

Simvastatin Treatment Improves Endothelial Function and Increases Fibrinolysis in Patients with Hypercholesterolemia

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Objectives: Statins reduce cardiovascular events by cholesterol-lowering as well as nonlipid-related actions. Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently identified independent risk factor of thrombosis. Endothelial dysfunction is also a strong predictor of cardiovascular events. The aim of this study was to assess the effects of simvastatin treatment on circulating TAFI concentrations and endothelial function in patients with hypercholesterolemia.

Methods: Thirty-five patients (19 female, mean age 48 ± 7 years) with hyperlipidemia were recruited into the study. Simvastatin was administered, 40 mg daily, for eight weeks to all subjects. Study subjects did not receive any medication except for lipid-lowering therapy during the follow-up period. Endothelial function was evaluated by flow-mediated dilation (FMD) from the brachial artery of the patients. Plasma lipid parameters, TAFI levels and endothelial function were measured before and after simvastatin treatment.

Results: Treatment with simvastatin showed a significant decrement in plasma total cholesterol, LDL cholesterol and triglyceride levels ($p < 0.05$). Plasma TAFI levels were also significantly decreased after simvastatin treatment [median 17.0 (range 0.4–93.7) mcg/mL versus median 6.9 (range 0.8–63.0) mcg/mL, $p < 0.001$]. Mean FMD was measured $7.7 \pm 2.5\%$ at baseline and significantly improved after treatment ($13.0 \pm 1.4\%$) ($p = 0.001$).

Conclusion: Our findings of decreased TAFI levels may reflect the beneficial effect of simvastatin treatment on fibrinolysis, and improved endothelial function may suggest the improved future cardiovascular events in hyperlipidemic patients.

Key words: simvastatin ■ TAFI ■ fibrinolysis ■ endothelial function ■ hypercholesterolemia

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INTRODUCTION

Hyperlipidemia can lead to coronary heart disease via the chronic acceleration of atherosclerosis, inflammation, and impaired fibrinolysis.¹ Medical interventions to prevent atherosclerosis progression and improve fibrinolytic function are required in hyperlipidemic patients. Hydroxymethylglutaryl-CoA-reductase inhibitors (statins) reduce cardiovascular events by cholesterol lowering as well as nonlipid related actions.^{2–10} It has been shown that simvastatin therapy not only improves endothelium-dependent vasodilation and prothrombotic state but also reduces oxidant stress and inflammation markers in hypercholesterolemic patients.^{5,6,8,10}

Thrombin activatable fibrinolysis inhibitor (TAFI), a recently identified independent risk factor of thrombosis, attenuates fibrinolysis by delaying the lysis of clots mediated by all the fibrinolysis activators.^{11–14} Elucidation of the critical regulatory molecule of fibrinolysis, such as TAFI, in hyperlipidemic patients could help better understanding of the pathobiology of the enigmatic prethrombotic state associated with hyperlipidemia.¹³ Furthermore, alterations of the hemostatic molecules, including TAFI, after simvastatin administration may be of assistance for the improvement of the pharmacological management of hyperlipidemia.

Endothelial function is impaired in several clinical conditions such as hyperlipidemia.^{15,16} Moreover, endothelial dysfunction measured by flow-mediated dilation is reported as one of the early markers of atherosclerosis and an independent predictor of

future cardiovascular events.^{17,18}

The aim of this study was to assess circulating TAFI levels and endothelial function in hyperlipidemic patients and to test the effects of simvastatin on TAFI concentrations and endothelial function.

METHODS

The study was performed in a prospective cohort design. The study population consisted of 35 hyperlipidemic patients who had not been treated with lipid-lowering medications before. All patients had a fasting low-density-lipoprotein (LDL) cholesterol >130mg/dl after six weeks of the National Cholesterol Education Program Diet.¹⁹ Patients with known or suspected coronary artery disease (CAD) such as typical chest pain, coronary atherosclerosis documented with coronary angiography, history of myocardial infarction, percutaneous coronary angioplasty (PTCA) and coronary artery bypass graft surgery (CABG) were excluded from the study. Other exclusion criteria were valvular heart disease, chronic atrial fibrillation, hypertension, diabetes mellitus, malignancy, chronic liver disease, hypothyroidism, chronic kidney disease, connective tissue disorders, pregnancy, obesity (body mass index >30kg/m²) and treatment with antiinflammatory or anticoagulant drugs. Eligible subjects underwent a comprehensive medical assessment, including documentation of the detailed history, physical examination, anthropometric assessment and measurement of the essential lab-

oratory variables. All of the subjects were given simvastatin, 40 mg daily, for eight weeks. Study patients did not receive any medication except for lipid-lowering therapy during the follow-up period. The local ethics committee approved the study, and all patients granted written informed consent.

