Effect of low-density lipoprotein apheresis on patients with peripheral arterial disease. Peripheral Arterial Disease LDL Apheresis Multicenter Study (P-LAS)

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***Aim*. The effectiveness of low-density lipoprotein (LDL) apheresis for patients with peripheral arterial disease (PAD) was investigated to confirm a hypothesis based on subjective evidence that the amelioration of blood rheology would be the most contributing factor for improvement in clinical symptoms. Evaluation of the severity of intermittent claudication is difficult because of the lack of an accurate parameter to assess muscle ischemia during exercise, thus we objectively evaluated by non-invasive near-infrared spectroscopy (NIRS) on a treadmill in this study.**

***Methods*. Thirty-one patients with PAD were evaluated for hemostatic function and physiological parameters such as ankle-brachial pressure index (ABI), maximum tolerated walking distance (MTWD) and recovery time (RT) or recovery ability index (RAI) on NIRS. Laboratory tests included plasma assays of total cholesterol, LDL-cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and fibrinogen. The change in red-cell filtration rate was evaluated for the improvement of microcirculation. Statistical analysis was performed using the paired Student's t-test with Bonferroni's correction.**

***Results*. A significant improvement in ABI and MTWD was observed after average 9.6±0.8 sessions of LDL apheresis treatment and the amelioration of microcirculation in ischemic muscle was objectively evaluated as significant improvement in RAI on NIRS. Rest pain was improved in all 5 patients with Fontaine's classification III or IV. A severe ulcer refractory to usual medications was dramatically diminished in the area by 10 sessions of LDL apheresis and fully healed 5 months after the final LDL apheresis treatment followed by medication. No angiographical change was observed in the arterial occlusive lesions in any patients.**

***Conclusion*. The effectiveness of LDL apheresis on the improvement in physiological parameters such as ABI, MTWD and clinical symptoms in patients with PAD was confirmed. The severity of intermittent claudication was objectively evaluated using non-invasive NIRS. The RT or RAI was useful parameter to evaluate the improvement in the ischemic symptoms of the extremities.**

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Key words: **Lipoproteins, LDL - Apheresis, LDL - Fibrinogen - Red cell filtration rate - Spectroscopy, Near-Infrared - Recovery Ability Index.**

Hyperlipidemia, especially the increase of low-density lipoprotein (LDL) cholesterol, is one of the important risk factors in peripheral arterial occlusive disease. LDL apheresis treatment for coronary artery disease in patients with familial hypercholesterolemia has yielded regression of coronary atherosclerosis [1-4] and reduction in the incidence of coronary disease events.[5] Theoretically, this treatment is effective for other arteriosclerotic disease. This multicenter study was designed to investigate the effect of LDL apheresis on clinical symptoms and biological or objective variables in 31 hyperlipidemic patients with peripheral arterial disease (PAD). Evaluation of the severity of intermittent claudication is difficult because of the lack of an accurate parameter to assess muscle ischemia during exercise, thus we used by non-invasive near-infrared spectroscopy (NIRS) on a treadmill.[6-11]

Materials and methods

Patients

We enrolled 31 patients (70±1.8 years old, mean±SE; range: 47-86 years; 20 men, 11 women)

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with PAD (Fontaine's classification: II, 26; III, 4; IV, 1) in this study. They had hypercholesterolemia (total cholesterol ≥220 mg/dL) even after dietary treatment and medication.

LDL apheresis

LDL apheresis was performed with a Liposorber LA-15 system (Kaneka Co. Ltd., Japan), installed in a reciprocative double-column (150 mL) system equipped with an MA-01 machine (Kaneka Co. Ltd., Japan), once a week for 10 weeks, treating 3 000-5 000 mL of plasma on each session. The column contained cellulose beads connected with dextran sulfate as an adsorbent that bound and removed LDL. When one column is saturated, the machine switches to the other, and the saturated column is automatically reactivated using hypertonic saline. This cyclic process of adsorption and elution makes it possible to remove LDL, very low-density lipoprotein (VLDL), and triglyceride selectively without affecting circulating levels of high-density lipoprotein (HDL) and high-molecular proteins such as albumin. About 70-80% of total cholesterol and LDL cholesterol can be removed per session when 4 000 mL of plasma is treated. Medication used prior to apheresis was continued without change during the study.

