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Consistent Lowering of Clotting Factors for the Treatment of Acute Cardiovascular Syndromes and Hypercoagulability: A Different Pathophysiological Approach

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Abstract: Hypercoagulability is a key contributor to acute cardiovascular syndromes and to various microcirculatory disorders. The use of heparin-mediated extracorporeal low-density lipoprotein/fibrinogen precipitation (HELP) apheresis makes a controlled, immediately effective reduction of clotting factors possible, and induces subsequent positive effects on plasma viscosity, erythrocyte aggregation, and microcirculation. Oxygen supply to an ischemic artery can thus be increased without hemodilution, qualifying the HELP system as a possible therapeutic tool in the treatment of acute cardiovascular syndromes and microcirculatory disorders. **Key Words:** Atherosclerosis—Cardiovascular diseases—Cholesterol—Coagulation—Fibrinogen—Low-density lipoprotein apheresis—Thrombolysis.

For many years, low-density lipoprotein (LDL) cholesterol was in the limelight of discussions about atherosclerosis, casting a shadow on the thrombogenic aspects of atherogenesis. In recent years, scientific interest increasingly focused on the clotting system in order to find therapeutic strategies for preventing the acute cardiovascular syndromes. This paper describes the overall effect of heparin-mediated extracorporeal LDL/fibrinogen precipitation (HELP) apheresis on the clotting system in patients with ischemic heart disease: The significance of these observations is to show the deescalation of a chronic hypercoagulable status, which allows a new therapeutic approach for the treatment of acute and chronic atherothrombotic complications.

HELP apheresis is similar to other LDL apheresis procedures in regard to removal of LDL cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and lipoprotein(a) (Lp[a]) but different in that it uses a natural anticoagulant, heparin, as the medium for precipitation of the lipoproteins. The drastic reduction of plasma fibrinogen also depends on the use of heparin. Clinical efficacy and safety have been established by numerous controlled studies and through the treatment of more than 1,000 patients (>180,000 treatments) over the last 15 years (1–11). We and others previously reported partial effects of the HELP system on clotting factors and hemorrheology (1–13), but the overall picture regarding the hemostatic balance deserves a special article because of the arising chances for the treatment of atherothrombosis.

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PATIENTS

We studied 18 patients with severe ischemic heart disease who were treated with HELP apheresis at weekly or biweekly intervals, on average for a period of 4 years. Two patients suffered from three-vessel coronary artery disease (CAD) and were prescribed regular HELP apheresis after partial reocclusion of their coronary bypass grafts. The remaining 16 patients were enrolled after heart transplantation because of pronounced hypercholesterolemia for secondary (n = 9) or primary (n = 7) prevention of

transplant-associated CAD. Of these patients, 14 had a history of ischemic cardiomyopathy, and two of dilated cardiomyopathy. Extracardiac features of atherosclerosis were present in 4 patients: 2 patients had experienced strokes and transient ischemic attack (TIA) episodes associated with carotid atherosclerosis, and 2 others suffered from peripheral arterial disease.

The mean age of all the patients was 56 ± 12 years. Two heart transplant (HTX) patients were women. All the patients received 100 mg aspirin daily, except 1 patient who was treated with warfarin because of peripheral arterial occlusive disease (Warfarin was withdrawn 1 week prior to measurement of the clotting factors, and was replaced by subcutaneous heparin). None of the patients had a history of venous thrombosis or of platelet disorders. All the patients received antihypertensive medication (ACE inhibitors, diuretics) and lipid-lowering therapy with simvastatin (15 to 20 mg/d), respectively. The immunosuppressive treatment of the HTX patients was cyclosporine A, prednisone, and azathioprine. Three patients had acquired diabetes after transplantation and were treated with tolbutamide.

During an observation period of 5 years on regular HELP treatment, the patients experienced neither clinical or angiographic progression of atherosclerosis nor acute cardiovascular complications. All patients gave informed consent to the study.

HEPARIN-MEDIATED EXTRACORPOREAL LDL/FIBRINOGEN PRECIPITATION APHERESIS

The HELP system has been in clinical use since 1984. It is an extracorporeal system (B. Braun AG, Melsungen, Germany) in which the first step of the circuit is plasmapheresis for the separation of plasma from blood cells and platelets, which are not affected by the procedure. Heparin and acetate buffer are added to the plasma, lowering the plasma pH to 5.12, which leads to the precipitation of the atherogenic blood constituents, such as LDL cholesterol, Lp[a], and fibrinogen. The efficacy of precipitation with this system is 100% (1–3).

