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Antioxidant effects of atorvastatin in patients with stable coronary artery disease

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Abstract

Objective. To assess the effect of atorvastatin on antioxidant enzyme activities in blood plasma and tissue in patients with stable coronary artery disease and postinfarction cardiosclerosis.

Materials and methods. The study included 122 patients with coronary artery disease (CAD) and 20 healthy controls. The following blood plasma parameters were assessed by generally accepted measurement tools: lipid profile, lipid peroxidation (LPO) products — diene conjugates (DC), thiobarbituric acid reaction products (TBA-RP), enzymatic antioxidant glutathione peroxidase (GP), erythrocyte superoxide dismutase (SOD), plasma activity of the antioxidant ceruloplasmin/transferrin system (AOS CP/TF) — by the electron paramagnetic resonance method. Endothelial function was investigated by ultrasound assessment of endothelial-dependent flow-mediated vasodilation (EDFMD) by the D. Celermajer et al. method.

Results. Patients with stable CAD and dyslipidemia showed the intensification of LPO processes, therefore, DC increased by 77 %, TBA-RP — by 58 %, and the impairment of enzyme regulation of reactive oxygen species (ROS): the decrease of AOS CP/TF by 33 %, SOD by 25 % and GP by 39 % compared with the control group. After the prescription of 20–40 mg of atorvastatin per day for 6 months in combination with complex cardiovascular therapy, the level of SOD increased by 16 %, GP — by 60 %, the activity of AOS CP/TF — by 12.5 %, the level of DC decreased by 40 %, TBA-RP — by 32 %, EDFMD improved by 36 %.

Conclusion. Atorvastation in combination with complex cardiovascular pharmacotherapy has antioxidant and antiperoxide activity and improves endothelial function in patients with stable CAD with manifestations of oxidative stress.

Keywords: atorvastatin, coronary artery disease, dyslipidemia, antioxidant protection, endothelial dysfunction.

Conflict of interest: none declared.

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Introduction

It is generally accepted that dyslipidemia (DLP) is one of the main risk factors (RF) for the development of atherosclerosis and coronary artery disease (CAD). Low-density lipoprotein cholesterol (LDL–C) plays a pivotal role in the pathogenesis of atherosclerosis, and oxidative modification of LDL that occurs due to action of free radicals and reactive oxygen species (ROS), exacerbates endothelial dysfunction and damage in patients with CAD that contributes to the atherosclerotic plaque (AP) destabilization. Numerous studies have shown that the reduction of atherogenic lipids levels, primarily LDL–C, during treatment with HMG-CoA reductase inhibitors — statins, directly correlates with the decrease of cardiovascular and all-cause mortality [1].

Along with antihyperlipidemic properties, statins also have pleiotropic and lipid-independent effects on all types of vascular cells and leukocytes [2]. HMG-CoA reductase inhibitors block the proliferation and migration of smooth muscle cells from media to intima layers during the AP formation [3], increase the activity of endothelial nitric oxide synthase (eNOS) [2, 4], have antioxidant properties, activate NO, and reduce the production of AT_1- receptors [5]. Statins reduce: the activity of NAD(P)H oxidase subunits, inflammation processes, the expression of cell adhesion molecules and the activity of macrophage migration [6], the development of matrix metalloproteinases and tissue factor [7].

It has been established that oxidative stress increases in patients with CAD [8]. Along with the disease development, an imbalance in the system of lipid peroxidation — antioxidant defense system occurs; thus, the assessment of endogenous antioxidant defense system and antioxidant medication properties are still highly relevant. Along with tissue antioxidant enzymes, the plasma antioxidant system (AOS)

ceruloplasmin/transferrin (CP/TF) also inactivates lipid peroxidation. During the oxidation of ions from Fe2+ to Fe3+, CP promotes its incorporation into apotransferrin. Due to this reaction, direct LPO inducers — Fe2+ ions, are eliminated from blood plasma. Thus, the development of superoxide anion radicals, that regenerate during non-enzymatic oxidation of Fe2+ ions, is prevented.

Literature data on antioxidant effects of statins are contradictory [9], however, further researches on its role into the oxidative stress manifestations and the activity of the AOS CP/TF in patients with CAD are still highly relevant and scientifically based. The investigation of statin therapy effects on the pathogenesis of atherosclerosis, including endothelial dysfunction, lipid metabolism, oxidative stress, NO metabolism, antioxidant defense system, will expand the understanding of its mechanisms of action, efficacy and safety in the reduction of atherosclerotic complications.