Measurements

Clinical symptoms, ankle-brachial pressure index (ABI), and maximum tolerated walking distance (MTWD) on a treadmill were assessed on the day before the first LDL apheresis and after a series of 10 LDL apheresis procedures. The optrodes of a near-infrared spectrometer (Non Invasive Oxygenation Monitor OM-100A, Shimadzu Co., Japan) were positioned on the posterior aspect of the calf. The accuracy of this 3-wavelength spectrophotometer has been reported.[11] The light guide and the near-infrared detector were applied to the skin 4 cm apart, using a bandage. NIRS measurement of oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb) were made continuously during a resting period in the standing position, during a walking period with exercise and during a recovery period after exercise in the standing position. The time between the end of exercise and the intersection of the curves of recovering oxy-Hb and deoxy-Hb was measured (recovery time: RT) on NIRS spectrum. The exercise on the treadmill was performed by the multistage loading method, which consisted of a 12% gradient at a speed that increased from 1.6 km/h to a maximum of 3.2 km/h by 0.8 km/h per minute for as long as each patient was capable, and the MTWD was measured. Blood specimens to measure serum lipid levels were obtained immediately before and after the 1st and 10th apheresis treatment. Serum fibrinogen levels and red-cell filtration rate were measured immediately before the 1st and after the 10th apheresis, and also at 1 week after the final apheresis treatment. Red-cell filtration rate was measured according to the method by Reid *et al.*,[12] and the toetip photoplethysmogram waveform was recorded by Dyna Pulse SDP-100 (Fukuda Denshi, Japan). The wave height of each toetip photoplethysmogram was measured on every toetip of the ischemic limb and the mean value was used for comparison. We defined change of more than 10% as significant. With the informed consent of all patients, conventional arteriography or intravenous digital subtraction angiography (IV-DSA) was performed before the 1st and after the 10th apheresis treatment. Therapeutic results were investigated at 1 week after a series of 10 LDL apheresis procedures.

Statistical analysis

We used the paired Student's t-test compare differences in variables and values were expressed as mean±SE (standard error of the mean). A P-value less than 0.05 was considered to indicate a statistically significant difference. For duplicate comparison in changes of serum fibrinogen and red-cell filtration rate, we used the paired Student's t-test with Bonferroni's correction and considered a P-value less than 0.025 to indicate a significant difference.

Results

An average of 9.6±0.8 (mean±SD) LDL apheresis sessions were performed during a period of 7±1 weeks. Total cholesterol and LDL cholesterol were reduced after a session of LDL apheresis, however no significant change in serum level of HDL cholesterol was observed (Table I). There were no significant changes in hematocrit, globulin and albumin. Changes in serum fibrinogen immediately before, after the apheresis and 1 week after the final apheresis treatment were measured in 20 patients.

TABLE I.—*Change in serum lipid levels before and after a session of LDL apheresis treatment.*

	Before (mg/dL)	After (mg/dL)	Change (%)	P-value
Total cholesterol	279±8.46	186±5.3	33.3	<0.0001
LDL cholesterol	197±9.51	117±5.47	40.8	<0.0001
Triglyceride	174±17.5	122±13.1	29.9	<0.0001
HDL cholesterol	44±2.9	47±2.6	-7	NS

N=26; NS: not significant; HDL: high density lipoprotein.

