Jpn J Apheresis 13(2): 75-77, 1994

Evaluation of Low-Density Lipoprotein Apheresis with a Dextran Sulfate Cellulose Column

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Among several different modalities developed for removal of low-density lipoptotein (LDL), dextran sulfate (DS) cellulose column (Liposorba, Kanegafuchi Chemical Industry, Osaka, Japan) is most frequently used for LDL apheresis in Japan. The merit of the DS column is its clear specificity for apolipoprotein B, through which serum LDL can be lowered without limitation. The long-term treatment of hypercholesterolemia by this LDL apheresis method yielded regression of atherosclerotic lesions in 30-40% of patients.[1] The basis for this regression might be related to the fact that intensive removal of LDL by apheresis causes excessive exclusion of cholesterol from other than intravascular cholesterol reserve. The aim of the present study was to verify this hypothesis. Moreover, the distribution volume of (VLDL+LDL)-cholesterol was measured based on the single pool model and was compared with the sum of intravascular plasma volume and the volume of the extracorporeal circuit. The most frequent side effects of LDL apheresis with the DS column is hypotension. The mechanism of hypotension might be related to the increase in plasma levels of bradykinin which is released from high-molecular-weight kininogen (HMWK) by the action of kallikrein converted from plasma prekallikrein (PK).[2] The conversion of PK to kallikrein is related to the activation of initial contact phase of the intrinsic coagulation pathway, in which a complex of factor XII, PK, and HMWK is formed on the surface with negative electrostatic charges. We examined the ability of the DS column used for LDL apheresis to activate this process. Since nafamostat mesilate, a protease inhibitor, inhibits the activity of kallikrein, the effect of nafamostat mesilate on bradykinin generation was also examined.

Subjects and Methods

1. Cholesterol balance during LDL apheresis. Subjects consist of 7 patients with severe hypercholesterolemia. The mean serum cholesterol level of these patients before apheresis was 280± 83 mg/dl (mean±SD). For LDL apheresis, we used a computer-controlled machine equipped with one plasma filter and two DS columns (MA01, Kanegafuchi Chemical Industry). The total treated volume of plasma was 3,000-6,000 ml, and it took 3-4 hours for one apheresis session. Hemoglobin, hematocrit, and serum concentrations of albumin, total cholesterol, and Lp(a) were measured before and after LDL apheresis. The decrease of intravascular reserve of cholesterol and Lp(a) was calculated from serum concentrations and intravascular plasma volume before and after apheresis. Intravascular plasma volume was calculated according to the body structure. The amount of plasma components removed during LDL apheresis was determined by the decrease in intravascular reserve. The loss of these components in the discarded fluid was also determined by measuring these concentrations in the discarded fluid and the discarded volume. For the measurement of distribution volume of (VLDL+LDL)-cholesterol, serum concentrations of total cholesterol, and HDL-cholesterol were measured at 0-ml, 1,000-ml, 2,000-ml, and 3,000-ml plasma treatment during LDL apheresis. The concentrations of (VLDL+LDL)-cholesterol were obtained by subtracting those of HDL-cholesterol from those of total cholesterol. The distribution volume of (VLDL+LDL)-cholesterol was calculated based on the single pool model and compared with the sum of intravascular plasma volume and the volume of the extracorporeal circuit.

2. Drawbacks of DS column. To investigate the effects of DS on initial contact phase of intrinsic coagulation pathway, LDL apheresis with a DS column was undertaken using two different types of anticoagulants, heparin or a protease inhibitor, nafamostat mesilate (Futhan, Torii Yakuhin Co.). Nafamostat mesilate inhibits the action of kallikrein to release bradykinin from HMWK, whereas heparin has no such effect. These anticoagulants were continually in-

fused from the inlet side of the plasma filter. The samples for determination of the bradykinin level were taken from the blood circulation circuit before the filter and the plasma before and after the DS column. In LDL apheresis using heparin, the blood samples for measurement of factor XII, HMWK and PK were taken before and after apheresis, and before and after the DS column at the 1,000-ml plasma treatment.

