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Effects of heparin-mediated extracorporeal low-density lipoprotein precipitation beyond lowering proatherogenic lipoproteins—reduction of circulating proinflammatory and procoagulatory markers

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Abstract

In addition to hypercholesterolemia, proinflammatory and prothrombotic markers have been suggested to play an important role in atherogenesis. We examined whether heparin-mediated extracorporeal low-density lipoprotein precipitation (HELP) therapy modulates the circulating levels of proinflammatory and prothrombotic markers. Twenty-two coronary heart disease (CHD) patients undergoing regular HELP-apheresis (18 males, 4 females, mean age 57.3 ± 10.9 years) were enrolled in this study. A single HELP therapy treatment significantly decreased the circulating levels of high sensitivity C-reactive protein (hs-CRP), soluble vascular adhesion molecule-1 (sVCAM-1), soluble E-selectin, lipopolysaccharide binding protein (LBP), endothelin-1 (ET-1), and monocyte chemoattractant protein-1 (MCP-1) on average by 67, 37, 24, 27, 24, and 15%, respectively. Prothrombotic factors including fibrinogen, tissue factor (TF), soluble CD40 ligand (sCD40L), and homocysteine were decreased by 66, 27, 16, and 22%, respectively. In accordance with previous studies HELP therapy reduced total cholesterol, low density lipoprotein (LDL) cholesterol, and Lp(a) mass by 50, 61, and 62%, respectively. Our data suggest that simultaneous reduction of proinflammatory and prothrombotic factors together with atherogenic lipoproteins by HELP-apheresis may contribute to improvement of endothelial dysfunction and thereby inhibit progression of atherosclerotic lesions and stabilize the existing plaque.

Keywords: HELP; Atherosclerosis; Adhesion molecules; Monocyte chemoattractant protein-1; Tissue factor; Soluble CD40 ligand; Lipopolysaccharide binding protein; Endothelin-1

1. Introduction

Various factors are involved in inflammatory and coagulatory processes during atherosclerosis. It is now widely accepted that endothelium plays a central role in regulation of vascular tone, modification of lipoproteins, inflammation, thrombogenesis and transformation of circulating monocytes into foam cells [1]. Injured endothelium expresses adhesion molecules (AMs), which mediate adhesion of leukocytes to endothelial cells and initiates the onset of atherogenesis [2]. Release of chemokines such as monocyte chemoattractant protein-1 (MCP-1) facilitates monocytes to penetrate into intima. Activated T-lymphocytes and macrophages interact with vascular cells in injured intima and secrete cytokines and growth factors which promote proliferation of smooth muscle cells [3]. Recently CD40 ligand (CD40L) has been suggested to play a crucial role in coronary heart disease (CHD). Both membrane bound and soluble forms of this ligand interact with CD40 resulting in an inflammatory response [4,5]. Elevated soluble CD40L (sCD40L) levels have been reported in patients with unstable angina, hypercholesterolemia and restenosis after angioplasty [6–8]. CD40L stimulates macrophages which express procoagulatory tissue factor (TF). During plaque ruptures TF triggers

Abbreviations: sVCAM-1, soluble vascular cellular adhesion molecule-1; sE-selectin, soluble endothelial selectin; MCP-1, monocyte chemoattractant protein-1; Hs-CRP, high sensitive C-reactive protein; ET-1, endothelin-1; LBP, lipopolysaccharide binding protein; TF, tissue factor

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thrombus formation which results in acute complication of atherosclerosis [9]. Endothelin-1, the most potent vasoconstrictor, may further stimulate the release of cytokines and chemokines [10]. Several studies have demonstrated an association between a variety of chronic bacterial infections and atherosclerotic cardiovascular disease [11,12]. Lipopolysaccharide binding protein (LBP) is secreted by liver during inflammation and endotoxemia. LPS-LBP complex after recognition by CD14+ cells activates intracellular signaling of genes for proinflammatory cytokines and chemokines [13]. This may further promote the atherosclerosis process.

