Selective Removal of Low Density Lipoprotein by Plasmapheresis in Familial Hypercholesterolemia

Shinji Yokoyama, Rikurou Hayashi, Makoto Satani, and Akira Yamamoto

Plasma lipoproteins were selectively removed from familial hypercholesterolemic patients by using two types of plasmapheresis: double-membrane filtration and selective adsorption of very low density lipoproteins (VLDL) and low density lipoproteins (LDL). In both techniques, plasma was separated from blood cells by using hollow-fiber filters, and 100% of the VLDL and LDL was recovered in the filtrate. In double-membrane filtration, the second hollow-fiber filter trapped 84% of LDL + VLDL, 48% of high density lipoprotein (HDL), 24% of albumin, and 46% of the remaining plasma protein. By treating 3 liters of plasma from a patient weighing 60 kg, 60% of the LDL and 30% to 40% of the HDL were removed as a result of an exponential decay of each component with the respective trapping coefficients. When dextran sulfate-cellulose was used as a LDL sorbent, there was only loss of LDL and VLDL, and no loss of any other major plasma component or of HDL. The sorbent column (400 ml) was saturated with 7.5 g of LDL cholesterol by treatment with 3.5 liters of plasma; the maximum reduction of LDL cholesterol was thus about 300 mg/dl for the patient weighing 60 kg. No serious side effects were observed during the long-term trials (19 to 27 months for four patients on double-membrane filtration and 10 months for the two patients on dextran sulfate-cellulose treatment).

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Plasma low density lipoprotein (LDL) has been recognized as a primary risk factor for developing atherosclerotic vascular lesions, especially in coronary arteries. Reduction of the plasma LDL level has been shown to decrease the incidence of ischemic heart attack in hyperlipoproteinemic patients. On the other hand, the plasma high density lipoprotein (HDL) level is inversely correlated, in epidemiological studies, to the incidence of coronary heart disease independently of plasma LDL contribution. Thus, the risk of coronary heart disease must be considered from both of these aspects.

Familial hypercholesterolemia is a common hereditary disorder and is characterized as a genetic deficiency of the function of the specific cell surface receptor for LDL. Because of their elevated levels of plasma LDL, these homozygotes develop fatal, premature, atherosclerotic vascular lesions in their first or second decade of life, and some heterozygotes develop such lesions in their third or fourth decade. Some oral drugs substantially lower plasma LDL levels in heterozygotes when used in appropriate combinations. For homozygotes, however, oral drugs have little effect on plasma LDL levels, with the exception of probucol, which has some effect. Therefore, direct removal of LDL from the patients’ plasma by plasmapheresis is the best treatment for homozygotes and for those heterozygotes for whom oral drugs are not effective.

Although the major pathogenic factor of atherosclerosis in patients with familial hypercholesterolemia is the increase of plasma LDL, we often encounter substantially reduced levels of HDL which may be an additional risk factor. Thus in plasmapheretic treatment, it is important to distinguish LDL from HDL upon removal of lipoproteins. It is also important to
preserve the other plasma components, including albumin, that are unnecessarily wasted in nonspecific plasmapheresis, such as plasma exchange. Therefore, in patients with familial hypercholesterolemia, selective removal of LDL has been practiced experimentally since 1975 by using heparin agarose affinity gel, anti-LDL-antibody-agarose gel and LDL precipitation with heparin in low pH. On the basis of these achievements, we have been trying to develop other conventional plasmapheresis techniques for selective removal of LDL: double-membrane filtration and a hard material for selective LDL adsorption. In this paper, we describe the specificity of these two techniques in removing LDL in comparison with the removal of other plasma components such as HDL. All patients described had familial hypercholesterolemia and agreed to receive these treatments.

Methods

Plasma Separators, Membrane Filters, and Adsorbent

Hollow-fiber membrane filters were used as plasma separators. These included: polyvinylalcohol filters (Plasmacure, Kuraray, Osaka) with an average pore size of 0.2 μm and an effective surface area of 0.6 m²; cellulose acetate filters (Plasmaflo, Asahi Medical, Tokyo) with the same average pore size and the same effective surface area for double membrane filtration; polysulfone hollow-fiber filters (KNS 05, Kanegafuchi Chemical Industry, Osaka) with an average pore size of 0.2 μm and an effective surface area of 0.5 m² for selective LDL adsorption. The second filters for double-membrane filtration were hollow-fiber membrane filters of copolymer of ethylenevinyl-alcohol (EVAL) (Evaflux 4A, Kuraray, Osaka) with an average pore size of 0.03 μm and an effective surface area of 2.0 m²; and cellulose diacetate (CDA) filters (Cascadeflo, Asahi Medical, Tokyo) with approximately the same pore size and surface area. Dextran sulfate cellulose beads (LA01, Kanegafuchi) were autoclaved at 121° C for 20 minutes and used as a specific LDL sorbent packed in the column with a volume of 400 ml.

