LDL-apheresis; Potential Procedure for Prevention and Regression of Atheromatous Vascular Lesion

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Nine patients with familial hypercholesterolemia (FH), 6 with homozygotes and 3 with heterozygotes, were treated with long term repetitive LDL-apheresis. The techniques are simple plasma exchange with human albumin solution, double membrane filtration, and selective LDL-adsorption by dextran sulfate-cellulose gel. The average term was 3.5 years except for the two homozygotes for whom the treatment was only initiated in our facility. Plasma total cholesterol levels were controlled between pretreating level, 330 to 500 mg/dl, and posttreatment level, 100 to 160 mg/dl, by biweekly treatments. All patients showed remarkable improvement of cutaneous and tendinous xanthomas. One homozygous patient died at 31 years old of myocardial infarction after 2 years of treatment. A homozygous patient who has been treated since 5 years old for 6 years was reexamined by angiography and was shown to have atheromatous lesions regressed in the aortic valve region and in the left renal artery.

PLASMA low density lipoprotein (LDL) is the major carrier of cholesterol in human blood plasma and elevation of its concentration has been recognized as one of the primary risk factors for development of atheromatous vascular lesions, especially in coronary arteries. Reduction of plasma LDL level has also been shown to prevent development of coronary heart diseases (CHD) in hypercholesterolemic subjects. The epidemiological data from the Framingham study shows that the prevalence of CHD increases almost linearly at plasma total cholesterol concentration (TC) of 220 mg/dl. For those who had TC below 220 mg/dl, the yearly prevalence was 0.3%, and it increased to 1.4% year at 300 mg/dl of TC. These values are equivalent to the results of primary prevention study by The Lipid Research Clinic in which the decrease of yearly prevalence is from 1.4% to 0.4% by reducing plasma TC level from 300 to 200 mg/dl with cholate-adsorptive resin among the male subjects with average age of 48 years old. Mortality from CHD in this study was about 1/3 of the prevalence. On the basis of these results, one might be able to calculate the contribution of hypercholesterolemia to CHD deaths among Japanese by using the mortality statistics and the distribution profile of plasma cholesterol. Of 18,000 CHD deaths per year among males aged from 50 to 70 years old, about 3,500 (20%) was estimated to be due to hypercholesterolemia. Thus, we may be able to save these lives by controlling plasma cholesterol level below the threshold, 220 mg/dl.

However, whether or not further reduction of LDL level can lead atheromatous vascular lesion to regress needs to be studied. It is now possible to reduce LDL cholesterol by drug treatment to below the threshold level of atherosclerosis.

Key words: LDL-apheresis Coronary heart disease Hypercholesterolemia Familial hypercholesterolemia

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1116 Japanese Circulation Journal Vol. 51, September 1987
development. However, we may have to reduce LDL further than that level in order to attain the regression of atheromatous vascular lesion.

In addition to this, there are some patients with severe hypercholesterolemia who resist drug treatments. Among those are homozygotes and a part of heterozygotes of familial hypercholesterolemia (FH), who genetically lack LDL receptor, the major catabolic site of plasma LDL. Elevation of LDL in these patients generally rise to the level where the mortal CHD develops.

In order to reduce the LDL level of these patients, direct LDL-removal techniques have been used in several laboratories. These techniques include plasma exchange and selective or semi-selective LDL removal such as double membrane filtration or selective LDL adsorption. First, plasma exchange was used to treat homozygous patients of FH and then other selective LDL removal techniques were applied to FH patients including some heterozygotes. The repetitive long-term treatment of homozygous FH patients has been shown to extend their life span by preventing or retarding development of coronary artery lesions. We have also been developing new techniques of LDL-apheresis such as double membrane filtration and selective LDL-adsorption by dextran sulfate-cellulose and have been treating homo- and heterozygous FH patients with these new procedures. These techniques are now established and available to reduce LDL to the level of FH patients out of danger of atherosclerosis. The techniques are also applicable in the reduction of LDL to the level where we may expect a regression of atheromatous lesion.

