Artificial Organs
26(2):133–139, Blackwell Publishing, Inc.

Low-Density Lipoprotein Apheresis: Clinical Results with Different Methods

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Abstract: In 40 patients (22 women, 18 men) suffering from familial hypercholesterolemia resistant to diet and lipid lowering drugs, low-density lipoprotein (LDL) apheresis was performed over 84.9 ± 43.2 months. Four different systems (Liposorber, 28 of 40, Kaneka, Osaka, Japan; Therasorb, 6 of 40, Baxter, Munich, Germany; Lipopak, 2 of 40, Pocard, Moscow, Russia; and Dali, 4 of 40, Fresenius, St. Wendel, Germany) were used. With all methods, average reductions of 50.6% for total cholesterol, 52.2% for LDL, 64.3% for lipoprotein (a) (Lp[a]), and 43.1% for triglycerides, and an average increase of 10.3% for high-density lipoprotein (HDL) were reached. Severe side effects such as shock or allergic reactions were very rare (0.5%) in all methods. In the course of treatment, an improvement in general well being and increased performance were experienced by 39 of 40 patients. Assessing the different apheresis systems used, at the end of the trial, there were no significant differences with respect to the clinical outcome experienced with the patients' total cholesterol, LDL, HDL, and triglyceride concentrations. However, to reduce high Lp(a) levels, the immunoadsorption method with special Lp(a) columns (Lipopak) seems to be most effective: –59% versus –25% (Kaneka) – (Baxter), and –29% (Dali). The present data demonstrate that treatment with LDL apheresis of patients suffering from familial hypercholesterolemia resistant to maximum conservative therapy is very effective and safe even in long-term application. **Key Words:** Familial hypercholesterolemia—Low-density lipoprotein apheresis—Liposorber system—Immunoadsorption—Lipoprotein (a) apheresis—Dali system—Coronary heart disease.

High serum concentrations of low-density lipoprotein cholesterol (LDL-C) increase the risk of the development and progression of coronary heart disease (CHD) (1–6). In recent years the mode of interaction of cholesterol bearing lipoproteins with cells has been elucidated. It is suggested that elevated lipid concentrations in the serum lead to their accumulation in the intima of arteries which results in the development of atherogenic plaques. These alterations seem to be accompanied by changes in vessel tone and endothelial regulation (7–23).

More recent studies have demonstrated the termination of progression and even regression of coronary atherosclerosis as a consequence of lipid lowering intervention (13–19). With the introduction of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, definitive reductions in serum cholesterol levels could be achieved (24–36). However, in some patients cholesterol levels cannot be controlled adequately by appropriate diet and maximal drug therapy. All patients with homozygous familial hypercholesterolemia (FH) and patients with severe heterozygous FH belong to this specific group (23,37,38).

Surgical procedures such as portocaval shunt, ileal bypasses, and liver transplantation can be used to circumvent the disstrous effects of severe hypercholesterolemia, but these measures are associated with a high rate of severe complications and a high morbidity rate (39–41).

Plasma exchange, first described by De Gennes (42) and first used clinically by Thompson (43), is commonly used in these patients. Using plasma exchange implies nonspecifically removing all important and toxic plasma components. A recent development in this field is the introduction of several semiselective and selective LDL apheresis techniques that are far more selective than the plasma exchange procedure (44–50).

Four different LDL apheresis systems were used in our study: dextran sulfate adsorption for 28 of 40 (Liposorber, Kaneka, Osaka, Japan), immunoad-

Received March 2001; revised May 2001.
Presented in part on the 65th birthday of Prof. Dr. Horst Klinkmann, on May 7, 2000, Rostock, Germany.
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sorption for 6 of 40 (Therasorb, Baxter, Munich, Germany), LDL hemoperfusion for 4 of 40 (Dali, Fresenius, St. Wendel, Germany), and the immunoadsorption system with special anti-lipoprotein (a) (anti-Lp[a]) columns for 2 of 40 patients (Lipopak, Pocard, Moscow, Russia).

The purpose of the present investigation was to assess the efficacy of regular long-term treatment with LDL apheresis in primary and secondary prevention of atherosclerosis with its complications of coronary HD, stroke, and peripheral vascular disease. Furthermore, the clinical utility and safety of different apheresis methods were evaluated.

