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Selectins and Monocyte Chemotactic Peptide as the Markers of Atherosclerosis Activity

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Summary

The role of adhesive selectin molecules in the process of atherogenesis is an open question. These molecules are known as markers of atherosclerosis activity, however, only some biological mechanisms are known up to now. In this study we examined the levels of soluble forms of E-, P-selectin and monocyte chemotactic protein (MCP-1) in the process of extracorporeal cholesterol elimination by LDL-apheresis. We measured the levels of sE-, sP-selectin and MCP-1 in the plasma before and after LDL-apheresis and in the washout solution from immunoabsorption columns Lipopak. Eighty measurements were performed repeatedly in 6 patients with severe familial hypercholesterolemia (FH) on long-term LDL-apheresis treatment. Before the procedure P-selectin levels were 204±179 ng/ml, E-selectin 32.1±33.7 ng/ml, MCP-1 323.8±121 pg/l, whereas after the procedure we found P-selectin levels 131.6±34 ng/ml, E-selectin 33.1±51 ng/ml, and MCP-1 200.4±15 pg/l. Levels of P- selectin were increased in the blood of patients with FH in spite of long-term intensive extracorporeal LDL-elimination, documenting thus the activity of atherosclerosis. The levels of P-selectin and MCP-1 decreased significantly after the hypolidemic procedure and could be used as another marker showing the effectivity of the extracorporeal LDL-cholesterol elimination (immediately after the procedure), and, after further verification, may serve as a marker for controlling the therapy efficacy.

Key words

Atherosclerosis • LDL–apheresis • Selectin • Adhesive molecules

Introduction

Clinical consequences of atherosclerosis, i.e. ischemic heart disease, vascular cerebral stroke and lessions of the peripheral arteries are the leading cause of morbidity in the industrial countries. Therefore, a number of researchers (Malbohan *et al.* 2002, Šejda *et al.* 2002, Šindelka *et al.* 2002) as well as our team have focused on hitherto unclear pathophysiological mechanisms, which could be used in the prevention and therapy of atherosclerosis. Our present effort was made to influence severe familial hyperlipidemia by LDL-apharesis.

Endothelial dysfunction appears to contribute to the pathogenesis of atherosclerosis, both in the early stages of lesion formation and later during the disease when patients have already developed clinical symptoms.

Recent studies have also linked endothelial dysfunction of the coronary and forearm circulation with subsequent cardiovascular events. It therefore seems plausible that the reversal of endothelial dysfunction by effective hypolipidemic therapy may be an important mechanism for achieving its therapeutic benefits.

Familial hypercholesterolemia (FH) and familial combined hyperlipidemia (FCH) are genetic disorders, which are associated with a high incidence of severe cardiovascular complications in young people. When the dietary measures together with combined medicamentous therapy are without any result (about 5 % of patients), it is necessary to use extracorporeal lipoprotein elimination. Several methods exist which we have described previously (Bláha *et al.* 1998). Our working group has been using LDL-apheresis, which is an effective method to save the life in homozygous FH and to improve health status in heterozygous FH.

In the present paper we measured the levels of soluble forms of E-, P-selectin and monocyte chemotactic protein (MCP-1) during the treatment with extracorporeal cholesterol elimination by LDL-apheresis and examined whether it may serve as a marker for monitoring the intensity of therapy.

Methods

We used the method of LDL-apheresis based on immunoadsorption: plasma is pumped through a sepharose gel with coupled sheep antibodies against human apoprotein B 100, which forms the main protein component of LDL-cholesterol (Bláha *et al.* 2001).

