European Journal of Clinical Investigation (1989) **19**, 30–37

Improved haemorheology associated with a reduction in plasma fibrinogen and LDL in patients being treated by heparin-induced extracorporeal LDL precipitation (HELP)*

P. SCHUFF-WERNER†, E. SCHÜTZ*†, W. C. SEYDE‡, TH. EISENHAUER§, G. JANNING§, V. W. ARMSTRONG† & D. SEIDEL†, †Department of Clinical Chemistry, §Department of Nephrology and Rheumatology, ‡Department of Anaesthesiology, University Clinics, Robert-Koch-Strasse 40, D-3400 Goettingen, FRG

Received 15 April 1988 and in revised form 16 August 1988

Abstract. Heparin-induced Extracorporeal LDL-Precipitation (HELP) is an effective procedure for the elimination of both plasma LDL and fibrinogen.

In 10 adult patients with severe type II hyperlipoproteinemia, a single HELP treatment of 3 l plasma led to an acute decrease in the average plasma viscosity (PV) from 1·30 to 1·1 mPas. At the same time, an even more marked decrease in the mean erythrocyte aggregation rate from a pathological value of 7·9% to a value of 3·7% (normal range < 5%) was observed. Long-term studies on five patients demonstrated a lasting improvement in these two haemorheological variables. The acute rheological changes were also accompanied by an improvement in polarographically determined muscle oxygen tension. Mean oxygen tension values measured in both the m.biceps brachii and the m.tibialis anterior in five patients before and after a single HELP treatment increased from 30 ± 4 to 37 ± 7 mmHg and from 27 ± 2 to 31 ± 3 mmHg respectively.

These results may provide an explanation for the rapid improvement in patients' clinical symptoms such as angina pectoris and in stress electrocardiogram which have been observed during HELP therapy.

Keywords. Atherosclerosis, haemorheology, LDL-cholesterol, heparin-induced-LDL-apheresis, plasma viscosity, erythrocyte aggregation, blood fluidity, tissue oxygen tension.

Introduction

Elevated plasma levels of low density lipoproteins (LDL) are strongly linked with an increased risk for coronary heart disease (CHD) [1–3]. In the heterozygous form of familial hypercholesterolemia (FH), which can be associated with hyperfibrinogenemia [4], severe atherosclerosis often occurs before the fourth or fifth decade of life. Persons with the homozygous form of this disease have markedly increased LDL-cholesterol concentrations and usually suffer death from myocardial infarction before the age of thirty [5].

Treatment of FH by diet and drug therapy alone is often ineffective. Encouraging results have, however, been obtained by Thompson and co-workers [6] who treated five homozygotes by regular plasma exchange for a mean of 8·4 years. They observed an improved survival of the treated individuals over their untreated siblings. In conventional plasma exchange, the patients' plasma has to be replaced by donor plasma or plasma protein fraction (PPF) with the risks of transmitting infectious diseases or inducing adverse immunological reactions to the foreign proteins [7,8]. In the case of PPF substitution there is also an unspecific loss of other plasma proteins, in particular the immunoglobulins. It is therefore not surprising that methods have been developed to selectively remove LDL in order to return the patient's own lipoprotein-free plasma.

Heparin-induced Extracorporeal LDL-Precipitation (HELP) is based on the observation of Burstein and Scholnick [9] that lipoproteins can be differentially precipitated by heparin at acid pH in the absence of divalent cations. Seidel and Wieland [10,11] extended these findings to develop a specific system for both the determination of LDL-cholesterol in serum and plasma as well as the extracorporeal elimination of LDL from plasma based on their specific precipitation by heparin at pH 5·12.

In addition to LDL Armstrong *et al.* [12] have demonstrated that a limited number of other plasma proteins are also precipitated to varying degrees by the HELP procedure. Of particular interest was the observation that fibrinogen is as effectively eliminated as LDL by HELP [13,14]. This is one of the major differences to other LDL-apheresis procedures such as immunoadsorption with immobilized anti-LDL antibodies [15] or specific adsorption with dextran sulfate [16].

* This paper includes part of the dissertation of E. Schültz.

Correspondence: P. Schuff-Werner, Department of Clinical Chemistry, University Clinics, Robert-Koch-Strasse 40, D-3400 Goettingen, FRG.

Although LDL-cholesterol is one of the major risk factors in atherogenesis, it is becoming increasingly clear that the frequency of cardiovascular complications is significantly higher in patients with elevated fibrinogen levels [17–21]. Furthermore, both fibrinogen and LDL are known to affect haemorheological parameters such as plasma viscosity and red blood cell aggregation [22–26], which may contribute to disturbances in microcirculation. The co-precipitation of fibrinogen and LDL might therefore be of therapeutic value in the treatment of FH.

