Atorvastatin Reduces Plasma Levels of Factor VII Activity and Factor VII Antigen in Patients with Hyperlipidemia

Eriko Morishita1, Shinji Minami2, Chizuko Ishino3, Masanori Kanno3, Chika Uotani3, Hidesaku Asakura1, Tamotsu Matsuda2, and Shinji Nakao2

1Department of Laboratory Sciences, School of Health Science, Kanazawa University, Kanazawa, Japan.
2Department of Internal Medicine (III), School of Medicine, Kanazawa University, Kanazawa, Japan.
3Department of Internal Medicine, NTT Kanazawa Hospital, Kanazawa, Japan.
4Department of Internal Medicine, Inami General Hospital, Toyama, Japan.

Atorvastatin is a powerful new synthetic 3-hydroxy 3-methylglutaryl-coenzyme A reductase inhibitor currently in clinical use. Its effects on plasma levels of factor VII were examined in 30 hyperlipidemic patients. After 12 weeks of atorvastatin treatment, factor VII activity (FVIIc) and factor VII antigen (FVIIag) levels had decreased by 13% (p < 0.0001) and 12% (p < 0.0001), respectively. The decreased concentrations of serum triglycerides correlated with decreases in FVIIc levels (r = 0.54, p = 0.0023) and FVIIag levels (r = 0.59, p = 0.0006) at 12 weeks of treatment with atorvastatin. No significant changes were seen in activated factor VII (FVIIa) levels. Plasma concentrations of fibrinogen were slightly, but not significantly, increased at 12 weeks. No significant changes were seen in plasminogen activator inhibitor-1 levels. The effects of atorvastatin on FVII may contribute to a decreased thrombotic potential, resulting in fewer thromboembolic events, including a reduction in coronary heart disease. J Atheroscler Thromb. 2001; 9: 72-78.

Key words: Atorvastatin, Hyperlipidemia, Factor VII, Triglyceride

Introduction

Clinical trials have demonstrated that lipid lowering therapy, especially with 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA reductase inhibitors; statins), significantly reduces the risk of cardiovascular events (1,2). These benefits have been ascribed to the plasma LDL-cholesterol lowering effect of statin therapy. Moreover, the effects of statins may involve nonlipid mechanisms such as plaque stabilization due to a decreased lipid content of the lesions (3), improved endothelial function (4,5) and a decreased tendency toward platelet thrombus formation (6). These alternative (to the LDL-cholesterol lowering effect) mechanisms could partly account for the reduction in coronary events achieved with statin therapy.

Blood lipid levels have been shown to be associated with levels of certain hemostatic parameters (7). In hyperlipidemic patients, prothrombotic status has been reported to be characterized by an increase in factor VII (FVII), fibrinogen and plasminogen activator inhibitor-1 (PAI-1) levels (8). On the other hand, FVII (9,10), fibrinogen (11), and PAI-1 (12,13) are important factors for determining the risk of coronary heart disease. Therefore, the aim of this study was to evaluate changes in these factors in hyperlipidemic patients undergoing treatment with atorvastatin, a powerful statin currently in clinical use (14).

Study Design, Subjects and Methods

Patients
We have studied 30 patients with primary hyperlipidemia (serum total cholesterol concentrations ≥ 5.7
mmol/l, or serum triglyceride concentrations ≥1.7 mmol/l (male/female: 19±11, mean age: 55±9 years). The following groups were ineligible to participate in the study: women of childbearing age, patients with severe hepatic or renal disease or hypothyroidism, patients who took drugs known to affect blood coagulation or fibrinolysis, and patients who had sustained a major coronary ischemic incident or stroke previously.

At initial screening, patients completed a detailed questionnaire about their medical and treatment history. A fasting blood sample was taken for laboratory analysis. Following this visit and a 4-week single-blind placebo run-in period, eligible and compliant patients received a 10 mg daily dose of atorvastatin. Patients were subsequently re-examined every 4 weeks by taking blood samples just before breakfast. The hemostatic parameters were measured at baseline and 12 weeks. The study protocol was approved by the Ethics and Research Committee at the University of Kanazawa. Informed consent was obtained from all patients.

Serum lipids, plasma fibrinogen, factor VII activity (FVIIc) and antigen (FVIIlag), activated factor VII (FVIIa), and PAI-1 antigen levels were also determined in age- and sex-matched controls (n=35, male/female: 24±11, mean age: 51±9 years). These subjects, recruited from hospital staff and relatives, were apparently healthy according to their medical history and a physical examination.

