Heparin Reduces Overcirculation-Induced Pulmonary Artery Remodeling Through P38 MAPK in Piglet

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Background. **Artery remodeling is the principal change of pulmonary artery hypertension. Heparin has been shown to inhibit vascular smooth muscle cell proliferation. We hypothesized that heparin may modulate vascular remodeling in pulmonary artery hypertension, and explored the mechanism.**

Methods. **A localized overcirculation-induced artery remodeling was created in piglets by anastomosing the left lower lobe pulmonary artery (LLLPA) to the thoracic aortic artery. Piglets were treated with heparin or saline for 4 weeks. Hemodynamic data were collected, and histology of the lung was assessed. We investigated the expressions of several candidate genes in lung and further observed the involvement of P38 mitogen-activated protein kinases (MAPK). The effects of heparin on the growth of cultured pulmonary arterial vascular smooth muscle cell and P38 MAPK expression were further determined under various conditions.**

Results. **Four weeks after the shunt setup, overcirculation caused significant LLLPA remodeling, pressure increase, and pulmonary vascular resistance increase, and LLLPA flow reduction compared with that immediately after the shunt setup. Heparin reduced the LLLPA remodeling, pressure, and pulmonary vascular resistance, and increased the LLLPA flow compared with that not heparin treated. Shunt and heparin treatment did not change the piglet's systemic hemodynamics. Shunt increased the expression of P38 MAPK and heparin decreased its expression in the shunted LLLPA. Both heparin and P38 MAPK inhibitor suppressed VSMC growth and P38 MAPK expression in the cultured VSMC, but they did not present additive effects when the two treatments were combined.**

Conclusions. **Heparin reduces overcirculation-induced pulmonary artery remodeling through a P38 MAPK-dependent pathway.**

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Pulmonary artery hypertension (PAH) is characterized by small pulmonary artery (PA) wall remodeling, causing lumen stenosis and increased pulmonary vascular resistance. Although much effort has been devoted to understanding this disorder, the mechanism remains poorly understood [1, 2]. One principal characteristic of the remodeling is the prominent accumulation of vascular smooth muscle cells (VSMC) that orchestrates this process. The VSMC have been one of the key targets to prevent and treat occlusive vessel diseases [3]. Heparin is clinically used as an antithrombotic agent. Our previous studies have indicated that heparin inhibited pulmonary vascular remodeling in rodent models [4, 5].

We developed a localized overcirculation-induced PA remodeling model by anastomosing the left lower lobe pulmonary artery (LLLPA) to the thoracic aortic artery in piglets. We then administered heparin and observed the effects of heparin on the remodeling of LLLPA, cardiac hemodynamics, and P38 mitogen-activated protein kinases (MAPK) expression in the tissue and cultured VSMC.

Material and Methods

Animals

Growing Yorkshire piglets were studied at the age of 4 months and weighing approximately 40 kg to 44 kg. All the procedures were performed in accordance with protocols approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital, Harvard University, and complied with the "Guide for the Care and Use of Laboratory Animals" (NIH publication 78-23, revised 1996).

Overcirculation Setup

Piglets were anesthetized with intravenous sodium thiopental (10 mg/kg) and then intubated and ventilated with fraction of inspired oxygen of 0.4, tidal volume of

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Abbreviations and Acronyms

FCS = fetal calf serum
LLL = left lower lobe
LLLPA = left lower lobe pulmonary artery
LLLPpa = left lower lobe pulmonary artery pressure
LLL PVR = left lower lobe pulmonary vascular resistance
MAPK = mitogen-activated protein kinases
PA = pulmonary artery
PAH = pulmonary artery hypertension
PVR = peripheral vascular resistance
VSMC = vascular smooth muscle cells
WT% = wall thickness percentage

15 mL/kg, and respiratory rate of 11 breathes per minute. Anesthesia was maintained with propofol (165 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The thoracotomy was performed through the left fourth intercostal space, and the LLLPA was dissected and then connected to the thoracic aortic artery by end-to-side anastomosis. The lungs were reexpanded, and the pleural air was evacuated before tightly closing the chest. The piglets (5 piglets in each group) received subcutaneous morphine twice daily for 3 days. The patency of the shunt was checked daily by auscultation and finally confirmed by histologic examination at the time of euthanasia.