The LDL-apheresis system consists of primary plasma separation performed with a classic continuous blood cell separator Cobe-Spectra (USA) working at whole blood flow rates from 50 to 80 ml/min and plasma flow rates of 30 to 50 ml/min and a differential plasma separation system consisting of a device (Adsorption-desorption Automat, Medicap, Germany) automatically performing the repetitive, cyclic loading and desorption of two columns (Fig. 1). The biochemistry of the adsorber system led to a loading capacity of 5 g LDL-cholesterol per column. Our clinical approach permits to load and desorb each pair of columns as often as it is necessary for the treatment. The treatment time thus varies according to the pretreatment cholesterol level of the patient (up to 4 h). The adsorber columns (Lipopak, Pocard, Russia) contain sheep antibodies against apoprotein B coupled to sepharose 4B after bromcyanide activation. Beside the advantages of LDL-apheresis due to the specificity of elimination, the safety of the system was confirmed by many laboratories. The peripheral veno-venous access avoids all potential risks of a central venous or arterial access. The LDL-cholesterol can be decreased to the desired normal or subnormal level without a loss of normal plasma proteins or HDL-cholesterol (Bláha *et al.* 2001). Long-term treatments lead to a steady-state between synthesis rate and removal after five to seven aphereses, if the treatments are performed regularly. The number of days under 5 mmol/l is supposed to be crucial for the regression of atheromatosis (Bláha *et al.* 2001).

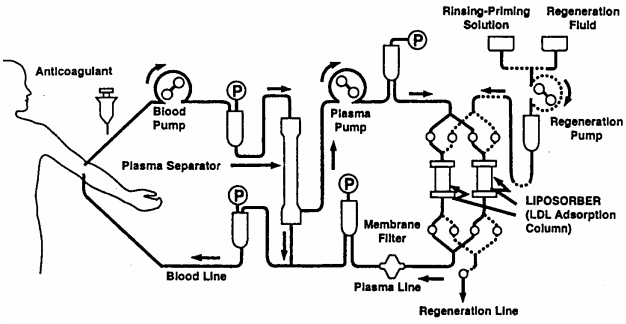

Fig. 1. The scheme of LDL-adsorption

We measured the levels of sE-, sP-selectin and MCP-1 in plasma before and after LDL-apheresis and in the washout solution from absorption columns. Eighty measurements were repeatedly carried out in six patients with severe familial hypercholesterolemia (FH) in long-term LDL-apheresis treatment. The method was the same in all cases: ELISA; producer: RDS (USA), with the sets: Parameter human sP-selectin, Parameter human sE-selectin, Quantikine human MCP-1.

Statistical analysis

Analyses were performed with procedures available in SPSS (Sigma Stat 2.03, San Rafael, USA, 1997). For the statistical evaluation of the differences between the level before and after procedure, the standard paired t-test and/or Wilcoxon signed rank test were employed.

Results

Serum lipoproteins

Acute treatment with LDL-apheresis induced a significant decrease in total plasma cholesterol, very low-

density lipoprotein (VLDL) cholesterol, intermediate density (IDL) cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triacylglycerols (TAG) in serum (Fig. 2).