Heparin is a sulfated polysaccharide with a long-chain polymer structure, which makes it ideal for such precipitations. It has the highest negative charge of any naturally occurring polymer, due to esterification with numerous sulfate groups, and thereby provides many binding sites for anionic substances (14).

In the next step of the procedure, the heparin precipitate is cleared from the plasma by passing it through a polycarbonate filter and then through an anion-column adsorber to remove any soluble heparin. In the final step, plasma neutralization to physiologic pH and fluid balance are achieved by means of bicarbonate dialysis and ultrafiltration. In one session, lasting 2 h, a plasma volume of approximately 3 L is passed through the system. The duration and quantity of plasma treated can be varied according to the individual treatment goal (4).

ASSAY PROCEDURES

Blood samples were drawn immediately before and after HELP treatment, and clotting factors were determined by standard immunological and functional assays (Roche AG, Mannheim, Germany, and DADE-Behring AG, Marburg, Germany). Plasma fibrinogen concentrations were determined according to Clauss (15). Procoagulant variables measured were fibrinogen; prothrombin; factors V, VII, and VIII; von Willebrand factor (WF); factors IX, X, XI, XII, and XIII; and plasminogen activator inhibitor-1 (PAI-1). The fibrinolytic parameters analyzed were plasminogen and tissue plasminogen activator (t-PA), and inhibitor proteins C, S, and antithrombin (AT). All clotting factors were measured at two time points for every patient.

Erythrocyte aggregability and plasma viscosity were determined as described previously (12). LDL cholesterol was quantified by the enzymatic CHOD-PAP method (Roche AG, Mannheim, Germany) after precipitation of LDL from cholesterol with dextran sulfate (Immuno AG, Heidelberg, Germany). Lp[a] was measured by nephelometry (Immuno AG).

All patients were investigated for inherited resistance to activated protein C with the COA-TEST APC Resistance V (Chromogenics, Milan, Italy). Positive test results were confirmed by DNA analysis and demonstration of factor V Leiden mutation (Arg 506 ⊃ Gln), according to the method of Zöller et al. (16).

STATISTICS

Changes of the laboratory variables before and after HELP apheresis were compared with Wilcoxon's matched pairs signed rank test. The same test was applied for the assessment of the intraindividual variability of hemostaseological parameters between two subsequent HELP sessions. p values >0.05 were considered to indicate statistical significance.

RESULTS

Hypercoagulability in patients with ischemic heart disease

Due to the advanced stages of atherosclerosis, patients exhibited chronically elevated plasma concentrations of the clotting factors, as expected. In particular, fibrinogen (413 ± 124 mg/dl), factor VIII (195 ± 48%), von Willebrand factor (193 ± 78%), and factor VII (136 ± 43%) were significantly increased compared to apparently healthy controls (17–24) (Table 1). The highest plasma fibrinogen and von Willebrand factor levels were found among the heart transplant patients suffering from transplant-associated arteriosclerosis.

Short-term effects of HELP apheresis on clotting factors

Table 1 shows the rate of removal of the clotting factors by HELP apheresis. All clotting factors were coprecipitated by heparin, resulting in significant decreases of their plasma concentrations ($p < 0.01$). Accordingly, there were changes in the global tests, i.e., the prothrombin time (INR: 1.2 vs. 1.7) and the activated prothrombin time (35 ± 12 vs. 72 ± 22 s). Factor V is highly homologous to factor VIII (25), and both were eliminated by apheresis with similar efficacy (–57%).

Apheresis decreased PAI-1 concentrations by 17% (3.68 ± 1.27 and 3.07 ± 1.33 U/ml) and t-PA levels by 31% (4.49 ± 1.46 vs. 2.92 ± 0.88 ng/ml). For the clinical relevance of this, it has to be remembered that PAI-1 and t-PA have much shorter half-lives (seconds) than the other coagulation factors.