PATIENTS AND METHODS

Since 1989, a total of 40 patients suffering from severe FH (4 homozygous and 36 heterozygous FH) were treated with LDL apheresis, diet, and lipid lowering drugs for 84.9 ± 43.2 months (range 1–121 months). Before LDL apheresis, all patients were treated with diet and lipid lowering drugs. At the onset of the trial, 38 of 40 patients were regarded as refractory to conventional therapy measurements because the goal of treatment, lipoprotein levels according to the guidelines recommended by the European Atherosclerosis Society (52), was not reached even after treatment periods of up to 6 years (range 2–6 years). In 5 of 40 patients, use of lipid lowering drugs was discontinued as a result of severe side effects (constipation, diarrhea, gastrointestinal discomfort, vomiting, and nausea).

Table 1 shows the characteristics of the 40 patients studied. Before applying LDL apheresis, 38 of 40 patients had CHD with severe angina pectoris symptoms, a history of myocardial infarction, or coronary artery bypass graft (CABG).

CHD was diagnosed after a history of myocardial infarction substantiated by electrocardiogram (ECG, Minnesota Rating Scale 1.2–2 [52]), creatine kinase isoenzymes, or by coronary angiography. Further, CHD was diagnosed by an ECG showing signs of possible myocardial ischaemia (Minnesota Rating Scale 1.3, 4.1–4, 5.1–3, or 7.1 [52]) or by coronary angiography if one of the epicardial vessels containing stenosis measured a 50% or greater loss in diameter or 3 vessels had stenoses of at least 30%. Manifestations of peripheral vascular and cerebrovascular disease were taken with a history of transient ischemic attack, stroke, intermittent claudication, rest pain, limb amputation, or arterial surgery. To assess improvement in symptoms of CHD, the World Health Organization (WHO) chest pain questionnaire was used (53). Systolic and diastolic blood pressure were measured on the left or the right arm with the subject seated for 5 to 10 min and the arm at heart level. Arterial hypertension was defined as blood pressure of 160/90 mm Hg or more or normotension under antihypertensive treatment. Diabetes mellitus was diagnosed according to the criteria of WHO (54). Body mass index (BMI) was calculated as the square of weight (in kilograms) per height (in meters). Smokers were defined as persons smoking more than 1 cigarette per day for at least 1 year before the beginning of the study. All other patients were classified as nonsmokers. Smoking habits were recorded by interview.

TABLE 1. *Main clinical features of the 40 patients studied treated by LDL apheresis*

	n
Coronary heat disease	38
Myocardial infarction	34
Percutaneous transluminal angioplasty	35
Corona-arterio-venous bypass	9
Stroke	8
Claudication	37
Arterial hypertension	40
Type II diabetes mellitus	14

Treatment modalities

Besides LDL apheresis, all patients received a diet; 35 of 40 patients received lipid-lowering drugs (fibrates [gemfibrozil 400–1,000, bezafibrate 200–800, and fenofibrate 100–500 mg/day], HMG-CoA reductase inhibitors [simvastatin 5–40, lovastatin 10–80, and pravastatin 10–40 mg/day], colestiopol 15–30 mg/day, colestyramine 400–1,000 mg/day, and niacin acid 600–12,400 mg/day).

Four different LDL apheresis systems were used: dextran sulfate adsorption for 28 of 40 (Liposorber, Kaneka), immunoadsorption for 6 of 40 (Therasorb, Baxter), LDL hemoperfusion for 4 of 40 (Dali, Fresenius), and the immunoadsorption system with special anti-Lp(a) columns for 2 of 40 patients (Lipopak, Pocard). In all the methods, plasma is obtained by a primary separation system. Blood is withdrawn from the patient via peripheral venous access and circulates through the extracorporeal circuit in which hollow-fiber separators separate the plasma from the cellular blood components.

In the Liposorber system, plasma is perfused through columns containing cellulose microplates on which dextran sulfate (molecular weight 4,500) has been immobilized. Dextran sulfate adsorbs from plasma all cholesterol containing apolipoprotein B (ApoB) (total cholesterol, LDL, very-low-density lipoprotein, ApoB, triglycerides, and Lp[a]). Adsorption occurs between the ApoB component and dex-

tran sulfate (55,56). In the immunoadsorption systems, heteroclonal antibodies against the protein component of human LDL cholesterol (ApoB 100) or especially against Lp(a) (immunoadsorption system from Lipopak) obtained from sheep are equivalently bound to Sepharose gel (57). The direct adsorption of the lipoproteins LDL and Lp(a) from whole blood (Dali) is a relatively new LDL apheresis technique that does not need a primary cell/plasma separation step. The adsorber is made of the negatively charged polyacrylate ligands with the positively charged ApoB moiety of LDL and Lp(a). These lipoproteins are retained selectively on the column (58).

