Effect of Extracorporeal Low-Density Lipoprotein Elimination on Circulating Cell Adhesion Molecules in Patients With Hypercholesterolemia

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Levels of circulating cell adhesion molecules (cCAM) may be useful markers for stratifying cardiovascular disease severity or prognosis.[1–4] Furthermore, cCAM levels are elevated in subjects with insulin resistance, type 2 diabetic patients, hypertensive patients, and in smokers.[5–11] The reported effects of hypercholesterolemia on cCAM levels, however, has been controversial.[12–15] Sampietro and colleagues[12] reported that familial hypercholesterolemia was associated with elevated levels of cICAM-1 and cE-selectin. They found a decrease in the levels of these soluble CAMs immediately after extracorporeal low-density lipoprotein (LDL) elimination, which is capable of preventing the progression and initiating the regression of atherosclerosis in hypercholesterolemic patients by inducing functional improvement, which may precede anatomic changes.[16,17] According to their hypothesis, plasma cholesterol regulates soluble CAM expression in familial hypercholesterolemia and soluble CAMs may serve as markers of efficacy in lipid-lowering trials. Futhermore, they concluded that the beneficial effects of LDL elimination on cardiovascular risk may be partially attributable to the downregulation of endothelial CAMs.[12] Such an effect, if at least partially mediated by downregulation of endothelial CAM expression, would, however, presume a long-term effect. In contrast, a number of studies have shown that hypercholesterolemia is not correlated with increased cCAM levels.[13,15] Therefore, the aim of the present study was to assess both the short- and long-term effects of LDL apheresis on the levels of circulating intercellular cell adhesion molecule (cICAM-1), circulating vascular cell adhesion molecule (cVCAM-1), and cE-selectin in patients with familial hypercholesterolemia.

Eight hypercholesterolemic patients (4 men, 4 women; average age 52 ± 15 years) were recruited at 3 LDL apheresis centers (Department of Internal Medicine IV of the University of Leipzig, Department of Metabolic Research of the Medical Faculty Carl Gustav Carus Dresden, and the Vogtland Clinic Plauen GmbH, Germany). The underlying hyperlipoproteinemia was heterozygous familial hypercholesterolemia. Patients had proved to be refractory to cholesterol-lowering drug therapy. A cholesterol-lowering diet equivalent to the American Heart Association step I diet and cholesterol-lowering drug therapy with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors atorvastatin and lovastatin was continued during the period of LDL apheresis treatment. All patients included in the study had various degrees of cardiovascular disease. The presence and severity of coronary artery disease was documented by coronary angiography. Patients with acute myocardial infarction, secondary hyperlipidemia, impaired hepatic or renal function, hypertension, diabetes mellitus, obesity, clinically manifest infections, connective tissue disease, malignancy, or who were on anti-inflammatory or cytotoxic drugs were excluded from the study. The Ethical Committee of the University of Leipzig approved the project plan and the patients gave informed consent. The method used for extracorporeal LDL elimination was DALI (Direct Adsorption of Lipids, Fresenius St. Wendel, Germany). In this LDL apheresis system, the elimination of LDL and lipoprotein(a) (Lp(a)) is performed not in plasma but in human whole blood by adsorption onto polyacrylate-coated polyacrylamide beads.[18,19]

LDL apheresis treatment was performed at weekly intervals. To test the long-term effects of extracorporeal LDL elimination, laboratory measurements were performed at baseline before the LDL apheresis procedure, 1 month after therapy, and 2, 3, and 6 months after LDL apheresis treatment. Furthermore, repeated measurement were performed immediately before and after each apheresis procedure in 10 patients.