In this report, we would like to describe our experiences with LDL-apheresis treatment of FH patients with some evidences of regression of atheroma, indicating the potential role of this technique for the future treatment of coronary heart diseases.

**MATERIALS AND METHODS**

**Patients:** The list of the patients are shown in the Table I. The patients 1 to 7 are treated biweekly in our facility for long term. Patient 8 was treated 8 times in our facility before she was transferred to the other hospital for the regular treatment and 3 times again when she entered our hospital for delivery. Patient 9 was treated 3 times for initiation of treatment before she was transferred elsewhere. All the patients were shown to have premature vascular atheromatous lesions mostly by angiography. Cutaneous xanthomas were remarkable in most of the patients except for patients 6 and 7. Thickening of Achilles tendon was shown in the all except for the patient 3. Anginal episodes were reported with the patients 1, 4, 8 and 9 and ischemic changes in electrocardiogram were also seen with these patients. Patient 7 underwent aorto-

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**Table I** LIST OF THE PATIENTS TREATED IN NATIONAL VASCULAR CENTER WITH LDL-APHERESIS

<table>
<thead>
<tr>
<th>Sex</th>
<th>Type</th>
<th>BY</th>
<th>Start</th>
<th>TC</th>
<th>n</th>
<th>Pre</th>
<th>Post</th>
<th>Drug</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>HM</td>
<td>1974</td>
<td>11/15/80</td>
<td>720</td>
<td>156</td>
<td>436 ± 49</td>
<td>138 ± 21</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>HM</td>
<td>1971</td>
<td>03/01/83</td>
<td>638</td>
<td>82</td>
<td>412 ± 41</td>
<td>152 ± 18</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>HM</td>
<td>1979</td>
<td>09/14/83</td>
<td>670</td>
<td>69</td>
<td>500 ± 67</td>
<td>153 ± 34</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>HM</td>
<td>1954</td>
<td>02/07/83</td>
<td>483</td>
<td>62</td>
<td>366 ± 30</td>
<td>152 ± 31</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>HT</td>
<td>1952</td>
<td>03/24/83</td>
<td>550</td>
<td>91</td>
<td>456 ± 42</td>
<td>160 ± 18</td>
<td>P, C, M</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>HT</td>
<td>1947</td>
<td>12/20/83</td>
<td>528</td>
<td>70</td>
<td>447 ± 44</td>
<td>176 ± 29</td>
<td>DF</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>HT</td>
<td>1930</td>
<td>02/15/83</td>
<td>350</td>
<td>66</td>
<td>323 ± 12</td>
<td>101 ± 5</td>
<td>C, P</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>HM</td>
<td>1959</td>
<td>05/25/82</td>
<td>780</td>
<td>11</td>
<td>422 ± 46</td>
<td>189 ± 45</td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>HM</td>
<td>1954</td>
<td>03/27/86</td>
<td>464</td>
<td>3</td>
<td>378 ± 95</td>
<td>130 ± 32</td>
<td>M</td>
</tr>
</tbody>
</table>

**BY** = the year of birth; **Start** = the day the treatment started; **TC** = plasma total cholesterol before entering the treatment; **n** = the number of the treatment in National Cardiovascular Center by the end of August, 1986; **Pre** and **Post** = the average total cholesterol level before and after each treatment (mean ± SD). **P** = probrucol; **C** = cholestyramine; **M** = compactin (ML236B) or its derivative. **PE** = plasma exchange; **DF** = double membrane filtration; **DS** = dextran sulfate-cellulose. **GB** is the grass beads ornelt for non-specific adsorption only used experimentally for short term. **HM** = homozygote of FH; **HT** = heterozygote of FH.
coronary bypass operation and LDL-apheresis was started afterwards in order to prevent re-obstruction of the venous graft.