HELP-apheresis efficiently removes LDL, Lp(a) and fibrinogen in patients with familial hypercholesterolemia (FH), CHD, cardiac bypass surgery patients, heart transplantation (HTX), cerebral infarction, myocardial ischemia and patients with sudden hearing loss [14–17]. Recent studies also report reduction of circulating levels in AMs [18,19] and high sensitivity CRP (hs-CRP) [20,21] by HELP-apheresis. Since atherogenic lipids, proinflammatory and procoagulatory markers are actively involved in atherosclerotic process, their simultaneous removal may thus be beneficial. The aim of the present study was to examine whether HELP therapy would reduce the circulating levels of E-selectin, MCP-1, LBP, ET-1, TF, CD40L, and homocysteine.

2. Methods

2.1. Patients

Twenty-two patients suffering from familial heterozygous hypercholesterolemia, advanced CHD, after heart transplantation and peripheral occlusive vascular disease were included in this study (Table 1). They were treated with regular HELP-apheresis at weekly intervals for a period of over 6 months. Exclusion criteria were impaired renal function, diabetes mellitus, cardiovascular events within the last 3 months and clinical signs of infectious disease. This study was approved by the ethics committee of University of Munich and informed consent was obtained from each patient.

Table 1
Characteristics of patients

N (number)	22
Gender (male/female)	18/4
Age (years)	57.3 ± 10.9
Heterozygous FH	2
CHD	14
Peripheral vascular disease	2
HTX	4
Statins	17
Statins & Fibrates	1
Cyclosporin A	4
Beta-blocker	15
ACE inhibitors	21
Calcium antagonists	1
Aspirin	20

Venous blood samples for obtaining serum and plasma were centrifuged at 1000 × *g* and 4 °C for 15 min. Lipids and fibrinogen were measured immediately after sampling. Plasma and serum samples for measurement of other parameters were aliquoted and stored at −80 °C until use.

2.2. HELP-apheresis

The procedure for HELP therapy has been described previously [14]. In brief, venous blood was drawn from cubital vein catheter and blood cells were separated from plasma which was mixed with acetate-heparin buffer (pH 4.85) (1:1 v/v ratio) to precipitate fibrinogen, LDL and Lp(a). Excess of heparin was removed by anion-exchange filter. Physiological pH was restored by biocarbonate dialysis and extra fluid was removed by ultrafiltration. Plasma was then mixed with cell-rich blood fraction and returned back to the patients. The average plasma volume treated was 3300 ml per session.

2.3. Laboratory parameters

Concentrations in serum of total, VLDL, LDL, and HDL-cholesterol, Lp(a) mass, apo AI, apo B, triglycerides, and plasma fibrinogen were measured by standard routine methods on Automatic Analyzer (Hitachi 911 and STA-R, Roche, Germany). Quantitative measurement of sVCAM-1, sICAM-1, sE-selectin, endothelin-1, MCP-1, and sCD40L in serum was performed by solid phase sandwich enzyme-linked immunosorbent assay (ELISA) from R&D systems (Minneapolis, USA) according to the manufacturer's protocol and limits of detection were less than 2.0, 0.35, 0.1, 1.0, 5.0 ng/ml, and 10 pg/ml, respectively. Tissue factor was measured in sodium citrate plasma by IMUBIND ELISA kit (Loxo GmbH, Dossenheim, Germany) with a detection limit of 10 pg/ml. LBP in serum was measured by chemiluminescence sandwich immunoassay (IMMULITE®, DPC, USA) with a detection limit of 0.2 μg/ml. Homocysteine levels in plasma were measured by fluorescence polarization immunoassay (FPIA) [IMx®, Abbott, USA] with a detection limit of <0.5 μmol/l. Hs-CRP was measured by immunoturbidimetry (COBAS INTEGRA, Roche, Germany) with a detection limit of 8.5 μg/dl.

