The Changes in Plasma Lipoproteins in a Case of Heterozygous Familial Hypercholesterolemia after Plasmapheresis

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The changes in plasma lipoproteins after plasmapheresis were estimated in a case of heterozygous familial hypercholesterolemia. Two and a half liters of plasma were exchanged with the same volume of Plasmanate. The effects of cholestyramine on the changes in plasma lipoproteins were also studied after the second plasmapheresis. Fasting plasma was collected serially, and plasma VLDL, IDL, LDL₁ ($1.019 < d < 1.045$), LDL₂ ($1.045 < d < 1.063$), HDL₂ ($1.063 < d < 1.125$) and HDL₃ ($d > 1.125$) were separated by ultracentrifugation. The cholesterol and apoprotein contents in these lipoprotein-fractions were analyzed. Apoprotein AI, AII, B, CII and E were measured by single radial immunodiffusion. VLDL-C and plasma apoprotein CII and E increased very rapidly. The IDL-C increase was slightly slower than that of VLDL-C. LDL₁-C continued to increase for three weeks, but LDL₂-C reached a plateau around a week after plasmapheresis and declined thereafter. The change of apoprotein B was similar to that of cholesterol in VLDL, IDL, LDL₁ and LDL₂. Cholesterol amounts in HDL₂ and HDL₃ changed in a parallel manner, as did apoprotein AI and AII. HDL₂ and HDL₃ reached their preplasmapheresis levels in a week. Cholestyramine did not inhibit the increase of VLDL, IDL, LDL₁ or LDL₂ in this case.

Key Words: Plasmapheresis, Familial hypercholesterolemia, Lipoproteins, LDL subfractions, HDL subfractions.

Familial hypercholesterolemia (FH) is an inherited disease of an autosomal dominant trait¹⁾. The incidence of FH of the heterozygous form is said to be one per 500 population^{1, 2)}. The most serious complication of FH is coronary heart disease (CHD). FH is highly resistant to medication. Plasmapheresis³⁻⁶⁾, portacaval shunt⁷⁻⁹⁾ and partial ileal bypass¹⁰⁾ have been applied to treat this condition. Plasmapheresis has been used for the treatment of FH of both the homozygous and heterozygous forms. The disappearance of xanthoma and the improvement of coronary stenosis have already been reported as a result of relatively long-term plasmapheresis¹¹⁾. However, there have been no reports on acute

changes in subfractionated plasma lipoproteins after plasmapheresis.

In normal subjects, very low density lipoprotein (VLDL) is converted to low density lipoprotein (LDL) via intermediate density lipoprotein (IDL) by the delipidation by lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) in the blood circulation. VLDL is the sole precursor of LDL in normal subjects^{12, 13)}. However, in FH a part of LDL has recently been said to be secreted directly to the plasma from the liver. The rates of direct LDL secretion are variable in individual FH patients^{14, 15)}.

Plasmapheresis has the advantage of facilitating study of the interrelationship between lipoproteins

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without the use of radioisotopes. The aim of this study was to estimate the changes in subfractionated plasma lipoproteins in an FH patient after plasmapheresis in order to study the interrelationship between them.

MATERIALS AND METHODS

Subject

A 30-year-old male who was admitted to Tokai University Hospital for the evaluation of coronary stenosis was plasmapheresed twice. His mother's plasma lipid levels were normal, but those of his father were unknown. He has a sister who suffers from hypercholesterolemia and xanthomatosis. Upon admission, plasma total cholesterol, triglyceride and high density lipoprotein (HDL) cholesterol levels were 533 mg/dl, 146 mg/dl and 30 mg/dl, respectively. LDL-binding to his 72 hours' cultured peripheral lymphocytes was 60% of normal control. ECG at rest was normal, and Holter monitoring ECG revealed slight ST depression on exercise. Fifty percent stenosis of the left anterior descending (LAD), 30% stenosis of the right coronary arteries and diffuse narrowing of the LAD artery were found by coronary arteriography.