The decrease in concentration depends on the amount of plasma passed through the system in one session; here, 3 L of plasma. Figure 1 describes exemplarily the elimination kinetics of the clotting factors for fibrinogen, WF, and antithrombin in 1 patient (1.68-m height, 77-kg body weight; estimated plasma volume of 3.003 L) who suffered from transplant-associated arteriosclerosis after cardiac transplantation 11/13/1991. Fibrinogen and WF showed nearly linear parallel decreases, depending on the amount of plasma treated, whereas antithrombin elimination already reached the maximum after 1-L plasma treatment. More intensive treatment up to 3.5 L of plasma caused no relevant further antithrombin loss.

A factor V Leiden mutation that might have further increased the risk of thromboembolism was excluded in all but 1 patient who was a heterozygous carrier of the mutation.

TABLE 1. *Removal of clotting factors by HELP-apheresis (Mean concentrations from 18 patients with ischemic heart disease)*

Factor	Units	Before HELP	After HELP	Δ in %
Fibrinogen	(mg/dL)	413 ± 124 (406)	173 ± 71 (159)	–58%
Prothrombin	(%)	102 ± 16 (98)	46 ± 8 (46.5)	–55%
Factor V	(%)	115 ± 19 (113)	50 ± 9 (49)	–57%
Factor VII	(%)	136 ± 43 (131)	92 ± 32 (89)	–32%
Factor VIII	(%)	195 ± 48 (193)	83 ± 32 (80)	–57%
von Willebrand	(%)	193 ± 78 (179)	85 ± 33 (78)	–56%
Factor IX	(%)	160 ± 31 (161)	88 ± 18 (92)	–45%
Factor X	(%)	103 ± 15 (105)	54 ± 12 (54)	–48%
Factor XI	(%)	117 ± 26 (122)	51 ± 13 (48)	–56%
Factor XII	(%)	114 ± 24 (110)	56 ± 10 (57)	–50%
Factor XIII	(%)	114 ± 31 (116.5)	63 ± 19 (64.5)	–45%
Plasminogen	(mg/dL)	118 ± 19 (116)	59 ± 13 (60)	–50%
Antithrombin	(%)	117 ± 21 (122)	88 ± 13 (91)	–25%
Protein S	(%)	106 ± 9 (110)	69 ± 22 (64)	–35%
Protein C	(%)	113 ± 27 (118)	59 ± 16 (57)	–48%

The clotting factor concentrations are expressed as mean ± standard deviations. Median concentrations are listed in parentheses.

Long-term effects of HELP apheresis on the clotting profile

The long-term effect of regular HELP treatment depends on frequency and intensity of apheresis treatment. Regarding frequency, weekly or biweekly intervals effectively prevented atherothrombotic

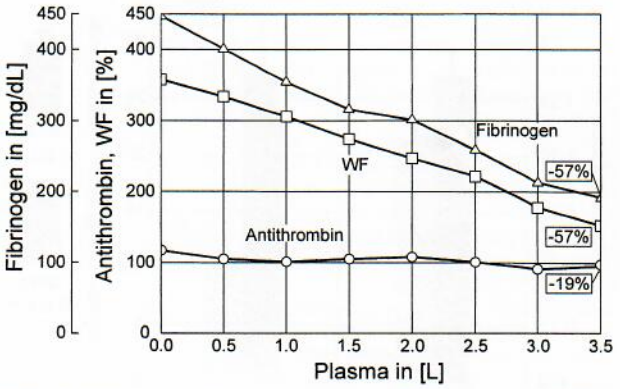

FIG. 1. Elimination kinetics of plasma fibrinogen, von Willebrand factor, and antithrombin during HELP apheresis in one patient are shown. Blood samples were drawn from the patient's cubital vein while he was treated with HELP apheresis.

complications in all patients over the last 4 years, respectively. Regarding intensity, a common treatment covers 3 or 4 L of plasma, depending on the patient's body weight (above or below 90 kg).

The degree of reduction of each clotting factor depends on the respective half-life and synthesis rate, the latter of which may vary considerably according to various acute-phase stimuli. Given the half-life of plasma fibrinogen, which is 4 days, a weekly treatment reduced the plasma fibrinogen concentrations of the patients by 48%, on average. Regular treatment induced no rebound effect onto the fibrinogen concentration. Figure 2 shows the fibrinogen-lowering effect of regular apheresis treatment in a heart transplant patient who was admitted to the apheresis treatment after a transplant-associated arteriosclerosis had been diagnosed. Regular biweekly apheresis reduced his plasma fibrinogen concentration on average from 6.1 g/L to normal levels of 3.1 g/L. During the entire treatment period, he experienced no progression of the heart disease.