Procedure: Three major techniques were used for the removal of LDL from the patients' plasma; plasma exchange, double membrane filtration and LDL-adsorption by dextran sulfate-cellulose gel. Plasma was separated from blood cells by hollowfiber filters except for a few occasions with centrifuge instruments. The cellulose acetate hollowfiber filter (Plasmaphlo, Asahi Medical) was used in plasma exchange therapy with 5% human albumin solution for infant patients with body weight below 30 kg. Standard volume exchanged was 1.01 for the patient with below 25 kg weight and 1.51 for those above 25 kg (about 1 plasma volume). Cellulose acetate (Plasmaphlo, Asahi Medical) or polyvinylalcohol hollowfiber filters (Plasmacure, Kuraray) were used for plasma separation and the cellulose diacetate (Cascadeflo, Asahi, Medical) or ethylene-vinylalcohol copolymer (Evaflux 4A, Kuraray) filters were used for trapping plasma macromolecules in double membrane filtration. For selective LDL removal, dextran sulfate-cellulose (Lisosorbal, Kanegafuchi) was used as a sorbent in combination with the polysulfone hollowfiber (Sulflux, Kanegafuchi) as a plasma separator. The topological illustrations of the circuits are shown in Fig. 1. The standard volumes treated were 3.0 to 3.51 for adult patients. The details of these procedures were described elsewhere. Most of the patients were given oral drugs such as probucol, cholestyramine, compactin and its hydroxyl derivatives as listed in the Table I.

RESULTS AND DISCUSSION

Fractional Removal of LDL and Other Plasma Components.

Decrease of each plasma component during the operations should be expressed as exponential functions. In simple plasma exchange, every plasma component except for albumin decreases following the equation, $C = C_0 \times \exp (-\nu(V_f + V_e))$, where $C_0$ is the initial concentration of the plasma component after the extracorporeal circuit was equilibrated with plasma, $V_f$ and $V_e$ are intra- and extracorporeal circulation volume, and $\nu$ is the exchanged plasma volume. In double membrane filtration, the equation becomes $C = C_0 \times \exp (-\nu(V_f + V_e)/f)$, where $f$ is the fractional trapping ratio of each component in the second filter and $\nu$ is the plasma volume passed through the second filter. Consequently, the plot of $\ln(C)$ against $\nu(V_f + V_e)$ gives a straight line with the slope $f$ and with the extrapolated ordinate intercept $C_0$, as long as $f$ remains constant throughout the operation and there is no significant efflux of the component into plasma from any other pools during the time of the operation. It indeed gave good straight line for most of the component (LDL, high density lipoprotein (HDL), crude globulin fraction) in the case with dextran sulfate-cellulose.
cellulose, the plot for LDL deviates from the straight line with $f = 1$ as the column of the sorbent being saturated with LDL. When the column was switched to the new one before the plot deviates, C continued to decrease following the theoretical equation.

In standard procedure, described in the method section, approximately two thirds of

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Fig. 2. Long term treatment of the patient 1 with LDL-apheresis. Upper and lower lines in each panel respectively indicate the levels before and after each treatment. AG indicate the time of angiography.

Fig. 3. Regression of cutaneous xanthoma in the patient 1 after 4-year treatment. Upper 2 pictures were taken before entering the treatment.
LDL was removed in each operation, with the same fractional expense of HDL and other components in plasma exchange, with a 50% loss of HDL and crude globulin fraction in double membrane filtration, and without significant loss of those in the LDL-adsorptive procedure. Due to the rebound increase, the LDL level reached 70% of the original level one week after the treatment and came back to the original level two weeks after the treatment as the steady state with biweekly treatment. The rebound rate of HDL was, if there was any loss, much higher than LDL and reached the original level after 4 to 6 days with overshooting to some extent. The use of probucol or compactin derivative was somehow effective on homozygotes resulting in a reduction of the pretreatment level of LDL by 10%. The pre- and posttreatment levels of plasma total cholesterol in each patient are listed in Table I.