Plasmapheresis

Venous blood was introduced from the brachial vein to a cell separator IBM-2997. The plasma was separated from blood cells by continuous centrifugation at 1,600 rpm. The separated plasma was discarded into a plastic bag. Blood cells were mixed with Plasmanate and returned to the patient through the femoral vein. ACD-A was used as an anticoagulant. Two and half liters of plasma were removed at the rate of 1,000 ml/hr.

Laboratory procedures

Fasting plasma was collected serially. VLDL, IDL, LDL₁ ($1.019 < d < 1.045$), LDL₂ ($1.045 < d < 1.063$), HDL₂ ($1.063 < d < 1.125$) and HDL₃ ($d > 1.125$) were harvested ultracentrifugally by the method of Havel et al.¹⁶⁾ Cholesterol and triglyceride levels in lipoproteins were estimated with a SMAC autoanalyzer¹⁷⁾. Apoprotein, the protein moiety of lipoproteins, was also measured. Plasma apoprotein AI, AII, B, CII and levels¹⁸⁾ were meas-

ured by single radial immunodiffusion (SRID). The immunodiffusion plates containing antibodies of apoprotein AI, AII, CII and E were supplied by Daiichi Chemical Company, Tokyo, Japan. The antibody plates of apoprotein B were from Eiken Chemical Company, Tokyo, Japan.

RESULTS

The changes in cholesterol amounts in VLDL and IDL are shown in Fig. 1. VLDL cholesterol returned to the value before plasmapheresis very rapidly, reaching a higher level on the following day. IDL cholesterol increased more slowly than VLDL cholesterol. IDL cholesterol increased very rapidly upon cholestyramine treatment.

Cholesterol levels in LDL₁ and LDL₂ are indicated in Fig. 2. The increase of LDL cholesterol levels after plasmapheresis was much slower than that of VLDL and IDL cholesterol. The increase in cholesterol was faster in LDL₁ than in LDL₂ at the early stage after plasma exchange. LDL₁ cholesterol needed three weeks to return to its preplasmapheresis value, while LDL₂ cholesterol required only a week. Cholestyramine did not show any inhibitory effects on the increase of LDL₁ or LDL₂ cholesterol levels in this case.

HDL₂ and HDL₃ cholesterol levels were very low in the patient. Plasma HDL₂ and HDL₃ cholesterol levels were 10 mg/dl and 8 mg/dl, respectively. HDL₂ and HDL₃ cholesterol increased in a parallel manner and reached a plateau at the seventh day after the first and the fourth day after the second plasmapheresis, respectively, (Fig. 3).

The changes in plasma apoprotein CII and E are shown in Fig. 4. Average plasma apoprotein CII and E levels were 4.3 mg/dl and 4.9 mg/dl, respectively, in 249 healthy subjects at the Health Counselling Center of Tokai University Hospital. Plasma apoprotein CII and E levels were 7 and 8 mg/dl, respectively, in the patient. Therefore, he had high plasma apoprotein CII and E levels. Plasma apoprotein CII and E levels returned very quickly to the values before plasmapheresis as did VLDL cholesterol. Apoprotein CII and E

Fig 1. Changes in VLDL and IDL cholesterol levels after plasmapheresis.

Fig 2. LDL₁ and LDL₂ cholesterol levels after plasmapheresis.

moved very similarly after plasmapheresis.

Plasma apoprotein B levels in VLDL, IDL, LDL₁ and LDL₂ before the first plasmapheresis were, 2, 8, 180 and 13 mg/dl, respectively. Apoprotein B in LDL₁ fell from 180 mg/dl to 26 mg/dl after two and a half liters of plasma exchange. The change in apoprotein B in VLDL, IDL, LDL₁ and LDL₂ was very similar to that in cholesterol in these lipoproteins (Fig. 5). The apoprotein B level increased more slowly in LDL₂ than in LDL₁ at the early stage after

plasmapheresis and reached at peak earlier in LDL₂ than in LDL₁. Cholestyramine did not prevent the increase of apoprotein B in VLDL, IDL, LDL₁ or LDL₂ in this case.