Effects of HELP apheresis on hemorheological parameters

After HELP apheresis, plasma viscosity and erythrocyte aggregability were significantly diminished by 19% and 60%, respectively (Fig. 3). These effects derived mainly from the drastic reduction of plasma fibrinogen (–58%), and to a much lesser extent from that of LDL cholesterol (–53%). The effects on plasma viscosity and erythrocyte aggregation were still present on the following day after apheresis: plasma viscosity was 14% lower, and erythrocyte aggregation was 28% lower.

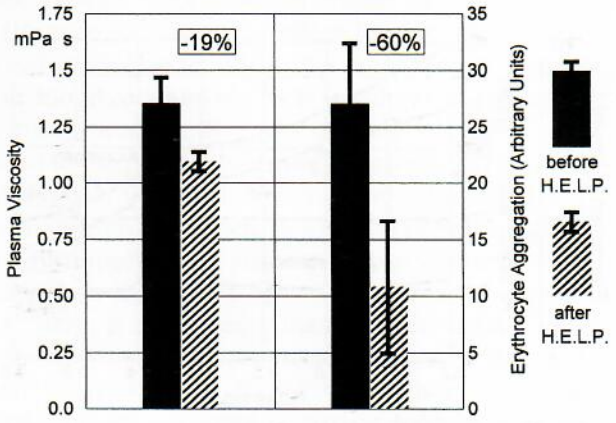

FIG. 2. Shown is reduction in plasma viscosity and erythrocyte aggregation by HELP apheresis: mean concentrations of 18 patients with ischemic heart disease (concentrations expressed as mean ± standard deviations).

Effects of HELP apheresis on the lipoprotein profile

Despite receiving the maximal tolerable doses of statins, all the patients were hypercholesterolemic, which was the reason for the regular apheresis treatment. HELP apheresis reduced total plasma cholesterol on average from 236 ± 41 mg/dl to 126 ± 24 mg/dl (–47%); LDL-cholesterol from 151 ± 41 mg/dl to 71 ± 25 mg/dl (–53%); VLDL-cholesterol from 42 ± 17 mg/dl to 18 ± 11 mg/dl (–57%); nonfasting triglyceride levels from 240 ± 88 mg/dl to 127 ± 66 mg/dl (–47%); and lipoprotein (a) from 57 ± 33 mg/dl to 25 ± 12 mg/dl (–56%). All these differences were statistically significant ($p = 0.001$). The mean high-density lipoprotein cholesterol (HDL-cholesterol) level was 43 ± 10 mg/dl, and was not acutely affected by this apheresis system.

DISCUSSION

The current study demonstrates the effects of HELP apheresis treatment in atherothrombotic diseases. The procedure enables the simultaneous reduction of both atherogenic lipoproteins and clotting factors, with similar efficacy. A single application of HELP apheresis reduced the plasma concentrations of each compound by 45% to 60% (excepting antithrombin), depending on their affinity for heparin, resulting in significant reductions of plasma viscosity and erythrocyte aggregability.

Figure 4 illustrates in detail that HELP apheresis reduces the availability of all participants in the coagulation cascade. The procedure also exerted an effect on the last step of the cascade, i.e., formation of the fibrin plug. Plug formation depends primarily on the fibrinogen concentration, whereas plug propagation and stabilization depends mainly on the availability of thrombin and factor XIII (27). Figure 5 shows that the HELP apheresis interfered with this process directly, by removal of fibrinogen, and indirectly, by removal of the precursors of thrombin and activated factor XIII. As fibrinogen and factor XIII have slower synthesis rates and longer half-lives (viz. 3 to 5 days) compared with the other coagulation factors, their removal may have a longer-lasting effect on thrombus stabilization. The removal of prothrombin should diminish the positive feedback activation of factors V and VIII (Fig. 4).

