epithelial cells, sensitisation could not be induced by co-culturing with amniotic cells from the original donor.

Furthermore, serum samples collected 1 month after implantation from these four volunteers were found by immunofluorescence not to react against the amniotic cells cultured in vitro, although the cells continued to express the specific antigen.

Although our results show no acute rejection of the amniotic epithelial cells, further studies are needed to establish whether these cells can be successfully transplanted without immunosuppression to treat patients with appropriate inborn errors of metabolism.

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Requests for reprints should be addressed to M. A., Paediatric Research Unit, Guy's Hospital Medical School, Guy's Tower, London Bridge, London SE1 9RT.

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APPLICATION OF SPECIFIC EXTRACORPOREAL REMOVAL OF LOW DENSITY LIPOPROTEIN IN FAMILIAL HYPERCHOLESTEROLAEMIA

W. STOFFEL H. BORBERG
V. GREVE

Institut für Physiologische Chemie und Medizinische Klinik der Universität zu Köln, West Germany

Summary A highly selective method for the removal of apolipoprotein-B-containing serum lipoproteins (low density lipoproteins [LDL] and very-low-density lipoproteins [VLDL]) from hypercholesterolaemic patients by immunoadsorption in an extracorporeal system consists of separation of plasma from the blood cells by a blood-separation centrifuge, followed by the selective adsorption of LDL from the plasma on anti-LDL-'Sepharose'. The LDL-free plasma effluent, from which high-density lipoproteins (HDL) and other plasma proteins are not removed, is returned to the patient with the blood cells. Three patients with familial hypercholesterolaemia, one homozygous and two heterozygous, were treated repeatedly during a period of 9 months. No undesirable side-effects or changes in clinical, chemical, haematological, or immunological parameters have yet been observed. The new procedure has several advantages over treatments currently used; it is non-invasive, more specific, and less costly and lowers LDL to a greater degree.

Introduction

WE have demonstrated that apolipoprotein-B-containing lipoproteins (low-density lipoproteins [LDL] and very-low-density lipoproteins [VLDL]) can be removed specifically and efficiently in vitro and in vivo from pig blood by a combination of extracorporeal plasma separation and LDL immunoadsorption.[1] This technique has been applied over the past 9 months to three patients with familial hypercholesterolaemia; one patient was homozygous and two were heterozygous.

The efficiency and safety of extracorporeal removal of LDL are described here, and its advantages over plasma exchange (plasmapheresis) in the treatment of patients with familial hypercholesterolaemia are summarised. Plasma exchange treatment has been discussed by Thompson.[2]

Patients and Methods

Patients

Patient 1 was a 28-year-old male heterozygote. His body weight was 73 kg and he underwent treatment for 3 months. Patient 2 was a 37-year-old female heterozygote who weighed 68 kg and underwent treatment for 9 months, and patient 3 was a 15-year-old homozygous boy whose body weight was 47 kg. His period of treatment was 3 months.

Methods

The chemical procedures for the measurement of lipids and lipoproteins have been described previously.[1] Human LDL was purified by standard procedures;[3] it was proved to be homogeneous by agarose electrophoresis,[4] immunodiffusion,[5] and immunoelectrophoresis.[6] Human LDL was covalently bound to 'Sepharose' Cl-4B for mass production of monospecific antibodies against human LDL from sheep immune serum. Anti-LDL was coupled to cyanogen-bromide-activated sepharose Cl-4B as previously described.[1] For the treatment of patients the following changes to the published procedure were made: two silanised, glass columns (internal diameter, 10 cm; length, 8 cm) with sintered glass filters (pore size, 20–40 μm) were each packed with 400 ml anti-LDL-sepharose. The blood flow-rate was adjusted to 60–80 ml/min, and the plasma flow-rate during treatment was adjusted to approximately 30 ml/min. Coagulation was prevented by treatment with heparin (input rate, 40 units/min; not more than 10 000 units per treatment) or a combination of heparin (2500 units before the run) and citrate (acid-citrate-dextrose, United States Pharmacopeia formula B) added to blood in a ratio 1:8. The two columns were used in rotation; while LDL was being desorbed from one column (out of the circuit) the other (in circuit) served as immunoadsorbent. Carrot juice was given to patients the evening before treatment so that movement of LDL through the column could be monitored by the orange colour of carotenes in the LDL. The dead volume of the entire system, consisting of an IBM continuous-flow blood-cell-separator centrifuge (model IBM 2997), tubing, and anti-LDL column volumes, was about 450–500 ml. Before switching columns from adsorption to desorption, the plasma in the dead volume of the LDL-loaded column was displaced into the patient with 400–500 ml saline. LDL-desorption was performed with 1 mol/l glycine/HCl buffer pH 2·8–3·0, as previously described. The working capacity of each column was about 2 g total LDL-cholesterol (or about 1 g apolipoprotein-B or 4 g LDL). During treatment the first immunoadsorbent column was saturated within about 30 min, the second and third in about 45 min, and the fourth in 60 min.

