The effects of atorvastatin on antioxidant/antiinflammatory properties of HDLs in hypercholesterolemics

Gülşen AKALIN ÇİFTÇİ[1,]*, İpek ERTORUN[2], Aysen AKALIN[3], İbrahim Özkan ALATAŞ[2], Ahmet MUSMUL[4]

[1]Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey
[2]Department of Medicinal Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, Eskişehir, Turkey
[3]Department of Endocrinology, Faculty of Medicine, Eskisehir Osmangazi University, Eskişehir, Turkey
[4]Department of Biostatistics, Faculty of Medicine, Eskisehir Osmangazi University, Eskişehir, Turkey

Background/aim: Hypercholesterolemia is characterized by changes in lipid profile, nitric oxide pathway, and oxidative stress markers, but functions of high-density lipoprotein (HDL) were not well established in hypercholesterolemic subjects treated with atorvastatin. In this study, we aimed to evaluate effects of atorvastatin treatment on functionality of HDL, oxidative stress, and endothelial functions in hypercholesterolemic subjects.

Materials and methods: Thirty patients (20 females, 10 males) aged from 40 to 60 years and diagnosed as hypercholesterolemic were included. Patients were treated with 10 mg/day atorvastatin for 3 months. Markers of endothelial functions, namely asymmetric dimethylarginine (ADMA), homocysteine, and nitric oxide (NO), and markers of oxidative status, namely malondialdehyde (MDA), antioxidant potential (AOP), paraoxonase 1 (PON1), and arylesterase, were measured. Before and after atorvastatin treatment, glucose, lipid parameters, and antioxidant/antiinflammatory HDL levels were also measured.

Results: ADMA and homocysteine levels were decreased whereas NO levels were increased with atorvastatin therapy. MDA levels were decreased but AOP, PON1, and arylesterase levels and antiinflammatory characteristics of HDLs were increased. Furthermore, lipid profiles of the patients improved with atorvastatin therapy.

Conclusion: Hypercholesterolemia is a cause of oxidative stress, endothelial dysfunction, and proinflammatory HDL levels. Atorvastatin is a beneficial pharmacological modulator of impaired antiinflammatory HDL-C levels, endothelial functions, and oxidative status against atherosclerosis indicating pleiotropic effects of statins.

Key words: Antioxidant/antiinflammatory high-density lipoprotein, atorvastatin, hypercholesterolemia, oxidative stress

1. Introduction

Hypercholesterolemia is one of the established risk factors in the pathogenesis of atherosclerosis and subsequent coronary heart disease (1). Endothelial function is impaired by the decrease in synthesis and release of endothelium-derived relaxing factors or by the inactivation after reaction with superoxide radicals (2–4). Nitric oxide (NO), which is a potent endogenous vasodilator, is generated by NO synthase (NOS) in the endothelium. Decreased NO production impairs endothelium-dependent vasodilation and also accelerates the development of vascular events (5).

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS and a marker for atherosclerosis in circulation; it may be a novel risk factor for endothelial dysfunction and coronary artery diseases. It is synthesized with N-methyl transferases from proteins (5,6).

Elevated levels of homocysteine are an independent risk factor for cardiovascular diseases. Homocysteine may contribute to enhanced oxidative inactivation of NO by its redox activity, which arises from the formation of disulfides and the generation of hydrogen peroxide and superoxide anion (7). Increased levels of homocysteine have been shown to inhibit the activity of dimethylarginine dimethylaminohydrolase, which is responsible for the metabolism of ADMA (7) and causes an accumulation of ADMA (5). Deterioration in the homocysteine metabolism may also induce oxidative stress. Homocysteine increases expression of TNF-α, which increases oxidative stress. S-adenosylmethionine, an intermediate in homocysteine metabolism, is a source of methyl groups that are involved in ADMA (5).

Paraoxonase 1 (PON1), which catalyzes the hydrolysis of biologically active lipids in oxidized low-density

* Correspondence: gakalin@anadolu.edu.tr

lipoprotein (8), is one such protein that has been reported to possess both antioxidant and antiinflammatory properties (8). Homocysteine thiolactone, which is a both toxic and cytotoxic metabolic product of homocysteine, is detoxified by the homocysteine thiolactonase activity of PON1 (7). Hence, PON1 prevents protein homocysteinylation, the process involved in atherogenesis.