We have, therefore, investigated the influence of extracorporeal LDL and fibrinogen elimination on rheological parameters in patients with FH and CHD. We were especially interested as to whether the long-term effects of regular weekly treatment on plasma viscosity and erythrocyte aggregation might offer an explanation for the rapid improvement in CHD symptoms such as angina pectoris reported by several of our patients after only 6–12 weeks of HELP treatment.

Patients and methods

Patients

The rheological investigations described in this study were performed on 10 patients (nine males and one female) aged from 30 to 51 years with heterozygous FH and angiographically documented CHD who had begun regular weekly HELP treatment.

Heparin-induced extracorporeal LDL-precipitation (HELP)

The HELP procedure is described in detail elsewhere [12,13]. Briefly, plasma is obtained by filtration of whole blood through a 0·55 μ filter and is then mixed continuously with an equal volume of a 0·2 M sodium acetate buffer (pH 4·85), containing 100 i.u. ml^{-1} of heparin (Braun-Melsungen AG, Melsungen, FRG). Precipitation occurs at a final pH of around 5·12. The suspension is continuously recirculated through a 0·4 μ polycarbonate filter from which a LDL- and fibrinogen-free plasma is obtained which is then passed through an anion exchange filter to adsorb excess heparin. Finally, physiological pH is restored by bicarbonate dialysis and excess fluid is removed by ultrafiltration before the plasma is mixed with the blood cells and returned to the patient. All filters and connecting tubes are sterile disposable systems intended for single use only (HELP-LDL-Apheresis™, Braun-Melsungen AG, Melsungen, FRG). Plasmapheresis, filtration, dialysis and ultrafiltration are carried out with a combined system especially developed for HELP-treatment (Secura Plasmat™, Braun-Melsungen AG, Melsungen, FRG).

Clinical chemical analyses

Total protein content was measured by the biuret method (Boehringer, Mannheim, FRG). Total cholesterol was estimated by the CHOD-PAP method (Boehringer, Mannheim, FRG). LDL-cholesterol was determined by a precipitation procedure based on dextran sulfate [27] using a commercially available test kit (Immuno, Heidelberg, FRG). HDL-cholesterol was also measured using a commercial test kit (Boehringer, Mannheim, FRG). Fibrinogen and plasminogen were measured by radial-immunodiffusion (Partigen™, Behring AG, Marburg, FRG).

Haemorheological parameters

RBC count and haematocrit were estimated in the routine haematology laboratory using a Coulter counter. Plasma viscosity was determined in a capillary tube plasma viscosimeter (KSPV-4, Rheomed GmbH, Aachen, FRG) as described by Jung *et al.* [28]; the time taken by a plasma bolus to travel a defined distance under constant pressure in a thermally stabilized capillary (37°C; 0·8 mm) was measured and the dynamic viscosity then calculated in mPas. Erythrocyte aggregation was measured using the Myrenne Erythrocyte Aggregometer (MA-1, Myrenne GmbH, Roetgen, FRG) following the method of Schmid-Schönbein [29,30]. Briefly, RBC were washed in isotonic saline (0·9%) and adjusted in autologous plasma to a haematocrit of 35%. Twenty microlitres of the test sample were placed on a conical plate subjected to high shear rate rotation. The formation of RBC aggregates caused an increase in light transmission through the sample. The integral over 5 s was compared to a given maximum aggregation value and expressed in percentages.

Measurement of tissue oxygen pressure

Tissue oxygen pressure (pO_2) in skeletal muscle (m. biceps bracchii and m. tibialis anterior) was measured polarographically in five patients, immediately before and 30 min after HELP treatment. Measurements were made at 200 different locations in the muscle tissue using a needle electrode and a KIMOC pO_2-Histograph (G.M.S., Lübeck, FRG) [31,32]. Haemorheological parameters (erythrocyte aggregation and plasma viscosity) were determined simultaneously. The data were analysed by the two-sample Kolmogoroff-Smirnov test.

Results

Acute changes in plasma viscosity and erythrocyte aggregation

Plasma viscosity and erythrocyte aggregation were determined in 10 patients who were beginning HELP therapy. These parameters were measured both before and after the first four HELP treatments. The results are presented in Fig. 1 as the mean values for each of the weekly treatment procedures. In addition to the