Results and Discussion

1. Cholesterol balance during LDL apheresis. The decrease in intravascular reserve of total cholesterol, Lp(a), and albumin was 3.91±0.77 g, 392±124 mg, and 6.09±1.55 g, respectively. On the other hand, the removed amount of these plasma components in the discarded fluid was respectively 4.53±0.78 g, 397±124 mg, and 7.56 ±0.80 g. Although there were no significant differences in Lp(a) and albumin between the discarded amount and the decrease in intravascular reserve, the discarded amount of cholesterol was significantly ($P<0.05$) greater than the decrease in intravascular reserve of cholesterol (Fig. 1). This finding suggests that cholesterol removed during LDL apheresis originates from not only intravascular reserve, but also extravascular tissue including cholesterol synthesized in the liver. The distribution volume of (VLDL+LDL)-cholesterol calculated based on the single pool model was 3,065±280 ml, and was significantly greater than the sum of the intravascular plasma volume and extracorporeal circuit volume, 2,633 ±199 ml. There was a close correlation ($r=0.998$) between these two values (Fig. 2). The results indicate that the amount of cholesterol synthesis might not be negligible during LDL apheresis or the single pool model might not be justified for the precise analysis of cholesterol kinetics.

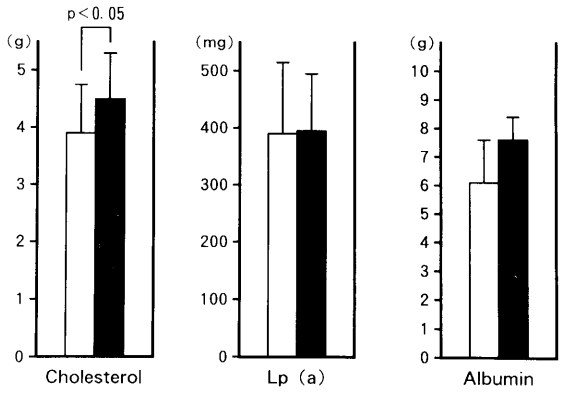

Fig. 1 Comparison between the decrease in intravascular reserve (open column) and discarded amount (closed column). The discarded amount of cholesterol is significantly ($P<0.05$) greater than the decrease in intravascular reserve. There is no significant difference between intravascular reserve and discarded amount in Lp(a) and albumin.

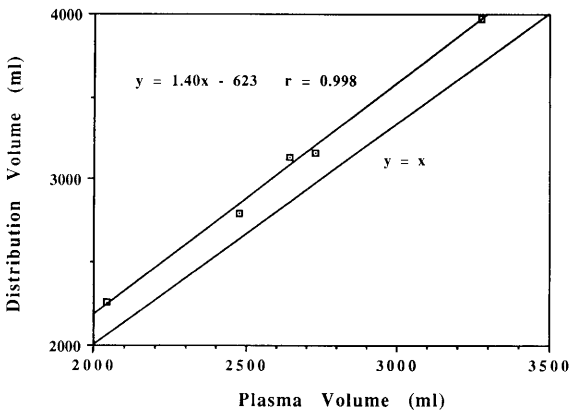

Fig. 2 Correlation between intravascular plasma volume and (VLDL+LDL)-cholesterol. There is a highly significant correlation between the plasma volume and distribution volume of (VLDL+LDL)-cholesterol. The latter value is consistently greater than the former value.

2. Drawbacks of DS column. The mechanism of cholesterol adsorption is electrostatic binding between the positive charges of apolipoprotein B of LDL and the negative charges of DS. However, the activation of initial contact phase in the coagulation pathway will be elicited by a negatively charged surface such as high-molecular-weight DS which results in bradykinin generation. Although low-molecular-weight DS is used for LDL apheresis, there have been few reports regarding the ability of this material to

