Journal of Clinical Apheresis 4:78–81 (1988)

Removal of Low-Density Lipoproteins (LDL) and Fibrinogen by Precipitation With Heparin at Low pH: Clinical Application and Experience

D. Seidel, V.W. Armstrong, P. Schuff-Werner, and T. Eisenhauer

Department of Clinical Chemistry and Medicine, University Hospital Göttingen, Federal Republic of Germany

The clinical application of a new extracorporeal procedure (HELP) for the selective elimination of low-density lipoproteins and fibrinogen by heparin precipitation at acid pH is described. Plasma, obtained by filtration of whole blood through a 0.2 μm filter is continuously mixed with an equal volume of an acetate buffer (pH 4.85) containing heparin. After removal of the precipitated heparin complex by filtration, excess heparin is adsorbed to a specially developed filter, and the clear plasma filtrate is subject to bicarbonate dialysis/ultrafiltration to restore physiologic pH and remove excess fluid. The calculated efficiency for the elimination of low-density lipoproteins and fibrinogen from plasma by HELP is 100% and is therefore comparable to conventional plasmapheresis. However, the HELP system shows a high degree of specificity, with over 80% of total protein being returned to the patient. A total of over 350 treatment procedures have now been performed. Patient compliance and acceptance have been excellent, and no major complications have been observed. The system is therefore suitable for the treatment of severe hyper-β-lipoproteinemia; its use for the treatment of coronary heart disease is currently under investigation in a prospective multicenter study in which treatment efficiency will be controlled by coronary angiography on 45 patients treated with HELP over a period of 2 years.

Key words: hypercholesterolemia, extracorporeal plasma treatment, LDL-apheresis, fibrinogen

INTRODUCTION

Familial hypercholesterolemia is inherited as an autosomal dominant disease. The extensive studies by Goldstein and Brown and their colleagues [1] have revealed that this disorder is caused by mutations in the gene encoding the low-density lipoprotein (LDL) receptor. In its rare homozygous form, cholesterol levels are massively elevated, due to the elevation of LDL cholesterol in plasma; they frequently reach values of 1,000 mg/dl or more. Severe atherosclerosis generally develops in childhood, and death from myocardial infarction occurs before the age of 30. In its heterozygous form (1:500), subjects only have one deficient gene and total LDL receptor activity is approximately 50% of normal. Cholesterol levels are in the range of 300–500 mg/dl, and coronary artery disease usually occurs in the fourth decade of life.

Treatment of familial hypercholesterolemia by diet and drug therapy alone is often ineffective. Encouraging results have, however, been obtained in the treatment of this disorder by plasma exchange [2, 3].

Conventional plasma exchange requires replacement of the patient's own plasma with a donor plasma or, more usually, a plasma protein fraction. This can cause problems due to the introduction of foreign protein and the transmission of infectious diseases. Ways have therefore been sought to selectively eliminate LDL followed by the return of the patient's own plasma. We have now developed a procedure for the continuous elimination of LDL and fibrinogen from plasma based on their precipitation at low pH in the presence of heparin [4–7]. This procedure hase been named HELP: heparin-induced extracorporeal LDL precipitation.

PROCEDURE FOR HELP

The flow scheme for HELP is illustrated in Figure 1. Plasma is obtained by filtration of whole blood through a 0.2-μm filter. This is then mixed continuously with a 0.3 M acetate buffer (pH 4.85) containing 100 IU/ml of heparin. The flow rates of plasma and buffer are normally identical and are in the range of 20–30 ml/min. Precipitation occurs at a final pH of 5.12 in a precipitation chamber, after which the suspension is recirculated through a 0.4-μm polycarbonate membrane filter. An LDL- and fibrinogen-free filtrate is obtained from this filter, which is then passed through a heparin adsorber to remove excess heparin. This adsorber is an ion exchange resin capable of completely binding the polyanion hepa-

Address reprint requests to Dr. D. Seidel, Dept. of Clinical Chemistry and Medicine, University Hospital Göttingen, Robert-Koch-Str. 40, 3400 Göttingen, FRG.

rin at a pH of 5.12; plasma proteins are not retained at this pH. Finally, the buffer-plasma mixture is subject to bicarbonate dialysis/ultrafiltration to restore physiologic pH and remove excess acetate and water before the LDL- and fibrinogen-free plasma is mixed with the blood cells from filter 1 and returned to the patient. The various parts of this procedure (tubing filters, etc.) are all sterile, disposable systems intended for single use only.

The total extracorporeal volume of the system, excluding filter 1 and the blood tubing, amounts to approximately 1,100 ml, of which half will be plasma and half buffer during treatment. For the treatment of a homozygous child, we alter the ratio of buffer to plasma to 3:1 in order to reduce the total amount of plasma in the extracorporeal system. The buffer heparin concentration is then lowered from 100 to 50 IU/ml. Prior to each treatment the system is washed and primed with an isotonic salt solution. Patients are therefore not subjected to fluid depletion at the start of each HELP treatment. For one treatment (usual plasma volume 3,000 ml), approximately 2 hours are required, and patients can be treated on an outpatient basis under well-standardized conditions, providing reproducible results with regard to LDL and fibrinogen elimination.

