Long-Term Hemostatic Effects of Cholesterol-Lowering Therapy with Atorvastatin

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Key Words

Hemostatic system · Hypercholesterolemia ·
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Abstract

This study assessed hemostatic effects of an HMC-CoA reductase inhibitor, atorvastatin, on different parameters in 32 hypercholesterolemic patients of both sexes. In the patients and in 25 control subjects, plasma levels of tissue-type plasminogen activator, plasminogen activator inhibitor (PAI-1), D-dimer, prothrombin fragment 1 + 2 (F1 + 2), total cholesterol, triglycerides and fibrinogen had been measured. All these parameters were evaluated in patients after 6 and 12 months of treatment with atorvastatin at a dosage of 20 mg/day. This treatment significantly lowered the total cholesterol level in all patients. Moreover, after 6 months of atorvastatin treatment, PAI-1 and F1 + 2, which were both increased at baseline, were significantly reduced. This reduction continued after 12 months. The present results show that a reduction of hemostatic abnormalities, which exist in hypercholesterolemia, may be another important effect of the atorvastatin therapy.

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Introduction

Hypercholesterolemia is the most important predictive factor for coronary artery disease [1]. The correlation between total cholesterol levels and the LDL cholesterol level in particular and the risk of coronary artery disease events has been demonstrated in men and women of all ages [2–4]. Hemostatic abnormalities have been described in hypercholesterolemic patients and a possible interrelationship between hypercholesterolemia and hemostatic changes may play a role in the increased thrombotic risk of these patients [5, 6].

Statins, 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, are widely used in the treatment of hyperlipidemia. It has been shown that long-term treatment with statins is safe and improves survival in patients with coronary heart disease. These effects may be correlated with other beneficial properties besides their cholesterol-lowering effect [7]. The results of the Cholesterol and Recurrent Events trial (CARE) indicate that patients with myocardial infarction may benefit from lipid-lowering therapy with a statin, even if the cholesterol level is within an acceptable Northern European limit (<5.3 mmol/l).

Many abnormalities of hemostasis have been described in hyperlipidemias and, in the class of lipid-lower-

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ing drugs, some statins have a rapid preventive action on myocardial infarction and coronary deaths after only several months' treatment [8, 9]. In men with hypercholesterolemia, a 3-month simvastatin treatment is accompanied by a marked reduction of thrombin generation [10]. Atorvastatin increases endothelial nitric oxide synthase levels in human platelets of hyperlipidemic subjects [11] and there are conflicting data about the effects of atorvastatin on plasma fibrinogen [12–17].

Material and Methods

Patients and Controls

We studied 52 patients, admitted to the Department of Internal Medicine of Messina University, with type IIa hyperlipoproteinemia, having fasting total plasma cholesterol of >230 mg/dl and plasma triglyceride levels of <150 mg/dl. After having given their written informed consent, we performed a complete checkup and excluded from the study the subjects (n = 20) with apparent atherosclerotic diseases, diabetes, metabolic or inflammatory disease, impaired functions of liver and kidney, any circulatory disorders or being treated with drugs known to affect the hemostatic system (anticoagulants, heparin, aspirin). This last point was essential to avoid a possible confounding factor linked to the action of these drugs on the hemostatic system, and moreover the aim of our study was to evaluate a possible cardiovascular protection exerted by atorvastatin. In the remaining 32 patients, plasma levels of fibrinogen, total cholesterol, triglycerides, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1), D-dimer (DD) and prothrombin fragment 1 + 2 (F1 + 2) were evaluated at baseline and after 6 and 12 months of therapy with 20 mg/day of atorvastatin. Moreover, baseline results of patients were compared with those obtained in 25 healthy control subjects matched for sex, age and body weight recruited from the nursing school of Messina. The study protocol was approved by the Ethic Committee of the University of Messina School of Medicine. Basic personal characteristics of the patients and controls are presented in table 1.