In all the systems the blood flow was between 30 and 120 ml/min; the perfusion flux was between 10 and 40 ml/min. Treatment lasted on average 2.9 ± 1.1 h (range 0.5–6.5 h). The treatment volume depended mainly on the quality of vascular access. The median was 4.8 L/session (between 0.5 and 8.7 L/session). Anticoagulation was effected using nonfractionated heparin in 30 of 40 patients (4.002 ± 2.096 IU [range 2.000–8.000 IU]) and low molecular heparin in 6 of 40 patients (2.010 ± 801 IU [range 2.000–3.000 IU]). In 4 of 40 patients treated with the Dali system, acid citrate dextrose-A in a concentration of 1:20/1:40 ml was used.

Patients were studied after a 12 h testing period. Serum samples were taken preapheresis and postapheresis. The time-averaged lipid lowering is the mean value over the time between aphereses (59–61). According to these findings, the interval values (mean of value pre- and post-LDL-apheresis) were used for evaluation. To maintain the time-averaged lipoprotein levels at a desirable level according to the guidelines recommended by the European Atherosclerosis Society (51), LDL apheresis must be repeated between once per week and/or every 3 to 4 weeks. On average, there were 32.8 ± 12.5 (range 13–61) LDL aphereses per patient per year. Lipid concentrations reached during the study period were compared with long-term average values obtained over a period of 14.3 ± 12.1 (range 2–48) months before the trial.

Laboratory methods

Serum total cholesterol was measured using CHOP-PAP reagents, triglycerides were measured by an enzymatic method (GPO-PAP), and Lp(a) was measured by ELISA. High-density lipoprotein (HDL) was estimated after precipitation with phosphotungstate. LDL-C was derived by the Friedewald formula (62). Turbidimetry was used to establish the fibrinogen concentrations.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science. All data except for triglycerides and Lp(a) concentrations were expressed as mean ± standard deviation. The triglyceride and Lp(a) levels were expressed as medians because their distribution departed substantially from normal. For comparisons the chi-squared test, Fisher's exact test, Student's *t*-test, Wilcoxon's rank sum test, and Wilcoxon's rank test were used. Significance was defined at the 0.05 level.

RESULTS

Before LDL apheresis, mean lipoprotein levels were 476.4 ± 74.9 mg/dl (n = 207) for total cholesterol, 295.5 ± 71.6 mg/dl (n = 197) for LDL, 38.9 ± 7.8 mg/dl (n = 187) for HDL, 508.2 ± 212.8 mg/dl (n = 183) for triglycerides, 52.4 ± 11.3 mg/dl (n = 97) for Lp(a), and 273.2 ± 46.7 mg/dl and 472.8 ± 121.2 mg/dl (n = 152) for fibrinogen. Over the total study period of 84.9 ± 43.2 months (range 1–134) with LDL apheresis, mean reductions ($p < 0.05$) of 50.6% for total cholesterol, 52.2% for LDL, 64.3% for Lp(a), 43.1% for triglycerides, 39.5% for ApoB, and 26.7 % for fibrinogen were reached (Table 2).

Clinical outcome

During the trial an improvement in general well being and increased performances were experienced in 39 of 40 patients. On average, increased performance was reached 2 to 3 months after the beginning of LDL apheresis. At the start of the treatment, 38 of 40 patients were suffering from CHD and intermittently from angina symptoms.

Presently, 37 of 40 patients are free of symptoms. A reduction of 60% to 100% of nitrate medication was observed in 32 of 38 patients who received this therapy at the beginning of the trial. In the course of

TABLE 2. *Lipoproteins (mg/dl) in 40 patients (n = 3,640 LDL apheresis treatments)*

Lipoprotein	LDL apheresis		Mean percentage changes (%)
	Before	After	
Total Cholesterol (mg/dl)	471.4 ± 74.9	206.5 ± 57.3	–50.6
LDL (mg/dl)	295.5 ± 71.6	132.4 ± 64.4	–52.2
HDL (mg/dl)	38.9 ± 7.8	42.9 ± 12.8	+10.3
Triglycerides (mg/dl)	508.2 ± 212.8	289.6 ± 152.3	–43.1
Lp(a) (mg/dl)	52.4 ± 11.3	18.7 ± 7.8	–64.3
ApoB (mg/dl)	273.2 ± 46.7	165.2 ± 57.3	–39.5
Fibrinogen (mg/dl)	472.8 ± 121.2	345.7 ± 102.4	–26.7