Two columns were assigned to each patient and were used in successive treatments; some have been in use for 9 months without any loss of LDL-binding capacity. Between treatments the columns were stored at 4°C under 0·02% NaN_3 in saline saturated with chloroform. The columns were rinsed thoroughly with saline (3–4 litres) before use.

Strict measures were taken to ensure sterility. Before each treatment the saline-wash was tested for bacterial contamination and for pyrogens (rabbit and *Limulus* test). All patients were repeatedly tested by the passive anaphylaxis test[7] for human anti-sheep-immunoglobulin antibodies, and hepatitis A and B antigens. Informed consent was obtained in accordance with regulations set by the ethics commission of the medical faculty of the University of Cologne.

Results

The zygosity of the patients was determined by examination of the family trees and by receptor-testing the cultured fibroblasts of patients. The cultured fibroblasts of the siblings of patient 2 and the parents of patient 3 were also tested. All three patients were resistant to the usual treatments (lipid-lowering diets and drugs). Treatment intervals varied from 1 to 5 weeks at the convenience of the patients, but there was a tendency for cholesterol levels to decrease most when treatments were carried out at 2-week intervals. The percentage removal of LDL-cholesterol ranged from 54 to 76% (see table).

No side-effects such as fever, chills, syncope, arrhythmias, or electrocardiographic changes, were observed in any of the three patients. Routine tests of blood chemistry showed no changes, and there were no changes in concentrations of the cellular elements of the blood.

Fig. 1 presents the time-course data for changes in concentrations of total cholesterol, LDL plus VLDL cholesterol, and total protein during a single LDL-removal treatment lasting 3 h in patient 2. Total cholesterol was reduced from 470 mg/dl to 112 mg/dl, whereas HDL concentrations (not given) did not change. The temporary changes in total protein concentration were probably caused by dilution by infusion to the patient of the anticoagulant-containing saline in the dead volume of the system (about 450–500 ml, approximately 10% of the plasma volume). The total protein concentration returned to the original levels within 2–4 h of the treatment.

CLINICAL DATA AND RESULTS

Patient	Total plasma cholesterol levels					Removed‡		
	Steady state* (mg/dl)	Before treat-ment† (mg/dl)	After treat-ment (mg/dl)	Mean between treat-ments (mg/dl)	Interval between treat-ments (weeks)	LDL (g)	Choles-terol (g)	Re-moved choles-terol (%)¶
1	260±30	260	115	203	3	9	4·4	56
		290	130	176	2	9·5	4·8	55
		221	99	169	3	7·5	3·6	55
		238	108			7·5	3·6	55
2	470±15	472	112	223	2	14	7·0	76
		334	153	283	4	11	5·3	54
		412	180	285	3	15	7·3	56
		390	138	247	1	11	5·3	65
		355	113	241	2	11·5	5·7	68
		368	146	251	2	10	5·0	60
		356	133	251	2	12	6·3	63
		369	158	241	2	10	4·7	57
		324	119	207	1	11	5·4	63
		294	95	197	1	9	4·3	68
		298	112	278	5	7·5	3·7	62
		443	164			13	6·4	63
3	500±20	448	171	228	0·5	11	5·3	62
		285	88	183	2	8	3·9	69
		278	103	150	0·5	10	4·9	63
		196	53			6	3·0	74

*Lowest level under medical management. †Immediately before extracorporeal removal of LDL. ‡LDL and cholesterol concentrations recovered from the columns upon regeneration of the immunoadsorbent. ¶Removed cholesterol as a percentage of total cholesterol before treatment.