Blood Sampling and Assay Methods

Blood samples were obtained by antecubital venipuncture; blood was drawn between 8 a.m. and 11 a.m. after an overnight fast and a 10-min rest. Blood was mixed with 1/10 vol 0.13 mol/l trisodium citrate, placed directly into refrigerated vacutainers, immediately placed on ice, and centrifuged within a few minutes at 2,000 g for 20 min at 4°C and frozen at –80°C for about 1 month until assayed. Plasma levels of t-PA, PAI-1, DD and F1 + 2 were measured by enzyme-linked immunosorbent assays. In particular, t-PA was evaluated by Asserachrom t-PA (Diagnostica Stago, Asnières-sur-Seine, France, normal range 1–12 ng/ml), PAI-1 by Asserachrom PAI-1 (Diagnostica Stago, normal range 4–43 ng/ml), DD by Asserachrom DD (Diagnostica Stago, normal values <500 ng/ml) and F1 + 2 by Enzygnost F1 + 2 micro (Behringwerke, Germany, normal range 0.2–1.1 nM). A double sample was evaluated for each subject.

Plasma levels of total cholesterol [18], triglycerides and fibrinogen were determined by automated routine procedures.

Table 1. Basic personal characteristics of patients and controls

	Patients	Controls
Subjects	32	25
Age ^a , years	55 ± 3	56 ± 2
Male/female	18/14	15/10
BMI ^a , kg/m ²	23 ± 3	22 ± 3
Current smokers	7	5

^a Values are expressed as mean ± SD.

Table 2. Basal levels of lipidic and hemostatic parameters in patients and controls

	Patients	Controls	p <
Total cholesterol, mg/dl	308 ± 56	190 ± 35	0.001
Triglycerides, mg/dl	135 ± 12	128 ± 15	n.s.
Fibrinogen, mg/dl	302 ± 20	290 ± 18	n.s.
t-PA, ng/ml	7.9 ± 3.0	7.4 ± 3.1	n.s.
PAI-1, ng/ml	23.8 ± 8.1	15.3 ± 8.8	0.01
DD, ng/ml	248 ± 55	225 ± 48	n.s.
F1 + 2, nM	0.35 ± 0.08	0.19 ± 0.07	0.002

Values are expressed as mean ± SD.

Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). Student's unpaired t test was adopted to compare hypercholesterolemic patients with healthy subjects. The statistical evaluation of data after treatment was carried out with ANOVA for repeated measures (on trial factor) and, if differences were found, Scheffé's F test was used to evaluate the effects of atorvastatin on the percent change from baseline in the cholesterol profile and the hemostatic markers. Pearson's correlation coefficient was calculated to evaluate the correlation between two variables.

Results

Data are presented in tables 2 and 3. At baseline, total plasma cholesterol levels were significantly higher in patients than in controls (p < 0.001), whereas no significant difference was detected in plasma triglycerides levels among study subjects. The plasma PAI-1 and F1 + 2 levels were also significantly higher in hypercholesterolemic patients than in normal subjects. On the other hand, there were not significant differences, for t-PA, DD and fibrinogen between patients and controls. All patients tolerated

Table 3. Changes in lipidic and hemostatic parameters by atorvastatin

	Baseline	6 months	12 months	p<
Total cholesterol, mg/dl	308 ± 56	238 ± 38	225 ± 30	0.002
Triglycerides, mg/dl	135 ± 12	132 ± 10	136 ± 14	n.s.
Fibrinogen, mg/dl	302 ± 20	325 ± 19	320 ± 14	n.s.
t-PA, ng/ml	7.9 ± 3.0	7.4 ± 3.8	7.8 ± 2.9	n.s.
PAI-1, ng/ml	23.8 ± 8.1	20.1 ± 7.0	20.0 ± 6.8	0.01
DD, ng/ml	248 ± 55	229 ± 42	235 ± 48	n.s.
F1 + 2, nM	0.35 ± 0.08	0.29 ± 0.06	0.25 ± 0.07	0.001

Values are expressed as mean ± SD.

therapy with atorvastatin well and none of the them had to be withdrawn from the study because of side effects.