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, are widely used for hypercholesterolemia treatment and have cardioprotective and antioxidant effects regardless of their lipid lowering effects. Some statins and their metabolites also act as free radical scavengers, reducing reactive oxygen species (ROS) formation (7). Statins increase serum PON1 activity, improve endothelial function, enhance the stability of atherosclerotic plaques, and suppress vascular inflammation (9).

The aim of our study was to examine the short-term protective effect of statins on endothelial dysfunction and atherosclerosis processes in patients with hypercholesterolemia. For evaluating endothelial dysfunction, ADMA, NO, and homocysteine levels; oxidant/antioxidant status; and lipid profile, especially the functionality of high-density lipoprotein (HDL), were determined.

2. Materials and methods

Thirty patients (20 females, 10 males) with hypercholesterolemia aged from 40 to 60 years and admitted to the Endocrinology Department of the Eskişehir Osmangazi University Medical School were included in the study. The study inclusion criteria for patients were to carry two or more risk factors for coronary heart disease (hypertension, smoking, diabetes, physical inactivity, unhealthy diet, obesity, elevated blood cholesterol, excessive stress) and low-density lipoprotein cholesterol (LDL-C) levels in the range of 130–300 mg/dL, or to carry one or no risk factor for coronary heart diseases and LDL-C levels in the range of 160–300 mg/dL.

Before and 3 months after the statin treatment after 12 h of overnight fasting, blood samples were collected from each patient in both heparinized and polymer gel containing tubes to obtain serum and plasma, respectively. Thirty patients were orally administered atorvastatin (10 mg/day) for 3 months. Other lipid-lowering drugs and over-the-counter agents such as niacin or omega-3 were not allowed. Blood samples were collected in the same manner. A control group consisted of 30 healthy subjects who were not on any medication and did not smoke. Patients with established atherosclerotic vascular disease or diabetes and patients with lipid disorders other than hypercholesterolemia were not included in the study.

Markers of endothelial functions, namely ADMA, homocysteine, and NO, and markers of oxidative status, namely malondialdehyde (MDA), antioxidant potential (AOP), PON1, and arylesterase, were measured. Before and after atorvastatin treatment, lipid parameters and antioxidant/antiinflammatory HDL levels were also measured.

Blood samples were centrifuged at $1097 \times g$ for 10 min (Jouan MR 22). Serum, total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), apolipoprotein (Apo A1), and apolipoprotein (Apo B) were immediately measured with Roche Diagnostic kits in a Modular Systems (Roche Diagnostics) analyzer according to the manufacturer's instructions. Lipoprotein(a) (Lp(a)), Apo A1, and Apo B levels were measured by immunoturbidimetric method with Roche Diagnostic kits in a Modular Systems (Roche Diagnostics) analyzer according to the manufacturer's instructions. Part of the serum and plasma samples were frozen at –80 °C (Jouan VX350 series, Thermo Electron) without delay until the analysis of other parameters.

Nitrate and nitrite, which reflect the best index of total NO production, are the final and stable end products of NO in vivo. Nitrate was assayed by a slight modification of the cadmium-reduction method, as defined by Cortas and Wakid, and absorbance was read against the blank at 540 nm after 30 min (10). ADMA levels were measured according to the Jones and Wu method with minor modifications and results were given as µmol/L (11,12). Homocysteine and MDA levels were measured with a Chromsystems Diagnostic commercial kit (Munich, Germany) using an Agilent HP 1100-Model HPLC system equipped with an autosampler and a fluorescence detector. Serum AOP was measured by a method described by Durak et al. (13). AOP values were assessed from the difference between MDA levels of control and sample studies and expressed as U/h. PON1 activity was measured with a fully automated paraoxonase activity measurement kit (Rel Assay Diagnostics) in a Modular P (Roche Diagnostics) analyzer according to the manufacturer's instructions. The enzyme activity was expressed in U/L. Arylesterase activity was measured with a fully automated arylesterase measurement kit (Rel Assay Diagnostics) in a Modular P (Roche Diagnostics) analyzer according to the manufacturer's guidelines. The enzyme activity was expressed in U/L. Antiinflammatory properties of HDL were determined by the change in fluorescence intensity resulting from oxidation of DCFH by LDL in the presence of tested HDL (14). HDL isolation from subject samples was carried out by dextran sulfate precipitation method (14).