Procedure

A double-membrane filtration circuit was used with a KM 8500 system (Kuraray) as shown in Figure 1 A. Blood was withdrawn at a rate of 100 ml/min by using a peristaltic pump from the forearm vein or the arteriovenous fistula and is returned to the vein.

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Figure 1. Topologic schemes of the extracorporeal circuit. A. The circuit for double-membrane filtration. P1 is a pump for blood withdrawal; P2 is a pump for plasma separation; P3 is a pump for draining trapped plasma and for infusing supplementary albumin solution. The shaded areas indicate the whole blood and blood-cell-rich portion. Arrows indicate the direction of the blood plasma flow. B. The circuit for specific LDL-adsorption plasmapheresis. Blood is withdrawn from the arteriovenous fistula and is returned to the vein.
riovenous fistula formed in the forearm of the patients. Plasma was separated through the filter at a rate of 30 ml/min by using a peristaltic pump and was passed through the second filter. Then the filtrate was infused into the patient with the cell-rich portion of the blood that remained unfiltered by the first filter. The solution trapped by the second filter was discarded at a rate of 3 ml/min to prevent plugging the filter; a 5% albumin solution in physiological saline was synchronously infused into the patient as a supplementary solution. To avoid confusing the results by changing the colloid osmotic pressure of the plasma, the circuit was filled with a 5% albumin solution in physiological saline before the operation when the detail of removal kinetics was studied. The total extracorporeal circulating plasma volume was about 400 ml when the extrafibrous space of the first filter was sufficiently evacuated.

For the sorbent system, the circuit was mounted on a KEM21 system (Nikkiso, Tokyo) as shown in Figure 1B. The rates of blood withdrawn (90 ml/min) and plasma filtration (30 ml/min) were the same as those in double filtration. The whole plasma separated through the filter was simply passed through the sorbent column with a downward flow, and the eluate was infused into the patient with the blood cell-rich portion. A single column was used for a single treatment until saturation. The total extracorporeal circulating volume was about 500 ml including the column inclusion volume of 350 ml. The circuit was not filled with albumin solution, but with physiological saline containing 5 mM Ca++ before the operation.

The whole operation was performed on-line. In all cases, the circuit of the secondary treatment of plasma was opened 10 minutes after the circuit of the plasma separator was equilibrated with blood. Heparin (2000 unit/2.0 ml) was injected at the beginning of the operation and then was continuously infused throughout the operation at a rate of 30 unit/min. The procedure was approved by the Committee for Clinical Trials of the Cardiovascular Center.

**Assay of Plasma Component**

Concentrations of several plasma components (total cholesterol, HDL cholesterol, total protein, and albumin) were monitored in venous or shunt-blood plasma immediately after separation through the plasma separator and in plasma immediately after the second filter, or sorbent column. Total cholesterol was measured by the enzymatic method with a Determiner TC5 assay kit (Kyowa Medics, Tokyo). HDL cholesterol was measured by a combination of LDL precipitation with heparin-Ca++ and an enzymatic method with use of an assay kit, HDL-C kitN (Nihon Shoji, Osaka). Total protein was measured by the biuret method with use of a TPSset assay kit (Daiichi Pure Chemical, Tokyo). Albumin was measured by the bromcresol purple method with use of a Dia-color AB kit (Toyobo Company Limited, Osaka). Very low density lipoprotein (VLDL) and LDL fractions were represented by total cholesterol minus HDL cholesterol.

Immunoglobulin A, G, and M, and complements C3 and C4 were measured with laser immunometry. Immunoglobulin E was measured with radioimmunoassay in the blood plasma before and after treatment (before returning the blood plasma in the extracorporeal circulation) in the clinical chemistry laboratory of the National Cardiovascular Center Hospital. The number of blood cells and hematocrit were counted in the hospital clinical hematology laboratory; standard blood biochemistry tests for electrolytes and for renal, liver, and thyroid (T3, T4, and TSH) functions before and after the treatment were also done in this laboratory. Plasma apolipoproteins (apo) A-I, A-II, B, C-I, C-II, and E were determined before and after treatment by a single radial immunodiffusion method by using 1% agarose gel plate that contained specific goat antisera provided by Dai-i-ichi Pure Chemicals (Tokyo).

