The Effects of Recombinant Tissue-Type Plasminogen Activator (rt-PA) on Canine Cadaver Lung Transplantation

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Abstract: The intrapulmonary thrombi that form after the cessation of circulation are thought to be one of the major causes of graft function failure. We evaluated the effect of recombinant tissue-type plasminogen activator (rt-PA) in a canine cadaver lung transplant model. Donor dogs were killed by the intravenous administration of pancuronium bromide without heparinization, and left for 2h at room temperature. The donor lungs were then flushed with low potassium dextran glucose (LPDG) solution, being subjected to a total ischemic time of 3h. Following left lung transplantation, the contralateral pulmonary artery of the recipient dogs was ligated. In group 1 (n = 6), chloride solution was administered from the main pulmonary artery for 90 min, commencing 15min prior to reperfusion. In group 2 (n = 6), 2.5 µg/kg per min of rt-PA, and in group 3 (n = 6), 5.0 µg/kg per min of rt-PA, were continuously infused in the same manner as in group 1. Lung function, including arterial blood gases and pulmonary hemodynamics, was measured for 3h. The side effects of rt-PA were evaluated by measuring the prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, alpha2-plasmin inhibitor (α2-PI), plasminogen, and fibrin/fibrinogen degradation product (FDP). All of the animals in the three groups survived throughout the observation period. The group 3 animals had significantly better gas exchange than the group 1 animals, and the pulmonary hemodynamics were significantly better in the group 2 and 3 animals than in the group 1 animals. The FDP levels in the group 2 and 3 animals were significantly higher than those in the group 1 animals, while the PT and APTT were significantly prolonged in the group 3 animals. These findings led us to conclude that rt-PA improves early lung function, particularly pulmonary hemodynamics.

Key Words: lung transplantation, non-heart-beating donor, recombinant tissue-type plasminogen activator

Introduction

Lung transplantation has become an effective method of palliating selected patients with end-stage lung disease; however, the shortage of suitable donors remains a serious problem. To increase the size of the pulmonary donor pool, we investigated lung transplantation using lungs harvested from cadavers after the cessation of circulation.1-3 The reasons and mechanisms for poor cadaver lung function are controversial; however, intrapulmonary thrombi that has formed after the cessation of circulation are thought to be one of the major causes of early graft function failure. In the present study, we evaluated the effects of administering recombinant tissue-type plasminogen activator (rt-PA) infusion at the time of reperfusion in a canine cadaver lung transplantation model.

Materials and Methods

Preparation of the Donors

A total of 18 adult mongrel dogs weighing from 6.0kg to 12.0kg were used as donors. The donor animals were premedicated with an intramuscular injection of atropine sulfate (0.05mg/kg) and ketamine hydrochloride (5mg/kg), and anesthetized with intravenous thiamylal sodium (20mg/kg). They were then intubated and mechanically ventilated with 100% oxygen. Intravenous pancuronium bromide (0.5mg/kg) was administered and mechanical ventilation was stopped. Cardiac arrest was ascertained by electrocardiogram. The animal was secured on the table in the supine position and left at room temperature for 2h.

The main pulmonary artery was opened through a median sternotomy and a 20-Fr catheter was inserted 90min following cardiac arrest. After the left atrial appendage was incised, the postmortem thrombi in the pulmonary artery and left atrium were suctioned and
extracted using forceps. Ventilation was established at a tidal volume of 35 ml/kg, a respiratory rate of 15 breaths/min, and a positive end-expiratory pressure (PEEP) of 5 cmH₂O. The donor lungs were flushed in situ with 50 ml/kg of 4°C LPDG solution from a bag hung 50 cm above the chest, 120 min after cardiac arrest. Simultaneous topical cooling was achieved by immersing the lungs in cold saline. At the completion of the flush, the trachea was stapled, leaving the lungs well inflated with 100% oxygen. The heart-lung block was excised and placed in a plastic bag containing cold LPDG solution, then preserved at 4°C for 3 h.