HELP apheresis reduced not only the concentrations of procoagulant factors, but also those of plasminogen and inhibitor proteins S, C, and antithrombin (Table 1). This may limit the fibrinolytic potential of the procedure, although antithrombin was reduced to a lesser extent (–25%). On the other

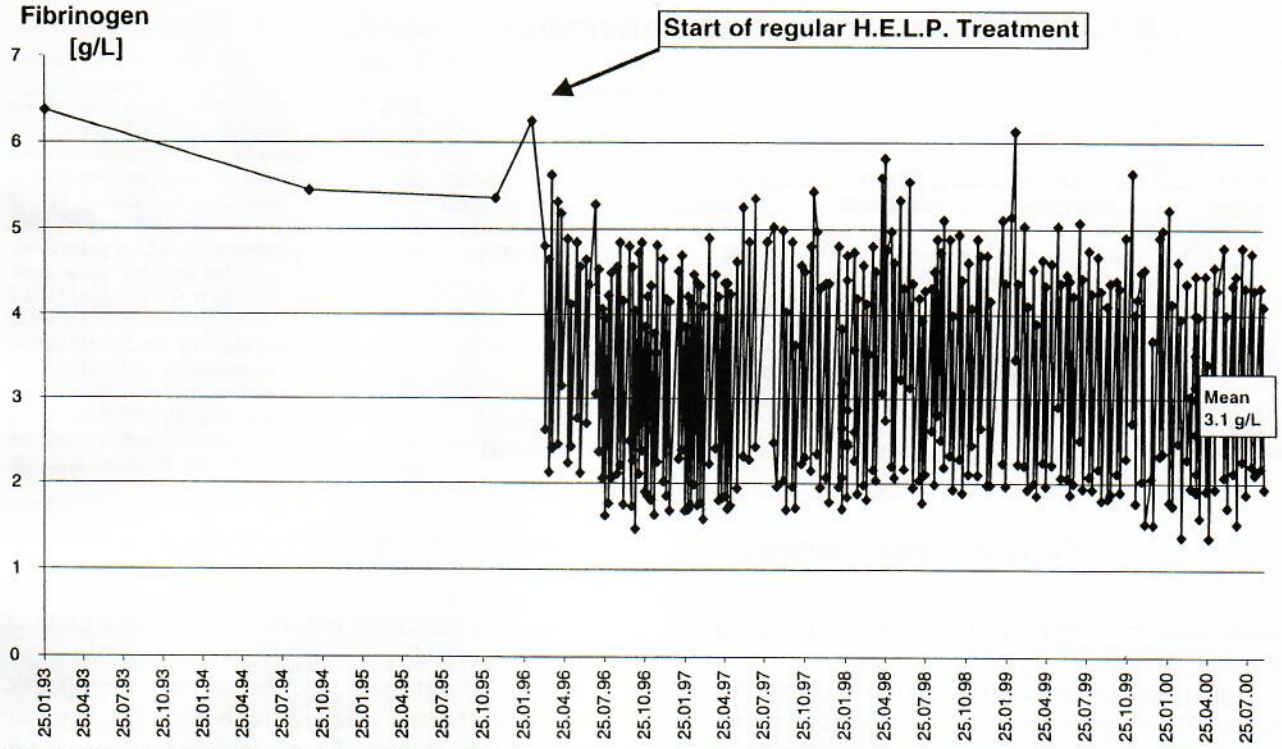

FIG. 3. Chronic monitoring of plasma fibrinogen in a heart transplant patient undergoing regular HELP apheresis. Regular HELP treatment lowered the plasma fibrinogen concentration from concentrations >6 g/L to an average of 3.1 g/L. Apheresis was performed in biweekly intervals.

hand, the concomitant removal of fibrinolytic factors may explain why, in 15 years of experience with the HELP system, bleeding complications never occurred, and this despite the common addition of oral anticoagulant medication (1–10).