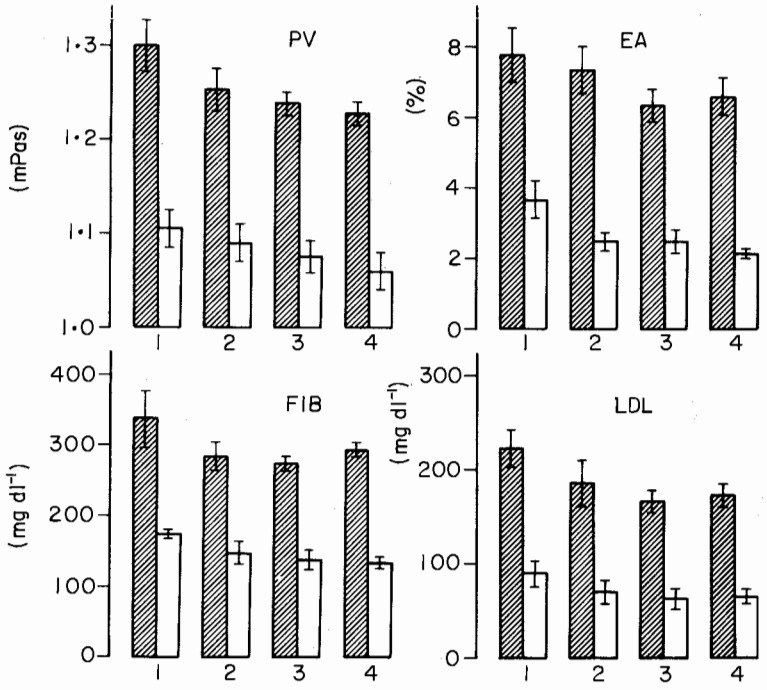

Figure 1. Acute changes in plasma viscosity (PV), erythrocyte aggregation (EA), fibrinogen (FIB) and LDL-cholesterol as measured before ▨ and after □ the first four HELP treatments performed at weekly intervals (week 1–4). The data are mean values ± SEM from 10 patients.

two rheological parameters, the corresponding mean LDL-cholesterol and fibrinogen concentrations are also illustrated for comparison. The plasma viscosities of our patients were generally in the upper normal range (normal range: 1·18–1·34 mPas). Each HELP treatment caused a significant decrease in viscosity to within the lower normal range and below. Over the first four weekly treatments, there was a gradual reduction in the average pretreatment plasma viscosity. The pretherapeutic RBC aggregability was above the normal range (< 5%) in most of our patients, indicating an abnormal rheological status. After a single HELP treatment the aggregation index (%) decreased markedly to within the normal range of < 5%. This acute reduction in RBC aggregation was a constant phenomenon in all of our patients treated with HELP (Fig. 1).

The rates of return of LDL-cholesterol, fibrinogen, plasma viscosity and erythrocyte aggregation were also followed over a period of 7 days between two

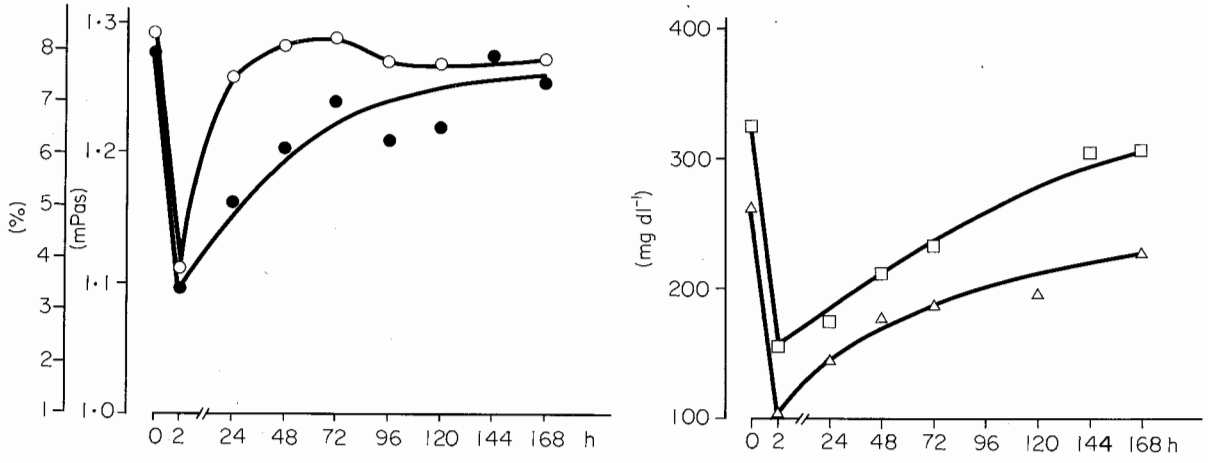

Figure 2. Rates of return of (a) plasma viscosity (○——○), (b) erythrocyte aggregation (●——●), (c) plasma fibrinogen (□——□) and (d) serum LDL-cholesterol (△——△) to pre-apheresis levels. The results represent the mean values as measured in five patients.

