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Effect of Atorvastatin on Cellular Adhesion Molecules on Leukocytes in Patients with Normocholesterolemic Coronary Artery Disease

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Abstract. *Background: Expression of cellular adhesion molecules on leukocytes plays a key role in coronary artery disease (CAD). The aim of the present study was to assess whether atorvastatin therapy has an impact on the expression of cellular adhesion molecules on leukocytes in patients with normocholesterolemic CAD. Patients and Methods: In 54 patients with CAD and atorvastatin treatment and 54 CAD patients without atorvastatin therapy, expression of CD40L, CD11a, CD11b, CD54, CD62L and CD41 on leukocytes was measured using flow cytometry. All patients were normocholesterolemic. Results: Atorvastatin treatment led to a significantly lower expression of CD40L, CD11b and CD54 on monocytes ($p<0.05$) and neutrophils ($p<0.05$). Expression of CD11a was significantly lower on monocytes ($p<0.05$) in atorvastatin-treated patients. Conclusion: The present results indicate that atorvastatin apparently improves chronic inflammation and may have a beneficial effect on hemostasis by reducing the expression of cellular adhesion molecules on leukocytes.*

Inflammation plays a key role in atherosclerosis and coronary artery disease (1, 2). Various adhesion molecules on leukocytes, endothelial cells and platelets are involved in leukocyte adhesion and subsequent migration of leukocytes into areas of inflammation (3-5). Expression of leukocyte adhesion molecules is induced by a number of endothelial and leukocyte activators, such as oxidized low density lipoprotein (6).

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Statins retard the progression of coronary atherosclerosis. Treatment with statins reduces morbidity and mortality caused by coronary artery disease (CAD) (7). Besides their lipid-lowering actions, these drugs improve endothelial function, enhance fibrinolysis and have antithrombotic actions (6). Their selective interaction with leukocyte function antigen 1 (LFA-1; CD11a) *via* binding to an allosteric site within LFA-1 has been demonstrated (8).

The effect of statins on adhesion molecules was investigated in hypercholesterolemic patients (6, 9-12). In these clinical trials, the control group often consisted of normolipemic healthy volunteers without CAD (9-11). Elevated markers of coagulation activation are found in plasma samples from patients with hypercholesterinemia indicating an ongoing prothrombotic state (10). Expression of adhesion molecules on leukocytes is up-regulated in these patients (11).

The aim of the present investigation was to assess if normocholesterolemic patients with proven CAD treated with atorvastatin show differences in the expression of cellular adhesion molecules on leukocytes compared with normocholesterolemic CAD patients without statin therapy.

Patients and Methods

Inclusion of patients was after informed consent and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the local Ethics Committee.

Data were a *post-hoc* analysis of a larger prospective, randomized, douple-blind study. A total of 108 patients undergoing elective coronary angiography were included. Patient characteristics are shown in Table I. Fifty-four patients had been taking atorvastatin for at least 8 weeks before admission to the hospital (mean dosage 30±11 mg/d; range 10-40 mg/d). The other 54 patients did not receive statin therapy before the angiography. Patients were matched according to their total cholesterol levels. All patients were normocholesterolemic (total cholesterol: 194±40 mg/dl in patients with atorvastatin treatment and 195±47 mg/dl in patients without statin therapy).

Table I. *Patient characteristics*.

	Atorvastatin n=54	Controls n=54
Age (years)	60.6±10.4	62.4±9.0
Gender (M/F)	35/19	39/15
Cholesterol (mg/dl)		
Total	194±40	195±47
LDL	121±22	124±25
HDL	47±18	43±12
CAD		
1-Vessel	20 (37%)	22 (40%)
2-Vessel	19 (35%)	16 (30%)
3-Vessel	15 (28%)	16 (30%)
Diabetes mellitus	13 (24%)	11 (20%)
Hypertension	42 (77%)	45 (83%)
Smoker	24 (44%)	21 (39%)
History of		
Myocardial infarction	4 (7%)	2 (4%)
Percutaneous coronary intervention	3 (6%)	2 (4%)
Coronary artery bypass graft surgery	0	0

LDL, Low density lipoprotein; HDL, high density lipoprotein; CAD, coronary artery disease.

Biochemical methods. Blood samples were drawn at admission to the hospital after an overnight fast by venipuncture using 3 ml sampling tubes containing EDTA (Sarstedt, Rommelsdorf-Nürnbrecht, Germany).