Blood samples, except those taken immediately after apheresis, were obtained after an overnight fast for measurement of serum lipids, apolipoproteins and lipoproteins, cCAMs, and other clinical chemistry parameters. Serum levels of ICAM-1, VCAM-1, and E-selectin were determined by monoclonal antibody-based enzyme-linked immunosorbent assays (R & D Systems, Europe Ltd., Abingdon, United Kingdom). All samples were tested twice. Cholesterol and triglyceride concentrations were determined using test kits from Roche Diagnostics Mannheim GmbH (Mannheim, Germany) (cholesteroloxidase–phenol, aminophenazone, peroxidase [CHOD-PAP] method). To determine serum high-density lipoprotein (HDL) cholesterol, we used the polyethylene glycol 20 000 precipitation method (Quantolip, Immuno GmbH, Heidelberg, Germany). LDL cholesterol was measured with the help of precipitation reagent polyvinyl

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TABLE 1 Pre- and Postapheresis Values of Serum Total Cholesterol, LDL Cholesterol, Circulating Intracellular Cell Adhesion Molecule-1 (cICAM), Circulating Vascular Cell Adhesion Molecule-1 (cVCAM), Circulating E-selectin, Immunoglobulin G (IgG), Immunoglobulin M (IgM), and Immunoglobulin A (IgA)

	Preapheresis		Postapheresis		
Parameters	Mean ± SD	Median	Mean ± SD	Median	Change
Chol (mmol/L)	7.24 ± 0.91	7.58	3.28 ± 0.63	3.30*	54% (48–62)
LDL cholesterol (mmol/L)	4.92 ± 0.92	4.89	1.12 ± 0.34	1.20*	77% (70–83)
cICAM-1 (ng/mg)	261 ± 78.7	248	233 ± 67.4	230*	10% (7–13)
cVCAM-1 (ng/ml)	439 ± 134	401	343 ± 112	331*	22% (16–28)
cE-selectin (ng/ml)	45.8 ± 15.8	43.5	45.3 ± 19.9	39.0†	3% (−5–10)
IgG (g/L)	9.09 ± 2.34	9.26	7.35 ± 2.21	7.01*	20% (14–25)
IgM (g/L)	1.22 ± 1.23	0.83	0.93 ± 1.05	0.59*	23% (12–33)
IgA (g/L)	2.05 ± 1.49	1.56	1.48 ± 0.78	1.32*	24% (16–32)

Data are given as mean values and standard deviations (SD) as well as medians based on the treatment of 10 patients. Percent changes are given, including 95% confidence intervals.

*$p < 0.05$ versus preapheresis, †$p > 0.05$ versus preapheresis using Wilcoxon's matched pair rank test.

sulfate (Roche Diagnostics Mannheim GmbH). Apolipoproteins A-I and -B, C-reactive protein, transferrin, α-haptoglobin, and immunoglobulins IgG, IgA, and IgM were analyzed immunonephelometrically using antisera obtained from Dade Behring AG (Marburg, Germany). Lp(a) was measured by electroimmunoassay using kits supplied by Immuno GmbH (Heidelberg, Germany).

Data were analyzed with SPSS (Statistical Package for the Social Science, SPSS, Inc., Chicago, Illinois) for Windows and S-PLUS (The Consulting Group Insightful Corp., Seattle, Washington). Comparisons of parameters before and after LDL apheresis treatment were performed using Wilcoxon's matched pair-rank test. A p value <0.05 by a 1-tailed test was considered statistically significant. For analysis of long-term effects of LDL apheresis, we used a fitted mixed effect regression model for repeated measurements with random intercept and missing covariates.

At initation of apheresis treatment, patients had elevated mean concentrations of serum total cholesterol, LDL cholesterol, and apolipoprotein-B (apo-B) (7.8 mmol/L, 5.5 mmol/L, and 1.6 g/L, respectively). Average Lp(a) concentration was 26.9 mg/dl (range 6.7 to 75.3). Mean HDL cholesterol and apo A-I levels were 0.9 mmol/L and 1.1 g/L, respectively, for men and 1.3 mmol/L and 1.5 g/L, respectively, for women. Patients' mean cCAM levels were within the reference range. One hundred fifty-eight samples from control subjects were evaluated. The mean ± 2 SD reference ranges for cVCAM-1, cICAM-1, and cE-selectin were 266 to 814, 93 to 381, 266 to 814, and 7 to 75 ng/ml, respectively. Because inflammatory diseases may increase cCAM levels, the serum concentration of C-reactive protein, α-haptoglobulin, and transferrin were measured. The concentrations of these acute-phase proteins were within the reference range (C-reactive protein <5.0 mg/L, α-haptoglobulin <2.8 g/L, transferrin >3.0 g/L).