TABLE I. Efficiency of Elimination of Various Plasma Lipoproteins and Proteins by HELP

Lipoprotein/protein	Efficiency %
LDL cholesterol	102 ± 13
Fibrinogen	97 ± 15
C-4 complement	97 ± 24
Plasminogen	84 ± 16
C-1 esterase inhibitor	82 ± 18
C-3 complement	59 ± 19
AT-III	44 ± 13
Total protein	22 ± 9
HDL cholesterol, albumin, IgG, IgA, IgM, transferrin, ferritin	< 18

EFFICIENCY AND SPECIFICITY OF HELP

The aim of any specific elimination procedure must be to achieve 100% elimination of the protein or lipoprotein in question. Random sampling during HELP after the precipitation filter (filter 2) revealed that the plasma was virtually free of LDL and fibrinogen.

It is important to note that fibrinogen is removed as effectively as LDL by HELP. The reduction in mean fibrinogen levels may indeed be highly beneficial, since it will lower the viscosity of the blood and may therefore improve perfusion of tissues in severe atherosclerotic vascular damage. Furthermore, fibrinogen and, in particular, its degradation products can both inhibit PGI_2 synthesis by endothelial and vascular smooth muscle cells [8], thereby facilitating platelet aggregation; they can also cause injury to endothelial cells [9]. Together with LDL, fibrinogen probably plays an important role in atherogenesis, and since it is often elevated in hypercholesterolemia it deserves therapeutic attention.

The data in Table I, in which the efficiency is expressed as the percent elimination of each protein or lipoprotein, were derived from 25 individual HELP procedures. The findings were generally in agreement with the results from in vitro studies.

LDL cholesterol, together with fibrinogen and C-4 complement, were quantitatively eliminated during HELP. Plasminogen was also precipitated to a large extent, whereas C-3 complement and AT-III were removed to a lesser extent. Other proteins investigated decreased only marginally during HELP.

CLINICAL APPLICATION OF HELP

At present, five patients with familial hypercholesterolemia, one homozygous child and four heterozygous adults, have been treated by HELP, for a total of ca. 300 treatment procedures (Table II). The frequency of treatment has averaged once every 9 days. Average pretreatment LDL cholesterol levels have been reduced by ca.

TABLE II. Average Pre- and Post-treatment LDL, and HDL Cholesterol and Fibrinogen Levels During HELP Therapy*

	Treatments		LDL cholesterol (mg/dl)				HDL cholesterol (mg/dl)			Fibrinogen (mg/dl)			
Patient	No.	Duration (weeks)	Before therapy	Pre-treatment	$\bar{X}$	Post-treatment	Before therapy	Pre-treatment	Post-treatment	Before therapy	Pre-treatment	$\bar{X}$	Post-treatment
J.C.	36	44	796	421	(298)	175	22	22	14	375	321	(240)	158
E.S.	64	72	323	212	(140)	67	44	54	46	395	256	(180)	103
H.E.	50	68	365	261	(178)	95	60	67	58	410	282	(207)	132
P.K.	41	56	252	157	(117)	76	50	48	45	360	283	(220)	157
C.K.	36	42	240	157	(100)	42	50	45	38	260	209	(147)	84

*Concentrations presented for LDL, HDL, and fibrinogen are mean values; $\bar{X}$ = represents the calculated mean between the values before and after the treatments.