In this study we aimed to assess the role of atorvastatin in the correction of oxidative stress and endothelial dysfunction in patients with CAD and DLP.

Materials and methods

The study included 122 patients with CAD, functional classes (FC) II–III of angina pectoris, post-infarction cardiosclerosis with DLP types IIa and IIb according to WHO classification (65 men and 57 women) aged from 36 to 72 years old, average age — 58.3±7.9 years. All the participants signed written informed consent to participate in research. Average time since CAD diagnosis was 5.1±6.7 years. The control group included 20 healthy participants (10 men and 10 women), average age — 46.8±6.7 years.

Non-inclusion criteria were: the intake of antihyperlipidemic and antioxidant medications, the presence of myocardial infarction less than 6 months before the study, the presence of IIb-III stages of heart failure according to Strazhesko-Vasilenko classification, secondary hyperlipidemia, dysproteinemia, exacerbation of chronic diseases, hematopoiesis impairment, oncology.

Exclusion criteria were: the development of acute or exacerbation of chronic disease that required pharmacological treatment, patient's refusal to continue participation. The study has been approved the Ethics Committee of Moscow State University of Medicine and Dentistry named after A.I. Evdokimov (protocol № 4).

All study participants received diet No. 10 recommended by the Institute of Nutrition of the Russian Academy of Medical Sciences and basic treatment with beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, calcium antagonists and antiplatelet agents, underwent clinical and laboratory investigations, biochemical blood profile analysis, as well as instrumental examination in order to establish the diagnosis of CAD and identify exclusion criteria.

To assess the effect of atorvastatin as part of complex cardiovascular therapy on antioxidant enzymes activity and LPO process, we divided 122 patients with CAD into two groups. The 1st group included 102 patients with CAD (mean age 57.8±8.1 years) who received 20–40 mg of atorvastatin, the 2nd (comparison group) that included 20 patients with CAD (mean age — 60.7±7.2 years) who received only basic cardiovascular treatment due to their refusal to take statins. Both groups were comparable by gender, age, stage of CAD and laboratory data at baseline, and, therefore, were representative to determine treatment effectiveness (Table 1).

The determination of total cholesterol (TC), LDL–C and high-density lipoprotein cholesterol (HDL–C) was carried out by basic laboratory methods. The content of primary LPO products- diene conjugates (DC) was studied by the method of Iamamoto J. and determined spectrophotometrically by optical density at 232 nm, secondary thiobarbituric acid reaction products (TBA-RP) — by the method of Asakawa T., Matsushita S. modified by L.N. Shishkina with the addition of 10 μl of 0.01% solution of ionol in alcohol into the incubation medium. The amount of enzyme required for 50% inhibition of the reac-

Table 1. **Demographic data in patients with CAD and healthy controls**

Parameter	Group 1 (n=102)	Group 2 (n=20)	Control group (n=20)
Age, years	57.8 ± 8.1	60.7 ± 7.2	46.8±6.7
Men/women	52/50	13/7	10/10
The duration of CAD, years	5.1 ± 2.7	5.3 ± 2.8	—
Angina pectoris, II functional class	21(20%)	4 (20%)	—
Angina pectoris, III functional class	33 (32%)	7 (35%)	—
Arterial hypertension	83 (81%)	16 (80%)	—
CHF, I–IIa	54(53%)	11 (55%)	—
Smoking	23 (22%)	5 (25%)	5 (25%)
Genetic burden of CVD	61 (60%)	11 (55%)	14 (70%)
Overweight	10 (10%)	2 (10%)	1 (5%)

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tion of nitrotetrazolium blue by the superoxide anion radical generated during the oxidation of xanthine by xanthine oxidase at 560 nm using "Hitachi-557" spectrophotometer according to the method of Beauchamp C. and Fridovich I. was taken as a unit of superoxide dismutase (SOD) activity. Glutathione peroxidase (GP) activity was determined in conjugated glutathione reductase system for the oxidation of NADPH using tert-butyl hydroperoxide as a substrate according to the method of Lankin V. Z., Gurevich S. M.

Antioxidant activity (AOA) of AOS CP/TF in blood plasma was measured by the method of electron paramagnetic resonance (EPR). Endothelial function was studied using in B-mode of ultrasound with linear probe of 7.5 MHz with the assessment of endothelium-dependent vasodilation (EDVD) according to the method of Celermajer D. et al. (1992).

Statistical analysis of the obtained data was carried out using "Statistica 10" software. Quantitative variables, assuming their normal distribution, were compared using Student's t-test for two independent groups and using paired Student's t-test to compare studied parameters before and after treatment.

