Artificial Organs
22(2):135–139, Blackwell Science, Inc.

Lipoprotein(a) Apheresis in Severe Coronary Heart Disease: an Immunoadsorption Method

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Abstract: Lipoprotein(a) (Lp[a]) is associated with an increased cardiovascular risk. It is similar to low-density lipoprotein with an additional molecule of apo A covalently linked to apo B-100 by one disulfide bridge. Apo A is highly homologous to plasminogen. The kringle 4 motive of plasminogen is repeated between 10 and 40 times in apo (a). Currently, there is no drug therapy available to lower Lp(a). Since October 1993, we have carried out over 160 immunoadsorption treatments on 3 patients with elevated Lp(a) as their only risk factor and a history of myocardial infarction. Lp(a) was removed from plasma by sepharose coupled anti-Lp(a) columns. Lp(a) levels were lowered from above 170 mg/dl to below 30 mg/dl immediately after Lp(a) apheresis. To achieve this, the patient's plasma volume had to be treated 2 to 3 times. Nonspecific protein loss during column changes remained negligible. There were no serious unwanted effects during or after treatment. Minor circulatory problems (tachycardia, flush) occurred in 11% of the treatments but only with plasma flow rates above 55 ml/min. In 1 patient, coronary angiography after 2 years and in another patient after 1 year showed no progression. The third patient has not yet had repeat coronary angiography. Like the others, he reported subjective improvement after 1 year of apheresis. It is concluded that Lp(a) apheresis may retard progression of atherosclerosis in patients with selective Lp(a) elevation. Further studies to support this hypothesis are needed. **Key Words:** Lipoprotein(a)—Low-density lipoprotein—Atherosclerosis—Apheresis—Immunoadsorption.

For over 25 years trials have been conducted, showing that cholesterol lowering interventions can reduce cardiovascular risk and in patients with preexisting coronary heart disease, total mortality can be lowered (1–4). Today the most important alternative and addition to cholesterol-lowering drug therapy is lipid apheresis (5).

Another risk factor for atherosclerosis, lipoprotein(a) (Lp[a]), was first described by Berg in 1963 (6). Its electrophoretic mobility is mainly in the pre-β fraction with a density between 1.05 and 1.10 g/ml. Lp(a) consists of a low-density lipoprotein (LDL) particle covalently linked to the glycoprotein apo A by a disulfide bridge. Apolipoprotein A (apo A) is highly homologous to plasminogen (7,8): the kringle 4 motive of plasminogen is repeated between 10 and 40 times in apo A. Kringle 5 exists once (Fig. 1). The number of kringle 4 repeats accounts for the size of the molecule and is inversely correlated to the plasma concentration of Lp(a) (9). Its plasma levels vary up to 1,000-fold between individuals, and the population distribution is highly skewed in white and Asian populations. Lp(a) levels are determined by several autosomal alleles on chromosome 6 (7). Its atherogenic properties are not fully understood. Lp(a) has been found in atheromatous plaques. Several mechanisms have been postulated: blocking of plasminogen binding sites on fibrin clots, interaction with other coagulation proteins, and hepatic growth factor. Grainger showed that Lp(a) and apo A enhance proliferation of human smooth muscle cells in culture by inhibiting the activation of plasminogen to plasmin, thus blocking the proteolytic activation of transforming growth factor-β (TGF-β), an autocrine inhibitor of human vascular smooth muscle cells. The activation of TGF-β is inhibited in the aortic wall and serum of mice expressing apolipoprotein A as a consequence of apo (a) inhibition (11,12) (Fig. 1).

Since 1985, several studies have shown that Lp(a) is a major independent risk factor for atherosclerosis, increasing cardiovascular and cerebrovascular morbidity and mortality at a young age (10–16).

Received May 1997; revised October 1997.
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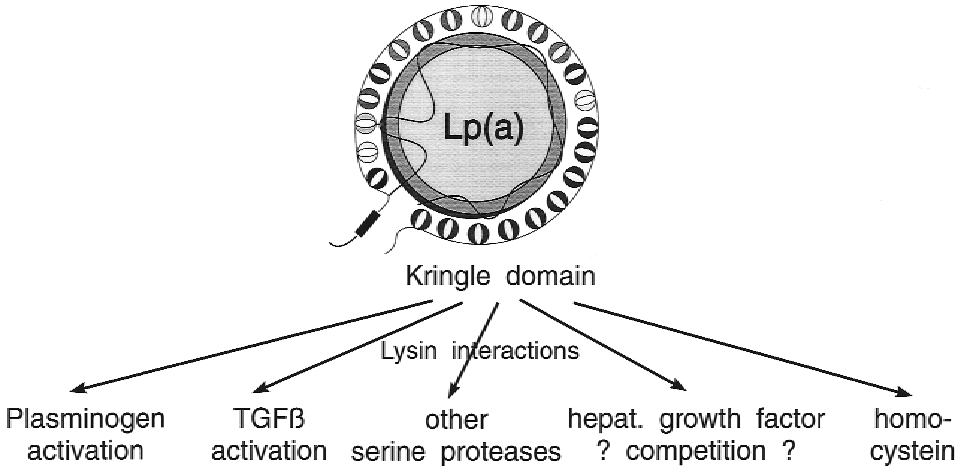