Methods

Venous blood samples were obtained from subjects who had fasted overnight. Blood was collected into siliconized glass tubes containing 3.8% (w/v) trisodium citrate. Plasma was isolated from whole blood by centrifugation at 2,500×g for 10 min at 4°C. Isolated plasma was stored frozen at −80°C until assayed. Serum for the measurement of cholesterol and triglycerides was prepared by allowing whole blood to clot in a siliconized glass tube at room temperature for 1 h followed by centrifugation at 1,200×g for 10 min.

Serum lipid levels were measured by standard enzymatic colorimetric methods using commercially available kits for total cholesterol (L-type Wako cholesterol: Wako, Tokyo, Japan) and triglycerides (L-type Wako TG-H; Wako). The level of high-density lipoprotein (HDL) cholesterol was measured using the same method after the very low-density lipoproteins and low-density lipoproteins (LDL) had been removed by precipitation with dextran sulphate and phosphotungstic acid in the presence of magnesium ions (Daichi Chemical, Tokyo, Japan). The level of LDL cholesterol was calculated using the Friedewald equation (16). Serum concentrations of lipoprotein (α) [Lp(α)] were assayed using an enzymelinked immunosorbent assay (ELISA) (Biopool AB, Umeå, Sweden). The level of glycosylated hemoglobin A1c (HbA1c) was measured using high pressure liquid chromatography.

The following hemostatic protein levels were measured at baseline and 12 weeks: prothrombin time (PT), fibrinogen, FVIIc, FVIIlag, FVIIla and PAI-1 antigen. The Clauss method was employed to determine the plasma fibrinogen concentration using an automatic recorder (CA-5000; Toa, Tokyo, Japan) (11). FVIIc was determined with a chromogenic prothrombin time-based assay using Owren's veronal buffer (Dade Behring Inc., Aguada, PR), human FVII deficient plasma (Baxter Diagnostics AG., Deerfield, IL) and thromboplastin reagent (Chromoquick; Hoechst, Tokyo, Japan). The FVIIc levels were expressed as percent activity compared with a commercially available standardized calibration plasma. The FVIIlag concentration was determined by an ELISA method using Asserachrom VII : Ag (Diagnostica Stago, Asnieres, France), normal range 76-123%. FVIIla levels were determined with an one-stage clotting assay using a recombinant truncated tissue factor, specific for FVIIla cofactor function (STACLOT VIIa, Diagnostica Stago). The plasma levels of PAI-1 were determined using a commercially available ELISA kit (Imulyse PAI-1; Biopool AB).

Statistical analysis

Data are presented as the mean±standard deviation (SD). Because normal distributions were not obtained for some parameters, the statistical significance of differences between controls and hyperlipidemic patients was assessed by nonparametric tests (Mann-Whitney U-test). The statistical significance of differences between values at 12 weeks of treatment and baseline was evaluated by Student's paired t-test. Correlations between different variables were determined using Spearman's rank correlation analysis. p values<0.05 were considered significant.

Results

Table 1 shows the lipids and fasting blood glucose levels at baseline and the 12-week mark in patients with hyperlipidemia, and age- and sex-matched control subjects. At baseline, serum levels of total cholesterol (p<0.0001), LDL-cholesterol (p<0.0001), and triglycerides (p<0.0001) were significantly higher in the patient group than the control group. There were no significant differences in serum HDL-cholesterol, Lp(a) and serum blood sugar levels between the patients and the control subjects.

Table 2 summarizes the hemostatic parameters in the two groups. With respect to the control subjects, the patients with hyperlipidemia had significantly higher levels of FVIIc (p<0.0001) and FVIIlag (p<0.0001). The levels of PAI-1 antigen was also significantly higher in the hyperlipidemic group (p<0.0003). There was no significant difference in plasma fibrinogen concentrations and FVIIla levels between the two groups.
Table 1. Serum levels of lipid and blood glucose in controls and hyperlipidemic patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=35)</th>
<th>Hyperlipidemic patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline 12 weeks</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.71±0.54</td>
<td>6.89±0.72*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.09±0.63**</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.83±0.53</td>
<td>4.36±0.77*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.85±0.64**</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.36±0.30</td>
<td>1.36±0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.56±0.40*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.09±0.34</td>
<td>2.47±1.46*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.45±0.79**</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/dl)</td>
<td>17±14</td>
<td>24±22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29±26</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>97±7</td>
<td>100±10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>104±19</td>
</tr>
</tbody>
</table>

Values are given as means±SD. *p<0.0001 from controls using the Mann-Whitney U test. **p<0.0005, ***p<0.0001 from baseline levels using Student’s paired t test.