Venous blood samples were obtained by the venipuncture of the large antecubital veins of the studied patients without stasis, after a 12-hour fast. The samples were then centrifuged immediately; the plasma was separated and stored at -80°C until the measurement of TAFI. TAFI levels were quantified by the ELISA method via using a commercially available assay (Imunoclone®). Fasting plasma total cholesterol, high-density-lipoprotein (HDL) cholesterol, LDL cholesterol, triglyceride, fibrinogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), creatinine, TAFI and other biochemical parameters were measured before and after eight weeks of simvastatin treatment.

Determination of Flow-Mediated Dilatation

Endothelial dysfunction was measured by flow-mediated dilatation (FMD) before simvastatin treatment and after eight weeks of treatment. The brachial artery was imaged with a System Five (GE Vingmed Ultrasound, Horten, Norway) cardiac ultrasound scanner and 7.5-mHz linear-array transducer. A pressure cuff was placed on the forearm

Table 1. Clinical characteristics and laboratory parameters of study patients before and after simvastatin therapy

	Baseline	After 8 Weeks of Simvastatin Therapy	P Value
<i>Anthropometric Measurements</i>			
Weight (kg)	76.75 ± 11.66	76.34 ± 11.89	NS
Body mass index (kg/m ²)	28.16 ± 4.02	28.00 ± 4.08	NS
Waist circumference (cm)	92.65 ± 9.98	92.25 ± 10.29	NS
<i>Blood Pressure (BP)</i>			
Systolic BP (mmHg)	129.71 ± 12.72	127.43 ± 10.74	NS
Diastolic BP (mmHg)	87.80 ± 8.24	86.29 ± 7.39	NS
<i>Metabolic Profiles</i>			
Fasting plasma glucose (mg/dl)	95.23 ± 13.78	91.54 ± 10.53	NS
Triglyceride (mg/dl)	174.57 ± 69.08	137.51 ± 69.47	0.008
Total cholesterol (mg/dl)	257.29 ± 22.06	177.89 ± 26.20	<0.001
LDL cholesterol (mg/dl)	166.71 ± 22.66	95.57 ± 25.39	<0.001
HDL cholesterol (mg/dl)	55.40 ± 14.34	55.91 ± 14.96	NS
VLDL cholesterol (mg/dl)	34.73 ± 13.77	27.03 ± 13.90	0.004
ALT (IU)	26.57 ± 13.96	24.34 ± 11.81	NS
AST (IU)	22.71 ± 5.67	23.11 ± 6.31	NS
Fibrinogen (mg/dl)	349.26 ± 61.20	313.06 ± 61.28	<0.001
Creatinine (mg/dl)	0.9 ± 0.1	0.8 ± 0.2	NS
CK (U/L)	45 ± 12	44 ± 21	NS

HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CK: creatine kinase

and inflated to exceed systolic blood pressure by 40 mmHg for 5 minutes, and the cuff was rapidly deflated. Using electrocardiographic triggering, end-diastolic images were measured at baseline and 2 minutes after cuff deflation. Brachial artery diameter (8–12-mm segment) was measured by two blinded sonographers. Baseline diameter was calculated as the average diameter from all baseline images measured. The 60-second diameter was calculated as the average of all images measured between 55- and 65 seconds after cuff deflation. FMD induced by reactive hyperemia was expressed as actual FMD [(60-second) – (baseline diameter) = FMD mm] and as relative change from baseline (FMDmm/baseline diameter = FMD%). The intraobserver and interobserver correlation coefficients for baseline and deflation diameters were 0.93 and 0.91, respectively.

Statistical Analysis

All analyses were performed with SPSS 10.0 for Windows®. The summary of results is expressed as mean \pm SD, unless otherwise stated. The paired sample t test was used for normally distributed variables and the Wilcoxon rank-sum test for skew distributed variables. Spearman analysis was used to identify correlations between changes in FMD, TAFI concentration and other study parameters. A p value <0.05 was considered as statistically significant.

RESULTS

Thirty-five patients (19 female, 16 male; mean age 48 ± 7 years) with hyperlipidemia were recruited into the study. Subjects were racially homogenous. None of the subjects were withdrawn from the study because of the adverse effects. Clinical characteristics and laboratory parameters of the study subjects before and after eight weeks of simvastatin treatment are shown in Table 1.