The reduction of serum fibrinogen after the 1st apheresis was 20.4% compared with before the apheresis, and a 10.7% of reduction was maintained at 1 week after the final apheresis treatment (Figure 1). The red-cell filtration rate increased immediately after the 1st apheresis, and the increased level (11% compared with before) was maintained at 1 week after the final apheresis (Figure 2). The results of changes in physiological measurements and clinical symptoms are summarized in Table II, where more than 10% of change of index, distance or wave height before and after the apheresis treatment in each subject was defined as "improved" or "worsened". The ABI increased in 60% (26/43) of the extremities observed, with a significant average change from 0.66±0.027 to 0.76±0.03 (15%, P<0.001). The MTWD increased in 70% (16/23) of the patients, with a significant average change from 160±18.5 to 190±22.6 m (20%, P<0.001). The RT on near-infrared spectroscopy decreased in 57% (8/14) of the patients, with a significant average change from 223±33.4 at baseline to 183±33 s (P=0.0217) after 10 sessions of LDL apheresis. Meanwhile, recovery ability index (RAI=RT/exercise time) was analyzed considering the multistage loading on treadmill exercise which had caused different loading for each patient.[13] The RAI showed significant improvement from 1.66±0.429 to 1.44±0.418 (P=0.0441). In 2 patients, although increases in MTWD, from 200 to 250 m (25%) and 280 to 330 m (18%), were observed, the change in ABI was only from 0.51 to 0.52 and 0.87 to 0.90, respectively. However, their RT on NIRS showed significant improvement from 221 to 197s (-11%) and from 144 to 96 s (-33%), respectively.

Rest pain improved in all 5 patients with Fontaine's classification III or IV accompanying with the decrease in their dose of an analgesic. One patient, suffered from both diabetes mellitus and rheumatism, had an ulcer on her anterior tibia, which showed severe resistance to usual medications such as prostaglandin or antiplatelet drugs and could not be decreased in size (75×25 mm) before applying LDL apheresis. An obstructive

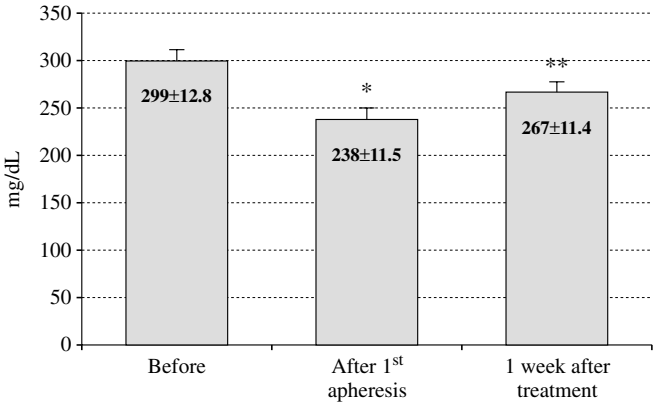

Figure 1.—Change in serum fibrinogen. *P<0.001 *vs* before, **P=0.003 *vs* before.

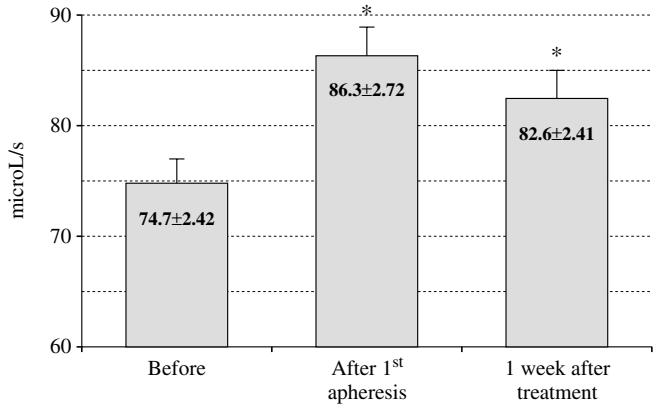

Figure 2.—Change in red-cell filtration rate. *P<0.001 *vs* before.

TABLE II.—*Change in physiological measurements and subjective clinical symptoms at 1 week after the final LDL apheresis treatment.*

	Observed extremities	Improved	Unchanged	Worsened
Physiological measurements				
ABI	43	26	16	1
MTWD	23	16	7	0
NIRS (RT)	14	8	5	1
Plethysmography	28	15	13	0
Subjective clinical symptoms				
Coldness	19	17	2	0
Numbness	11	7	4	0
Rest pain	5	5	0	0

ABI: ankle brachial pressure index; MTWD: maximum tolerated walking distance; NIRS (RT): recovery time on near-infrared spectroscopy.

occlusion in her superficial femoral artery was detected on the X-ray angiogram as shown in Figure 3. The ulcer decreased to 70×17 mm after 10 procedures of the apheresis, and further diminished to 16.5×17 mm after 2 further weeks of medication. It was fully healed at 5 months after the final apheresis treatment, while medication was continued, and showed no sign of recurrence during the 4 years follow-up period (Figure 4). No change was observed in the arterial occlusive lesions in any investigated patient after an average of 9±1 sessions of LDL apheresis.