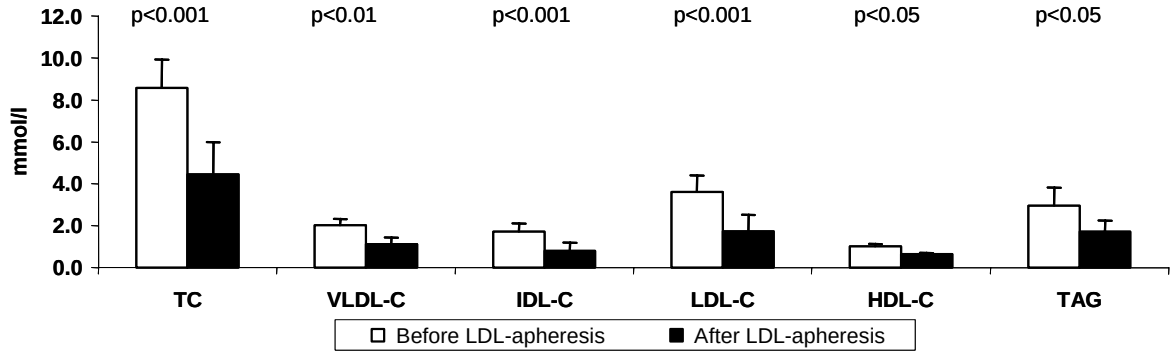

Fig. 2. Changes (means ± S.E.M.) in serum total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), intermediate density lipoprotein cholesterol (IDL-C), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triacylglycerols (TAG) (mmol/l) during treatment using LDL-apheresis. Blood samples from hyperlipidemic patients were analyzed before and after particular LDL-apheresis. Each bar represents data from all patients.

Table 1. Mean levels and range of selectins and MCP-1 in plasma

	Before procedure	**After procedure**	**P =**	**Normal levels**
P-selectin (ng/ml)	204.9 (60.9-420.4)	131.6 (34.9-429.9)	0.030	51-113
E-selectin (ng/ml)	32.1 (5.1-115.3	33.1 (2.4-179.9)	0.488	29-63
MCP-1 (pg/l)	323.8 (166.7-498.2)	200.4 (121.8-433.4)	0.011	200-722

Measurements of selectins and MCP-1 levels

We have found that there is an increased level of P-selectin in the blood of hypercholesterolemic patients in spite of long-term intensive treatment (regular procedures of extracorporeal LDL-cholesterol elimination). E-selectin at the baseline remained in the normal range. Immediately after the LDL-apheresis procedure both P-selectin and MCP-1 decreased significantly. The changes of E-selectin before and after this procedure were not significant (Table 1). Both the selectins and MCP-1 in the washout solution from the absorbers in the middle of washing period were minimal or not detectable.

Discussion

The knowledge of mechanisms leading to the development of atherosclerotic processes, especially their initial yet reversible phases, are important for both research and therapeutic practice. Characteristic histological picture of lipoid spots with subintimal accumulation of lipid-containing macrophages derived from blood monocytes, is well known. An increased adhesivity of monocytes to the vessel endothelium is one of the early changes (Kuijpers and Harlan 1993). Some studies have demonstrated that monocytes adhesion to vessel endothelium is stimulated by the exposure to an increased cholesterol level, especially LDL- and VLDL-cholesterol, both *in vitro* (Couffinhal *et al.* 1993) and *in vivo* (Joris *et al.* 1983). The unique treatment approach that makes possible to study the consequences of relatively fast changes of plasma and cell-membrane lipid homeostasis *in vivo* is LDL apheresis. This therapeutic method used in our department leads to a quick and significant decrease of pathologically increased LDL-cholesterolemia in patients with severe hypercholesterolemia, which could not be solved in another way. The levels of apo-B lipoproteins – LDL and VLDL – are reduced by more than 60 % during 3-4 h, while HDL content does not change (Kulschar *et al.* 1995 and our own experience).

The interaction between monocytes and endothelial cells is of crucial importance in the initial phase of the atherosclerotic plaque formation. LDL

shows a direct chemotactic activity as concerns monocytes and stimulates endothelial cells to produce MCP-1 (monocyte chemotactic peptide) (Kirkpatrick *et al.* 1995). Invasion of monocytes from the blood is an essential prerequisite for the plaque formation. Only some biological mechanisms, which take part in cytokine release and expression of adhesive molecules (CAMs) on the surface of participating cells, have been understood until now. The participation of activated endothelial cells and activated thrombocytes in the pathogenesis of atherosclerosis is still open question (Mantovani *et al.* 1997). For the determination of their activation, the levels of soluble forms of the adhesive E-selectin and P-selectin molecules could be measured in peripheral blood (Blann *et al.* 1997). These molecules which regulate the starting phase of leukocyte and thrombocyte adhesion are deposited in intracellular granules from which they are quickly transported on the surface after cell activation. E-selectin is found only in Weibel-Palade bodies of endothelial cells. It was proved that sP-selectin is located in both alpha-granules of thrombocytes and Weibel-Palade bodies of endothelial cells. Furthermore, sP-selectin in peripheral blood is exclusively released from alpha-granules of thrombocytes. The contribution of either platelets or endothelium could be estimated separately by simultaneous measurements of sP-selectin and sE-selectin during the therapeutic apheresis.