2.4. Statistical analysis

The results are given as mean values and standard deviations. Differences between pre- and post-apheresis values are given as percentage. Data were analyzed using SPSS software (Version 11.5, SPSS Inc., Chicago, IL). Statistical significance of the data was evaluated by paired sample *t* test. Correlation coefficients were obtained by Pearson analysis. For all analyses, $P < 0.05$ was considered signif-

Table 2
Reduction in circulating lipids and lipoproteins by a single HELP-apheresis

Lipid profile (mg/dl)	Pre-HELP	Post-HELP	Reduction (%)
Total cholesterol	216.1 ± 38.1	107.0 ± 20.4	50.3
Triglycerides	232.7 ± 178.8	111.0 ± 100.4	53.3
LDL-cholesterol	128.6 ± 29.9	49.8 ± 17.1	61.4
HDL-cholesterol	47.6 ± 14.4	37.8 ± 8.6	17.0
VLDL-cholesterol	36.8 ± 33.1	17.3 ± 11.3	38.5
Apo AI	150.8 ± 27.3	119.2 ± 26.0	21.3
Apo B	109.2 ± 23.6	42.1 ± 12.3	61.8
Lipoprotein(a)	91.3 ± 48.2	32.5 ± 18.0	61.7

Reductions were statistically significant ($P < 0.001$).

icant. The possible hemodilution effects were corrected by the hematocrit (Hct) values before and after each therapy.

3. Results

A single HELP therapy treatment reduced circulating levels of total cholesterol, LDL-C, VLDL-C, Lp(a), triglycerides, apo B, and apo AI by 50, 61, 39, 62, 53, 62, and 21%, respectively ($P < 0.001$). A small but significant reduction in HDL-C was also noted (Table 2). These observations are in accordance with previous studies [14,15].

Next we examined the effects of HELP therapy on proinflammatory markers. A reduction in circulating levels of sVCAM-1, sE-selectin, and hs-CRP by 37, 24, and 67%, respectively, was noted ($P < 0.001$). No significant reduction in sICAM-1 levels was observed. HELP therapy reduced the circulating levels of MCP-1, ET-1, and LBP by 15, 24, and 27%, respectively ($P < 0.01$, Table 3). Thssese reductions are independent of pre-apheresis levels.

HELP therapy drastically reduces circulating levels of fibrinogen and improves blood viscosity [22]. It has also a potential for lowering levels of coagulatory factors [23]. We therefore examined if HELP therapy would modulate concentrations of TF, CD40L and homocysteine which play a vital role in thrombotic events. Circulating levels of fibrinogen, TF, CD40L and homocysteine were reduced by 66, 27, 16, and 22%, respectively (Table 4). The effects of HELP therapy on sCD40L differ from those observed on other markers measured in this study. In some patients no reduction but an increase in sCD40L was noted ($n = 7$). We therefore divided patients into three groups according to their pre-apheresis sCD40L concentrations to examine whether initial levels correlate with the reduction by HELP therapy (group 1: >7.0 ng/ml, group 2: 4.0–7.0 ng/ml, and group 3: 1.0–4.0 ng/ml). HELP therapy reduced sCD40L in groups 1 and 2 by 35 and 30%, respectively ($P < 0.05$). However, an increase which was not significant was noted in group 3 (ca. 21%) (Fig. 1).

No correlation between pre-HELP LDL levels and proinflammatory markers was observed (data not shown). However pre-apheresis levels of fibrinogen showed a significant correlation with TF, sVCAM-1, and LBP (Fig. 2a-c) ($r = 0.477$, 0.423, 0.427, respectively, $P < 0.05$).

Table 3
Modulation of circulating proinflammatory markers by a single HELP-apheresis

Proinflammatory parameters	Pre-HELP	Post-HELP	Reduction (%)	*P*-value
sVCAM-1 (ng/ml)	674.8 ± 185.8	426.5 ± 151.5	37.0	<0.001
sICAM-1 (ng/ml)	148.1 ± 37.5	153.9 ± 40.9	−6.2	NS
sE-selectin (ng/ml)	31.1 ± 11.0	24.0 ± 9.8	23.6	<0.001
MCP-1 (pg/ml)	409.9 ± 124.2	346.9 ± 110.0	15.0	<0.001
ET-1 (pg/ml)	1.8 ± 0.5	1.3 ± 0.3	24.4	<0.001
LBP (μg/ml)	7.1 ± 3.8	4.7 ± 2.1	26.7	<0.01
Hs-CRP (mg/dl)	0.5 ± 1.24	0.17 ± 0.4	66.9	<0.001

NS: not significant.