Table 1 shows serum concentrations of total and LDL cholesterol, cCAMs, and immunoglobulins before and immediately after a single apheresis treatment. Reductions of cICAM-1 and cVCAM-1 were 10%, and 22%, respectively. Similar reductions in the levels of immunoglobulins were observed. In contrast, cE-selectin levels were unaffected.

To evaluate the long-term effect of LDL apheresis on the levels of cICAM-1, cVCAM-1, and cE-selectin, preapheresis levels of cCAMs at baseline, after 1 month, and after 2, 3, and 6 months of apheresis treatment were measured. For each parameter separate mixed-effect regression models for repeated measurements with missing covariates were fitted. A significant slope was not observed for cVCAM-1, cICAM-1, or cE-selectin. The following regression coefficients b and p values were found: cVCAM-1 ($b = 3.1$, $p = 0.34$), cICAM-1 ($b = 7.3$, $p = 0.41$), and cE-selectin ($b = 1.1$, $p = 0.06$).

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In contrast to E-selectin, the CAMs ICAM-1 and VCAM-1 belong to the immunoglobulin superfamily. In the present study, a reduction in levels of IgG, IgA, and IgM in the same order of magnitude as the reduction of cICAM-1 and cVCAM-1 was observed. Therefore, the decrease of cCAM levels may be attributed to absorption onto the extracorporeal circulation components of the apheresis system. According to the present study, a shift of the LDL cholesterol levels from ≈5.0 to ≈1.1 mmol/L was, therefore, not associated with a change in cCAM levels. Different apheresis systems could effect cCAM levels in different ways. Therefore, in our own investigations, the short-term effect of dextran sulfate absorption (Kaneka Co., Europe N.V., German Branch, Weisbaden, Germany) on cCAMs was tested in 4 patients. However, no difference was found in the 2 apheresis systems used.

The present study focused on the question of whether there is a long-term effect of LDL apheresis on cCAMs levels. Apheresis was standardized such that a postapheresis LDL cholesterol level of 1.0 to 1.4 mmol/L was achieved. Rebound curves of cholesterol and LDL cholesterol showed an increase up to ≈80% of initial values within 1 week. After this interval, LDL apheresis treatments were repeated. During the 6-month study period, no changes in cICAM-1, cVCAM-1, and cE-selectin

levels were detected. The data of the present study suggest that extracorporeal LDL removal does not effect cCAM levels. This is also supported by our cross-sectional data[10] and findings that 3 months of therapy with standard doses of 3 different statins (atorvastatin, simvastatin, and pravastatin) does not lower cCAM levels, despite substantial reductions in cholesterol levels. Therefore, it is concluded that changes in LDL cholesterol are not associated with changes in cCAMs.

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Frequency of Subacute Resumption of Isthmus Conduction After Ablation of Atrial Flutter

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Achievement of complete bidirectional conduction block in the right atrial isthmus, bordered by the tricuspid valve annulus and the inferior vena cava, is an accepted predictor of long-term efficacy after atrial flutter ablation.[1–6] However, in follow-up studies performed in patients at 1 to 12 months after successful ablation,[1,2,5,7] isthmus conduction resumes in as many as 50% and is associated with a 10% incidence of recurrent atrial flutter during the first year after ablation.[1,3,5] The time course for resumption of isthmus conduction is unknown. Therefore, we sought to determine the incidence of resumption of isthmus conduction within the first 24 hours after successful atrial flutter ablation and to determine whether early repeat ablation would preclude long-term recurrence of atrial flutter.

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We evaluated 75 consecutive patients (61 men [81%], mean age 63 ± 13 years [range 19 to 86]) referred for ablation of isthmus-dependent atrial flutter. Left ventricular function was reduced (≤50%) in 28 patients (37%). The most common associated cardiovascular diseases were coronary artery disease (n = 14), valvular heart disease (n = 10), and hypertension (n = 9). However, no predisposing condition for development of atrial flutter could be identified in 27 patients (36%).

Atrial flutter was the only documented arrhythmia in 50 patients (67%). A history of both atrial flutter and atrial fibrillation was present in 14 (18%). How-

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