Table 1. Effect of repeated HELP treatment at weekly intervals on erythrocyte aggregation as determined in five patients before the 1st, 4th, 8th and 12th treatment

Changes in plasma viscosity during the course of HELP treatment

Patients	Before	%	4 Wk	%	8 Wk	%	12 Wk	%
1 H.Sp.	1·36	100	1·19	87·5	1·18	86·8	1·22	89·7
2 F.Wa.	1·37	100	1·25	91·2	1·25	91·2	1·18	86·1
3 F.-J.Wi.	1·28	100	1·25	97·7	1·22	95·3	1·24	96·9
4 H.Se.	1·36	100	1·23	90·4	1·27	93·4	1·26	92·6
5 V.Jä.	1·45	100	1·19	82·1	1·28	88·3	1·22	84·1
$\bar{X}$	1·36	100	1·22	89·8	1·24	91·0	1·22	89·9
SD	0·06		0·03	5·7	0·04	3·5	0·03	5·1
Median	1·36	100	1·23	90·4	1·25	91·2	1·22	89·7

individual apheresis procedures. The data are presented as the mean values measured in five different patients (Fig. 2). LDL-cholesterol, fibrinogen and erythrocyte aggregation showed a gradual return to their pre-apheresis levels while plasma viscosity showed a more rapid increase within the first 24 h after apheresis.

Long-term effects on plasma viscosity and erythrocyte aggregation

Rheological parameters were also measured at 4-week intervals during the first 3 months of HELP therapy in five patients. Determinations were made prior to each monthly HELP procedure. As can be seen from Table 1, pretreatment plasma viscosity values decreased in all patients during the first 4 weeks from $1{\cdot}32 \pm 0{\cdot}8$ mPas to $1{\cdot}22 \pm 0{\cdot}4$ mPas and remained relatively stable thereafter. The decreases in the pretreatment erythrocyte aggregability indices showed rather more variation between individual patients (Table 2). However, there was a consistent improvement in the erythrocyte aggregability during long-term HELP therapy at weekly intervals; the aggregation index decreased from a mean of $8{\cdot}32 \pm 2{\cdot}45\%$ to $6{\cdot}6 \pm 1{\cdot}57\%$ after 4 weeks and to a mean of $6{\cdot}37 \pm 1{\cdot}38$ after 8 weeks therapy.

The effects of long-term HELP therapy on LDL-cholesterol and fibrinogen levels as well as on plasma viscosity and erythrocyte aggregation are presented in Fig. 3 for one individual on HELP treatment for almost 6 months. The measurements illustrated were obtained immediately prior to an apheresis. Pretreatment fibrinogen levels decreased during the first 3 weeks of therapy and remained fairly constant thereafter. Similar decreases were also observed for LDL-cholesterol and the two rheological parameters. Interestingly, an increase in LDL-cholesterol after approximately 11 weeks treatment due to a prolonged interval between aphereses was also accompanied by an increase in erythrocyte aggregation and plasma viscosity.

Despite the relatively rapid rate of return of plasma

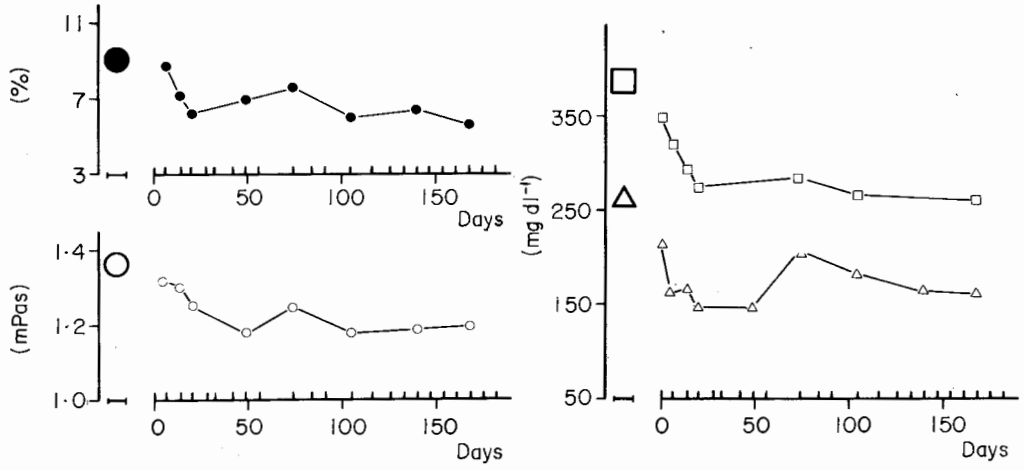

Figure 3. Effect of long-term HELP treatment on (a) plasma fibrinogen concentration (□——□), (b) serum LDL-cholesterol levels (△——△), (c) plasma viscosity (○——○) and (d) the erythrocyte aggregation index (●——●) in a 51-year-old male suffering from CHD and FH. The values shown were measured directly before each HELP treatment. The vertical dashes indicate the individual HELP treatments.