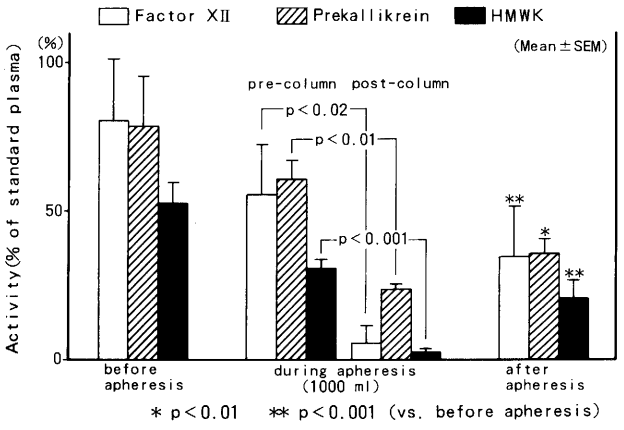

Fig. 3 Changes in factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) during LDL apheresis using a DS column. The activity of each factor was significantly decreased by passing of plasma through the DS column. It was significantly lower after apheresis than before apheresis.

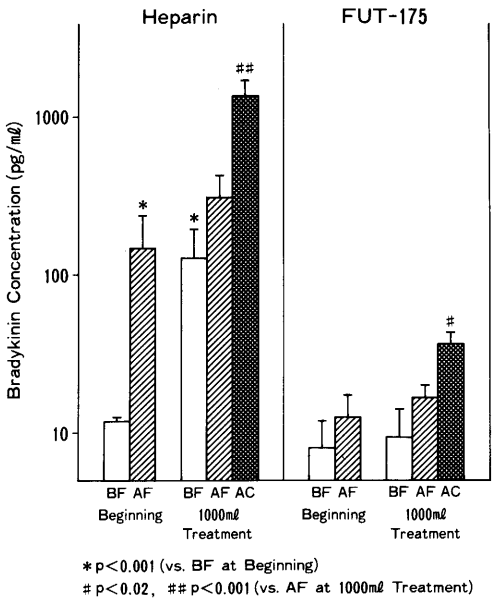

Fig. 4 Bradykinin generation during LDL apheresis with heparin and nafamostat mesilate (FUT-175). The bradykinin concentration is shown on a log scale in the ordinate. BF, blood before filtration through the plasma filter; AF, plasma after filtration through the plasma filter; AC, plasma after passing through the DS cellulose column. In the LDL apheresis with heparin, the bradykinin concentration rose after filtration of plasma through the filter at the beginning of apheresis, and bradykinin concentration in BF significantly ($P<0.001$) increased after 1,000-ml plasma treatment as compared with the beginning. Moreover, it markedly increased by passing through the DS column. In LDL apheresis with FUT-175, a minimal increase of bradykinin concentration was observed only after passing through the column.

activate the contact phase. The present study demonstrated this activation by showing the reduction of factor XII, PK, and HMWK (Fig. 3) as well as the generation of bradykinin by the passing of plasma through the DS columns (Fig. 4). Plasma levels of bradykinin increased tenfold in systemic circulation after 1,000-ml plasma treatment. Nafamostat mesilate inhibits the activities of kallikrein with IC_{50} values on the order of 10^{-6} M. The present study demonstrated that nafamostat mesilate inhibits bradykinin generation during LDL apheresis. At 1,000-ml plasma treatment, a small but significant decrease in systolic blood pressure was observed only during apheresis with heparin. Since blood pressure and blood bradykinin level did not change significantly with nafamostat mesilate, the decrease in blood pressure with heparin might be explained by the vasodilatory action of bradykinin. Almost all of the bradykinin generated during LDL apheresis will be rapidly destroyed by angiotensin-converting enzyme (ACE) in plasma or endothelial cells. When patients are given ACE inhibitors for the treatment of hypertension or heart failure, the breakdown of bradykinin is inhibited, which in turn augments the increase in the blood bradykinin levels. The rise of blood bradykinin levels may cause an allergic reaction or hypotension in some patients who are taking ACE inhibitors. In such patients whose blood pressure is markedly lowered by LDL apheresis, it is recommended to stop ACE inhibitors or to use nafamostat mesilate as an anticoagulant.

References

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