The sorbent column was washed after the operation with 0.4 M NaCl solution to elute the lipoproteins bound to the gel. The total bound cholesterol was estimated by measuring the total amount of cholesterol in the eluate.

**Patients**

The patients treated by plasmapheresis all had familial hypercholesterolemia (Table 1). Diagnoses were based on pedigree analysis and on receptor assay of skin fibroblasts. Every patient received oral drug therapy first, and those for whom this therapy was unsuccessful received plasmapheresis. Patient 1 had received simple plasma exchange therapy for 1 year from 11 years of age until his body weight exceeded 40 kg when he received double-filtration therapy. Patient 2 had received a coronary bypass operation 1.5 years before starting double-membrane filtration plasmapheresis. All drug regimens failed to reduce this patient's plasma cholesterol level below 450 mg/dl even though she was a heterozygote. Patient 3 was first treated with a glass-bead sorbent system and then by double filtration followed by dextran sulfate-cellulose treatment. Patient 4 received double-membrane filtration plasmapheresis therapy for 1.5 years and was then switched to specific LDL-adsorption therapy.

Angiography showed that Patients 2 and 4 had atherosclerotic lesions in the coronary arteries. Patient 3 had an angina attack when exercising. Angiography showed that Patient 1 did not have atherosomatous lesions in the coronary arteries but did have them in the aortic root, the ascending aorta, and the cervical arteries. All patients had cutaneous xanthomas, tendon thickening, and arcus corneas resulting from elevated plasma cholesterol levels. As a standard procedure, the patients were treated at 2-week intervals. Drug regimens were combined with plasmapheresis treatment to help reduce the rate of increase in lipoprotein concentration after treatment.
Table 1. Patients with Familial Hypercholesterolemia Treated with Plasmapheresis

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yrs), sex</th>
<th>BW (kg)</th>
<th>Type</th>
<th>Xanthoma</th>
<th>IHD</th>
<th>Drug*</th>
<th>Drug†</th>
<th>PP duration (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14M</td>
<td>47</td>
<td>Homo</td>
<td>++</td>
<td>−</td>
<td>Probucol 620</td>
<td>Probucol 360-130</td>
<td>1.7(PE-DF)</td>
</tr>
<tr>
<td>2</td>
<td>58F</td>
<td>52</td>
<td>Hetero</td>
<td>+−</td>
<td>+</td>
<td>Probucol 450</td>
<td>Probucol 330-100</td>
<td>1.7(DF)</td>
</tr>
<tr>
<td>3</td>
<td>32M</td>
<td>61</td>
<td>Homo</td>
<td>+</td>
<td>+</td>
<td>Probucol 480</td>
<td>Probucol 340-130</td>
<td>1.7(DF-AD)</td>
</tr>
<tr>
<td>4</td>
<td>31M</td>
<td>65</td>
<td>Hetero</td>
<td>++</td>
<td>+</td>
<td>Cholestyramin 540</td>
<td>Compactin 500-200</td>
<td>1.7(DF-AD)</td>
</tr>
</tbody>
</table>

Diagnoses of all patients were based on the LDL-receptor assay of skin fibroblasts. PP = plasmaphoresis; BW = body weight; IHD = ischemic heart disease; NT = no treatment (plasma cholesterol pretreatment level); PE = plasma exchange; DF = double-membrane filtration; AD = LDL adsorption. *Numbers indicate the lowest cholesterol level achieved by the drug. †Numbers indicate the levels of cholesterol before and after the plasmapheresis operation, with a 2-week postoperative interval on the oral drug regimens. +− = mild xanthoma; + = moderate xanthoma; ++ = severe xanthoma.

Results

All the hollow-fiber membrane filters used as plasma separators showed 100% efficiency for filtration of LDL and VLDL. However, when Intralipid (Pharmacia Fine Chemicals, Piscataway, New Jersey), triglyceride particles stabilized by phospholipid with the size of large chylomicrons (larger than 500 nm in diameter), was infused to the patients, none of these hollow fibers let those particles pass and there was plugging. Therefore, membrane-filter plasma separators are not suitable for plasmapheretic treatment of hyperchylomicronemic patients.