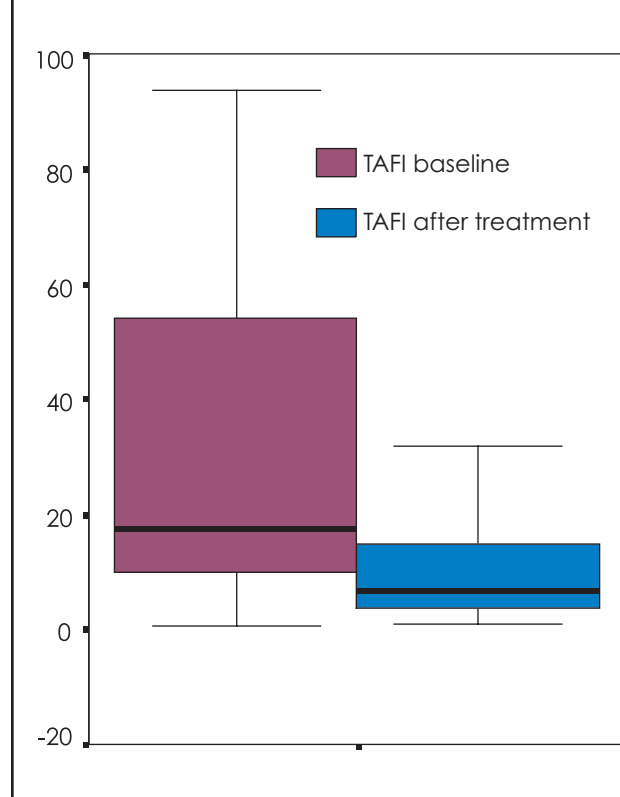
Plasma TAFI levels were significantly decreased after statin treatment [median 17.0 (range 0.4–93.7) mcg/mL versus median 6.9 (range 0.8–63.0) mcg/mL, $p < 0.001$, shown in Figure 1]. Mean FMD was measured $7.7 \pm 2.5\%$ at baseline and significantly improved after treatment ($13.0 \pm 1.4\%$) ($p = 0.001$). Mean percent change in flow mediated dilation ($5.1 \pm 2.9\%$) and plasma TAFI levels were not significantly correlated with baseline total cholesterol, LDL cholesterol, triglyceride, HDL cholesterol levels and also were not associated with the percent changes of these variables due to simvastatin therapy. Moreover, there was no statistically significant correlation between flow-mediated dilation and TAFI levels as well.

DISCUSSION

Hypercholesterolemia is associated with increased cardiovascular risk; however, the exact mechanisms have yet not been fully explained. The association between hypercholesterolemia and a prothrombotic state has always been suggested.^{20,21} In this study, simvastatin treatment significantly decreased plasma TAFI concentrations and improved endothelial function in hypercholesterolemic patients.

The benefit of statin therapy is not solely due to their cholesterol-lowering properties. It extends beyond that and involves pathways such as the coagulation and inflammation systems.²⁴ Simvastatin was shown as an effective stimulator of local fibrinolytic activity, as it increases t-PA and decreases PAI-1 production in endothelial cells.²² TAFI plays a crucial role in the various critical interactions among coagulation, fibrinolysis and inflammation.²³ Since patients with high TAFI levels had a high risk of thrombosis, decreased TAFI concentrations may indicate a profibrinolytic activity.²⁴ Therefore, our finding of decreased TAFI levels after statin treatment may suggest a decreased risk of thrombosis in hyperlipidemic patients. Since we did not find any correlation between changes in TAFI levels and lipid parameters, this may be due to nonlipid effects of

Figure 1. Plasma TAFI concentrations of hypercholesterolemic patients before and after simvastatin treatment



statin therapy.

Plasma fibrinogen, a marker of inflammation, is one of the predictors of coronary artery disease. In accordance to the previous studies, we found a significant decrement in fibrinogen levels in hypercholesterolemic patients with simvastatin therapy. This anti-inflammatory effect may add an additional improvement in cardiovascular outcome with statin treatment.

The results of our study are in accordance with previous studies indicating an improvement of endothelial function by statin treatment. Beneficial effects of simvastatin treatment on endothelial function may be the result of decreasing LDL cholesterol concentrations. However, there are studies showing that endothelial dysfunction was improved with statin therapy without a significant reduction of cholesterol levels.^{25,26} Consistent with those previous studies, we found that improvement of FMD with statin treatment was not associated with any lipid parameter change. Therefore, this result may suggest that the beneficial effect on endothelial function is due to direct effects of simvastatin on the vascular endothelium.

Our findings of improvement of dyslipidemia as well as decreased TAFI levels and improved endothelial function with simvastatin therapy might reflect the decreased future cardiovascular events in hypercholesterolemic patients. Although we did not find any correlation between plasma lipoprotein changes and beneficial effects on FMD and TAFI concentrations in hypercholesterolemic patients treated with simvastatin, the effects of improved plasma lipid levels on those parameters cannot be excluded. The association among hypercholesterolemia, hemostatic system and endothelial function should be examined in further studies not only for identification of the pathogenesis of cardiovascular events but also for the better medical management of hypercholesterolemic patients.

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