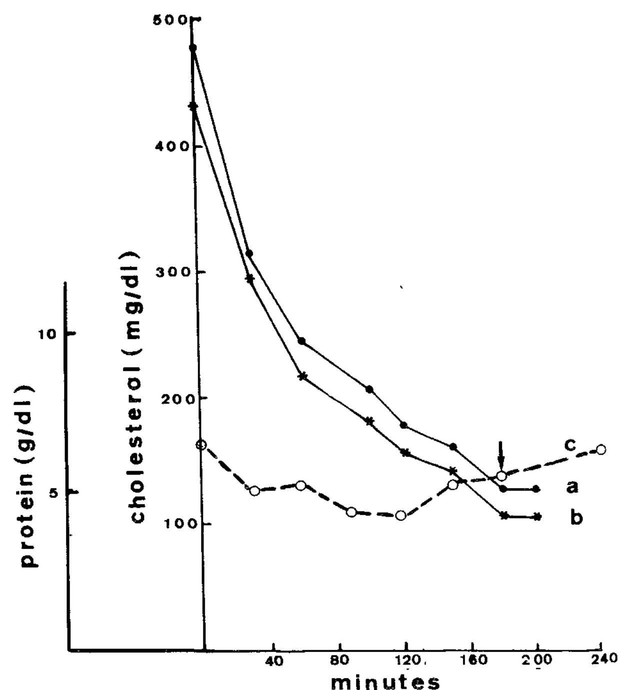

Fig. 1—Time-course of lipid and protein changes during extracorporeal removal of LDL in patient 2.

a = total cholesterol; b = LDL plus VLDL cholesterol; c = plasma protein. An arrow marks termination of treatment. Plasma samples were taken from the plasma line before entering the immunoadsorption column.

Fig. 2 shows the changes seen in two successive LDL-removal treatments of patient 2. After the first treatment, the total plasma cholesterol rose gradually to a new plateau in 8–10 days, after which the second treatment was carried out. Changes in the concentrations of HDL-cholesterol, triglyceride, and protein were acute and reversible and probably due to dilution.

Discussion

Plasma exchange, first introduced in 1975,[8] has been used to treat homozygous and heterzygous familial hyper-cholesterolaemia and has been found to be an effective alternative[9–13] to surgical treatment by portacaval shunt or partial ileal bypass.[3,14,15] The new approach described here offers the important advantage of the selective removal of LDL without removal of HDL, plasma proteins, or plasma solutes. We suggest the term "LDL,-apheresis" for the

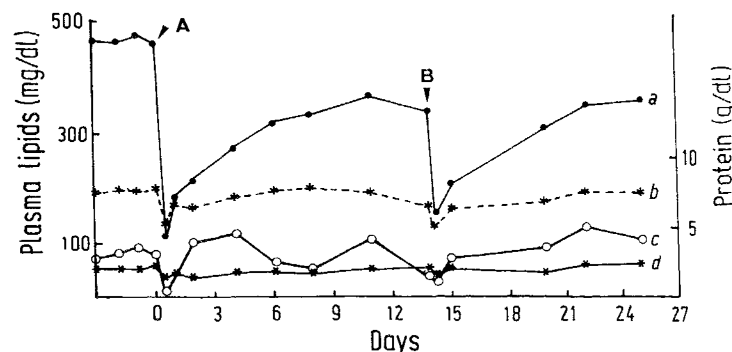

Fig. 2—Immediate and longer-term effects of extracorporeal removal of LDL in patient 2.

A and B are consecutive treatments, separated by 14 days. a = total cholesterol; b = total protein; c = triglycerides; d = HDL-cholesterol.