Figure 2 shows the efficiency of the second membrane filter, EVAL 4A, in trapping the plasma components. The value of the trapping coefficient $f$ was calculated as:

$$f = 1 - C/C_p$$

where $C_i$ and $C_p$ are the concentrations of plasma component after and before the filter, respectively and was shown as a function of plasma volume passed through the second filter during the actual plasmapheresis operation for Patient 4. Figure 2 shows that the average of the $f$ value was 84% for the LDL + VLDL fraction, 48% for HDL, 24% for albumin, and 46% for the rest of the plasma protein fraction. It is apparent that $f$ is a function of the molecular weight of the component. There was a slight tendency for the $f$ value to increase throughout the operation, which might represent some plugging of the second membrane filter. The other second filter, CDA membrane filter, was similar with respect to these parameters. Therefore, the data are described for the EVAL 4A filter.

If the removal of the plasma component simply depends on each $f$ value, the decay of the concentration of the component ($C$) should be expressed as an exponential function of the plasma volume that passed through the second filter $v$:

$$C = C_0 \times \exp\left(-fv/(V_i + V_e)\right)$$

where $C_0$ is the initial concentration of the component when the whole circuit in the device is equilibrated with the blood and plasma, and $V_i$ and $V_e$ are intra- and extracorporeal circulation volumes, respectively. Assuming that the total circulating blood volume is 8% of body weight, we calculated the $(V_i + V_e)$ value using the patient's hematocrit value and the $V_e$ value of the device. Therefore, the slope of $\ln C$ v.s. $v(V_i + V_e)$ plot gives $-f$.

Figure 3 shows some examples of this plot for the LDL + VLDL fraction, HDL, and the total plasma protein except for albumin in Patients 1 and 4. Each plot shows a straight line giving $f$ values consistent with
Figure 3. Decay of plasma components during double-membrane filtration. A. (LDL + VLDL) cholesterol; B. HDL cholesterol; C. The (total protein − albumin) fraction. The linearized form of the exponential decay function is \( \ln C = \ln C_0 - \frac{f v}{(V_i + V_e)} \) and this was used for \( \ln C \) vs. \( \frac{v}{(V_i + V_e)} \) plot in this figure. According to this plot, the slope gives the \( f \) value, and the y intercept gives theoretical \( C_0 \) values for the device equilibrated. • indicates Case 1; o indicates Case 4 as listed in the tables. All \( V_i + V_e \) values are available in Table 2. Experimental \( C \) values at \( v = 0 \) are the component concentrations before the device was connected to the patient. The lower values at \( v = 0 \) for Case 1 are the component concentrations immediately before the circuit for the second filter was opened. Straight lines indicate the least-square linear regression line for the experimental values. The initial 0.5 liter of the operation was omitted from the analysis because it included the process of dilution of plasma with the extracorporeal circuit. The solid lines are for the open circles and the broken lines are for the closed circles.

The initial part of the operation does not follow the theoretical equation shown in Figure 3, which represents the process of dilution of the plasma component with saline having filled the extracorporeal circuit prior to the operation. When the linear regression line of the plot was extrapolated to 0 volume, the ratio of the intercept \( (C_0) \) to the measured initial component concentration was consistent with the factor of dilution for LDL + VLDL and HDL (0.86 and 0.88, respectively) for both Patients 1 and 4. This was consistent with the \( D \) values discussed later in the text and in Table 2. The ratio was less for the plasma protein fraction (0.7), possibly indicating nonspecific adsorption of such components as fibrinogen and gammaglobulin by the surface of the circuit. Thus, double-membrane filtration removed roughly 60% of LDL, with 40% loss of HDL, when 3 liters of plasma were treated for the 60-kg patient according to the above equation. Albumin (20 g) filled the device before the operation, and 15 g was used as a supplement during the operation. This albumin consumption can be decreased, however, depending on the condition of the patient. Filling the device with albumin before the operation is not required when the patient is an adult with normal cardiac function. More LDL could be removed by treating more plasma, but of course more HDL would be lost and more supplementary albumin required.