Results

The comparative analysis revealed significant differences between composition of lipids, LPO processes, tissue and plasma antioxidant defense system in patients with CAD and from the control group (Table 2).

The activity of antioxidant enzymes: SOD (by 61 %) and GP (by 80 %) in erythrocytes significantly decreased in the vast majority of patients with CAD compared with the control group. The increase of the SOD/GP ratio indicated significant imbalance in the system of antioxidant enzymes in the erythrocyte towards oxidative stress in patients with CAD.

Table 2. **Laboratory data in patients with CAD and the control group (M±sd)**

Parameters	Patients with CAD (n=122)	Control group (n=20)
TC, mmol/l	6.4±0.9	5.3±0.9*
Triglycerides, mmol/l	1.7±0.3	1.4±0.4 *
LDL–C, mmol/l	4.7±0.9	3.0±0.8*
HDL–C, mmol/l	0.96±0.15	1.3±0.2*
AST, U/L	22.6±8.5	19.6±5.1
ALT, U/L	24.6±7.9	20.4±4.4*
Diene conjugates, nmol/ml	24.8 ± 5.1	14.0±2.7*
TBA-RP, nmol/mg	0.19 ± 0.04	0.12±0.04*
SOD, U/Ml	1772±523	2337±123*
GP, U/Ml	19.3 ± 6.7	31.4±3.3*
SOD/GP	92	74*
CP, conventional units	77.4±17.6	123.9±19.4*
CP/TF	0.8±0.1	1.2±0.3*

Note. * — differences between the parameters of patients with CAD and the control group are significant with p<0.001.

The changes of the amplitude of the EPR signals of the CP and TF reflects the dynamics of their paramagnetic properties and inherent activity. AOS of CP/TF was by 76 % lower in patients with CAD compared with the control group.

Thus, laboratory data of patients with CAD revealed DLP, accompanied by the intensification of LPO processes in the form of DC increase by 77 %, and TBA-RP increase by 58 % and the impairment of enzymatic regulation of ROS metabolism: the decrease of the AOS CP/TF activity by 33 %, SOD — by 25 % and GP — by 39 % compared with the control group.

Atorvastatin demonstrated antihyperlipidemic, antiperoxide and antioxidant effectiveness in the vast majority of examined patients with CAD and DLP during 6-month treatment in combination with basic therapy (Table 3).

After 3-month treatment with atorvastatin, 56 % of patients from group 1 achieved target lipid levels, and 44 % of patients required the increase of the dose up to 40 mg/day. The medication was well tolerated by most patients. Dyspeptic phenomena occurred in 5 % of patients during the first week of treatment and disappeared further on their own. After 6-month treatment, 83 (81 %) patients from group 1 achieved target levels of TC, 75 (74 %) — LDL–C, and none of the patients from group 2. The results correspond to the data of multicenter studies on lipid-lowering effect of statins. The required correction of antihyperlipidemic therapy was carried out in accordance with the current National Clinical Guidelines.

Atorvastatin as part of combination therapy for CAD reduced the severity of LPO processes and contributed to the increase of antioxidant defense system activity in 91 % of patients from group 1. SOD activity increased by 16 % after 6-months treatment, GP — by 60 %, AOS CP/TF — by 12.5 %. The level of DC decreased by 40 %, and TBA-RP — by 32 %. After 6 months of treatment, groups of patients with CAD who received and did not receive atorvastatin differed by lipid profiles, LPO products and the activity of tissue and plasma antioxidant enzymes (see Table 3). AST and ALT in both groups before and after treatment were within the reference values.

Table 3. **Clinical and laboratory data before and after 6-month treatment of patients with CAD (**M±**SD)**

Parameters	Group 1 (n=102)		Group 2 (n=20)	
	At baseline	After 6 months of treatment	At baseline	After 6 months of treatment
TC, mmol/l	6.3± 0.9	4.3±0.5**	6.5±0.8	6.1± 0.7** ⁺
LDL–C, mmol/l	4.6±0.9	2.4±0.5**	4.7±0.8	4.2±0.8** ⁺
HDL–C, mmol/l	0.96±0.2	1.14±0.1**	1.03±0.15	1.07±0.13
TG, mmol/l	1.7±0.4	1.5±0.2**	1.6±0.3	1.5±0.3
TBA-RP, nmol/mg	0.19±0.04	0.13±0.02**	0.21±0.04	0.23±0.04* ⁺⁺
Diene conjugates, nmol/ml	24.5±5.0	14.8±3.0**	26.3±4.9	28.7±4.4* ⁺⁺
SOD, U/Ml	1805±507	2101±414**	1605±583	1570±488⁺⁺
GP, U/Ml	19.7±6.9	31.5±6.5**	17.3±5.5	14.3±3.3* ⁺
CP, conventional units	78.3±18	92.9±16**	73.0±17.2	72.0±15.5⁺⁺
CP/TF	0.8±0.1	0.9±0.1**	0.79±0.1	0.75±0.1*⁺⁺
AST, U/L	21.9±8.2	24.5±8.7	26.1±9.3	23.8±6.1
ALT, U/L	24.9±7.8	29.3±9.8*	23.5±8.6	19.7±5.5⁺
EDVD, Δ%	7.3±3.9	9.9±2.3*	7.2± 4.0	8.3± 3.6