Table 2. Effect of repeated HELP treatment at weekly intervals on plasma viscosity as determined in five patients before the 1st, 4th, 8th and 12th treatment

Changes in erythrocyte aggregation during the course of HELP-treatment								
Patients	Before	%	4 Wk	%	8 Wk	%	12 Wk	%
1 H.Sp.	9·0	100	9·1	101·1	7·7	85·6	8·7	96·0
2 F.Wa.	9·2	100	6·2	67·4	6·9	75·0	7·6	82·6
3 F.-J.Wi.	6·7	100	6·0	89·6	5·3	79·1	4·2	62·7
4 H.Se.	11·0	100	9·6	87·3	6·5	59·1	6·3	57·3
5 V.Jä.	13·1	100	6·0	45·8	6·9	52·7	7·8	59·5
$\bar{x}$	9·8	100	7·4	78·2	6·7	70·3	6·9	71·6
SD	2·4		1·8	21·8	0·9	13·9	1·7	16·9
Median	9·2	100	6·2	87·3	6·9	75·0	7·6	62·7

viscosity to pre-apheresis levels after a single apheresis procedure, the long-term results show that a sustained reduction in plasma viscosity as well as in the other three parameters can be obtained through regular treatment by HELP-LDL-apheresis.

Changes in oxygen tension of peripheral muscle tissue

To ascertain whether the improved post-treatment rheological status might not also be reflected in an improved microcirculation, muscle oxygen tension was determined before and after HELP treatment at 200 different locations in both the m.biceps bracchii and m.tibialis anterior. An approximately Gaussian distribution of pO_2 values was observed for each particular muscle and patient. The results are presented as the cumulative frequency curves for the mean pO_2 values obtained from measurements in five patients. In both the m.biceps bracchii (Fig. 4a) and m.tibialis anterior (Fig. 4b) HELP treatment caused a significant shift ($P < 0{\cdot}05$, Kolmogoroff-Smirnov test) in the cumulative frequency curves to higher pO_2 values. This shift was somewhat more pronounced for the m.biceps bracchii (mean from 30 ± 4 to 37 ± 2 mmHg). In the m.tibialis anterior the mean pO_2 value increased from 27 ± 2 to 31 ± 3 mmHg. The hatched area represents the difference integral between the pre- and post-treatment curves. In the case of the m.tibialis anterior the greatest improvement was observed in the range of the lower pO_2 values the maximum differences of 16% occurring at 20 mmHg while for the m.biceps bracchii a maximum difference of 32·5% was found at 30 mmHg.

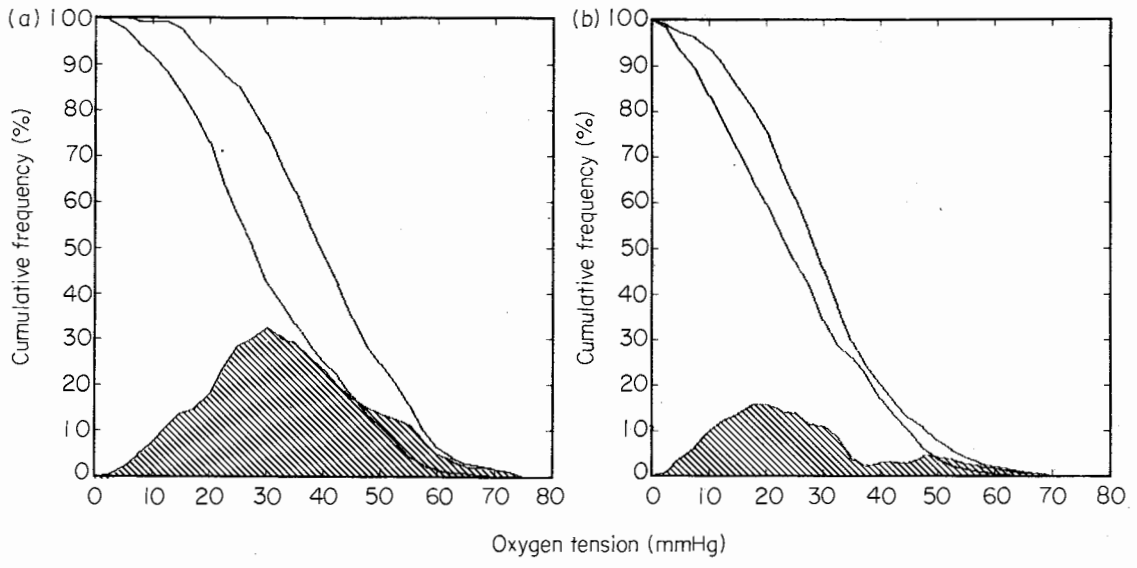

Figure 4. Cumulative frequency of muscle oxygen tension (total of 1000 single determinations) in five patients before (left curve) and after (right curve) a single HELP treatment (3000 ml plasma treated). The measurements were performed in the m.biceps bracchii (a) and the m.tibialis anterior (b). The hatched area represents the difference integral between both curves, showing the maximum different for m.biceps bracchii at pO_2 values of 30 mmHg (32·5%) and for m.tibialis anterior at pO_2 values of 20 mmHg (16%) respectively.