LDL: low-density lipoprotein, HDL: high-density lipoprotein, Lp(a): lipoprotein a, ApoB: apolipoprotein B.

the trial, 1 female patient died of an acute myocardial infarction after a treatment period of 16 months, 3 weeks after the last LDL apheresis. In the other subjects no further cardiac complaints, strokes, or symptoms of peripheral vascular disease were manifested and no CABG was done, even after long-term treatment.

Percutaneous transluminal angioplasties were performed only in 5 of 39 patients during the LDL apheresis to improve the effectiveness of the treatment.

LDL apheresis systems

Applying the dextran sulfate adsorption system in 28 of 40 patients (Liposorber), the immunoadsorption system in 6 of 40 patients (Therasorb), the LDL hemoperfusion system in 4 of 40 patients (Dali), and the immunoadsorption system with special anti-Lp(a) columns in 2 of 40 patients (Lipopak), the mean reductions of total cholesterol, LDL, and triglycerides showed no statistically significant differences ($p < 0.05$). However, all 3 systems, the Liposorber, the Therasorb, and the Dali system, showed a tendency greater toward reductions in total and LDL-C than the immunoadsorption system using special anti-Lp(a) a columns (Lipopak). With respect to Lp(a) levels, the immunoadsorption system with special anti-Lp(a) columns (Lipopak) was the most effective (Fig. 1).

Between 1989 and 1990, a total of 3,640 LDL apheresis treatments were performed in 40 patients. During this period, several adverse events classified as severe were observed. However, no deaths or life threatening or disabling adverse circumstances were seen.

Slight side effects of minor clinical relevance were documented in 12.5% of the LDL aphereses. These side effects were posttreatment venous bleeding, vomiting, hypoglycemia, and hypotension. Serious but not life-threatening side effects were observed in 14 LDL aphereses (0.4%). These included hypotensive episodes in 5 treatments and allergic reactions in 9 LDL aphereses. In these cases, LDL apheresis was discontinued, and adverse effects were managed without major problems. Also, there were no differences in the frequency of side effects among the 4 different apheresis systems used (Table 3).