**Long Term Effect of LDL-Apheresis**

No significant side effect was experienced except for occasional hypovolemic symptoms such as decrease of blood pressure, which were managed by infusion of electrolytes solution or human albumin solution.

White blood cell count generally increased after the treatment probably because of complement activation. It was almost doubled in double membrane filtration but to much less extent in either plasma exchange or LDL-adsorption. In the last case, activated complements (C₃a and C₅a) were almost completely absorbed by dextran sulfate-cellulose.

A typical profile of the plasma total and HDL-cholesterol level in the long term treatment is shown in the Fig. 2. The figure is of patient 1, who started to receive plasma exchange therapy when she was 5 years old. After 4 years when her body weight exceeded 30 kg, the procedure was switched to double filtration. During 6
years of the treatment, her body weight increased from 17 kg to 38 kg and her height from 115 cm to 155 cm, and the first menorrhagia was seen at 11 years old. There were no signs of mental or emotional disturbance with her. Cutaneous xanthomas decreased remarkably within 2 years as shown in Fig. 3. Achilles tendon thickness also decreased to normal level after 4 years' treatment.

With long term treatment, cutaneous xanthoma diminished remarkably in all of the cases after 2 to 3 years treatment, especially with the help of probucol®. Achilles tendon thickness also typically decreased to normal level in most of the patients. No signs of growth disturbance were seen in any children treated.

Vascular Lesions

Posttreatment vascular lesion was assessed in patient 1 who had received the longest treatment 5 years by angiography. Perfusion of the left coronary arteries were delayed and 90% stenosis was demonstrated at the entrance. Roughness of the aortic wall was observed especially in the ascending part to the extent of stenosis in the supravalvular regions and these findings were also found in the left subclavian and both common carotid arteries. Substantial aortic regurgitation was observed probably because of xanthomatous infiltration in the valve itself and perivalvular regions. Roughness of the aortic wall was also observed in the abdominal aorta and a clear imaging defect was seen in the left renal artery (Fig. 4).

In the angiograms taken after 5 years' treatment, there were no signs of further development of atheromatous change in the coronary arteries. The wall of aorta was smooth in comparison with the pretreatment angiogram. The most remarkable changes were improvement of aortic valve regurgitation and disappearance of the atheromatous lesion in the left renal artery (Fig. 4). Thus, some atheromatous lesion clearly can be regressed by lowering LDL level even in the homozygotes of FH patients. The average TC level maintained in these patients was some 300 mg/dl with the maximum 436 and the minimum 138 mg/dl. But this level does not seem to be low enough to regress coronary lesion although it seems to prevent further atherosclerotic development.

Patient 4 died of myocardial infarction after 2.5 years' treatment in spite of remarkable decrease in both cutaneous and tendon xanthomas and subjective improvement in the severity and frequency of anginal episodes. Autopsy revealed that the embolism was due to the rupture of atheroma in the left coronary artery. His TC level was between 366 and 152 mg/dl, and we might have been able to observe some regression in vascular lesions because subjective complaint of anginal episodes had been substantially improved after two years' treatment. However, no angiographic assessment was done before his entering treatment due to refusal by the patient. The myocardial infarction attack seemed to occur after substantial physical exercise.

LDL-apheresis was thus shown to be a powerful procedure to reduce LDL to the level which no other drug treatment could attain. By using this technique, severe hypercholesterolemic patients such as FH can be managed and the development of the mortal coronary disease can be prevented. It seems to be possible to induce atheromatous lesion in certain conditions. As we mentioned in the INTRODUCTION section, a few thousand deaths in Japan every year can be avoided by managing the plasma TC level. And for some of them regression of coronary atheromatous lesions may be achieved by intensive reduction of LDL level for the limited period of time by using LDL-apheresis.

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1986