**Preparation of the Recipients**

The recipient animals were sedated in the same manner as the donors. Anesthesia was maintained with a 50:50 mixture of nitrous oxide and oxygen and 0.5%–1.0% halothane. A 5-Fr Swan-Ganz catheter (American Edwards Laboratories, CA, USA) was placed in the main pulmonary artery from the external jugular vein to measure pulmonary artery pressure (PAP), central venous pressure (CVP), and cardiac output (CO). A 5-Fr lung water catheter (model HE-2900, Elecath Laboratories, NJ, USA) was placed in the abdominal aorta from the right femoral artery. An MTV-1100 lung catheter (Nihon Kohden Laboratories, Tokyo, Japan) was used to estimate the extravascular lung water (EVLW) and aortic pressure (AoP). Arterial and central venous blood gases were measured. After these baseline measurements (BL) had been obtained, a thoracotomy was performed through the left fifth intercostal space followed by pneumonectomy of the left side. The right pulmonary artery was encircled by a 1-0 silk suture to permit measurement of only the left lung function after transplantation. Left lung transplantation was performed using previously described techniques, with the order of anastomosis as follows: left atrium, pulmonary artery, bronchus. During implantation, the donor lung was wrapped with ice-soaked saline sponges. A 5-Fr catheter was inserted into the left atrial appendage to measure left atrial pressure (LAP).

At that point, each animal was randomly assigned to one of three study groups. In group 1, being the control group (n = 6), chloride solution was administered from the main pulmonary artery for 90 min, commencing 15 min prior to reperfusion. In group 2 (n = 6), 2.5 μg/kg per min of rt-PA (low dose), and in group 3 (n = 6), 5.0 μg/kg per min of rt-PA (high dose), were administered in the same manner as in group 1.

**Measurement of Lung Function**

The right pulmonary artery was ligated 15 min after reperfusion, forcing the recipient to be dependent on the transplanted lung for gas exchange. The time point 0 was defined as 15 min after ligation of the right pulmonary artery, and the 3-h assessment period was started. Both lungs were ventilated with a tidal volume of 20 ml/kg, a respiratory rate of 15 breaths/min, a PEEP of 5 cmH₂O, and an inspired oxygen fraction of 1.0. If metabolic acidosis occurred, intravenous sodium bicarbonate was infused to maintain a base excess of between 0 and 5 mEq/l. The chest was not closed, but the lung was loosely covered with saline-moistened sponges. AoP, PAP, LAP, and CVP were continuously recorded. Arterial and central venous blood gases were taken after 15, 30, 45, 60, 90, 120, 150, and 180 min, while CO and EVLW were measured after 15, 30, 60, 120, and 180 min. Pulmonary vascular resistance (PVR) (dynes/s cm⁻²) was calculated by the equation PVR = (Mean PAP – LAP)/CO × 79.92. EVLW was determined by the thermal-saline indicator dilution method. After the final measurements, the animals were killed by the intravenous administration of potassium chloride solution. The left upper and lower lobes were used to measure the wet to dry ratio, which was determined by the weight difference in the specimen before and after drying for 3 weeks at 90°C.

**Indicators of Hemostasis and Fibrinolysis**

Blood samples were collected at three time points, namely at the time of baseline measurement, then after 60 min, and 180 min. Blood was withdrawn from the lung water catheter directly into a 3.13% sodium citrate solution (9:1, v/v). Plasma samples were obtained within 60 min by centrifugation at 3000 × g for 10 min, and stored at −20°C. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using an automated fibrinometer (Model CA-4000 Toa Medical, Hyogo, Japan). Fibrinogen levels were determined using the method of Clauss. This assay measures the rate of fibrin clot formation after the addition of excess thrombin. Plasma plasminogen activity and α2-plasmin inhibitor (α2-PI) activity were measured by the method of Friberger and Knos using a chromogenic substrate, S-2251 (Kabi Diagnostics, Stockholm, Sweden). The results are expressed as a percentage of the activity in relation to that in pooled normal plasma, taken as 100%. The fibrin/fibrinogen degradation product (FDP) levels were measured by the latex agglutination method.

**Statistical Analysis**

All values are presented as means ± SEM. Statistical analyses were performed using an analysis of variance (ANOVA). Significance was defined as a P value of less than 0.05.