Discussion

The HELP procedure provides an effective alternative to conventional plasmapheresis for the treatment of familial hypercholesterolemia. It shows the same efficiency in lowering LDL plasma cholesterol but retains a high degree of specificity and has the advantage that the patients are not exposed to foreign compounds as with other LDL-apheresis techniques [12].

In contrast to other selective procedures for extracorporeal LDL elimination such as immunoadsorption with immobilized anti-LDL antibodies [15] or specific adsorption with dextran sulfate [16], HELP also effectively eliminates fibrinogen. This might be of clinical importance since there is now a large body of evidence linking elevated plasma levels of fibrinogen and an increased risk for myocardial and cerebral infarction [17–19]. Other studies have pointed out that the severity and extent of coronary lesions is correlated with the degree of fibrinogen elevation [20,21]. It is also of interest to note that FH can be associated with hyperfibrinogenemia [4].

The deposition of fibrinogen in the arterial intima is an early finding and might even precede LDL deposition [33]. Smith *et al.* [34,35] demonstrated that developing atheromatous lesions contain fibrinogen, presumably derived from the plasma, which has been converted in the plaque to fibrin. They also pointed out that fibrin accumulation is even greater than lipoprotein deposition in the fibrous plaques. It is well known that elevated fibrinogen levels increase blood viscosity [22–24] as well as inducing increased erythrocyte [25,26] and thrombocyte aggregation [36]. These properties may not only lead to an increased risk of thrombus formation but may impair the microcirculation, especially if haemodynamics are also perturbed [37].

Some of our patients, especially those with significantly impaired haemorheology have reported relief from severe angina attacks and an improved exercise capacity after a short period (6–12 weeks) on HELP treatment. Kilpatrick *et al.* [38] also noted a rapid improvement in whole blood and plasma viscosity after conventional plasmapheresis using plasma protein fractions which did not contain either fibrinogen or LDL to replace the patients own plasma. Although they were unable to relate this improvement in viscosity to an increase in lower-limb blood flow in further studies Rubba *et al.* [39] and Postiglione and Thompson [40] demonstrated a persistent improvement in arterial blood flow by venous occlusion plethysmography in FH patients after conventional plasmapheresis. Our present results confirm the beneficial effects on haemorheological parameters of eliminating both fibrinogen and LDL. Furthermore, we have also been able to show that these improvements are accompanied by an increase in muscle oxygen tension *in vivo*.

Whether coronary blood flow is increased by a reduction in viscosity is not known. Using indirect measures to estimate myocardial blood flow, Gordon *et al.* [41] found it to be correlated with plasma or blood viscosity. Cerebral blood flow does appear to be related to blood viscosity as shown by Humphrey *et al.* [42] in patients with paraproteinaemia. When the cerebral blood flow was plotted against the venous haematocrit only a weak correlation was found, whereas the flow correlated clearly to blood viscosity. The same relation has been reported for lower limb blood flow rates [43] after normovolaemic haemodilution. It is also of interest to note that in patients who have undergone vascular surgery, prolonged or excessive increases in fibrinogen levels have been shown to correlate with failure of arterial surgery [44].

Conventional methods to lower fibrinogen levels such as treatment with streptokinase or urokinase [45] or with the venom of the Malayan pit viper (Ancrod) can reduce effectively plasma fibrinogen levels, but they are limited by antibody formation and bleeding complications. Such a fibrinolytic therapy generates large amounts of fibrinogen degradation products (FDP) which are known to form fibrin monomer-fibrinogen complexes; these complexes may increase plasma viscosity in the early phase of treatment thus favouring thrombo-embolic complications [46]. FDP also influence prostaglandin metabolism, thus interfering with thrombocyte aggregation [47]. Under HELP we have found no evidence of fibrinogen degradation.

The fact that regression of atherosclerotic lesions may be induced by a dramatic reduction of circulating LDL-cholesterol has been shown recently after extracorporeal immunoadsorption [48,49] or after combined drug treatment [50]. In addition, atherosclerosis in rhesus monkeys regressed when high cholesterol diet for 2 years was followed by normal diet and LDL plasma levels returned to normal [51].

The HELP system is an efficient procedure for the treatment of CHD associated with FH. Besides its efficacy in lowering LDL-cholesterol, heparin-induced extracorporeal LDL precipitation may be helpful in the management of hyperfibrinogenemia, especially in patients who have been immunized against conventional agents. We are currently investigating the potential of this procedure which leads to a reduction in both plasma LDL and fibrinogen levels for inducing a regression of atherosclerosis in a multicentre study involving 45 patients.

Acknowledgments

The skilful technical assistance of Karin Gottsmann, Margit Stix and Karolin Fuchs is greatly appreciated.

This study was supported by the Bundesministerium für Forschung und Technologie (Förderkennzeichen 01 ZJ 8502) and the Deutsche Forschungsgemeinschaft (SFB Organprotektion, Teilprojekt A/6).