The treatment significantly lowered the plasma total cholesterol concentration from 308 ± 56 mg/dl at baseline to a concentration of 238 ± 38 mg/dl ($p < 0.002$) after 6 months and 225 ± 30 mg/dl ($p < 0.002$) after 12 months, but the therapy did not modify the plasma levels of triglycerides.

After 6 months of treatment PAI-1 and F1 + 2 were significantly reduced (PAI-1 from 23.8 ± 8.1 to 20.1 ± 7.0 ng/ml, $p < 0.01$; F1 + 2 from 0.35 ± 0.08 to 0.29 ± 0.06 nM, $p < 0.001$). These changes were also maintained after 12 months (PAI-1 20.0 ± 6.8 ng/ml, $p < 0.01$, and F1 + 2 0.25 ± 0.07 nM, $p < 0.001$). Fibrinogen did not change in any patients during the study. No relation was found between lipid levels and hemostatic variables.

Discussion

Therapy of hypercholesterolemia with statins has been shown to reduce the incidence of cardiovascular events both in primary and secondary prevention studies [19, 20]. It has been proposed that statins could exert this protective effect on the cardiovascular system, not only lowering total cholesterol and in particular LDL cholesterol levels, but also interacting with the hemostatic system. In fact, alterations of hemostatic markers are reported in hypercholesterolemic patients [5, 6] and a reduction of these changes may be necessary for effective prevention of cardiovascular diseases. Reports of the effects of treatment with HMC-CoA reductase inhibitors on blood coagulation in patients with hypercholesterolemia are conflicting. Tsuda et al. [21] and Jay et al. [22] reported significant decreases in plasma fibrinogen after administration of pravastatin; Koppensteiner et al. [23] and Koenig et al. [24] found that the plasma fibrinogen level remained

unchanged after treatment with lovastatin; Wierzbicki et al. [13] reported the effect of atorvastatin in increasing fibrinogen; Dangas et al. [25] found that levels of PAI-1 in both male and female hyperlipidemic patients and levels of F1 + 2 only in women were reduced by pravastatin.

In our hypercholesterolemic patients, at baseline, increased plasma PAI-1 and F1 + 2 levels were observed. These changes seem to be an expression of impaired fibrinolysis and of increased thrombin formation. Both alterations could indicate a trend in a thrombotic sense in hypercholesterolemic subjects.

Treatment with atorvastatin significantly lowered total cholesterol levels in all patients. Moreover plasma PAI-1 and F1 + 2 levels, which were elevated before treatment, decreased significantly. Our results are partially in accordance with other studies, even if conducted with another statin, pravastatin. In particular, Dangas et al. [26] showed that a 6-month treatment with pravastatin significantly improved the fibrinolytic profile in patients with and without coronary artery disease.

In our study, we did not show any correlation between total cholesterol reduction and hemostatic parameters. This is in accordance with another previous study of Dangas et al. [27] conducted in 57 hyperlipidemic patients treated with pravastatin that excluded LDL cholesterol reduction as an independent predictor of a reduction in thrombogenicity.

We decided not to enroll patients into this study who were being treated with drugs interfering with the hemostatic system and in particular with aspirin, because we wanted to excluded a possible confounding factor, but above all because only statins are shown to reduce the cardiovascular risk thanks to their direct antithrombotic effects reducing platelet adhesion to vascular endothelium, platelet aggregation, urinary excretion of thromboxane metabolites and the expression of tissue factor by monocytes [28]. Moreover, even if some authors, as for

example Dangas et al. [25–27], conducted their studies administering aspirin with statins to hypercholesterolemic patients, others treated such patients only with the inhibitor of HMG-CoA reductase, for example lovastatin, without aspirin [20].

In conclusion, the present results show that the reduction of hemostatic abnormalities, which exist in hypercholesterolemia, may be another important effect of atorvastatin, which may shed further light on the prevention of cardiovascular events.

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