Table 2 shows the average efficacies of double-membrane-filtration plasmapheresis of 3 liters of plasma in the four patients. The efficacy was expressed as the recovery of each plasma component after plasmapheresis and was calculated from the concentration before the device was connected to the patient \( (C_i) \) and that after the operation and before the blood and the plasma in the device \( (C) \) was returned. The latter value was divided by the diluting factor \( D \) obtained from the extracorporeal circulation volume \( (V_e, 0.4 \text{ liter}) \) and the patient's plasma volume \( (V_p) \):

\[
D = \frac{V_p}{V_i + V_e}
\]  

The ratio of \( C/D \) to \( C \) was obtained as the recovery. As shown in Table 2, removal of each plasma component was consistent with the predicted values calculated from the above equation by using the specific \( f, V_i \), and \( V_e \) values. Recovery of HDL was 1.5 to two times more than recovery of LDL. The loss of each immunoglobulin depended on its molecular weight.

Specific LDL—adsorption plasmapheresis was performed with dextran sulfate cellulose beads as described in Methods. This sorbent has been shown to bind in vitro to LDL and VLDL but not to HDL or other plasma components. The material was used for experimental plasmapheresis in hyperlipidemic rabbits (WHHL rabbits and diet-induced hyperlipidemic rabbits) and showed excellent selectivity and efficiency in removing LDL from animal blood plasma.
Table 2. Recovery of Plasma Components In Double-Membrane Filtration Plasmapheresis after Passage of 3 Liters of Plasma through the Second Filter

<table>
<thead>
<tr>
<th>Case</th>
<th>V (liters)</th>
<th>No. of assays</th>
<th>LDL chol</th>
<th>HDL chol</th>
<th>TP-Alb</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>5</td>
<td>0.39 ± 0.04</td>
<td>0.66 ± 0.13</td>
<td>0.61 ± 0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>23</td>
<td>0.33 ± 0.03</td>
<td>0.65 ± 0.04</td>
<td>0.63 ± 0.07</td>
<td>0.66 ± 0.08</td>
<td>0.51 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.50 ± 0.15</td>
</tr>
<tr>
<td>3</td>
<td>2.6</td>
<td>13</td>
<td>0.39 ± 0.02</td>
<td>0.71 ± 0.09</td>
<td>0.67 ± 0.08</td>
<td>0.69 ± 0.05</td>
<td>0.55 ± 0.07</td>
<td>0.36 ± 0.09</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>13</td>
<td>0.45 ± 0.03</td>
<td>0.66 ± 0.07</td>
<td>0.67 ± 0.07</td>
<td>0.67 ± 0.08</td>
<td>0.58 ± 0.07</td>
<td>0.39 ± 0.05</td>
<td>0.57 ± 0.09</td>
</tr>
</tbody>
</table>

D = diluting factor; V = the patient's plasma volume; LDL chol = low density lipoprotein cholesterol (total cholesterol minus HDL cholesterol); HDL chol = high density lipoprotein cholesterol; TP-Alb = total protein minus albumin; Ig = immunoglobulin. See text for the calculation method used for recovery. Values are means ± SE.

A flow rate compatibility test showed that the pressure gradient increased from 0 to 120 mm Hg linearly proportional to the plasma-flow rate within the range of 0 to 90 ml/min and to the water-flow rate from 0 to 120 ml/min in vitro, between the entrance and the exit of the 400 ml column. The pressure monitored at the chamber sited immediately before the column remained constant, with a plasma-flow rate of 30 ml/min throughout the operation (46 ± 8 mm Hg for 10 treatments). After fresh human plasma was passed through the column in vitro, adsorbed LDL and VLDL were eluted either with the linear gradient of NaCl concentration from 0.15 to 0.5 M or with 0.4 M NaCl solution. The recovery of adsorbed lipoproteins in the eluate was 100% with respect to cholesterol in both cases, and the washed column regained the original adsorption capacity by re-equilibrating with 0.15 M NaCl. Thus, the total amount of adsorbed lipoprotein was estimated by washing with 0.4 M NaCl solution after the operation.