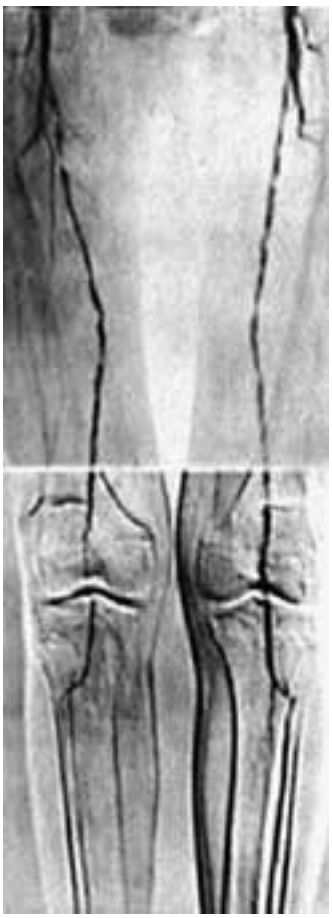

Figure 3.—Arterial angiogram of patient with ulcer.

Discussion

The effectiveness of LDL apheresis in improving limb ischemia in patients with PAD has been suggested to be mainly due to improvement in blood rheology:[14-21] reduction of plasma or blood viscosity,[15-19] improvement in the red-cell deformability.[16, 18] The significant reduction of serum fibrinogen and some coagulation factors [20, 21] as well as acute reduction of LDL would be the most effective factor to improve blood rheology. In the Edinburgh Artery Study,[22] it is reported that plasma fibrinogen levels are related to the future onset of PAD, providing further evidence of a possible role of elevated fibrinogen in the development of atherosclerotic disease.

As for other mechanisms related to the effectiveness of LDL apheresis, we can consider improvement of endothelium-dependent vasodilation,[23] improvement of peripheral microcirculation due to endogenous generation of bradykinin [24] or nitric oxide (NO) [23, 25, 26] as vasodilator materials, elevated production of PGI2 from vascular cells,[27] and reduction of adhesion molecules on mononuclear cells.[28] Thus, many studies have hypothesized based on the subjective evidence that improvement in blood rheology might be the most effective factor of LDL apheresis for PAD

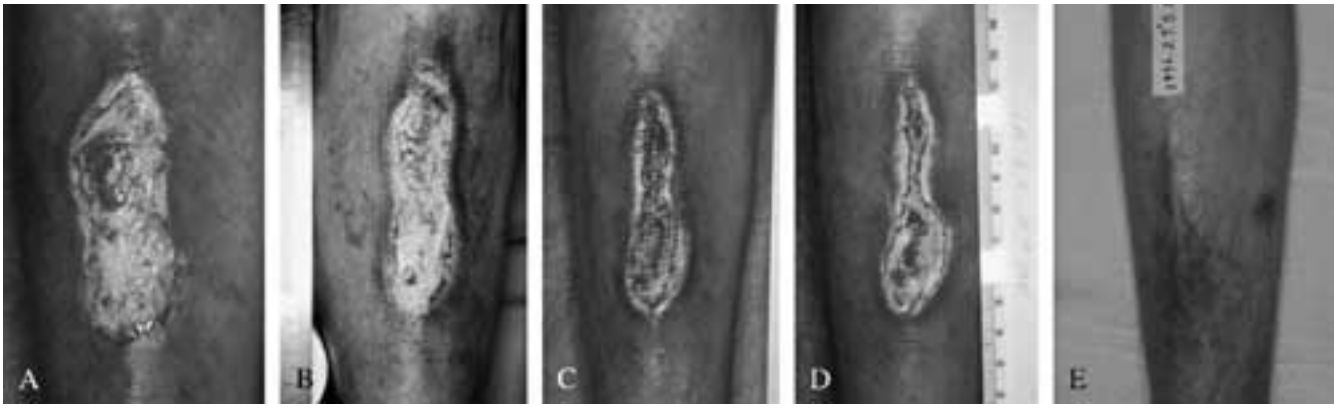

Figure 4.—Improvement of ulcer on the anterior tibia. A) Before apheresis; B) after 2nd; C) after 7th; D) after 10th; E) 5 months after 10th.