2.1. Statistical analysis

SPSS 13.0 for Windows was used for statistical analysis. Data are expressed as mean ± SD. Comparisons were performed by one-way ANOVA (Tukey post hoc analyses) for normally distributed continuous variables or Kruskal–Wallis H test for nonnormally distributed variables for several independent samples.

2.2. Ethics statement

All subjects were informed about the study protocol and written consent was obtained. Institutional ethics committee approval was received before the beginning of the study (30.06.09/40).

3. Results

In our study, hypercholesterolemic patients' lipid profile parameters, except HDL-C and Apo A1, were significantly increased. HDL-C and Apo A1 levels were significantly decreased when compared with controls (Table 1).

After atorvastatin treatment, TC, LDL-C, and Apo B levels (P < 0.001) and Apo (a) levels (P < 0.05) were significantly decreased (Table 1).

In our study, before treatment the group's ADMA, homocysteine, and MDA levels were significantly increased (P < 0.001), whereas NO, AOP, PON1, and arylesterase levels were significantly decreased compared to controls (P < 0.001) (Table 2).

On the other hand, after atorvastatin treatment, ADMA, homocysteine, and MDA levels were significantly decreased compared to hypercholesterolemic values (P < 0.001). NO (P < 0.001), PON1 (P < 0.001), and arylesterase (P < 0.01) levels were significantly increased compared to hypercholesterolemics. After treatment AOP levels were increased compared to hypercholesterolemic values, but this was not statistically significant (P > 0.05) (Table 2).

Antiinflammatory properties of HDL against LDL oxidation was measured by dichlorofluorescin assay.

Table 1. Lipid profiles of hypercholesterolemic patients before and after treatment (mean ± SD).

Parameters	Control	Hypercholesterolemic patients	
		Before treatment	After treatment
Triglyceride (mg/dL)	118.5 ± 60.6	173.0 ± 62.1*	148.2 ± 60.3*
Total Cholesterol (mg/dL)	196.4 ± 40.6	257.3 ± 52.4***	201.9 ± 34.9###
HDL-C (mg/dL)	52.4 ± 7.8	47.5 ± 10.8*	52.3 ± 9.3
LDL-C (mg/dL)	122.4 ± 30.3	171.6 ± 42.3*	130.9 ± 30.6###
Lp(a) (mg/dL)	39.5 ± 19.5	60.0 ± 25.5***	46.4 ± 12.4*,#
Apo (A1) (mg/dL)	162.2 ± 11.9	152.4 ± 22.7*	160.1 ± 18.6
Apo (B) (mg/dL)	84.8 ± 23.7	135.9 ± 17.8**	98.3 ± 25.2*,###

*P < 0.05 vs. control, **P < 0.01 vs. control, ***P < 0.001 vs. control, #P < 0.05 vs. study group before treatment, ###P < 0.001 vs. study group before treatment.

Table 2. The oxidative status and endothelial function parameters of hypercholesterolemic patients before and after treatment (mean ± SD).

Parameters	Control	Hypercholesterolemic patients	
		Before treatment	After treatment
ADMA (µmol/L)	0.41 ± 0.08	0.73 ± 0.18***	0.57 ± 0.12###
Homocysteine (µmol/L)	10.91 ± 2.8	14.8 ± 3.12***	12.4 ± 2.7###
NO (µmol/L)	20.8 ± 17.6	9.8 ± 9.2***	16.2 ± 3.6***,###
MDA (nmol/L)	0.05 ± 0.03	0.09 ± 0.02***	0.06 ± 0.03###
AOP (U/h)	10.08 ± 1.4	6.49 ± 3.9***	9.19 ± 2.8*
PON1 (U/L)	176.5 ± 31.3	126.8 ± 32.5***	164.3 ± 33.9*,###
Arylesterase (U/L)	439.5 ± 79.6	342.9 ± 66.5***	382.4 ± 80.2**,#

*P < 0.05 vs. control, **P < 0.01 vs. control, ***P < 0.001 vs. control, #P < 0.05 vs. study group before treatment, ###P < 0.001 vs. study group before treatment.