Figure 4 shows the typical decay curve of the plasma components as a function of the plasma volume that passed through the sorbent column when Patients 3 and 4 were treated. After the column was

Figure 4. Change in plasma component concentrations during LDL-adsorption plasmapheresis with dextran sulfate-cellulose beads. A. LDL + VLDL cholesterol. B. HDL cholesterol. C. The (total protein - albumin) fraction. D. Albumin. ○ indicate concentrations in plasma before separation from blood cells; • indicate concentrations in plasma after passage through the sorbent column. Concentrations in the plasma after plasma separation (immediately before the column) were similar to the concentrations in the plasma before separation. The data connected with solid lines are from Case 4 and those with broken lines are from Case 3. The lower data points at volume 0 are the component concentrations immediately before the column circuit was opened.
equilibrated with plasma, the concentrations of HDL, albumin, and total plasma proteins were similar before and after the column. Only the LDL + VLDL fraction was adsorbed until the binding capacity of the sorbent was saturated. Consequently, the plasma LDL + VLDL concentration specifically decreased, while the concentrations of HDL, albumin, and plasma protein fraction was first reduced as by further dilution of the plasma. The concentrations of these components then increased slowly throughout the operation, suggesting the re-concentration of circulating plasma to compensate for the decrease of plasma colloid osmotic pressure due to dilution. Consequently, the "intracorporeal" circumflex plasma volume was reduced by this process and might cause hypovolemic symptoms in some patients. When the device was filled with a 5% saline filled the extracorporeal circuit. Plasma protein (except albumin) showed a little more decrease than predicted by dilution even before the circuit of the column was opened. This might be due to nonspecific adsorption of plasma protein such as fibrinogen and gammaglobulin by the device's surface. After the column circuit was opened, the level of HDL, albumin, and plasma protein fraction was first reduced as by further dilution of the plasma. The concentration of these components then increased slowly throughout the operation, suggesting the re-concentration of circulating plasma to compensate for the decrease of plasma colloidal osmotic pressure due to dilution. Consequently, the "intracorporeal" circulating plasma volume was reduced by this process and might cause hypovolemic symptoms in some patients. When the device was filled with a 5% albumin solution before the operation, this re-concentration was prevented (data not shown).

Both from the decrease of the plasma LDL level, and from the cholesterol concentration in the eluate with 0.4 M NaCl from the column after the treatment, the column's saturation capacity for LDL cholesterol was estimated to be 7.5 g (18.8 g/liter gel), which is consistent with the saturation level of the gel for LDL obtained from an in vitro adsorption experiment. To reach the saturation, 3 to 4 liters of plasma must pass through the column; the reduction of the plasma LDL cholesterol level was about 300 mg/dl. This is close to that achieved by treatment of 3 liters of plasma by the double-membrane filtration system.

Table 3 shows the average recoveries of lipoproteins and crude plasma protein fractions by treatment with 3.5 liters of plasma in Patients 3 and 4. The values were calculated according to the same procedure as Table 2 by using the V, value of 0.5 l. HDL and albumin were not lost in treatment. The slight over-recoveries of these components may be the result of the re-concentration of the plasma described above. Other protein fractions were slightly reduced, by less than a few percent, showing nonspecific adsorption by the device.

Immunoglobulins and apolipoproteins are listed in Table 4. The loss of immunoglobulins was also less than a few percent. Recovery of apolipoproteins was consistent with the changes in lipoprotein subfractions. The average recovery of apo A-I and A-II was close to 100% in six treatments for each case. Recovery of apo B was slightly higher than recovery of total cholesterol - HDL cholesterol, reflecting a high cholesterol/apo B ratio in VLDL. The reductions of apo C-III, C-II, and E seem to be due to the removal of VLDL because 100% of HDL particles were recovered as indicated by HDL cholesterol, apo A-I and apo A-II. Higher recovery of apo C-II than of C-III and E may be due to transfer from VLDL to HDL during massive hydrolysis of VLDL triglyceride caused by injection of high doses of heparin. Thus, LDL-adsorption plasmapheresis with dextran sulfate-cellulose beads was shown to have excellent selectivity for removal of LDL and VLDL from patients with familial hypercholesterolemia. No significant changes were seen in the standard blood tests for electrolytes or renal, liver, and thyroid functions. Patients were each treated 14 times, and no sign of sensitization was observed; passive cutaneous anaphylaxis tests for dextran sulfate were all negative.