patients. A study on the role of LDL apheresis in postoperative care of bypass grafting for chronic arterial occlusion in patients with PAD [29] reported that the deteriorated ABI and intermittent claudication improved significantly after several applications of LDL apheresis.

Evaluation of the severity of intermittent claudication is difficult because of the lack of an accurate parameter to assess muscle ischemia during exercise. We evaluated by non-invasive NIRS on the treadmill.[6-11] In addition to observation of the significant increase in ABI and MTWD, we objectively investigated the RT or RAI on non-invasive NIRS to confirm the improvement of ischemic muscle, and significant changes in RAI on NIRS, which is reported to be unaffected by the amount of treadmill exercise,[13] supported the improvement of the oxygen metabolism in the extremities by repeated LDL apheresis treatment. Evaluation by RT or RAI on NIRS was useful in cases in which improvement of MTWD had been observed, while significant increase in ABI could not be detected. The cure of a severe ulcer refractory to medication was notable. The increase in ulcer size could not be suppressed with conventional medications such as prostaglandin or antiplatelet drugs, before we employed LDL apheresis. However, the ulcer size was gradually decreased with repeated apheresis and fully healed at 5 months after the final apheresis treatment followed by medications which had not been effective before the apheresis.

Kamimura *et al.*[30] reported the clinical course of a female PAD patient with intractable decubitus in her heel due to complete occlusion of the anterior tibial artery and who had been treated by a series of LDL apheresis sessions. The complete occlusion of the anterior tibial artery improved as shown on angiography, and the decubitus in her heel markedly improved after LDL apheresis therapy. These results may have been due to improvement in microcirculation by LDL apheresis, allowing delivery of sufficient drugs, oxygen or nutrition. Computerized quantitative analysis with angiography is reported to have shown improved graft stenosis and anastomatic stenosis in 2 of 6 patients following LDL apheresis for 1 year,[29] suggesting that long-term repeated LDL apheresis appears to be an efficient method to preserve graft patency and treat postoperative patients with deteriorated ABI. However, in our study no change was observed in the arterial occlusive lesions in all investigated patients after average 9±1 sessions of LDL apheresis. While, it is ethically difficult to perform a randomized controlled study on LDL apheresis, Kroon *et al.*[31] conducted aggressive lipid lowering study for 42 patients with coronary artery disease, randomly assigned to simvastatin plus LDL apheresis or simvastatin alone and followed for 2 years. Peripheral arterial end points were assessed with duplex ultrasonographic imaging of the femoral and tibial vessels. At the end of the study, the number of patients in the simvastatin-only group with hemodynamically important new stenoses in their peripheral vessels had increased from 6 to 13, as compared with a decrease from 9 to 7 patients in the simvastatin-plus-apheresis group (P=0.002). Recently, there are reports that serum levels of vascular endothelial growth factor (VEGF) significantly increased after LDL apheresis in parallel with an elevation of ABI,[32] and LDL apheresis caused an immediate rise in plasma levels of hepatocyte growth factor (HGF).[33] The improvement of ischemic symptoms due to LDL apheresis may be attributed partly to the angiogenic effects of these VEGF and HGF, however, further studies are requested to confirm the efficacy of long-term repeated LDL apheresis on the improvement in the arterial occlusive lesions.

Conclusions

The effectiveness of LDL apheresis on the improvement in physiological parameters such as ABI, MTWD and clinical symptoms in patients with PAD was confirmed. The severity of intermittent claudication was objectively evaluated using non-invasive NIRS and the RT or RAI was a useful parameter to evaluate the improvement in the ischemic symptoms of the extremities.

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