vs 127±76 mg/ml, p<0.05), concentrations decreased more rapidly with atorvastatin therapy after 2 weeks. There was a positive correlation between RLP-C and TG (r=0.63, p<0.01). The plasma concentrations of PAI-1 and t-PA did not change significantly after 3 months of therapy.

With respect to endothelial function, atorvastatin significantly increased FMD (5.6±2.1% vs 6.3±2.0%, p<0.01) after 2 weeks, and additionally it increased after 3 months.
(8.3±1.9%, p<0.0001). The % change in FMD was 23±26% after 2 weeks, and 66±61% after 3 months of therapy. Endothelium-independent vasodilation (nitroglycerin-induced dilation) did not differ significantly among the control group, patients at baseline, and patients after atorvastatin therapy. There was no correlation between these pleiotropic effects and the improvement in the lipid profile after 3 months of therapy.

**Table 2** Correlations Between Changes in the Pleiotropic Effects and Changes in the Lipid Profile

<table>
<thead>
<tr>
<th>Change after 2 weeks</th>
<th>Correlation coefficient</th>
<th>p-value</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔFMD vs ΔTC</td>
<td>-0.29</td>
<td>0.25</td>
<td>-0.07</td>
<td>0.77</td>
</tr>
<tr>
<td>ΔLDL-C</td>
<td>0.09</td>
<td>0.71</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>ΔTG</td>
<td>-0.12</td>
<td>0.64</td>
<td>-0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>ΔRLP-C</td>
<td>-0.22</td>
<td>0.38</td>
<td>-0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>ΔSmall-C</td>
<td>-0.13</td>
<td>0.65</td>
<td>0.03</td>
<td>0.91</td>
</tr>
</tbody>
</table>

**Discussion**

It has been reported that short-term atorvastatin therapy can improve endothelial function and decrease the concentration of C-reactive protein, supporting the usefulness of statins for treating acute coronary syndromes. However, other rapid pleiotropic effects of atorvastatin have not been reported. In the present study, atorvastatin therapy decreased the small, dense LDL-C concentration after 2 weeks. The concentration of small, dense LDL-C is higher in patients with coronary artery disease and Sasaki et al reported that atorvastatin decreased the concentration after 3 months. Our results indicate that atorvastatin can decrease the risks for coronary hear disease, such as the small, dense LDL-C concentration, much faster than has been previously reported. In our study, the small, dense LDL-C concentration at baseline was not different from that of control, but after 3 months of therapy it was lower than that of the control group. Tan et al indicated that small, dense LDL-C increased in normolipemic people, some of our controls had increased small, dense LDL-C. A possible mechanism for the decrease in small, dense LDL-C is removal from the circulation of the very low-density lipoprotein, remnant and intermediate density lipoprotein precursors of LDL. The precise mechanism of the remarkable decrease of small, dense LDL-C after atorvastatin therapy in this study remains unclear.

RLP-C is considered atherogenic and may contribute to adverse vascular effects. Increased RLP-C concentrations...
have been reported to be a significant and independent risk factor for coronary artery disease. \cite{12} Recently, Stein et al. reported that RLP-C concentrations are significantly reduced after 6 weeks of therapy with atorvastatin or simvastatin, but not with pravastatin. In the present study, atorvastatin significantly reduced the RLP-C concentration within 2 weeks, which is much faster than previously reported.\cite{12} Taken together, the results of the various studies, including the present one, indicate that the reduction of RLP-C may not be a common effect of the statins. The differential effect may be mediated by differences in TG-rich lipoprotein remnant kinetics, which is in keeping with our result of a positive correlation between RLP-C and TG.

TBARS are a marker of lipid peroxidation, and have been reported to be associated with the development of atherosclerosis.\cite{25} In our study, atorvastatin decreased the TBARS concentration within 2 weeks, so short-term therapy with atorvastatin may act as an antioxidant and contribute to the improvement of atherosclerosis.

Hypercholesterolemia is commonly associated with impaired endothelial function, and it has been proposed that the enhanced endothelial function conferred by statins contributes to the cardiovascular benefits of that therapy.\cite{26,28} Atorvastatin has been reported to improve endothelial function within 2 weeks, but the precise mechanism responsible for the beneficial effect of statins on the endothelium is controversial. Statins improve endothelial function via their lipid-lowering effect and via activation of endothelial nitric oxide synthase independent of their lipid-lowering action.\cite{29,30} We found that atorvastatin improved endothelial function within 2 weeks, independent of the lipid-lowering effect, based on the lack of correlation between the 2 parameters.

The rapid effects of atorvastatin on LDL subfraction distribution, RLP-C, oxidized LDL, and endothelial function observed in the present study suggest a much earlier benefit of atorvastatin therapy than has been previously reported.

PAI-1 and t-PA are important markers that are intimately linked to the risk of atherothrombosis. High concentrations of PAI-1 and decreased t-PA activity have been shown to be associated with the development of coronary heart disease.\cite{31} The concentration of PAI-1 has been reported to be unaffected by atorvastatin, whereas the activity of plasma t-PA activator is reportedly decreased by atorvastatin.\cite{24} In our study, neither fibrinolytic parameter was affected by atorvastatin after 3 months of therapy, which suggests that the early benefits of atorvastatin therapy are not mediated by improvements in endogenous fibrinolysis.

Aggressive cholesterol lowering with statins has been shown to be effective in treating atherosclerosis, but in the present study there was no correlation between the pleiotropic effects and improvement of the lipid profile. Previous studies indicate that several statin-induced pleiotropic effects are lipid-independent, which is in keeping with our results, and the cardiovascular benefits of statin therapy may be due not only to their lipid-lowering effects but also to their pleiotropic effects. It may be important to select a member of the statin class on the basis of both the lipid lowering and other pleiotropic effects of the agent, especially in patients with coronary artery disease.

**Study Limitations**

We did not investigate the effects of all statins or a placebo. It remains unclear whether the rapid pleiotropic effects in this study are specific to atorvastatin only or the entire statin class. It also remains unclear whether the rapid pleiotropic effects are unaffected by lipid lowering, such as the remarkable LDL-C decrease, though there was no correlation between the pleiotropic effects and improvement of the lipid profile. Furthermore, we did not investigate the temporal differences in the pleiotropic effects of long-term statin therapy. Further long-term trials that include all statins and a placebo are therefore needed.

**Conclusions**

Our results indicate that there are some beneficial effects of atorvastatin therapy within 2 weeks, including effects on LDL subfraction distribution and RLP-C, antioxidant effects, and alterations in endothelial function, which may be important in the treatment of cardiovascular diseases. In addition, there was no correlation between these rapid pleiotropic effects and the improvement in the lipid profile, which suggests that the pleiotropic effects are lipid-independent. We suggest that the use of statins should be based on the patient’s condition, as well as the lipid lowering and pleiotropic effects of the agent.

**References**