Hypercholesterolemia caused a significant decrease in antiinflammatory capacity of HDL compared to the control group (P < 0.001). Atorvastatin treatment increased HDL antiinflammatory capacities in hypercholesterolemic patients (P < 0.01) (Table 3).

4. Discussion

In the present study we demonstrated that 3 months of atorvastatin treatment markedly decreased oxidative stress and prooxidant/proinflammatory HDL levels and improved endothelial dysfunctions in the studied patients.

Over the last decades, emerging data have suggested that HDL functionality is more important than its quantity (15). Thus, studies have focused on investigating modulation of nonfunctional/proatherogenic HDL by pharmaceutical drugs. In particular, lipid-lowering drugs such as statins are mostly used for hypercholesterolemia (16). Thus, we studied the preventive role of atorvastatin on hypercholesterolemic patients against oxidative stress, endothelial dysfunction, and prooxidant/proinflammatory HDL functions.

Oxidative stress in the endothelium is known to contribute to the atherogenesis process. During atherogenesis, lipid peroxidation takes place in arterial macrophages and lipoproteins, which are rich in polyunsaturated fatty acid (7). Measurement of breakdown products such as MDA is the most common approach to determine the degree of lipid peroxidation induced by ROS (17). In this study, hypercholesterolemia increased MDA levels and decreased AOP levels compared to controls, indicating the presence of increased oxidative stress in hypercholesterolemia (P < 0.001). Since AOP reflects the total capacity of the enzymatic and nonenzymatic antioxidant defense system, a decrease in AOP demonstrates impairment in the total antioxidant defense system in hypercholesterolemia (17).

On the other hand, atorvastatin treatment decreased MDA levels (P < 0.001). The decreases in lipid peroxide levels may arise from decreased LDL-C levels by treatment with atorvastatin, since this drug may cause removal of 'aged LDL', which is more prone to oxidation. Bolayirli et

al. showed that antioxidant effects of statins were attributed to their reducing the generation of ROS by inhibition of vascular NAD(P)H oxidase, modification of redox homeostasis in LDL particles, inhibition of the respiratory burst of phagocytes, modulation of the RNA expression, antagonization of the prooxidant effect of angiotensin II and endothelin-1, increase of the upregulation of eNOS expression/activity and vascular NO, and binding to surface phospholipid of lipoproteins (7). Sathyapalan et al. showed that atorvastatin reduced MDA concentrations in patients with polycystic ovary syndrome, indicating pleotropic effects of atorvastatin, as in our study (18).

Its known that statins increase triglyceride-rich lipoprotein catabolism and decrease LDL-C levels (19). In our study, LDL-C and Apo B levels were significantly decreased, as indicated in the Atorvastatin Comparative Cholesterol Efficacy and Safety Study after 3 months of statin therapy (20). HDL-C and Apo A1 levels did not change because statins have fewer effects on Apo A1 in patients with hypercholesterolemia (20). In some studies it was shown that statin treatment increased Lp(a) levels (21,22), but in some studies controversial effects were reported, as in ours (23,24). Maron et al. showed that statin treatment did not affect Lp(a) levels (25). These results may be explained by individual mutational differences, as indicated by Bolewski et al. (26).

PON1 is an antioxidant enzyme that circulates in the blood in HDL particles and has arylesterase and lactonase activities. It protects against lipid peroxidation by its ability to hydrolyze lipids and degrade H_2O_2 (7). HDL is likely to be an important determinant of PON1 activity, which is responsible for antioxidant and antiinflammatory capacity. In support of this, we observed a direct relationship between mean HDL-cholesterol levels and PON1 levels in controls and hypercholesterolemics (8).

In this study, prooxidant/proinflammatory HDL, MDA, and AOP results show the presence of oxidative stress. Decreases in PON1 activities before treatment may depend on these oxidative stresses. Previous studies (7,8) suggested that oxidative stress depresses PON1 activity with inadequate antioxidant capacity,

Table 3. HDL score and percentage of pro- and antiinflammatory HDLs in groups.