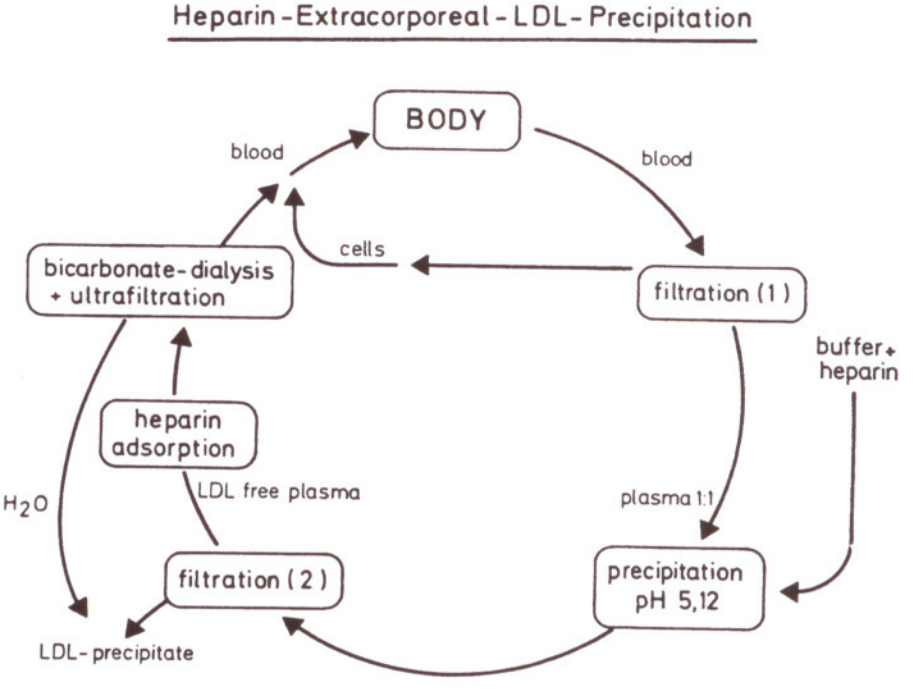

Fig. 1. Flow scheme of HELP.

50%, compared with the values prior to initiating therapy. The mean LDL cholesterol concentration to which the vascular wall is exposed will, of course, be even lower (ca. 40%), since the posttreatment values are as low as 22% compared with the values prior to the start of the HELP therapy. The data for fibrinogen are very similar to the LDL values. Plasma HDL concentrations are unaffected by the HELP procedure.

Despite long-term intensive treatment, pretreatment values of plasminogen, C-4 complement, C-3 complement, and C-1 esterase inhibitor have remained stable, indicating that treatment does not lead to a deficiency of these proteins. In the case of proteins that are not specifically precipitated by heparin at low pH, plasma concentrations at the end of HELP were generally in the range of 80-90% of the initial value. Samples taken 24 h after the end of treatment showed that these proteins have retained their original level.

We have as yet observed no ill-effects due to the HELP-therapy.

Special attention has been focused on the effect of HELP on hemostasis. Mean thrombin times and partial thromboplastin times at the end of a treatment procedure were 29.9 sec and 70.6 sec respectively, values that are typical for such extracorporeal procedures, in which heparin is employed as an anticoagulant. Plasma heparin levels at the end of treatment averaged 0.17 IU/ml. No bleeding complications have been observed. Plasma electrolyte concentrations were unaffected by HELP, and hematologic parameters were virtually unchanged at the end of each treatment and after more than 70 weeks of treatment.

Overall treatment tolerance has been very good, and no major complications have been observed. Minor complications have included moderate chills and transient shivering (n = 3 out of more than 300). No alterations in pulse rate, blood pressure, or body temperature were observed.

The present HELP procedure provides an alternative to conventional plasmapheresis for the treatment of familial hypercholesterolemia with the additional effect of lowering fibrinogen. While showing the same efficiency as the latter, it retains a high degree of specificity and has the advantage that the patient is not exposed to foreign proteins, with their attendant immunological problems. Furthermore, it displays a high degree of reproducibility, which should allow a consistent therapy independent of the clinic performing the treatment. Its use for the treatment of coronary heart disease is currently under investigation in a prospective multicenter

study in which treatment efficiency will be controlled by coronary angiography on 45 patients treated with HELP over a period of 2 years.

REFERENCES

1. 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.
2. Thompson GR: Plasma exchange for hypercholesterolemia. Lancet 1:1246–1248, 1981.
3. Thompson GR, Miller JP, Breslow JL: Improved survival of patients with homozygous familial hypercholesterolemia treated with plasma exchange. Br Med J 291:1671–1673, 1985.
4. Seidel D, Wieland H: Ein neues Verfahren zur selektiven Messung und extrakorporalen Elimination von low density Lipoproteinen. J Clin Chem Clin Biochem 20:684–685, 1982.
5. Armstrong VW, Windisch M, Wieland H, Fuchs C, Rieger J, Köstering H, Nebendahl K, Scheler F, Seidel D: Selective continuous extracorporal elimination of low-density lipoproteins with heparin at acidic pH. Trans Am Soc Artif Intern Organs 29:323–327, 1983.
6. 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 65:161–168, 1987.
7. Armstrong VW, Eisenhauer T, Noll D, Wieland H, Scheler F, Seidel D: Extracorporal plasma therapy: The HELP system for the treatment of hyper-β-lipoproteineima. In Vidhalm K, Naito HK (eds): Recent Aspects of Diagnosis and Treatment of Lipoprotein Disorders. Impact on Prevention of Atherosclerotic Diseases. Alan R. Liss, Inc., pp. 327–335.
8. Watanabe K, Ishida T, Yoshitami F, Tanaka K: Fibrinogen degradation products influence PGI_2 synthesis by cultured porcine aortic endothelial and smooth muscle cells. Atherosclerosis 51:151–161, 1984.
9. Watanabe K, Tanaka K: Influence of fibrin fibrinogen degradation products on cultured endothelial cells. Atherosclerosis 48:57–70, 1983.