Table 2. Plasma levels of markers of coagulation and fibrinolysis in controls and hyperlipidemic patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=35)</th>
<th>Hyperlipidemic patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline 12 weeks</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>ND</td>
<td>11.6±1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.4±1.03</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>242±53</td>
<td>264±58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>270±63</td>
</tr>
<tr>
<td>FVIIc (%)</td>
<td>93±19</td>
<td>141±26*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>122±23*</td>
</tr>
<tr>
<td>FVIIIc (%)</td>
<td>88±15</td>
<td>132±94**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>122±23*</td>
</tr>
<tr>
<td>FVII (mU/ml)</td>
<td>60±18</td>
<td>72±29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67±96</td>
</tr>
<tr>
<td>PAI-1 (ng/dl)</td>
<td>12.3±8.6</td>
<td>27.7±20.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.3±17.8</td>
</tr>
</tbody>
</table>

Values are given as means±SD. *p<0.0005, **p<0.0001 from controls using the Mann-Whitney U test. *p<0.0001 from baseline levels using Student’s paired t test. ND: not done

Effects of atorvastatin on serum lipid and blood glucose levels

As shown in Table 1, atorvastatin significantly reduced serum levels of total cholesterol, LDL-cholesterol, and triglycerides at 12 weeks compared to baseline values (p<0.0001). In contrast, HDL-cholesterol was increased at 12 weeks compared to the baseline (p<0.0005). Serum Lp (a) and fasting blood glucose levels did not change significantly following therapy.

Effects of atorvastatin on plasma hemostatic parameters

The effects of atorvastatin on plasma levels of hemostatic parameters are shown in Table 2. Treatment resulted in significant reductions in FVIIc (on average 13±13%; p<0.0001) and FVIIIc (12±12%; p<0.0001) after 12 weeks. No significant changes were observed in FVIIa levels however. Plasma concentrations of fibrinogen were slightly, but not significantly, higher after 12 weeks of treatment. No significant changes were seen in PT and PAI-1 antigen levels.

Relationships between serum lipid and FVIIc and FVIIIc levels

Although serum concentrations of triglyceride were strongly correlated with FVIIc (r=0.63, p<0.0005) and FVIIIc (r=0.63, p<0.0005) in the hyperlipidemic patients at baseline, we found no correlations between plasma levels of FVIIc (r=0.27, Fig.1A) and FVIIIc (r=0.23, Fig.1B) and serum triglycerides after treatment with atorvas-tatin. However, decreases in serum triglyceride concentrations correlated with decreases in plasma FVIIc levels (r=0.54, p=0.0023; Fig.2A) and FVIIIc levels (r=0.59, p<0.0006; Fig.2B). On the other hand, the decreases in FVIIIc did not correlate with the decreases in total cholesterol or LDL-cholesterol and were also unrelated to the changes in serum liver enzymatic activity (data not shown). Additionally, decreases in FVIIc did not correlate with decreases in total cholesterol, or LDL-cholesterol, and increases in HDL-cholesterol.

Discussion

We showed that atorvastatin significantly reduced plasma levels of FVIIc and FVIIIc, but not FVIIa, in all patients with hyperlipidemia, and decreases in plasma FVIIc and FVIIIc correlated with decreases in serum triglycerides.

About 99% of the FVII in the plasma of normal individuals exists as an inert zymogen while about 1% circulates in the activated form (FVIIa) (17). Activated factor X converts the zymogen of FVII into its enzymatically active form FVIIa. Methods for measuring plasma FVII levels can be grouped into those which measure FVII mass, FVIIc, or FVIIa. FVII mass is quantified by measuring the levels of FVII antigen. FVIIc is a measure of the ‘activity’ of plasma FVII in clot-based assays. Because of the limited conversion of FVII to FVIIa during assay, FVIIc measures an aggregate of FVII zymogen and preformed
Effects of Atorvastatin on Plasma Factor VII

Fig. 1. Correlations between serum triglyceride and plasma FVIIc (A) and FVIlag (B) at 12 weeks in patients taking atorvastatin.

Fig. 2. Correlations between changes in serum triglyceride and plasma FVIIc (A) and FVIlag (B) from baseline at 12 weeks in patients taking atorvastatin.