Flow cytometry. Expression of the following adhesion molecules on monocytes and neutrophils was measured by flow cytometry (EPICS XL-MCL; Coulter Electronics, Krefeld, Germany). Leukocytes were detected by using fluorescein-isothiocyanate (FITC)-conjugated anti-CD45 (leukocyte common antigen) (Becton & Dickinson Biosciences, Belgium). The following phycoerythrine (PE)-conjugated monoclonal antibodies were used for cell detection in fluorescence-activated cell sorting (FACS): Anti-CD40L (transmembrane protein of the tumor necrosis factor family), anti-CD62L (L-selectin) and anti-CD11a (integrin receptor LFA-1) (all from Coulter Immunotech, Krefeld, Germany); anti-CD11b (alpham subunit of the beta2-integrin Mac-1), anti-CD41, which detects the glycoprotein (GP) IIb-IIIa, anti-CD54 (ICAM-1) and mouse IgG1:FITC and IgG2a:FITC control antibody (Becton & Dickinson Biosciences). Whole blood (100 μl) was incubated with saturating concentrations of FITC-conjugated anti-CD45 and PE-conjugated monoclonal antibodies for 20 minutes at room temperature. Erythrocytes were lysed and leukocytes were fixed with a commercially available solution (FACSTM Lysing Solution; Becton & Dickinson Biosciences). Samples were then incubated for 10 minutes in the dark. Thereafter, samples were centrifugated at 200 ×g for 10 minutes, the pellet washed with phosphate-buffered saline (GIBCO, Karlsruhe, Germany), and recentrifuged. The pellet was then resuspended in phosphate-buffered saline and applied to the flow cytometer equipped with a 488-nm argon laser. Results are expressed as mean fluorescence intensity (MFI) of CD40L, CD11a, CD11b, CD54, CD62L and CD41 on monocytes and neutrophils.

Statistical methods. Statistical analysis was carried out by the statistical software package JMP™ (SAS Institute Inc., N.C., USA). Data are presented as mean values±standard deviation. Data were converted to a logarithmic scale for normal distribution before statistical analysis. MANOVA was used to analyze the significance of differences between patients receiving atorvastatin and patients without statin treatment. *P*-values of <0.05 were regarded as significant.

Results

Table I shows the patients' characteristics. No significant differences were detected between the two groups.

In atorvastatin-treated patients we found a significantly lower expression of CD40L, CD11b and CD54 on monocytes ($p<0.05$) and neutrophils ($p<0.05$) compared with patients without statin therapy. Expression of CD11a was significantly reduced on monocytes ($p<0.05$) in atorvastatin-treated patients, while no difference in CD11a expression on neutrophils was found (Figure 1). The expression of CD41 and CD62L on monocytes and neutrophils was not different between patients with atorvastatin therapy and those without (Table II).

Discussion

Normocholesterolemic patients with CAD treated with atorvastatin showed a significantly lower expression of CD40L, CD54, CD11a and CD11b on leukocytes compared to normocholesterolemic patients with CAD but without statin treatment.

CD54 (cICAM-1) is an immunoglobulin (Ig)-like cell adhesion molecule expressed by several cell types (*e.g.* leukocytes, endothelial cells). Major known functions of cICAM-1 relate to its role in cell adhesion and migration (13, 14). Higher CD54 expression on monocytes has been described in patients with CAD and relevant stenosis (15). Increased cICAM-1 expression may contribute to fibrinogen deposition and monocyte attachment, followed by subendothelial migration, which represents a crucial event in the development of atherosclerotic lesions (13, 16). Elevated expression of cellular ICAM-1 indicates a chronic inflammatory state in patients with chronic heart disease. Coagulation activation leads to conversion of ICAM-1-bound fibrinogen to fibrin, providing a potential role for ICAM-1 in hemostasis and thrombosis (13, 16, 17). Elevated expression of ICAM-1 and LFA-1 on monocytes has been shown in hypercholesterolemic patients (6). In hypercholesterolemic patients, simvastatin leads to a significant reduction of these adhesion molecules (6). In the present study, a significantly reduced expression of CD54 on monocytes and neutrophils was found in normocholesterolemic atorvastatin-treated patients with CAD, indicating an alteration of hemostasis and an anti-inflammatory effect of atorvastatin.