Groups	HDL score (FU)	>1	<1
		% Proinflammatory HDL	% Antiinflammatory HDL
Control	4.87 ± 0.99	6.7	93.3
Before treatment	8.86 ± 3.49[***]	66.7	33.3
After treatment	6.10 ± 2.61[*, ##]	23.3	76.6

*P < 0.05 vs. control, ***P < 0.001 vs. control, ##P < 0.01 vs. before treatment group.

excess oxidative stress, and inflammatory response, as evident in hypercholesterolemic patients. Furthermore, hypercholesterolemia is known to reduce PON1 activity through the interactions between the free sulfhydryl group of enzyme and oxidized lipids. In line with our findings, Hong et al. showed decreases in serum PON1 concentrations in high-cholesterol diet-induced hypercholesterolemia in rabbits (27). In our study, before and after atorvastatin treatment, the study group's PON1 levels were increased, indicating that atorvastatin as used in this study had antioxidant effects on hypercholesterolemia. PON1 activity was increased inversely proportionally to decreased lipid peroxides caused by ROS. It was shown that atorvastatin increased PON1 activities in different clinical and experimental studies, in line with our study (28–32), whereas some others failed to find any changes in serum paraoxonase activity following simvastatin or atorvastatin administration (16). Deakin et al. (33) explained that patients with the –108C allele, who already have higher PON1 levels, may further benefit from treatment with statins, indicating the importance of gene polymorphism among individuals.

The decreased activity of PON1 can depress the ability of circulating HDL particles to protect LDL from oxidation, to participate in the reverse cholesterol transport pathway, and to inhibit monocyte–endothelial cell interaction. All of these appear to be important in the inflammatory response in arteries that promotes atherogenesis. Beside this low PON1 activity, the tyrosine residues in Apo A1 may be modified by myeloperoxidase, which is a determinant of inflammatory state. Thus, Apo A1 levels and functions may decrease as a result of nonfunctional proinflammatory HDL against LDL oxidation and inability of HDL to promote cholesterol efflux by the ATP-binding cassette transporter A-1 pathway (34). All these events cause increasing proinflammatory HDL, which has proatherogenic characteristics as seen in our hypercholesterolemic patients (8). Decrease in Apo A1 levels may partly explain the nonfunctional HDL. On the other hand, statins may activate PPAR-γ receptors (33).

It was also reported that PPAR-γ activation affects ATP-binding cassette transporter A-1 expression, which is pivotal for HDL biogenesis (35).

In our study, atorvastatin treatment increased HDL's antioxidant/antiinflammatory capacity (P < 0.01), whereas it did not change HDL-C levels in hypercholesterolemics, indicating that this drug has an effect not only on PON1 activities but also on particle size, surface phospholipids, other antiinflammatory and antioxidant particles, and apoproteins, which are most important to hold and stabilize PON1 in HDL (8). It was reported that LCAT, ATP-binding cassette transporter, and platelet-activating factor acetylhydrolase have beneficial effects on HDL's protective effects against LDL oxidation beside its PON1 activity (15,36).

Increased homocysteine and ADMA and decreased NO levels are markers of endothelial dysfunctions as seen in our hypercholesterolemics. On the contrary, treatment with atorvastatin improved endothelial functions of these patients. Studies to date indicated that statins have no or little effect on ADMA and homocysteine levels, in contrast to our study (6,7). Our study group included different numbers of females (n = 20) and males (n = 10). The increased ADMA levels may thus be explained by beneficial effects of estrogen on the endothelium.

In conclusion, hypercholesterolemia is a cause of oxidative stress and endothelial dysfunction and prooxidant/proinflammatory HDL levels. Our results documented significant improvement in oxidative stress parameters and HDL's antiinflammatory capacity in hypercholesterolemic patients after atorvastatin treatment. Hence, this study shows that atorvastatin therapy is a beneficial strategy for improvement of HDL's antioxidant/antiinflammatory capacity and endothelial dysfunction against atherosclerosis.

Acknowledgment

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