Note. Differences between the parameters at baseline and after treatment are significant with * — p<0,05; ** — p<0,001. Intergroup differences are significant with ⁺ — p<0,0001, ⁺⁺ — p<0,001.

Endothelial function also improved along with LPO antioxidant defense system activity, according to the results of treatment in patients with CAD (see Table 3). It is remarkable that the changes were statistically significant in patients with CAD who received atorvastatin.

Discussion

The improvement of LPO antioxidant defense system parameters can be explained by the effect of key enzymes that are responsible for the synthesis and neutralization of ROS, eNOS and NAD(P)H-oxidase, in particular [2]. Several studies have proven early antioxidant effect of statins and their ability to restore the biological activity of NO. They contributed to the increase of vascular eNOS activity, decrease of ROS production in the vessels, and, therefore, improved endothelial function [2–5]. It has been shown that the antioxidant and anti-inflammatory effects of statins are closely associated with each other that are caused by the stimulation of the signaling transcription Lung-Kruppel-like factor 2 (LKLF/KLF2) that increase the activity of eNOS, thrombomodulin and anticoagulant properties of protein C, and decrease the effects of adhesion molecule genes-1 (VCAM-1) and plasminogen activator inhibitor-1 (PAI-1) expression that promote inflammation and thrombogenesis. [6, 10]. Literature data on the effect of statins on the parameters of oxidative stress in patients CAD are limited. Thus, atorvastatin and rosuvastatin at the dose of 80 mg and 40 mg per day, respectively, in 70 patients with myocardial infarction for 4 weeks showed significant decrease of LDL cholesterol, normalization of the total antioxidant status and oxidative stress index.

The study of 40 patients with DLP from Macedonian prescribed with rosuvastatin 20 mg/day showed that 67 % of patients had lower susceptibility of LDL to oxidation after rosuvastatin treatment (p = 0.03), and 53 % of patients — higher antioxidant capacity of HDL after treatment, however, the difference was not statistically significant (p=0.07). The increase of antioxidant potential of HDL during rosuvastatin treatment was more prevalent among men (OR=9.350; p=0.010) [12]. Atorvastatin as part of combination therapy of DLP contributed to the increase of antioxidant enzymes SOD, catalase, and CP activity, although these changes were not significant [13].

The reduction of oxidative stress and restoration of NO biological activity are the key mechanisms that explain beneficial effects of statins on endothelial dysfunction. The improvement of EDVD in the group of patients treated with atorvastatin can be explained not only by their antihyperlipidemic effect, but also by the ability to reduce the production of prooxidant enzymes and stimulate the synthesis of intermediates and enzymes involved in the neutralization of ROS and free radicals, which also have antioxidant properties [2].

Thus, this study has shown that atorvastatin improves endothelial function in patients with CAD and

DLP due to its antiperoxide and antioxidant properties, protective effect and the reduction of lipid peroxidation products by inactivation of lipid radicals, and the increase of tissue and plasma antioxidant enzymes activity.

Conclusion

The 6-month treatment with 20–40 mg/day of atorvastatin as part of combination therapy for cardiovascular diseases, showed its antioxidant and antiperoxide activity in 90 % of examined patients with CAD. Combination therapy in patients with cardiovascular diseases, including atorvastatin along with the achievement of target lipid levels contributes to the correction of the antioxidant status and significant reduction of endothelial dysfunction in patients with CAD and disturbances in lipid peroxidation — antioxidant defense system. This study suggests the need for integrated approach in the investigation of lipid profile and oxidative stress manifestations in patients with cardiovascular diseases. The obtained data on the pleiotropic properties of atorvastatin will expand the indications for its prophylactic use, considering its ability to reduce the risk of adverse prognosis, regardless of the lipid profile parameters.

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