Apoprotein AI levels were 20 mg/dl in HDL₂ and 44 mg/dl in HDL₃ before the first plasmapheresis. Apoprotein AII levels were 5 mg/dl in HDL₂ and 8 mg/dl in HDL₃. Total apoprotein AI and AII levels were 64 mg/dl and 13 mg/dl, respectively, which were about half of the normal values. Apoprotein AI and AII changed similarly

in both HDL₂ and HDL₃ (Fig. 6), reaching a plateau at the fourth day after plasmapheresis. The effect of cholestyramine on HDL increase after plasmapheresis was almost nil in this case. The increase of HDL to its preplasmapheresis level was much quicker than that of LDL.

DISCUSSION

The acute recovery process of plasma lipoproteins after plasmapheresis was investigated in a case of heterozygous FH. The increase of VLDL-C was very rapid. Plasma VLDL-C returned to its preplasmapheresis level on the day following plasmapheresis. VLDL-C continued to increase for a week and decreased thereafter. The increase of IDL-C was slower than that of VLDL-C. IDL-C

continued to increase up to two weeks after the first plasmapheresis. It is known that there is a good precursor-product relationship between VLDL and IDL in normal subjects. Cholesterol and apoprotein B in IDL reached their peaks one week later than those of VLDL. These delays might indicate the direct secretion of some IDL from the liver. The increase in LDL-C was much slower than that of VLDL-C and IDL-C. LDL₁-C continued to increase for three weeks until the second plasmapheresis, while LDL₂-C reached a plateau around a week after plasmapheresis and then declined. Therefore, we could not find good precursor-product relationships between IDL and LDL or between LDL₁ and LDL₂ in this FH patient. A part of IDL, LDL₁ and LDL₂

Fig 3. HDL₂ and HDL₃ cholesterol levels after plasmapheresis.

Fig 4. Chnages in plasma apoprotein CII and E after plasmapheresis.

Fig 5. Apoprotein B levels in VLDL, IDL, LDL₁ and LDL₂ after plasmapheresis.

Fig 6. Apoprotein AI and AII levels in HDL₂ and HDL₃ after plasmapheresis.

must be secreted directly from the liver. HDL₃ was demonstrated to be a precursor of HDL₂ in vitro^{19, 20}). However, the precursor-product relationship between HDL₂ and HDL₃ has not been demonstrated clearly in vivo. HDL₂-C and HDL₃-C changed in a parallel manner in this patient and did not show changes indicative of the precursor-product relationship. HDL₂-C, HDL₃-C, apoprotein AI, and apoprotein AII returned to their preplasmapheresis levels in a week after plasmapheresis.

Apoprotein CII and E increased very rapidly after plasmapheresis. The changes in plasma apoprotein CII and E were similar to the change

in VLDL-C. This is probably due to the fact that VLDL is the main carrier of apoprotein CII and E¹⁸). Apoprotein B is a major apoprotein in VLDL and IDL and almost the sole apoprotein in LDL. The change in apoprotein B in VLDL, IDL, LDL₁ and LDL₂ was very similar to that of cholesterol in these lipoproteins. Apoprotein AI and AII showed parallel changes in HDL₂ and HDL₃, as did cholesterol.

The effects of bile acids sequestering resin, cholestyramine¹) which is one of the most effective drugs for FH, were also investigated. The increase of VLDL-C, IDL-C and apoprotein CII and E was much faster and more marked on

cholestyramine. This is probably due to the fact that cholestyramine stimulated the excretion of bile acids into the feces and released the inhibition of cholesterol and VLDL synthesis by bile acids in the liver. Cholestyramine neither prevented the increase of LDL₁ and LDL₂ nor affected the increase of HDL₂ and HDL₃ in this case. The proper medication should be sought in order to prolong the interval between plasmapheresis for the long-term treatment of FH.

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