### Table 3. Recovery of Plasma Components in LDL Adsorption Plasmapheresis after Passage of 3 Liters of Plasma through the Sorbent Column

<table>
<thead>
<tr>
<th>Case no.</th>
<th>V,</th>
<th>D</th>
<th>No. of assays</th>
<th>LDL chol</th>
<th>ΔLDL (g)</th>
<th>HDL chol</th>
<th>Albumin</th>
<th>TP-Alb</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.6</td>
<td>0.84</td>
<td>9</td>
<td>0.42 ± 0.05</td>
<td>5.5 ± 0.6</td>
<td>1.02 ± 0.16</td>
<td>1.01 ± 0.03</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>0.86</td>
<td>9</td>
<td>0.48 ± 0.01</td>
<td>7.5 ± 0.9</td>
<td>1.02 ± 0.05</td>
<td>1.02 ± 0.05</td>
<td>0.88 ± 0.05</td>
</tr>
</tbody>
</table>

See Table 2 and text for definitions. The amount of LDL + VLDL cholesterol removed (ALDL) was calculated as (C, - C/D) x V,, where C, and C were as defined in the text. Values are means ± SE. LDL chol = total cholesterol minus HDL-cholesterol; TP-Alb = total protein minus albumin.

### Table 4. Recovery of Plasma Immunoglobulins and Apolipoproteins after 3 Liters of Plasma Treatment by Dextran Sulfate-Cellulose Beads

<table>
<thead>
<tr>
<th>No. of assays</th>
<th>IgA</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>3</td>
<td>0.98 ± 0.05</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>IgM</td>
<td>3</td>
<td>0.97 ± 0.02</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>6</td>
<td>1.06 ± 0.15</td>
<td>1.05 ± 0.15</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>6</td>
<td>1.10 ± 0.07</td>
<td>1.11 ± 0.15</td>
</tr>
<tr>
<td>Apo B</td>
<td>6</td>
<td>0.50 ± 0.05</td>
<td>0.57 ± 0.06</td>
</tr>
<tr>
<td>Apo C-II</td>
<td>6</td>
<td>0.64 ± 0.12</td>
<td>0.64 ± 0.17</td>
</tr>
<tr>
<td>Apo C-III</td>
<td>6</td>
<td>0.60 ± 0.02</td>
<td>0.69 ± 0.04</td>
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<tr>
<td>Apo E</td>
<td>6</td>
<td>0.44 ± 0.03</td>
<td>0.47 ± 0.06</td>
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</table>

Values are means ± se calculated as in Tables 2 and 3.
Changes in LDL (total cholesterol – HDL cholesterol) and HDL cholesterol levels in long-term trials are shown in Figure 5. Case 4 (Panel A) had received plasma exchange therapy several times before he was transferred to our facility, but the only detail of his clinical record available was the pretreatment level of plasma lipids. Case 3 (Panel B) had been taking probucol for 1 year before starting plasmapheresis therapy. The glass bead-sorbent system\(^{21,22}\) was first used in this case but proved inefficient with respect to adsorption capacity and lipoprotein selectivity. Double-membrane filtration and then dextran sulfate-cellulose sorbent were used in Cases 3 and 4. In both cases, LDL cholesterol levels were kept below 350 mg/dl by treatment at 2-week intervals. Figure 5 shows several LDL peaks, which were mostly caused by a long interval of non-treatment due to the holidays. Considering the time-course of the increase of LDL after the treatment,\(^{10,22}\) we find that the average concentration of LDL is a little higher than the midpoint of the values before and after treatment. It is, therefore, probably 280 mg/dl to 300 mg/dl for Case 3 and 300 mg/dl to 320 mg/dl for Case 4. Little increase in HDL has been seen so far, although this was observed by Lupien et al. after a long-term trial.\(^{23}\) Our results may be due to the oral drug regimens such as with probucol that we used. Frequencies of anginal attacks decreased in Cases 2 and 3. Cutaneous xanthomas and tendon thickenings were markedly improved in all four cases.

Discussion

There are some advantages in the sorbent system compared with the specific LDL-adsorptive materials described previously.\(^{14–23}\) The mechanical properties of the support material (cellulose beads) are preferable: they are autoclavable and withstand high flow rates (120 ml/min of water and 80 ml/min of plasma). The absence of organic crosslinks makes cellulose beads biologically less active than agarose beads. Because the adsorption capacity of the gel is as great as 18.8 g cholesterol/liter of wet gel, a single column is enough to reduce plasma LDL cholesterol level by 200 mg/dl to 300 mg/dl. Therefore, the extracorporeal circuit was simpler in this trial than in the previous LDL-apheresis system,\(^{21,22}\) which included the device for switching and regenerating the two columns. Table 5 compares the present procedure with the previous methods. (See references 18, 21, and 22 for details.)