Sixteen out of 18 patients studied here had undergone heart transplantation, which explains the remarkably high concentrations of clotting factors. Accordingly, Hunt et al. (29) had reported a significant increase of the plasma fibrinogen and factors VII and VIII concentrations after heart transplantation—the highest levels of clotting factors are observed in manifest transplant-associated arteriosclerosis. The regular use of immunosuppressive

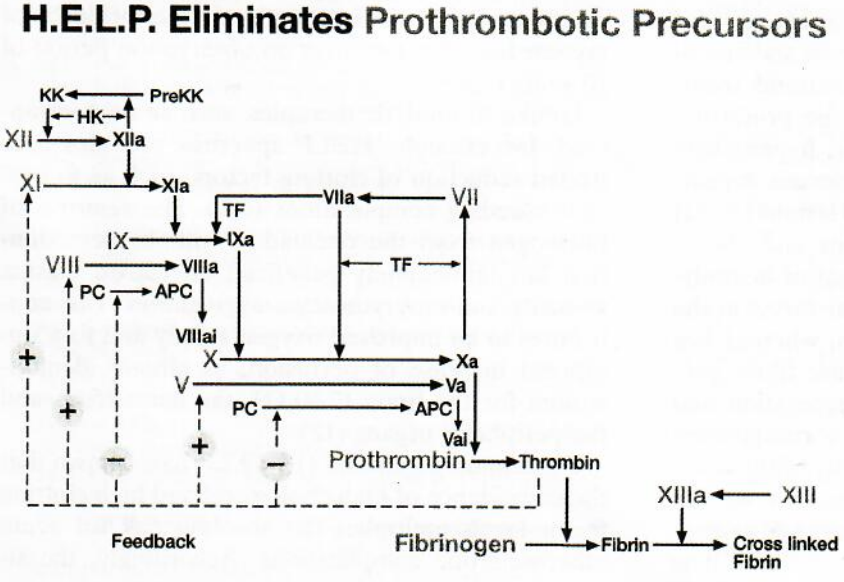

FIG. 4. The diagram shows regulation of the prothrombotic cascade with positive and negative feedback mechanisms (+/– symbols and dotted lines). The concentrations of all blue-colored clotting factors are reduced significantly by HELP apheresis. The arrows symbolize the process of transformation into the activated forms of each factor (X → Xa). Abbreviations are: KK: kallikreine, PreKK: pre-kallikreine, TF: tissue factor, PC: protein C, APC: activated protein C, Vai: deactivation of active factor V by activated protein C (adapted from Müller-Berghaus, Ref. 26).

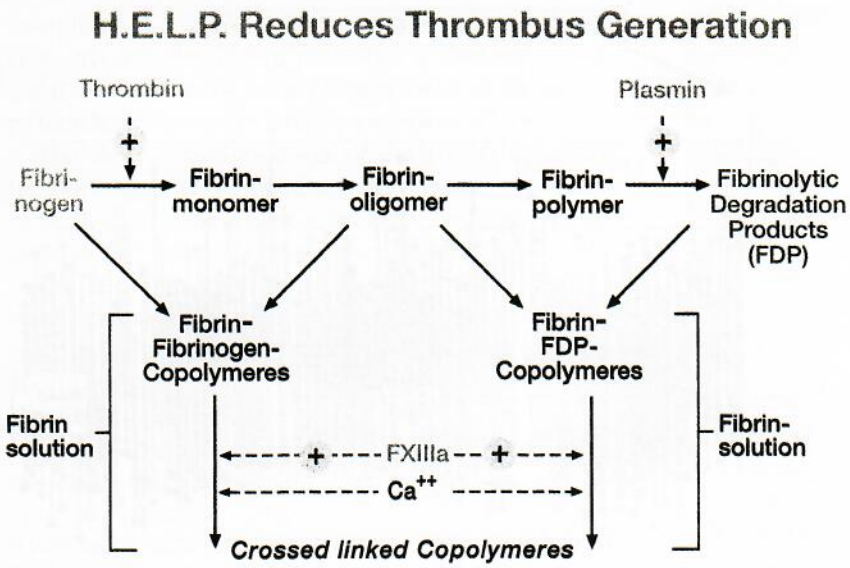

FIG. 5. Thrombus stabilization depends on the plasma concentration of fibrinogen. When the plasma fibrinogen concentration is low, the chance for an efficient cross-linking of the fibrin monomer is reduced. HELP apheresis removes plasma fibrinogen, and, additionally, prothrombin, which reduces the availability of thrombin and thus the generation of fibrin monomers. In parallel, HELP apheresis reduces the availability of activated factor XIIIa, and thereby further thrombus stabilization. The apheresis also reduces the availability of plasmin, and thus the production of fibrinolytic degradation products, a process that may reduce hemostatic activation (adapted from Müller-Berhaus, Ref. 28).

medication most likely contributes to the prothrombogenic condition.