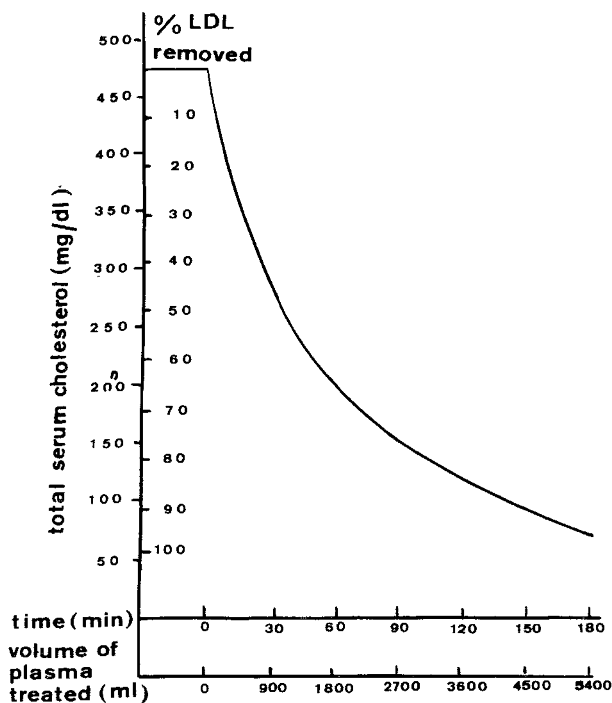

Fig. 3—Absolute concentration change and percentage removal of LDL-cholesterol.

Percentage removal was calculated on basis of no change in plasma volume during a single extracorporeal removal of LDL treatment and a plasma flow rate of 30 ml/min. The volume of plasma passed through the immunoadsorbent column is shown at the bottom of the figure.

technique. Extensive testing of the technique in vitro and in animals[1] demonstrated that it is more effective and more simple than the heparin-agarose affinity-column chromatography for LDL and HDL that has been used in dogs[16] and in man.[17-19] Since no foreign plasma constituents are required for maintenance of volume and osmotic pressure, our technique eliminates the possibility of transfer of infectious material, particularly hepatitis virus, which is a risk in any plasma substitution method.

The LDL-binding capacities of the anti-LDL-sepharose columns assigned to individual patients have not changed during up to 9 months' use. We have not observed damage to red blood cells or platelets, disturbances of electrolytes, infections, fever, sensitivity, or complement activation.

Several technical improvements should be sought in the future—e.g., lowering the dead volume of the system, increasing the speed of treatment, and regenerating the immunoadsorbent column automatically.

From a metabolic viewpoint, this technique must be evaluated in terms of changes in cholesterol pool sizes and cholesterol efflux rates. The ratio of free to total plasma cholesterol determined by the activity of lecithin-cholesterol acyl-transferase must be defined, as must erythrocyte and platelet functions. It is most important, however, to find out whether it leads to clinical changes such as regression of xanthomata, physiological changes in cardiac function, and morphological changes in occlusion of major arteries in patients with severe arteriosclerotic disease. The evidence for these signs of reversal of the atherosclerotic process in patients with long-standing hypercholesterolaemia has not yet been convincing with any mode of management. Our technique offers an attractive and feasible test of the lipid hypothesis.[20-23]

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Requests for reprints should be addressed to W. S., Institut für Physiologische Chemie der Universität Köln, Joseph-Stelzmann Str. 52, 5 Köln 4(Lindenthal), West Germany.

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"Mr Hobbs told me that the cause of his Lordship's death was trying an Experiment; viz. as he was taking the aire in a Coach with Dr Witherborne (a Scotchman, Physitian to the King) towards High-gate, snow lay on the ground, and it came into my Lord's thoughts, why flesh might not be preserved in snow, as in Salt. They were resolved they would try the Experiment presently. They alighted out of the Coach and went into a poore woman's house at the bottom of Highgate hill, and bought a Hen, and made the woman exenterate it, and then stuffed the body with Snow, and my Lord did help to doe it himselfe. The Snow so chilled him that he immediately fell so extremely ill, that he could not returne to his Lodging (I suppose then at Graye's Inne) but went to the Earle of Arundel's house at High-gate, where they putt him into a good bed warmed with a Panne, but it was a damp bed that had not been layn-in in about a yeare before, which gave him such a colde that in 2 or 3 dayes as I remember Mr Hobbes told me, he dyed of Suffocation."—Aubrey's Brief Lives (Frances Bacon: Viscount St Albans), edited by O. LAWSON DICK. Harmondsworth: Penguin Books, 1978, p. 179.