Dextran sulfate was covalently bound to the beads and used for the specific LDL-binding site. Although this material reportedly can sensitize the recipient when injected intravenously, the sorbent is kept apart from the blood cells in our system. In 28 procedures at 2-week intervals, for each of two patients, we did not observe any signs of sensitization. The white blood cell count decreased by 10% to 30% for the first 30 to 60 minutes of the operation, followed by an increase up to 130% probably caused by complement activation, although only marginal activation was indicated by the average recoveries of C3 and C4 (both 0.99) for three treatments of each patient. However, the change in white blood cell count was smaller than that observed in the double membrane-filtrations we have used in which the increase was up to 180%. Therefore, complement activation by the sorbent seems to be within a tolerable range, and the dextran sulfate-cellulose system is an effective, practical plasmapheresis method for the selective removal of atherogenic lipoproteins in patients with familial hypercholesterolemia.

The limitation of the single sorbent column system is the capacity of the sorbent beads to adsorb LDL. Seven grams of LDL cholesterol removal is close to a
Table 5. Comparison of Selective LDL Adsorption Plasmapheresis Procedures

<table>
<thead>
<tr>
<th>Plasma separator</th>
<th>Heparin-agarose*</th>
<th>Anti-LDL-antibody agarose†</th>
<th>Dextran sulfate-cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracorporeal volume (ml)</td>
<td>no (whole blood treatment)</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Additional infusion (ml)</td>
<td>—</td>
<td>300–500</td>
<td>—</td>
</tr>
<tr>
<td>Sorbent volume (ml)</td>
<td>100 (per bag)</td>
<td>400 x 2</td>
<td>400</td>
</tr>
<tr>
<td>Operation</td>
<td>intermittent (manual) (6–8 bags)</td>
<td>continuous (2 columns switching system)</td>
<td>continuous (single column)</td>
</tr>
<tr>
<td>Removal specificity</td>
<td>LDL, VLDL</td>
<td>LDL, VLDL</td>
<td>LDL, VLDL</td>
</tr>
<tr>
<td>Capacity of gel (g cholesterol/liter gel)</td>
<td>19</td>
<td>5</td>
<td>18.8</td>
</tr>
<tr>
<td>Treated plasma (ml/treatment)</td>
<td>150–700</td>
<td>5900</td>
<td>3500</td>
</tr>
<tr>
<td>Cholesterol reduction (mg/dl)</td>
<td>50–200</td>
<td>212</td>
<td>204 (Case 3)–239 (Case 4)</td>
</tr>
<tr>
<td>LDL-cholesterol removal (g)</td>
<td>1.3–5.2</td>
<td>5.6</td>
<td>5.5–7.5</td>
</tr>
<tr>
<td>Time (hrs)</td>
<td>—</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

*See references 16, 18, 33.†See references 19–21.

300 mg/dl reduction of its concentration in adult plasma. This is 60% of the original LDL cholesterol at 500 mg/dl, but it is only 33% if the original level of LDL cholesterol is 900 mg/dl. If a greater capacity for LDL removal is needed, the volume of extracorporeal circulation must be increased unless the volume-specific adsorption capacity increases. A system using switching between two columns21,22 may be useful for patients with very high plasma LDL levels.

Larger extracorporeal circulation volume causes more dilution of the plasma, resulting in reduction of the effective circulating plasma volume. Since patients with familial hypercholesterolemia probably have serious atherosclerotic lesions in their coronary arteries, aortic valves, and other vessels, we must be very careful about the risk of hypovolemia caused by this reconcentration of the plasma. To avoid this risk, we may have to fill the extracorporeal circuit with albumin before the operation. However, it should be noted that the patient's heart is still overloaded by the simple expansion of the total circulation volume due to the extracorporeal circuit.

Double-membrane filtration has some advantages in this respect. The extracorporeal circulation volume can be reduced to 300 ml by using commercially available half-size filter chambers with little decrease in filtration efficiency. Therefore, for children or for patients with low cardiac function, double filtration may be a better choice than the sorbent system. In addition, it is possible to remove more LDL by double filtration when the LDL concentration is very high, so this should be the first plasmapheresis treatment for a new patient. The combination of the two techniques reported here must be carefully chosen for the particular patient. For children, it is important to reduce the extracorporeal circulation volume as much as possible. When the body weight of the patient is less than 30 kg, a simple plasma exchange is still the best and safest technique because of the simplicity of control and the small extracorporeal circulation volume.

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Index Terms: familial hypercholesterolemia • plasmapheresis • low density lipoproteins
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