It should be noted that a special type of hypoten-

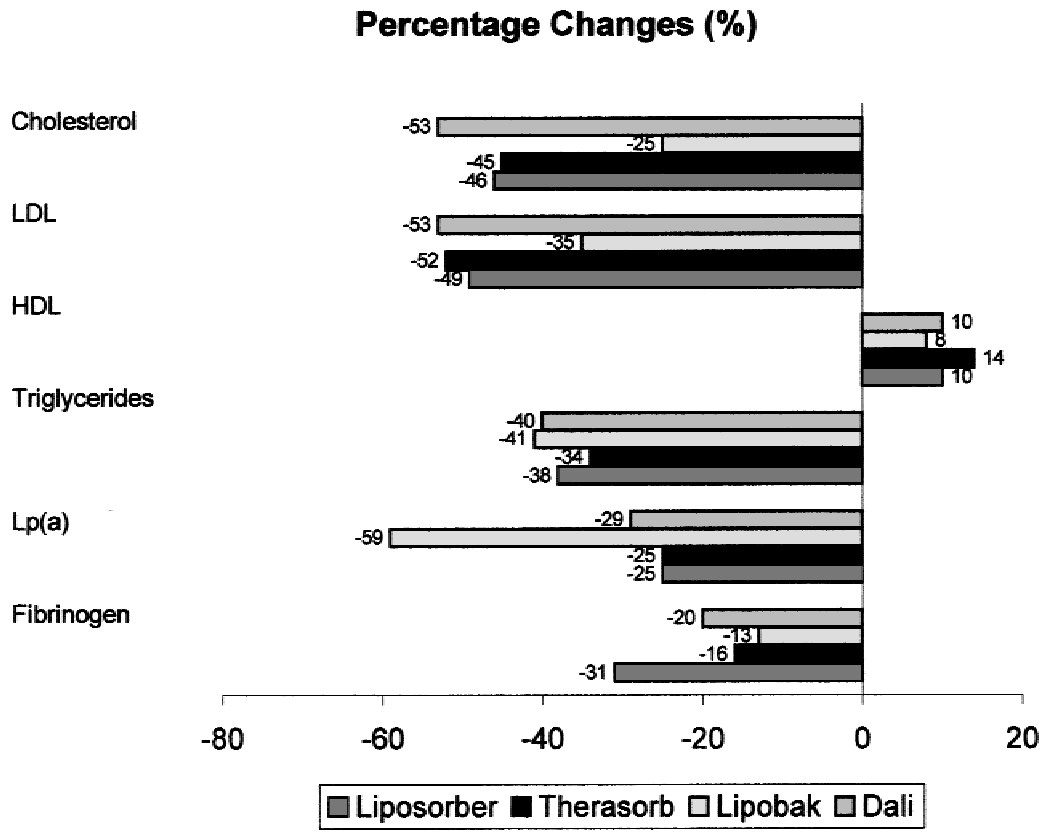

FIG. 1. The graph shows average percentage changes (%) in lipoproteins with 4 different LDL apheresis methods after a treatment period of 84.9 + 43.2 months. The average changes were calculated for both treatment periods [pretreatment time 1–6 years; LDL apheresis treatment time 1–9 years; LDL: low-density lipoprotein, HDL: high-density lipoprotein, Lp(a): lipoprotein a].

TABLE 3. *Side effects of long-term LDL aphereses (n = 3,640)*

Side effects	No. of patients affected (n)	Frequency (n)	Frequency (%)
Posttreatment venous bleeding	19	142	3.9
Vomiting	15	118	3.2
Hypoglycemia	6	110	3.0
Hypotension	14	87	2.4
Allergic reaction	4	9	0.25
Shock	2	5	0.15
Total	20	471	12.9

sive reaction caused by the release of bradykinin occurred in patients who had previously received concomitant ACE inhibitor treatment. This was first reported on by Olbricht et al. (63) followed by similar reports from Kroon et al. (64) and Keller (65).

This hypotensive reaction was sometimes associated with flushing, dyspnea, and/or bradycardia. Ten such episodes occurred in 8 patients who had taken ACE inhibitors. Subsequently, we decided that patients taking ACE inhibitors should not be treated with LDL apheresis.

DISCUSSION

Although numerous epidemiologic and intervention studies have shown a positive correlation between elevated serum lipoprotein levels and the development and progression of atherosclerosis with its complications (in particular CHD and peripheral vascular disease) (1–7), as yet no causal link has been established (14–18,66). Having exhausted all other conservative treatment possibilities, LDL apheresis has been shown to be very effective in the treatment of severe dyslipoproteinemia. The present reductions of serum lipid concentrations during treatment with different LDL apheresis methods are in agreement with the findings of other trials (67–78).

Assessing clinical outcome of the patients, there were no differences with respect to the 4 apheresis methods. All of the systems were safe and showed equivalent clinical efficacy even during long-term treatment, and all patients reported symptomatic improvement in angina, dyspnea, and claudication even after only a few apheresis sessions. It was suggested that immediate reduction of the LDL-C concentration with LDL apheresis promptly improves endothelium regulation and induces changes in coronary tone by an increase in endothelial derived relaxing factor (79–81). A decrease in plasma viscosity, which is caused by the removal of LDL and fibrinogen per liter), may improve vascular function and perfusion and leads to relief of ischemic symptoms.

With the dextran sulfate adsorption system, the immunoadsorption system, and the hemoperfusion system, there were no significant differences with respect to total cholesterol, LDL, HDL, Lp(a), and triglyceride concentrations at the end of the trial although reduction of fibrinogen levels was significantly greater using the immunoadsorption system. The most effective apheresis method in reducing high Lp(a) levels seems to be the immunoadsorption method with special Lp(a) columns. Elevated plasma concentrations of Lp(a) correlated highly with the incidence of premature cardiovascular and cerebrovascular disease (82–85). The strong association between Lp(a) and CHD may be caused by both Lp(a) atherogenesis and thrombogenicity; Lp(a) has been shown to inhibit fibrinolysis by competing with plasminogen for its binding sites on cell surfaces or on target molecules.

In conclusion, even after long-term treatment regimens, the Liposorber, Therasorb, and Dali systems showed the greatest efficacy (90). Only with respect to the elimination of Lp(a) was the Lipopak system more effective.

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