Nonetheless, every evaluation of the overall effect of the HELP procedure on the clotting system has to consider the dual function of the hemostatic system: "Under physiological conditions, the intricate hemostatic system is designed to maintain blood in a fluid state, but primed to react to vascular injury in an explosive manner to stem blood loss by sealing the defect in the vessel wall immediately" (12). Procoagulant factors are present in plasma in abundant concentrations, and are kept at bay only by the overabundance of the fibrinolytic system, which is the most potent proteolytic system in the body.

Under pathological conditions, i.e., with the development of atherosclerosis, this fragile hemostatic balance is gradually shifted toward chronic hypercoagulability (17,18,30) as a consequence of repeated vascular injuries. Injured endothelial cells and blood-derived monocytes release von Willebrand factor and tissue factor, thereby triggering the prothrombotic cascade and promoting a chronic hypercoagulant state (17,18). Advanced atherosclerosis is characterized by formation of complicated lesions (31,32) through incorporation of lipoproteins and microthrombi (32,33). With the development of hemodynamically relevant stenoses, high shear forces at the inner site provoke platelet activation, whereas low shear forces distal to the stenosis cause fibrin generation by enhancing erythrocyte aggregation and plasma viscosity (24,34). When such a complicated lesion erodes the vessel wall, the severity of the acute coronary syndrome depends on formation, extent, and persistence of the thrombus by increased expression of procoagulant factors, depressed fibrinolytic factors, and increased plasma viscosity and platelet aggregation (30,33).

Given this pathogenesis, the primary therapeutic goal is to prevent atherothrombosis (35); first, by stabilizing the atherosclerotic plaque by depriving it of plasma LDL-cholesterol (36,37); and second, by reducing the thrombogenicity (38) of the blood. The abundance of clotting factors, especially of fibrinogen, implies that only a drastic reduction of the latter may reverse the process, and the subsequent impairment of hemorheology and microcirculation.

The results of this study suggest that HELP apheresis is a highly effective tool for normalizing the disturbed homeostasis of coagulation, hemorheology, and lipoprotein metabolism in atherosclerosis. Our findings are in agreement with the results of previous clinical studies on this apheresis system, which had demonstrated an 85% reduction in the incidence of myocardial infarction over an observation period of 10 years (1,2,9).

Unlike fibrinolytic therapies, such as t-PA or ancrod, for example, HELP apheresis provides controlled reduction of clotting factors, such as to prevent bleeding complications (3,7). The removal of fibrinogen from the circulation without hemodilution has immediately beneficial effects on plasma viscosity and on erythrocyte aggregation. This contributes to an improved oxygen supply and to a significant increase of perfusion, as already demonstrated for the brain (7,10,11), the heart (5,6), and the peripheral organs (12).

Epidemiologic studies (17–19,23) have shown that the coincidence of high cholesterol and high clotting factor levels multiplies the absolute risk for acute atherosclerotic complications. Accordingly, the si-

multaneous reduction of both types of risk factors should decrease the occurrence of these complications more effectively.

Regarding the possible benefit of reversing hypercoagulability in atherogenesis, one can only adduce biochemical evidence because, to date, there are no anticoagulant drugs that can modify hypercoagulability in total. Compared with statins, HELP apheresis has much greater effects on erythrocyte and platelet aggregability, as well as on clotting factors. This presumably explains the clinical benefit observed in high-risk patients with generalized atherosclerosis and ischemic heart disease (1,2,4–8).

The current results strongly suggest that HELP apheresis treatment can improve the outcome of acute cardiovascular syndromes, because the formation and propagation of an occlusive thrombus depends mainly on fibrinogen and von Willebrand factor (27). It is reasonable to expect that the combination of HELP apheresis with thrombolytic therapy will permit the use of plasminogen activators at much lower doses than currently employed.

The work brought out here focuses primarily on the possible clinical implications of a short-term HELP apheresis as a kind of emergency treatment. The clinical benefits of a long-term application, seen in a variety of seriously ill cardiovascular patients, have been reported earlier (1–11).

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