FIG. 1. Represented is the Lp(a) molecule and its potential role in atherogenicity.

However, in 2 large case control studies in Finland and the U.S.A., Lp(a) was not associated with an increased cardiovascular risk (17,18). Both populations consisted of persons above 40 years of age without signs of cardiovascular disease at entry. The results of these studies indicate that high Lp(a) levels that have not led to symptomatic arteriosclerosis before the age of 40 years and thus also led to exclusion from this study might no longer play a role as a risk factor.

Thompson et al. conducted a study in which no difference was found between a group treated by biweekly LDL apheresis, lowering both LDL and concomitantly Lp(a), and a control group who took 3-hydroxy-3-methylglutaryl coenzyme a (HMG-CoA) reductase inhibitors as the only treatment to lower cholesterol without accompanying Lp(a) reduction. However, in this study Lp(a) levels were above 30 mg/dl for most of the interval between 2 apheresis sessions (19).

Currently, there is no drug therapy available to lower very high Lp(a) sufficiently. *N*-acetylcysteine has been shown to induce a dose-dependent reduction in Lp(a) levels of about 7% by causing dissociation of the apo A by cleavage of disulfide bonds (20–22). These reports have been contradicted by Wiklund (23), and in patients with very high Lp(a) levels, they can only be normalized by plasma exchange (24) or in parallel with LDL by various methods of lipid apheresis. Thus, Lp(a) is removed with LDL apheresis (25), by anti-apo B sepharose columns, or less efficiently by selective dextran sulfate cellulose columns (26) or heparin-induced extracorporeal LDL precipitation (HELP) (27–30). These methods all have the disadvantage of preferential removal of LDL that is abundant in comparison to Lp(a). In patients with normal cholesterol levels, LDL would be removed without bringing Lp(a) down to normal, or ideally low normal levels. It cannot be excluded that LDL apheresis might be harmful by lowering cholesterol too much. In 1991, Pokrovsky et al. presented a specific Lp(a) immunoadsorption column (31), which is at present the only means of reducing Lp(a) specifically.

METHODS

Immunoadsorption

Plasma separation was achieved with a Spectra cell separator (COBE, Munich, Germany) with a peripheral venous access on both arms at whole blood flow rates of 50–120 ml/min and a plasma flow rate of 20–50 ml/min. Anticoagulation was carried out with a combination of citrate and heparin to avoid citrate toxicity and to block the classic and alternative complement activation pathways.

Lp(a) was specifically removed from the plasma with sepharose coupled anti-LP(a) columns (31). Plasma treatment was carried out on a Citem 10 adsorption/desorption automat (Excorim, Lund, Sweden) that led plasma over a pair of 400 ml Lp(a) adsorption columns and desorbed the columns in turn with neutral saline buffer solution, glycine buffer of pH 2.4, and neutral buffer again. A flow chart of this procedure is shown in Fig. 2. During each apheresis session, 140–257% of patient plasma volume (4–9 L) was treated, which meant a treatment duration of 2–5 h.

Analytical methods

Lp(a) was measured by rocket gel electrophoresis (Immuno, Heidelberg, Germany). All other parameters were determined by standard laboratory procedures.

PATIENTS

Patient 1, U.H., is a 42-year-old man with selective Lp(a) elevation as the only risk factor for coronary

