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좌심실보조장치내면에서의 혈장단백질 흡착과 흡착된 피브리노젠의3차구조변화에대한 혈류의 영향

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THE FLUID DYNAMIC EFFECTS ON PLASMA PROTEIN ADSORPTION AND CONFORMATIONAL CHANGE IN LEFT VENTRICULAR ASSIST DEVICE (LVAD)

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INTRODUCTION

Plasma protein adsorption on the implanted prosthetic devices is strongly influenced by surface characteristics of materials and hemodynamics inside the device [1] and it would influence subsequent platelet adhesion and activation which lead to the thrombus formation at the blood-material interface.

Recently, the composition and molecular organization of adsorbed protein film are particular interest. Among the various plasma proteins, fibrinogen is known to be a major protein and it is believed that platelet reactivity is correlated with the antibody-detectable (i.e. conformationally intact) fibrinogen bound but not with the total amount of adsorbed fibrinogen [2]. We have studied the distribution of three major plasma proteins (fibrinogen, albumin, and IgG) quantitatively and conformational change in adsorbed fibrinogen using monoclonal antibodies (MoAbs) against various epitopes of fibrinogen inside the LVADs.

Finally we tried to elucidate the effect of wall shear stress on protein adsorption and its conformational change related to subsequent reactivity of platelets.

MATERIALS AND METHODS

In vitro flow visualization for the hydrodynamic LVAD was performed by a video camera (CCD, Hitachi) and an image processor (PC VISION PLUS) with a IBM PC.

The hydrodynamic LVADs were implanted in mongrel dogs of about 30 kg. We sectionalized the blood-contacted ventricle according to the level of shear rate after animal death. Adsorbed plasma proteins (fibrinogen, albumin, and IgG) of each segment were quantified by enzyme-linked immunosorbent assay (ELISA). LVAD was also circulated with human fibrinogen solution for 2 hrs at 37 °C. Heart rate was 60 and cardiac output was 1.4 L/min. Total fibrinogen adsorbed on each segment was quantified by ELISA and conformationally intact fibrinogen was quantified by RIA. Adsorbed fibrinogen was visualized by Immunogold-Silver Staining method. PU sheets were incubated with canine fibrinogen solution for 2 hrs at 37 °C. Then anti-fibrinogen antibody and Gold-labeled Protein-A were incubated with the surface. To measure the amount of conformationally intact fibrinogen, purified fibrinogen in phosphate-buffered saline (PBS, pH 7.4) was circulated in LVAD and the conformational change of adsorbed fibrinogen was observed by MoAbs (kindly supplied by Dr. Zaverio M. Ruggeri). The MoAbs are against fibrinogen domains specific to the platelet fibrinogen receptor (GPIIb-IIIa). They are Z69-8 (anti-fibrinogen dodecapeptide, gamma 400-411), 134B-29 (anti-fibrinogen peptide, alpha 566-580), and 155B-16 (anti-fibrinogen peptide, alpha 87-100). MoAbs were labeled with ^{125}I using the Chloramine-T method.

RESULTS AND DISCUSSION

A large vortex was developed in the center of the artificial ventricle at diastolic phase (Figure 1). The area near the outlet valve was considered as the lowest shear rate region. The shear rate in the top region of the ventricle was lower than that in the bottom region since there exists some flow separation points around the bottom area. We categorized eight specimens of the ventricle according to the level of shear rates. Polyurethane blood pump displayed different degrees of protein adsorption (Figure 2). Less proteins seem to be adsorbed in the region of the high shear stress. Overall result of MoAb-detectable fibrinogen adsorption according to the shear rates was shown in Figure 3. Less conformationally intact fibrinogen was adsorbed in the higher shear region and 134B-29 detectable fibrinogen was adsorbed much more than the other two Abs-detactable fibrinogen. These results were consistent with platelet adhesion pattern of previous report [1].

We found that less proteins were adsorbed in the higher shear region. The effect of shear level on fibrinogen adsorption and its conformational change was strongly dependent on the surface characteristics of materials. The MoAbs against alpha 566-580 (134B-29) was the most reactive with fibrinogen adsorbed on PU surface in this experiment.

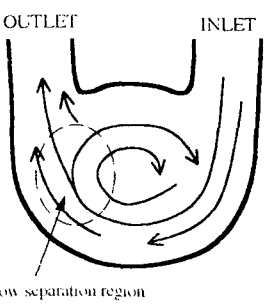

Figure 1. Schematic drawing of fluid path lines of LVAD's blood sac with flow visualization experiments

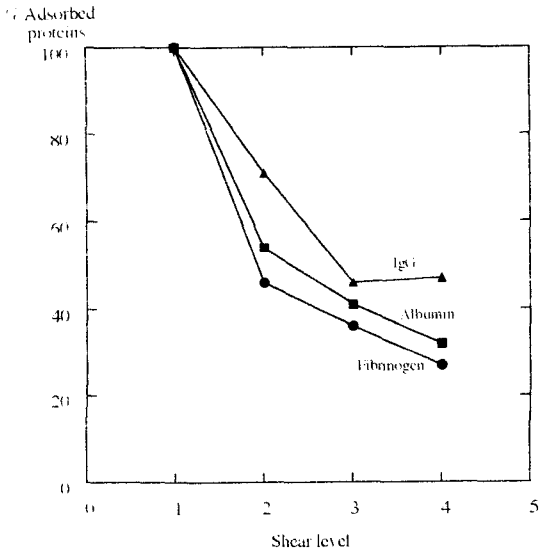

Figure 2. Relationship between plasma protein adsorption on polyurethane surface and the shear stress : Shear level : 4 > 3 > 2 > 1

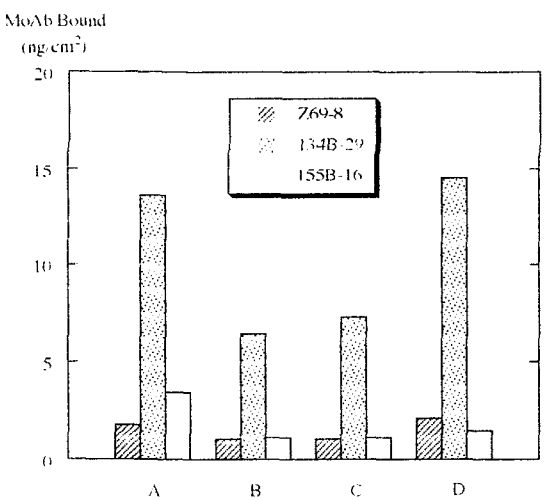

Figure 3. Effect of shear rate on the binding of antifibrinogen MoAbs to PU surfaces exposed to purified fibrinogen solution (Shear level : A < D << B = C)

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