룸부로키나제의 고정화에의한 항혈전성 향상에관한 연구

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ENHANCEMENT OF ANTITHROMBOGENICITY BY LUMBROKINASE IMMOBILIZATION IN TOTAL ARTIFICIAL HEART

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INTRODUCTION

Thrombotic obstruction is one of the most serious sequelae of artificial organs implanted into a living body. In 1983, Mihara et al.[1] extracted six fractions of strong and novel fibrinolytic enzymes (lumbrokinase, LK) from the earthworm, *Lumbricus rubellus*. These enzymes were very stable and showed greater antithrombotic activity than that of currently-used antithrombotics. In this work, we immobilized a LK fraction showing the least plasminogen-degrading activity on polyurethane (PU) surface to investigate its enzymatic activity and antithrombotic activity.

MATERIALS AND METHODS

The methanol extracted PU surface was treated with 3%(w/v) maleic anhydride methylvinyl ether copolymer (MAMEC) solution and finally incubated in a LK solution in PBS (pH 7.4). The surface was chracterized by ATR-FTIR, ESCA, and dynamic contact angle. The quantification of immobilized LK was performed by a dye-binding method. The immobilized LK activity was estimated by the fibrin plate method and caseinolytic activity assay. The stability against pH and temperature was also tested. The antithrombotic activity was evaluated by *in vitro* ^{125}I-fibrinogen adsorption in fresh whole blood and ^{99m}Tc-platelet adhesion tests. The distribution of radiolabelled fibrinogen and platelet was vidualized by autoradiography. The occlusion time through *ex vivo* rabbit A-A shunt was also determined.

RESULTS AND DISCUSSION

The immobilization of LK on PU surface was confirmed by IR and ESCA. The content of immobilized LK was 8 - 24 ug/cm^2, which was almost the same amount as that of immobilized albumin on the same surface. In contact angle measurement, LK-immobilized PU displayed hydrophilic property. The unit activity of immobilized LK was 4 - 18U/cm^2 and the relative activity ratio of immobilized LK to the soluble LK was about 30% (Table 1). The stability against pH and temperature was assessed by caseinolytic activity assay. Immobilized lumbrokinase was stable against various pH (4 - 10) and temperature (Figure. 1). Immobilized lumbrokinase showed a little activity even at 80 °C.

The ^{125}I-fibrinogen adsorption experiment in fresh whole blood showed that less fibrinogen was adsorbed on LK-immobilized surface than on PU and PU-MAMEC controls (Figure 2 and 3).

Although platelet adhesion was initially enhanced on LK-immobilized surface compared with PU-MAMEC surface, less platelets remained on LK-immobilized surface as time elapsed and showed reduced thrombogenicity (Figure 4).

The *ex vivo* occlusion time of untreated PU and PU-MAMEC surface were only 32 and 42 min, respectively. But that of LK-immobilized PU was extended to 140 min. These results suggested that the immobilized LK on PU surface might have decreased fibrinogen adsorption by digesting the adsorbed fibrinogen and

inhibited platelet adhesion/aggregation to the artificial surface as well , indicating that the LK-immobilized surface was highly antithrombogenic.

REFERENCES

1. Mihara H, et al., Thromb. Haemost., 50, 258,1983

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Table 1. Characteristics of Immobilized LK

Materials	Conc. (μg/cm²)	Activity (U/cm²)	Efficiency (%)
PU-MAMEC(I)-LK	7.6	4.0	23
PU-MAMEC(II)-LK	23.7	17.7	33

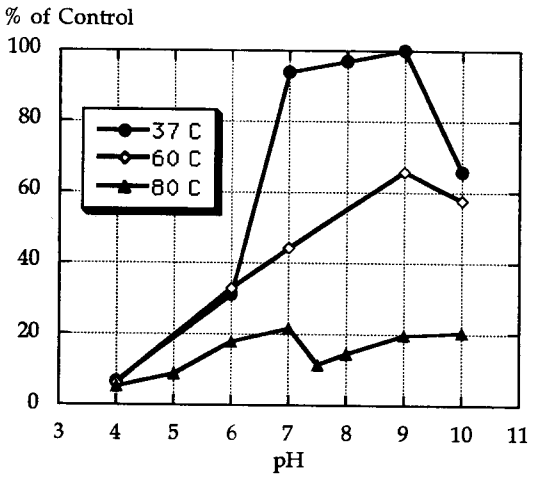

Figure 1. Stability of Immobilized LK against pH and Temperature

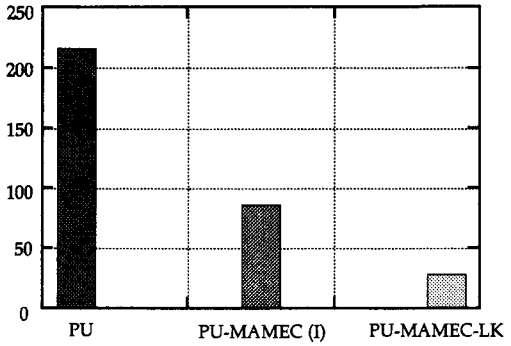

Figure 2. ^{125}I-Fibrinogen Adsorption

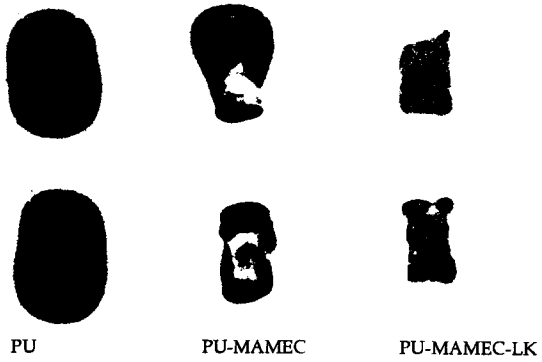

Figure 3. Autoradiography of ^{125}I-Fibrinogen Adsorption

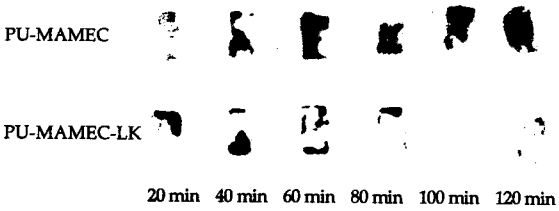

Figure 4. Autoradiography of ^{99m}Tc-Platelet Adhesion