FVIIa. Thus, the decrease in FVIIc may be due to decreased FVII zymogen, pre-existing FVIIa, or both (18). Since, in our study, plasma levels of FVIIc decreased but those of FVIIa did not, atorvastatin may lower FVII zymogen levels significantly compared to pre-treatment levels. Therefore, our results suggest that atorvastatin could affect FVII levels by inhibiting the synthesis of FVII. However, there has been no direct evidence of this in vitro or in vivo so far.

Levels of FVIIc and FVIlag correlate with fasting concentrations of blood lipids, in particular, levels of serum triglycerides (19). Recently, it has been reported that bezafibrate which is known to lower serum triglyceride concentrations, reduced FVIIc and FXc levels, and the rate of decrease in triglycerides and suppression of FVIIc were highly correlated (20). They claimed that the reduction in FVIIc and FXc can be attributed to an indirect effect of the lowering of triglyceride-rich lipoprotein levels. In the present study, reduced FVIIc and FVIlag levels correlated with reduced serum triglyceride concentrations. We also showed that atorvastatin significantly reduced triglyceride concentrations in addition to total cholesterol concentrations. Therefore, another possible explanation for the influence of atorvastatin on FVII levels could be an indirect effect of the lowering of triglyceride levels. Since FVII binds to triglyceride-rich lipoproteins in vitro (21), the reduction of these lipoproteins might be accompanied by an increased clearance of FVII.

FVIIc has been reported to be a significant predictor of the risk of fatal coronary events in middle-aged men (9, 10) and has been linked to other thrombosis disorders in a number of population-based studies (22). In contrast, an extended follow-up of the PROCAM cohort indicated that Vllc is not independently associated with coronary risk (23). On the other hand, an elevation of FVIIa has been demonstrated in some (24) but not all studies (25) of
patients with acute coronary syndrome, and plasma levels of FVIIIa were independently and inversely related to coronary risk (26). Hence, it remains unclear whether elevated plasma levels of FVIIIa or FVIIc, or both, are related to an increased risk of coronary heart disease, and whether plasma levels of FVIIIa are a better predictor than FVIIc or FVIIag. Although prospective and cross-sectional studies are needed to further evaluate the relevance of FVIIa or FVIIc measurements in predicting coronary heart disease, the reduction in FVIIc and FVIIag during atorvastatin treatment might in part contribute to the decreased risk of thrombosis.

An association between hyperlipidemia and increased levels of plasma fibrinogen has been reported (27,28). However, the present study using 10 mg of atorvastatin only showed a slightly, and non-significantly, increased fibrinogen concentration (a 6% rise after 26 weeks). Another study showed a 28% increase in plasma fibrinogen level in 22 patients with familial hypercholesterolemia treated with 60 mg of atorvastatin at 6 weeks (27). On the other hand, another study which is consistent with the present findings reported a 4% increase in plasma fibrinogen levels, not a significant change, after the treatment of 720 subjects with 10-20 mg of atorvastatin for 52 weeks (28). Thus, the effect may be associated with a dose response, or may be transient. However, the reason for the effect of atorvastatin on the plasma level of fibrinogen is at present unknown.

Attention has recently been directed to the role of the fibrinolytic system in hyperlipidemia. Specifically, studies have been performed to determine whether imbalances between fibrin deposition and lysis could trigger thrombotic events in hyperlipidemia. Fibrinolytic activity is dependent primarily on plasma concentrations of t-PA and PAI, especially PAI-1. Although a lowering of triglyceride levels through diet or the use of drugs has been shown to be associated with a decrease in PAI-1 levels, we found no concomitant lowering of triglyceride levels and PAI-1 levels by atorvastatin. Studies with other statins have reported an approximately 20% reduction in PAI-1 following pravastatin treatment (29), and 18% rise due to simvastatin treatment for 2 years (30). This discrepancy among studies could be related to differing study populations or baseline levels of PAI-1.

In summary, atorvastatin treatment in the hyperlipidemic patients was accompanied by decreases in plasma FVIIc and FVIIag. The effects of atorvastatin on hemostatic parameters appear to be far less marked than its lipid-lowering effects. However, atorvastatin may reduce the risk of acute coronary events in part by reducing the thrombogenic risk. The present study was a relatively small and uncontrolled one. In the future, a larger, randomized placebo-controlled trial that can assess more reliably the effects of atorvastatin on these hemostatic factors is needed.

References

Effects of Atorvastatin on Plasma Factor VII