References

1 Stamler J, Wentworth D, Neaton JD, for the MRFIT Research Group. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? JAMA 1987;256:2823–8.

2 Lipid Research Clinics Program. The Lipid Research Coronary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984;251:365–74.
3 Grundy SM. Cholesterol and coronary heart disease. A new era. JAMA 1986;256:2849–58.
4 Lowe GDO, Drummond MM, Third JLHC, Bremmer WF, Forbes CD, Prentice CRM, Lawrie TDV. Increased plasma fibrinogen and platelet-aggregates in type II hyperlipoproteinaemia. Thomb Haemostas 1979;42:1503–7.
5 Goldstein JL, Brown MS. Familial hypercholesterolemia. In: Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS, eds. The metabolic basis of inherited disease. 5th ed. New York: McGraw-Hill, 1983;672–712.
6 Thompson GR, Miller JP, Brestow JL. Improved survival of patients with homozygous familial hypercholesterolemia treated with plasma exchange. Br Med J 1985;291:1671–3.
7 Council on Scientific Affairs. Current status of therapeutic plasmapheresis and related techniques. Report of the AMA Panel on therapeutic plasmapheresis. JAMA 1985;253:819–25.
8 Huestis DW. Mortality in therapeutic haemapheresis. Lancet 1983;i:1043.
9 Burstein M, Scholnick HR. On the precipitation of serum lipoproteins by heparin. Nouv Revue fr Hemat 1974;14:131–6.
10 Seidel D, Wieland H. Ein neues Verfahren zur selektiven Messung und extrakorporalen Elimination von low density Lipoproteinen. J Clin Chem Clin Biochem 1982;20:684–5.
11 Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lip Res. 1983;24:904–9.
12 Armstrong VW, Windisch M, Wieland H *et al.* Selective continuous extracorporeal elimination of low-density lipoproteins with heparin at acidic pH. Trans Am Soc Artif Intern Organs 1983;29:323–7.
13 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 HELP system. Klin Wochenschr 1987;65:161–8.
14 Eisenhauer T, Schuff-Werner P, Armstrong VW, Talartschik J, Scheler F, Seidel D. Long-term experience with the HELP system for treatment of severe familial hypercholesterolemia. ASAIO 1987;10:395–7.
15 Stoffel W, Demant T. Selective removal of apolipoprotein B-containing serum lipoproteins from blood plasma. Proc Natl Acad Sci 1981;78:611–5.
16 Yokoyama S, Hayashi R, Satani M, Yamamoto A. Selective removal of low density lipoproteins by plasmapheresis in familial hypercholesterolemia. Arteriosclerosis 1985;5:613–21.
17 Wilhelmsen L, Svärdsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Eng J Med 1984;311:501–5.
18 Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA 1987;258:1183–6.
19 Meade TW, Chakrabarti R, Haines AP *et al.* Haemostatic function and cardiovascular death: early results of a prospective study. Lancet 1980;i:1050–4.
20 Kruskal JB, Commerford PJ, Franks JJ, Kirsch RE. Fibrin and Fibrinogen-related antigens in patients with stable and unstable coronary artery disease. N Eng J Med 1987;317:1361–5.
21 Leschke M, Motz W., Blanke H., Meier M., Kaffarnik H, Strauer BE. Der prädiktive Wert von rheologischen Parametern im Vergleich zu differenzierten Lipidparametern bei der Prävalenz der koronaren Herzerkrankung. Klin Wschr 1987;64(suppl IX):1146–7.
22 Leonhardt H, Arntz HE, Klemens UH. Studies of plasma viscosity in primary hyperlipoproteinaemia. Atherosclerosis 1977;28:29–40.
23 Dintenfass L, Kammer S. Plasma viscosity in 615 subjects. Effects of Fibrinogen, globulin, and cholesterol in normals, peripheral vascular disease, retinopathy, and melanoma. Biorheology 1977;14:247–51.
24 Seplowitz AH, Chien S, Smith FR. Effects of lipoproteins on plasma viscosity. Atherosclerosis 1981;38:89–95.
25 Rampling MW, Whittingstall P, Linderkamp O. The effects of fibrinogen and its plasmin degradation products on the rheology of erythrocyte suspensions. Clin Hemorheol 1984;4:533–43.
26 Merrill EW, Gilliland ER, Margetts WG, Hatch FT. Rheology of human blood and hyperlipemia. J Appl Physiol 1964;19:493–6.
27 Amstrong VW, Seidel D. Evaluation of a commercial kit for the determination of LDL-cholesterol in serum based on precipitation of LDL with dextran sulfate. Ärztl. Lab. 1985;31:325–30.