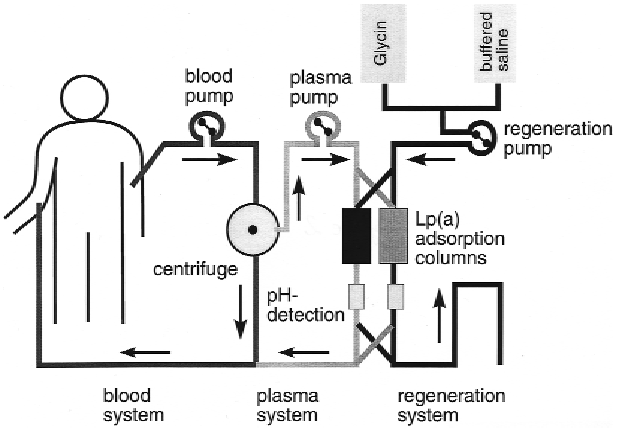

FIG. 2. The flow chart is of the Lp(a) immunoadsorption procedure. Plasma is separated by a continuously working blood cell separator (COBE Spectra). The plasma is processed automatically (Excorim Citem 10) on 2 immunoadsorption columns that are loaded and desorbed in turn.

artery disease. As he had already suffered 2 myocardial infarctions (MIs) at very young ages (31 and 35 years) even necessitating 1 resuscitation, it was urgent to prevent further cardiac events. At 35 years he underwent coronary angioplasty of the left anterior descending (LAD) coronary artery. A trial of nicotinic acid led to Lp(a) reduction of only 10% and was not tolerated by the patient due to abdominal pain and diarrhea. Lp(a) apheresis was started at the age of 40.

Patient 2, H.V., is a 53-year-old man who had suffered 2 successive MIs in January and April 1993 at age 51. He had a stent implantation in September 1993. His hypertension has been controlled by beta blockers and calcium antagonists for several years. He had only mild hypercholesterolemia. Because of poor venous access he had to have a Cimino-Brescia shunt on the left forearm. He has been on weekly Lp(a) apheresis since April 1994.

Patient 3, G.H., is a 48-year-old man who has been on weekly immunoadsorptions for 42 months. His first MI occurred at 43 years of age, and he underwent repeated PTCAs of the left anterior descending coronary artery and left circumflex artery after the event. Thallium scan showed exercise-induced anteroseptal and lateral ischemia as well as a reduction in posterolateral wall motion. He had no smoking history, and hypertension as well as hypercholesterolemia have been controlled by drugs for 7 years. His Lp(a) level is elevated.

RESULTS AND DISCUSSION

Immunoadsorption treatments

Since 1993, more than 290 Lp(a) apheresis sessions have been carried out on 3 patients. To lower Lp(a) below the risk limit (value of Lp(a) immediately after apheresis of less than 25 mg/dl), we had to treat 140–257% of patient plasma volumes (14.9 ± 4.5 L of blood volume and 6.4 ± 1.8 L of plasma treated with 4.5 ± 1.6 columns loaded with plasma), which meant an immunoadsorption duration of 185 ± 53 min. Lp(a) levels fell from 130.9 ± 44.5 to 28.5 ± 14.4 mg/dl (Fig. 3). Total cholesterol was lowered from 207.9 ± 24 to 145.5 ± 33.2 mg/dl and LDL from 132.7 ± 21.0 to 80.7 ± 25.6 mg/dl, and an HDL decrease can be accounted for by dilution (from 47.8 ± 5.5 to 36.5 ± 8.2 mg/dl after treatment) (Fig. 4). Nonspecific protein loss during column changes remained minimal if one takes into account that the values immediately after immunoadsorption reflect dilution effects due to infusion of 925 ± 275 ml of acid citrate dextrose buffer (ACDB) with heparin as anticoagulant and some of the rinsing buffer during the 2–5 column changes.

Protein and albumin levels changed from 70.40 ± 3.8 g/L and 48.3 ± 3.19 g/L, respectively, to 55.92 ± 3.6 g/L and 41.10 ± 5.2 g/L, respectively, after apheresis. This was paralleled by a fall in hemoglobin levels (13.9 ± 1.0 g/dl to 13.1 ± 1.4 g/dl), but the patients did not encounter any clinical problems from this dilution except from increased diuresis on the treatment day (Fig. 5).

Coagulation parameters were changed by heparin and citrate immediately after apheresis. Quick values were decreased from 62.5 ± 36.1 to 47.4 ± 31.8% and PTT was prolonged from 45.1 ± 15.7 to 91.4 ± 29.8 s. These pathologic values are explained by the fact that 1 of the patients is taking coumarole because of his anterior wall motility defect. There were no serious unwanted effects during or after treatment. For 10 treatments plasma exchange had to be carried out instead of immunoadsorption because of

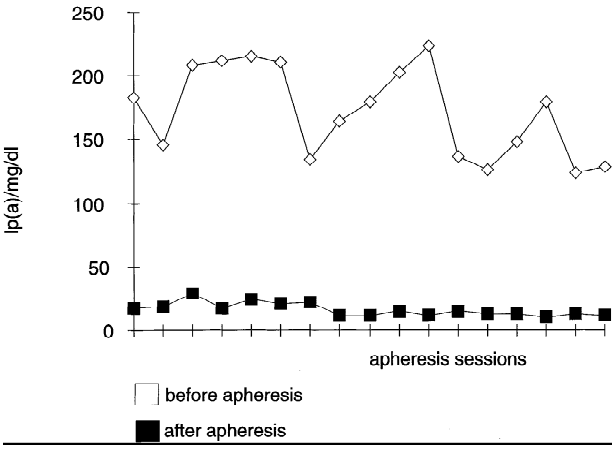

FIG. 3. Plotted are examples of Lp(a) values before and immediately after weekly Lp(a) immunoadsorption.

