# 룸브로키나제가 고정화된 폴리머 밸브의 in vivo 혈액적합성 평가

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# Evaluation of Blood compatibility at lumbrokinase immobilized polymer valves in vivo

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## ABSTARCTS

Lumbrokinase, potent fibrinolytic enzyme purified from earthworm, was immobilized onto polyurethane valves using photoreaction, photoreactive polyallyl-amine as a photoreactive linker. For evaluation of blood compatibility, lumbrokinase immobilized polymer valves were assembled into the total artificial heart (TAH). This TAH was implanted to 60kg healthy lamb for 1-3 days with the cardiac output 5 L/min. In the control lamb, the valves were untreated, in one other, only valves on the right were treated, and in the remaining animal, only those on the left. To facilitate the thrombus formation, low doses of heparin were administered. For evaluation of the immobilized lumbrokinase, thrombus formation, proteolytic and fibrinolytic activity was measured. This data shows that lumbrokinase-treated polyurethane valves lead to decreased thrombus formation in vivo, and that their biocompatibility is therefore higher than that of untreated valves.

Key Words: lumbrokinase, fibrinolytic enzyme, immobilization, total artificial heart

## INTRODUCTION

Thrombus formation is one of the major problems in blood contacting devices including total artificial heart, ventricular assist devices systems. In order to decrease the thrombus formation and increase the blood compatibility of the polyurethane, many attempts were tried to increase the blood compatibility. Current researches have been directed toward three aspects; endothelial cell culture, utilization of biologically active molecules, and chemical modification of polymer surfaces (1).

Lumbrokinase was purified from earthworm and has the stable, potent fibrinolytic activity compared with other agents. It also has the fibrin specificity compared with other proteolytic enzymes (2). And the biocompatibility of lumbrokinase immobilized polyurethane was analyzed using various methods in vitro (3).

In this study, biocompatibility of lumbrokinase treatment method was evaluated by in vivo experiment. For evaluation, treated polymer valve was fitted to the TAH and the TAH was implanted into the lamb. The thrombus formation and protein adsorption of polymer valve was examined.

## MATERIALS AND METHODS

*Immobilization of lumbrokinase*

Using air spray method, polymer valve was coated with synthesized photoreactive polyallyamine (0.5%). After air drying, UV light (254 nm) was illuminated for 5 min at 2.5 mW/cm$^2$. The valve was washed in phosphate buffered saline for one day and dried under vacuum conditions. Lumbrokinase at a final concentration of 100 ug/ml was added to the valves. EDC (1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide) at a final concentration of 0.03 M was added and the solution was incubated for 20 hours at room temperature. The valves were washed for 2 days. The sheet was dried in a vacuum.

*Total artificial heart experiment*

For total artificial heart implantation, a healthy 60kg lamb was selected. The lumbrokinase was immobilized to the polymer heart valves in each experiment. To facilitate the thrombus formation, heparin was not injected during six hours prior to autopsy. In all cases, the heart operated for between 1 and 3 days and in order to evaluate thrombus formation, it was treated with low

dose of heparin (500 U/kg/day).

*Evaluation of protein adsorption*

Primary antibody against fibrinogen, IgG was added into samples with the titer of 1:10,000, 1:5,000, respectively. The antibody was incubated for 2 hours at R.T washed with PBST. The secondary antibody against antimouse antibody conjugated with horse raddish peroxidase was added with the titer of 1:10,000 and the samples were incubated for 2hrs. For color development, substrate solution (1 mg ABTS and 0.05% hydrogen peroxide in 1 ml McIlvan buffer) was added and the sample was incubated for 10 minutes at room temperature. The optical density was measured at 405 nm.

## RESULTS AND DISCUSSION

Three cases of experiment were carried out to examine the antithrombotic activity of immobilized lumbrokinase in vivo (Figure 1).

In each of three experiments, the biocompatibility of lumbrokinase immobilized valve was evaluated by observing thrombus formation. To examine fibrinolytic activity during short-term experiments, low-dose heparin was used as an antithrombotic agent. In the control experiment, thrombi were observed in both inlet but not in outlet valves. In the second experiments, a large thrombus, 10 mm diameter and 1-2 mm thick, covered the whole of the left inlet valve. No thrombi were observed in other valves; of particular note in the fact that none were present in the lumbrokinase treated right inlet valve. In the third case, in which the TAH operated for 24 hours, minor thrombus was observed in the untreated right part but not in other valves.

Protein adsorption pattern after implantation was also examined in third case experiments. Two kinds of proteins were evaluated using EIA. Adsorbed fibrinogen in lumbrokinase immobilized polymer was decrease compared with that in control untreated valves. And adsorbed IgG was also decreased in lumbrokinase immobilized samples. This also reflects that lumbrokinase also decrease the fibrinogen adsorption by acting onto the fibrinogen or fibrin in vivo. This also has the correlation with the in vitro protein exposure data.

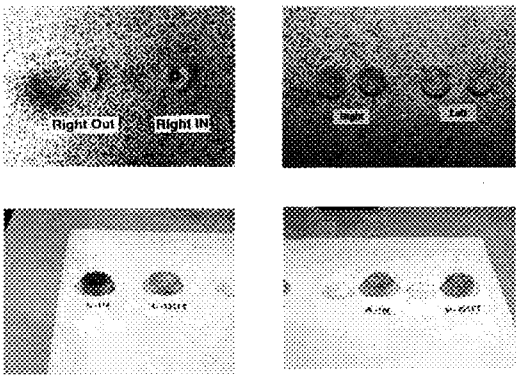


Figure 1. Thrombus formation of polymer valves after implantation into the lamb. (a) control, (b) left treated, (c) right treated valves.

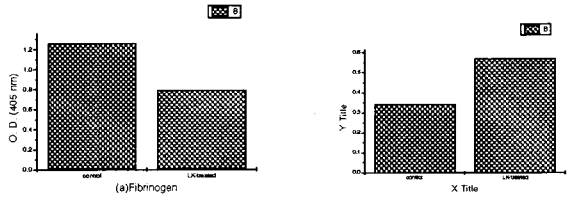


Figure 2. Protein adsorption after implantation. (a) fibrinogen, (b) IgG.

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