Hemodynamic Evaluation

Before the surgery was performed, the right femoral artery and right external jugular vein were cannulated for blood sampling and hemodynamic measurement as baseline data. The mean systemic blood pressure, heart rate, cardiac output, cardiac index, pulmonary vascular resistance arterial component, oxygen saturation of arterial blood (SaO_2%), hematocrit, pH, weight gain, mean PA pressure, and mean pulmonary wedge pressure were measured directly, calculated, or computed. The flow to the shunted LLL was measured with an electromagnetic flow probe. The LLLPA pressure (LLLPpa) was measured by direct needle puncture connected to a transducer. The shunted left lower lobe pulmonary vascular resistance (LLL PVR) and systemic peripheral vascular resistance (PVR) were calculated using the equation PVR = (Ppa − Ppw)/Q. Four weeks later, with the same anesthetic regimen as for the first procedure, the left femoral artery and left external jugular vein were cannulated for measurement. Then, the thoracotomy was performed through the left fifth intercostal space. The flow to the shunted lobe and the LLLPpa were measured again. The animals were euthanized with an overdose of anesthesia. The ratio of right ventricle to left ventricle plus ventricular septum was measured at death. The lungs were then carefully harvested and fixed by perfusion of 10% paraformaldehyde buffer (pH 7.4) through the trachea (there are 5 piglets in each group).

Heparin Treatment

The piglets were maintained on a superior vena cava infusion line stabilized on the dorsal skin of the neck. The piglets were intravenously given 5 mg/kg heparin (heparin sodium salt; MP Biomedicals, Solon, OH) twice a day for 4 weeks. The first loading dose of 10 mg/kg was administered 2 days after the surgery. In the placebo group, an identical volume of saline was injected (5 piglets in each group).

Elastin Van Gieson Staining and Histopathologic Analysis

For each animal, at least 10 blocks of lung tissue, randomly taken from the central and peripheral areas of lungs, were paraffin embedded. Each group included five piglets. We performed Elastin Van Gieson staining of the internal elastic lamina to accurately measure the wall thickness. The wall thickness was measured with an eyepiece micrometer and analyzed with image analysis software Image J, version 1.33u (National Institutes of Health) by two blinded investigators independently. Only the peribronchial pulmonary arterioles with a complete muscular coat were selected to undergo morphometry. The technique used to measure wall thickness percentage (WT%) is presented in Figure 1.

Western Blot Analysis

Protein was extracted from the piglet lung tissues or cultured piglet pulmonary VSMC (harvested during euthanasia). The VSMC grown in Petri dishes were serum starved for 48 hours and then stimulated with

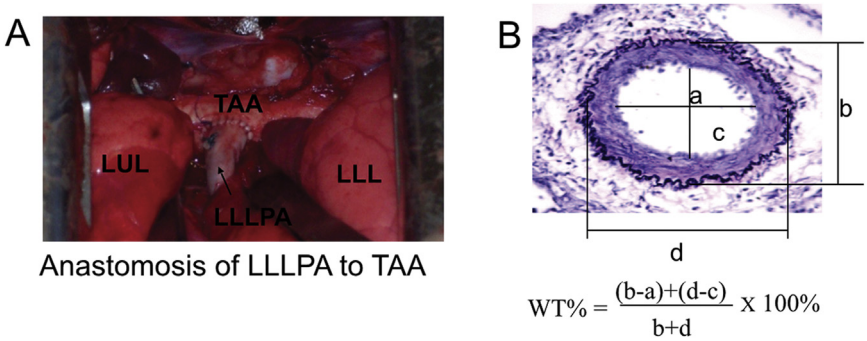

Fig 1. The left lower lobe pulmonary artery (LLLPA) to thoracic aortic artery (TAA) shunt setup and how to calculate the wall thickness percentage (WT%). (A) The LLLPA was connected to TAA by end-to-side anastomosis. (B) The approach used to calculate the vascular WT%. (LLL = left lower lobe; LUL = left upper lobe.)