Table 4
Reduction in procoagulatory factors by a single HELP-apheresis

Procoagulatory parameters	Pre-HELP	Post-HELP	Reduction (%)	*P*-value
Tissue factor (pg/ml)	280.0 ± 118.4	211.4 ± 113.8	26.5	<0.001
Fibrinogen (mg/dl)	339.3 ± 83.0	115.8 ± 43.7	66.1	<0.001
sCD40L (ng/ml)	5.25 ± 2.60	3.79 ± 2.39	16.0	<0.01
Homocysteine (μmol/l)	12.3 ± 4.4	9.6 ± 3.3	21.6	<0.001

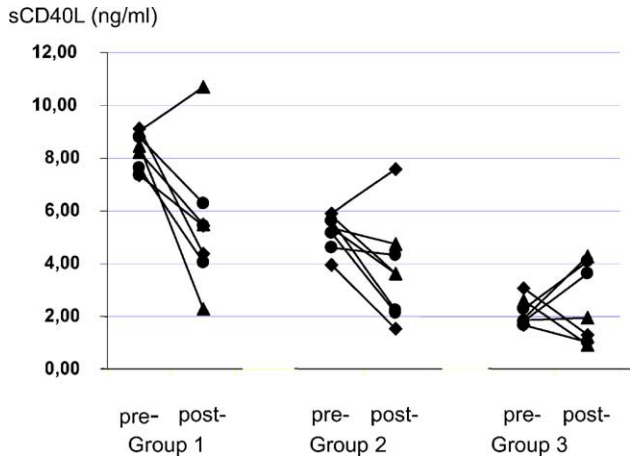

Fig. 1. Relationship between initial levels (pre-HELP) and reduction in sCD40L (post-HELP) by a single HELP therapy. Initial levels of sCD40L (immediately before HELP therapy) were divided into three groups (group 1: >7.0 ng/ml, group 2: 4.0–7.0 ng/ml, and group 3: 1.0–4.0 ng/ml).

4. Discussion

Important steps in initiation and progression of atherosclerosis plaque formation include accumulation of inflammatory cells within the intima, secretion of growth factors and transformation of lipid-loaded foam cells [3]. Adhesion molecules E-selectin, VCAM-1 and ICAM-1 play a major role in leukocyte adhesion to endothelium, which is detectable early in human and experimental plaque formation [2]. Soluble forms of AMs, CD40L and TF arise from proteolytic cleavage from cell membranes [6,24,25]. Expression of VCAM-1 was shown to be higher in human aorta with atheromatous changes and to correlate with soluble VCAM-1. It has also been reported that circulating levels of VCAM-1, but not ICAM-1, correlate with angiographically determined atherosclerotic area [26]. Reduction of both sVCAM-1 and sE-selectin by HELP-apheresis observed in this study may be beneficial for endothelium. In contrast to earlier reports [18,19] no reduction of sICAM-1 levels was noted. The removal of AMs by apheresis has been suggested to be dependent on molecular weight, being highest for P-selectin and lowest for ICAM-1 [19,27].

Atherosclerotic lesions accumulate inflammatory cells expressing CD40L, which is a transmembrane protein belonging to the TNF superfamily. Its receptor CD40, which belongs to the TNF receptor family, is expressed on B cells, monocytes, macrophages, dentritic cells and endothelial cells [4,5]. Ligation of CD40L with CD40 on monocytes/macrophages induces the synthesis of AMs, proinflammatory cytokines, chemokines (MCP-1) and TF. Tissue factor initiates the extrinsic coagulation cascade and is implicated in atherosclerotic plaque rupture [28]. In the present study HELP therapy reduced the levels of TF, MCP-1, and sCD40L, which may reduce the risk of thrombotic events in atherosclerosis.