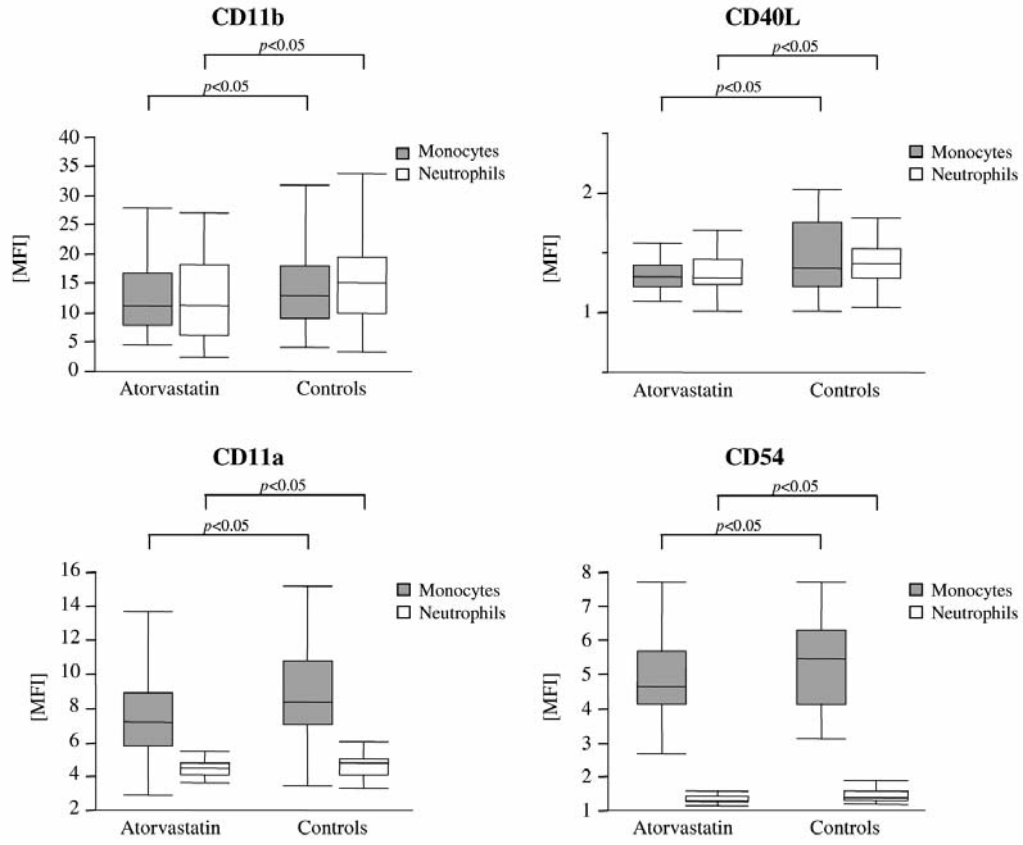

Figure 1. *CD54, CD11a, CD11b and CD40L levels on monocytes and neutrophils in patients with coronary artery disease treated with atorvastatin for at least 8 weeks (n=54) and in those without atorvastatin treatment (n=54). Results are expressed as mean fluorescence intensity (MFI) of CD54, CD11a, CD11b and CD40L on monocytes and neutrophils.*

The membrane-bound intregrin receptors LFA-1 (CD11a) and MAC-1 (CD11b) are ligands of cICAM-1 (CD54). LFA-1 (CD11a) mediates spreading and firm adhesion of leukocytes, followed by transendothelial migration (6). Up-regulation of CD11b has been found to enhance the procoagulant activity of leukocytes. CD11b binds to fibrinogen, induces tissue factor gene transcription in monocytes and activates factor X, initiating the coagulation cascade and thrombin generation (16-18). Recently, statins have been reported to selectively interact with LFA-1 *via* binding to an allosteric site within LFA-1 (8). In patients after coronary artery bypass surgery, CD11b expression on neutrophils was significantly lower in patients who were treated with atorvastatin before surgery (19). The data of the present study, which are in accordance with other investigations in hypercholesterolemic patients (6, 11), demonstrate anti-inflammatory properties of atorvastatin and a beneficial effect on hemostasis by reducing the expression of CD54, CD11a and 11b on leukocytes.

CD40 ligand (CD40L) is a trimeric, transmembrane protein of the tumor necrosis factor family and, together with its receptor CD40, an important contributor to the inflammatory processes that lead to atherosclerosis and thrombosis (20). CD40L plays a proximal role in a cascade of proatherothrombotic functions thought to be important in the pathogenesis of acute coronary syndromes (21). Both

Table II. *CD41 and CD62L on monocytes and neutrophils in patients with coronary artery disease treated with atorvastatin for at least 8 weeks (n=54) and in those without atorvastatin treatment (n=54). Results are expressed as mean fluorescence intensity (MFI) of CD41 and CD62L on monocytes and neutrophils. Data are presented as means±standard deviation.*

MFI	Atorvastatin	Controls
CD41		
Monocytes	12.5±7.6	13.6±7.3
Neutrophils	11.0±5.0	11.1±4.0
CD62L		
Monocytes	14.0±6.5	13.5±3.9
Neutrophils	33.1±10.8	31.1±10.6

CD40 and CD40L have been shown to be present in human atheroma (22). Blockade of CD40L-CD40 signalling pathway mitigates the development of atherosclerosis in mice (23). CD40/CD40L are involved in the stabilization of atherosclerotic plaques (24). In hypercholesterolemic patients increased levels of soluble CD40L have been found (12) and CD40L expression on platelets and monocytes is up-regulated in hypercholesterinemia (10, 25). Atorvastatin treatment leads to a significant reduction of CD40L expression on platelets in hypercholesterolemic patients without CAD (9,10). Oxidized LDL increases activation of the CD40/CD40L dyad and statins inhibit this effect *in vitro* (21). Controversial data are available on to whether down-regulation of CD40L depends on the lipid-lowering effect of statins, or not (9, 10, 12, 26, 27). The reduced expression of CD40L on monocytes and neutrophils in the normocholesterolemic atorvastatin-treated patients with CAD in the present study demonstrates the anti-inflammatory and antithrombotic effect of atorvastatin by altering the CD40/CD40L signalling pathway. By this mechanism, atorvastatin might improve atherosclerotic plaque stability. Our data give evidence that this effect is, at least in part, independent of the lipid-lowering effect.

The present results indicate that atorvastatin apparently improves chronic inflammation and may have a beneficial effect on hemostasis by reducing the expression of cellular adhesion molecules on leukocytes. This effect seems to be independent of the lipid-lowering action of atorvastatin.

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