28 Jung F, Roggenkamp HG, Schmidt J, Kiesewetter H. Das Kapillarschlauch-Plasmaviskosimeter: Methodik, Qualitätskontrolle und Referenzbereich. Biomed Technik 1985;30:152–8.
29 Kiesewetter H, Radtke H, Schneider R, Mußler K, Scheffler A, Schmid-Schönbein H. Das Mini-Erythrozyten-Aggregometer: Ein neues Gerät zur schnellen Quantifizierung des Ausmaßes der Erythrozytenaggregation. Biomed Technik 1982;27:209–13.
30 Schmid-Schönbein H, Gallasch G, Volger E., Klose HG. Microrheology and protein chemistry of pathological red cell aggregation (blood sludge) studied *in vitro*. Biorheology 1973;10:213–27.
31 Fleckenstein W, Heinrich R, Kersting T, Schomerus H, Weiss C. A new method for the bedside recording of tissue pO_2-histograms. Verh Dtsch Ges Inn Med 1984;90:439–43.
32 Kersting T, Reinhart K, Fleckenstein W, Dennhardt R, Eyrich K, Weiss C. The effect of dopamin on muscle pO_2 in healthy volunteers and intensive care patients. Europ J Anaesthesiol 1985;2:143–53.
33 Sadoshima S, Tanaka K. Fibrinogen and low density lipoprotein in the development of cerebral atherosclerosis. Atherosclerosis 1979;34:93–103.
34 Smith EB, Alexander KM, Massie IB. Insoluble 'fibrin' in human aortic intima. Atherosclerosis 1976;23:19–39.
35 Smith EB. Fibrinogen, fibrin and fibrin degradation products in relation to atherosclerosis. In: Fidge NH, Nestel PJ, eds. Atherosclerosis VII. Amsterdam: Excerpta Medica, 1986;459–62.
36 Peerschke EI, Zucker MB, Grant RA, Egan JJ, Johnson MM. Correlation between fibrinogen binding to human platelets and platelet aggregability. Blood 1980;55:841–7.
37 Schmid-Schönbein H. Interactions of vasomotion and blood rheology in haemodynamics. In: Lowe GDO, Barbenel JC, Forbes CD, eds. Clinical aspects of blood viscosity and cell deformability. Berlin: Springer, 1981;49–66.
38 Kilpatrick D, Fleming J, Clyne C, Thompson GR. Reduction of blood viscosity following plasma exchange. Atherosclerosis 1979;32:301–6.
39 Rubba P, Postiglione A, Scarpato N, Iannuzzi A, Mancini M. Improved reactive hyperemia test after plasma exchange in familial hypercholesterolemia. Atherosclerosis 1985;56: 237–42.
40 Postiglione A, Thompson GR. Experience with plasma-exchange in homozygous familiar hypercholesterolaemia. In: Widhalm K, Naito HK, eds. Detection and treatment of lipid and lipoprotein disorders of childhood. New York: AR Liss, 1985;213–20.
41 Gordon RJ, Snyder GK, Tritel H, Taylor WJ. Potential significance of plasma viscosity and haematocrit variations in myocardial ischaemia. Am Heart J 1974;87:175–82.
42 Humphrey PRD, Du Boulay GH, Marshall J *et al.* Viscosity, blood flow and haematocrit in patients with paraproteinaemia. Acta Neurol Scand 1980;61:201–9.
43 Yates CJP, Andrews V, Berent A, Dormandy JA. Increase in leg blood-flow by normovolaemic haemodilution in intermittent claudication. Lancet 1979;ii:166–8.
44 Postlethwaite JC, Goyle KB, Dormandy JA, Hynd JW. Improvement in experimental vascular graft patency by controlled defibrinogenation. Br J Surg 1977;64:28–30.
45 Marsh N. Fibrinolysis. Chichester: John Wiley & Sons, 1981;152–84.
46 Ernst E. Therapeutic defibrinogenation in peripheral vascular disease. Inter Angio 1985;4:373–7.
47 Watanabe K, Ishida T, Yoshitomi F, Tanaka K. Fibrinogen degradation products influence PGI_2 synthesis by cultured

porcine aortic endothelia and smooth muscle cells. Atherosclerosis 1984;51:151–61.
48 Hombach V, Borberg H, Gadzkowski A, Oette K, Stoffel W. Regression der Koronarsklerose bei familiärer Hypercholesterinämie IIa durch spezifische LDL-Apherese. Dtsch Med Wschr 1986;111:1709–15.
49 Saal SD, Parker TS, Gordon BR *et al.* Removal of low-density lipoproteins in patients by extracorporeal immunoadsorption. Am J Med 1986;80:583–9.
50 Blankenhorn DH, Nessim SA, Johnson RL, Sanmarco ME, Azen SP, Cashin-Hemphill L. Beneficial effects of combined cholestipolniacin therapy on coronary atherosclerosis and coronary venous bypass grafts. JAMA 1987;257:3233–40.
51 Eggen DA, Strong JP, Newman WP, Malcom GT, Restrepo C. Regression of experimental atherosclerosis lesions in rhesus monkeys consuming a high saturated fat diet. Arteriosclerosis 1987;7:125–34.