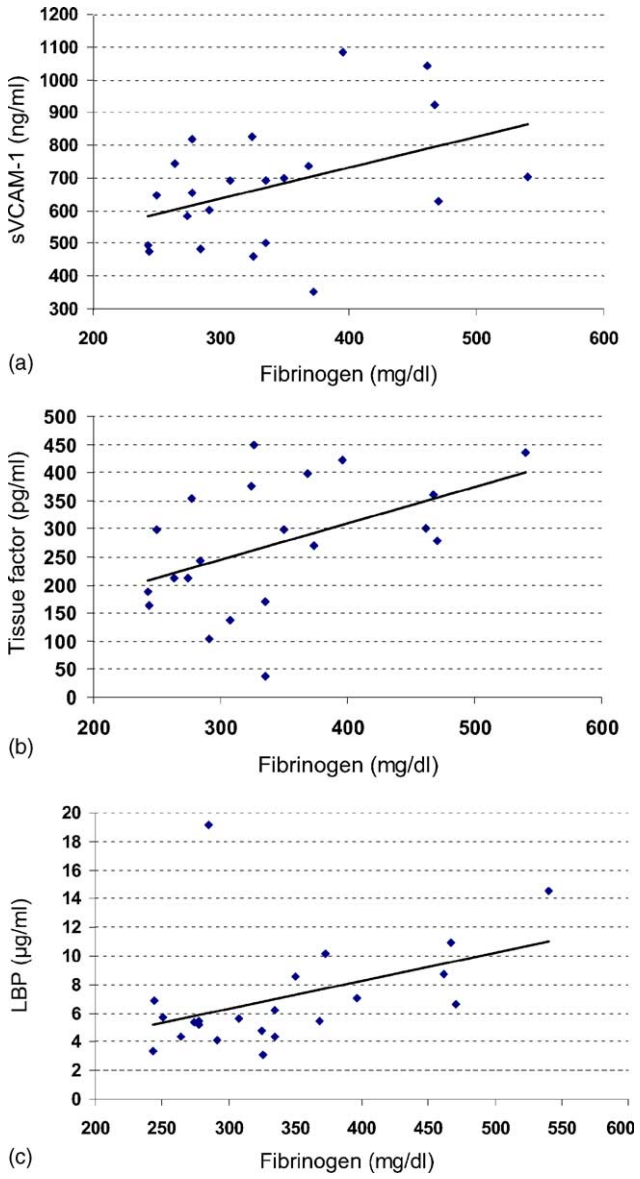

Fig. 2. Correlation between pre-HELP levels of plasma fibrinogen and serum sVCAM-1 (a), plasma fibrinogen and plasma TF (b), plasma fibrinogen and serum LBP (c). Pearson's corelation coefficients: $r = 0.423$, 0.477, 0.427, $P < 0.05$.

Endothelin-1 stimulates synthesis and release of cytokines and growth factors in experimental animal models and in vivo studies [10]. Thus, elimination of ET-1 reported in the present study by HELP-apheresis should not only decrease the direct vasoconstrictive injury to lesioned endothelium, but also attenuate the proinflammatory response in the atherosclerotic process.

Acute phase proteins, such as fibrinogen, CRP and LBP, are secreted by liver in response to inflammatory processes. Both fibrinogen and CRP are independent risk factors in CHD [29,30]. Fibrinogen, a central factor in thrombotic events, alters serum viscosity, platelet aggregation and thus hemorheology [22]. Recently, fibrinogen has been reported to stimulate chemokine (MCP-1) secretion in macrophages,

synovial fibroblasts and endothelial cells. It also alters morphology and proliferation of vascular endothelial cells [31]. Thus simultaneous removal of fibrinogen, TF, CRP and CD40L should prove beneficial in CHD patients.

LBP complexes with endotoxin (LPS) and this complex activates CD14+ cells and induces proinflammatory responses. Reduction of LBP by HELP therapy should diminish the LPS-mediated inflammatory response and deserves attention. Homocysteine is also an independent risk factor for CHD [32]. It has been reported that hyperhomocysteinemia stimulates the expression of MCP-1, VCAM-1, and E-selectin in vivo [33]. Thus reduction of homocysteine levels by HELP-apheresis shown in this study may reduce endothelial activation.