Pathologically increased concentrations of cell adhesion molecules were lowered after hypolipidemic treatment using LDL-apheresis as we have demonstrated for P-selectin and MCP-1. Other experiments were performed to reduce selectins by dextran sulfate columns (sICAM and sELAM – soluble intercellular and soluble leukocytic adhesive molecules) (according to Sampietro *et al.* 1997), to decrease of E-selectin, VCAM-1 and ICAM-1 by direct absorption of lipids, dextran sulphate adsorption or heparin extracorporeal low-density lipoprotein precipitation (Empen *et al.* 2002), and to lower plasma sVCAM-1, sICAM-1, and P-selectin during heparin extracorporeal low-density lipoprotein precipitation (HELP) (Pulawski *et al.* 2002). It is well known that oxidative LDL modification increases atherogenous properties. Monocyte incubation with oxidized forms of lipoproteins increases the production of cytokines by mononuclears and this leads to further increased expression of the endothelial adhesive molecules and also stimulates endothelial cells to the expression of chemotactic factors in further leukocytes (Frostergard *et al.* 1993). Lowering of LDL cholesterol level leads to a decrease of monocyte adhesion to endothelial cells (Cattin *et al.*1998).

Immediately after the LDL-apheresis procedure both P-selectin and MCP-1 decreased significantly. However, both the selectins and MCP-1 in the washout solution obtained from the absorbers in the middle of washing period were minimal or not detectable. Our measurements were made in the middle of washing procedure. It is possible that the selectins and MCP-1 are washed out in the beginning of the procedure as we have found for the LDL-cholesterol (Bláha *et al.* 2001). This might be the reason for minimal levels observed in the middle of washing period. Hence this experiment has to be repeated later in order to measure the levels of selectins and MCP-1 in the washed out solutions at the beginning of the procedure. Empen *et al.* (2002), who measured the concentrations of VCAM-1 and E-selectin in the outlets of the LDL-apheresis columns, found it significantly reduced compared to the concentration in the inlets. They conclude that the reduction of adhesion molecule levels observed during LDL-apheresis are at least partly due to their absorption onto the LDL-apheresis column, and that the extent of absorption depends on the principle of extracorporeal elimination.

The treatment using LDL-apheresis lowered pathologically increased LDL-cholesterol, however, such effect is transient and, depending on the type of disease, another increase of LDL-cholesterol makes it necessary to repeat the treatment periodically. The procedure of LDL-apheresis may be relatively long (approximately 4 h, during which the patient must stay in bed) and also relatively costly because of the need for optimal frequency of apheresis procedures. On the contrary, a reduced frequency of the separate treatments would provoke the progression of atherosclerosis. Therefore, the markers predicting the effectivity of therapy are needed. We successfully monitored thrombocyte activity (Bláha *et al.* 2004), but the evaluation of the size of the thrombocytes failed in spite of other reports (Bröijersen *et al.* 1994). The investigation of selectins requires a specialized, adequately equipped laboratory with the experienced staff.

However, the extracorporeal LDL-elimination can only be performed in specialized appropriately equipped centers in which it is also possible to measure the selectins and MCP-1. This examination is not too expensive and might be useful for monitoring the hypolipidemic intervention.

In conclusion, the levels of P-selectin and MCP-1, which significantly decrease after the hypolidemic procedure, could be used as another markers showing the effectivity of the extracorporeal LDL-cholesterol elimination (immediately after the procedure), and, after further verification, may serve as a marker for controlling the therapy efficacy.

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