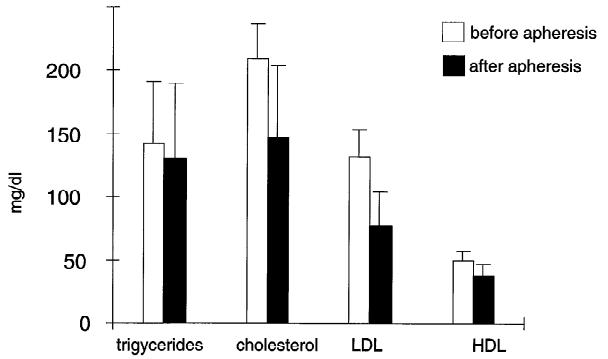

FIG. 4. The graph shows the mean values and standard deviations of the reductions of triglycerides, cholesterol, LDL, and HDL by Lp(a) apheresis.

a glass column housing was broken and new columns were not immediately at hand or because there was a (falsely positive) contamination in the sterility test. Tachycardia and flush occurred in 11% of the treatments with plasma flow rates above 55 ml/min. They were easily reversed by interrupting the treatment and decreasing the plasma flow rate afterwards.

Clinical results

Lp(a) apheresis sessions have been carried out on patients with a history of myocardial infarction in our department since October 1993. Patient 1 has been on apheresis for almost 5 years now. During that time his cardiac status has been stable. The patient reports a subjective improvement in overall physical performance. Coronary angiography 16 months after institution of Lp(a) apheresis showed a slight improvement with a reduction of stenosis of the LAD coronary artery from 53 to 36%. Ejection fraction and regional wall motility were unchanged.

Patient 2 has not yet had a repeat angiogram, which is planned in 8 months. However, a stress test and clinical investigation after 1 year of treatment showed improved exercise tolerance. Again the patient reports an overall improvement in his well-being.

Patient 3 has had a repeat angiography for unexplained dorsal chest pain after 1 year and another angiography after 3 years of treatment. There was no change compared to the previous angiogram. The complaints after 1 year have been interpreted as having been caused by cervical and thoracic spondylosis.

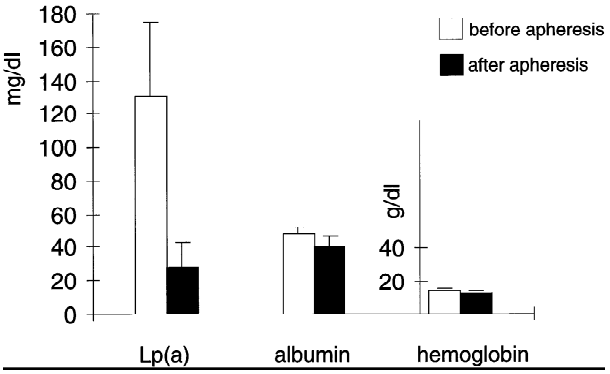

FIG. 5. The graph shows the mean values and standard deviations of the reductions of Lp(a), albumin, and hemoglobin by Lp(a) apheresis.

CONCLUSION

Lp(a) can be lowered from extremely high levels to normal values without removing other lipoproteins or serum components. The Lp(a) rise to preapheresis concentrations varies in our patients from 3–5 days. So far our patients have tolerated Lp(a) apheresis very well. Serious side effects have not been observed.

Because all 3 patients are stable under Lp(a) apheresis, this is interpreted to indicate that Lp(a) apheresis may retard progression of atherosclerosis in patients with selective Lp(a) elevation. Coronary angiographies after 1 and 3 years showed no significant changes in 2 patients. Further studies on more patients to support this hypothesis are needed. We think the ideal group to prove the efficacy of Lp(a) apheresis are patients with cardiovascular disease before age 50 in whom Lp(a) is the only or predominant risk factor, e.g., nonsmoking, normotensive, normolipemic patients who show symptomatic arteriosclerosis at young ages.

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