Various therapeutic approaches such as administration of antibodies and drugs have been tried to lower concentrations of CD40, CD40L, homocysteine, and TF [34–36]. Such treatments may partially improve the chronic and acute inflammatory situation. However, atheroslcerosis is a complex process involving lipoproteins, proinflammatory and prothrombotic events. Our study stresses the role of fibrinogen as a major factor. Indeed circulating levels of fibrinogen correlate with pre-apheresis levels of TF, VCAM-1 and LBP. Thus, simultaneous reduction in proatherogenic lipoproteins, fibrinogen, TF, CD40L, VCAM-1 and LBP should prove beneficial in arresting the progression of atherosclerosis and probably induce regression of existing atherosclerotic plaque. However, further studies are needed to establish long term beneficial effects of maintaining consistently lower levels of proatherogenic, prothrombotic and proinflammatory factors in CHD patients.

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References

[1] Steinberg D. Atherogenesis in perspectivre: hypercholesterolemia and inflammation as partners in crime. Nat Med 2002;8:1211–7.
[2] Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. Am J Hypertens 2001;14:44S–54S.
[3] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340(2):115–26.
[4] Buchner K, Henn V, Grafe M, der Boer OJ, Becker AE, Krozek RA. CD40 ligand is selectively expressed on CD4+ T cells and platelets: implications for CD40-CD40L signalling in atherosclerosis. J Pathol 2003;201(2):288–95.
[5] Mach F, Schönbeck U, Sukhova GK, et al. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. Proc Natl Acad Sci USA 1997;94:1931–6.
[6] Aukrust P, Muller F, Ueland T, et al. Enhanced levels of soluble and membrane bound CD40 ligand in patients with unstable angina: possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. Circulation 1999;100:614–20.
[7] Semb AG, van Wissen S, Ueland T, et al. Raised serum levels of soluble CD40 ligand in patients with familial hypercholesterolemia: downregulatory effect of statin therapy. J Am Coll Cardiol 2003;41:275–9.
[8] Cipollone F, Ferri C, Desideri G, et al. Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty. Circulation 2003;108(22):2776–82.
[9] Tremoli E, Camera M, Toschi V, Colli S. Tissue factor in atherosclerosis. Atherosclerosis 1999;144(2):273–83.
[10] Kowala MC. The role of endothelin in the pathogenisis of atherosclerosis. Adv Pharmacol 1997;37(2–3):299–318.
[11] Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997;350(9075):430–6.
[12] Davidson M, Kuo CC, Middaugh JP, et al. Confirmed previous infection with Chlamydia pneumoniae (TWAR) and its presence in early coronary atherosclerosis. Circulation 1998;98(7):628–33.
[13] Schumann RR, Latz E. Lipopolysaccharide-binding protein. Chem Immunol 2000;74:42–60.
[14] Eisenhauer T, Armstrong VW, Wieland H, Fuchs C, Scheler F, Seidel D. Selective removal of low density lipoproteins (LDL) by precipitation at low pH. First clinical application of the H.E.L.P.-system. Klin Wochenschr 1987;65:161–8.
[15] Seidel D, Armstrong VW, Schuff-Werner P. for the HELP Study Group. The HELP-LDL-apheresis multicenter study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. Part I. Evaluation of safety and cholesterol-lowering effects during the first 12 months. Eur J Clin Invest 1991;21:375–83.
[16] Lees R, Holmes N, Stadler R, Ibrahim SF, Lees AM. Treatment of hypercholesterolemia with Heparin-Induced Extracorporeal Low-Density Lipoprotein Precipitaion (HELP). J Clin Apheresis 1996;11:132–7.
[17] Suckfüll M. Fibrinogen and LDL apheresis in treatment of sudden hearing loss: a randomised multicentre trial. Lancet 2002;360:1811–7.
[18] Empen K, Otto C, Brödl U, Parhofer KG. The effects of three different LDL-apheresis methods on the plasma concentrations of E-Selectin, VCAM-1 and ICAM-1. J Clin Apheresis 2002;17:38–43.
[19] Pulawski E, Mellwig KP, Brinkmann T, Kleesiek K, Horstkotte D. Influence of single low-density lipoprotein apheresis on the adhesion molecules soluble vascular cellular adhesion molecule-1, soluble intercellular adhesion molecule-1, and P-Selectin. Ther Apher 2002;6(3):229–33.
[20] Moriarty PM, Gibson CA, Shih J, Matias MS. C-reactive protein and other markers of inflammation among patients undergoing HELP LDL apheresis. Atherosclerosis 2001;158:495–8.
[21] Wieland E, Schettler V, Armstrong VW. Highly effective reduction of c-reactive protein in patients with coronary heart disease by extracorporeal low density lipoprotein apheresis. Atherosclerosis 2002;162:187–91.
[22] Schuff-Werner P, Schutz E, Seyde WC, et al. Improved haemorheology associated with a reduction in plasma fibrinogen and LDL in patients being treated by heparin-induced extracorporeal LDL precipitation (HELP). Eur J Clin Invest 1989;19(1):30–7.
[23] Jaeger BR, Goehring P, Schirmer J, et al. Consistent lowering of clotting factors for the treatment of acute cardiovascular syndromes and hypercoagulability: a different pathophysiological approach. Ther Apher 2001;5(4):252–9.
[24] Leca G, Mansur SE, Bensussan A. Expression of VCAM-1 (CD106) by a subset of TLR (γ δ-bearing lymphocyte clones: involvement of a metallo-protease in the specific hydrolytic release of the soluble form. J Immunol 1995;154:1069–77.
[25] Sturk-Maquelin KN, Nieuwland R, Romijin FP, Eijsman L, Hack CE, Sturk A. Pro- and non-coagulant forms of non-cell-bound tissue factor in vivo. J Thromb Haemost 2003;1(9):1920–6.

[26] Semaan HB, Gurbel PA, Anderson JL, et al. Soluble VCAM-1 and E-selectin, but not ICAM-1 discriminate endothelial injury in patients with documented coronary artery disease. Cardiology 2000;93:7–10.
[27] Yamane S, Matsugane T, Motohashi K, et al. Double filtration plasma apheresis maintains normal adhesion molecule levels. Ther Apher 1998;2:224–7.
[28] Schonbeck U, Libby P. CD40 signaling and plaque instability. Circ Res 2001;89:1092–103.
[29] Lowe G, Rumley A, Norrie J, et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the west of Scotland Coronary Prevention Study. Thromb Haemost 2000;84:553–8.
[30] Folsom AR, Pankow JS, Tracy RP, et al. Investigators of the NHBLI Family Heart Study. Association of C-reactive protein with markers of prevalent atherosclerotic disease. Am J Cardiol 2001;88:112–7.
[31] Seeger FH, Blessing E, Gu L, Bornhold R, Denger S, Kreuzer J. Fibrinogen induces chemotactic activity in endothelial cells. Acta Physiol Scand 2002;176:109–15.
[32] Welch GN, Loscalzo J. Homocysteine and atherosclerosis. New Engl J Med 1998;338:1042–50.
[33] Wang GP, Woo CWH, Sung FL, Siow YL, Karmin O. Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia. Arterioscler Thromb Vasc Biol 2002;22:1777–83.
[34] Inwald DP, McDowall A, Peters MJ, Callard RE, Klein NJ. CD40 is constituively expressed on platelets and provides a novel mechanism for platelet activation. Circ Res 2003;92(9):1041–8.
[35] Vermeulen EG, Rauwerda JA, van den Berg M, et al. Homocysteine-lowering treatment with folic acid plus vitamin B6 lowers urinary albumin excretion but not plasma markers of endothelial function or C-reactive protein: further analysis of secondary end-points of a randomized clinical trial. Eur J Clin Invest 2003;33:209–15.
[36] Carraway MS, Welty-Wolf KE, Miller DL, et al. Blockade of tissue factor: treatment for organ injury in established sepsis. Am J Respir Crit Care Med 2003;167:1200–9.