Pulmonary hypertension due to left heart disease causes intrapulmonary venous arterialization in rats

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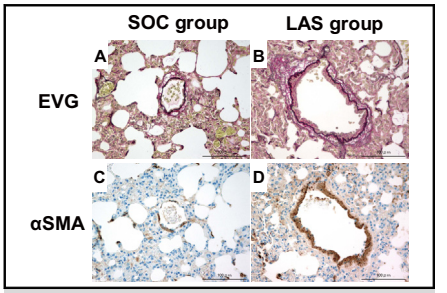
ABSTRACT

Objective: A rat model of left atrial stenosis–associated pulmonary hypertension due to left heart diseases was prepared to elucidate its mechanism.

Methods: Five-week-old Sprague–Dawley rats were randomly divided into 2 groups: left atrial stenosis and sham-operated control. Echocardiography was performed 2, 4, 6, and 10 weeks after surgery, and cardiac catheterization and organ excision were subsequently performed at 10 weeks after surgery.

Results: Left ventricular inflow velocity, measured by echocardiography, significantly increased in the left atrial stenosis group compared with that in the sham-operated control group (2.2 m/s, interquartile range [IQR], 1.9-2.2 and 1.1 m/s, IQR, 1.1-1.2, $P < .01$), and the right ventricular pressure-to-left ventricular systolic pressure ratio significantly increased in the left atrial stenosis group compared with the sham-operated control group (0.52, IQR, 0.54-0.60 and 0.22, IQR, 0.15-0.27, $P < .01$). The right ventricular weight divided by body weight was significantly greater in the left atrial stenosis group than in the sham-operated control group (0.54 mg/g, IQR, 0.50-0.59 and 0.39 mg/g, IQR, 0.38-0.43, $P < .01$). Histologic examination revealed medial hypertrophy of the pulmonary vein was thickened by 1.6 times in the left atrial stenosis group compared with the sham-operated control group. DNA microarray analysis and real-time polymerase chain reaction revealed that transforming growth factor-β mRNA was significantly elevated in the left atrial stenosis group. The protein levels of transforming growth factor-β and endothelin-1 were increased in the lung of the left atrial stenosis group by Western blot analyses.

Conclusions: We successfully established a novel, feasible rat model of pulmonary hypertension due to left heart diseases by generating left atrial stenosis. Although pulmonary hypertension was moderate, the pulmonary hypertension due to left heart diseases model rats demonstrated characteristic intrapulmonary venous arterialization and should be used to further investigate the mechanism of pulmonary hypertension due to left heart diseases. (J Thorac Cardiovasc Surg 2017;154:1742-53)



The medial wall of the PV was thickened in the LAS group.

Central Message

We prepared a PH-LHD rat model based on hemodynamics by increasing the left atrial and pulmonary venous pressure.

Perspective

PH-LHD is the most frequent cause of PH. Although PAH has been intensively investigated, PH-LHD remains unclear because of the lack of an appropriate PH-LHD animal model. In this study, we generated a novel PH-LHD rat model. TGF-β and endothelin-1 were significantly increased in this model. Further investigation will reveal a new mechanism and treatment for PH-LHD.

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Pulmonary hypertension (PH) due to left heart disease (LHD) is the most common form of PH. PH-LHD belongs to the second group in the World Health Organization classification that was updated in Nice.[1,2] Primary LHD consists of heart failure (HF) with reduced or preserved ejection fraction and severe left-sided valvular diseases, such as mitral valve stenosis. It has been recently reported that the presence of PH-LHD in patients with left

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Abbreviations and Acronyms

ET = endothelin
HF = heart failure
HR = heart rate
IQR = interquartile range
LA = left atrium
LAS = left atrial stenosis
LHD = left heart disease
PA = pulmonary artery
PAH = pulmonary arterial hypertension
PCNA = proliferating cell nuclear antigen
PDE = phosphodiesterase
PDE-5 = phospodiesterase-5
PH = pulmonary hypertension
PV = pulmonary vein
PVS = pulmonary vein stenosis
RV = right ventricular
SMA = smooth muscle actin
SOC = sham-operated control
TGF = transforming growth factor
VEGF = vascular endothelial growth factor

Scanning this QR code will take you to supplemental figures, tables, and videos for this article.

HF is associated with a worse prognosis than in those without PH-LHD.[3] Despite its prevalence and significance, there has been little research on PH-LHD. Moreover, no effective drug regimen has been established, and treatment is usually for the underlying LHD. For example, phospodiesterase-5 (PDE-5) inhibitors are clinically used for patients with PH-LHD[4-6]; however, the efficacy of PDE-5 inhibitors against PH-LHD is controversial[7] because the use of pulmonary vasodilators elevates left atrial pressure in those with PH-LHD, which may worsen congestive HF.

In addition, the pathophysiology of PH in PH-LHD should be different from that of pulmonary arterial hypertension (PAH), in which there is remodeling of the pulmonary artery (PA).[8] A main cause of the lack of clarity surrounding the treatment and mechanism in PH-LHD could be the absence of an appropriate PH-LHD animal model. The objective of this study is to establish a PH-LHD rat model based on the hemodynamic mechanism accompanying left atrial stenosis (LAS)-induced left atrial pressure elevation and observe changes in the lung to elucidate the mechanism of PH-LHD.

MATERIALS AND METHODS

Experimental Design

Five-week-old Sprague-Dawley rats were purchased from the same trader (Sankyo Labo Service Corporation, Tokyo, Japan) and installation date, and randomly divided into LAS and sham-operated control (SOC) groups. For example, we purchased 2 rats for a 1-day surgical procedure and then divided them into 1 for LAS and 1 for SOC. The protocol of this study is shown in Figure 1. Animals were intubated with Angiocath 18G under 2% isoflurane anesthesia, and respiration was managed using a Harvard rodent ventilator (Harvard Apparatus, Holliston, Mass), in which the tidal volume was set at 10 μL/g, and the respiratory rate was set at 100/min in accordance with a previous study.[9] The thymus was removed by lateral thoracotomy through the fifth intercostal region. Because the thymus was stuck with the epicardium, we needed to remove the thymus. The heart was identified, and an interrupted suture was applied to the left atrial appendage using 5-0 Prolene (Johnson and Johnson, New Brunswick, NJ) in the LAS group (n = 5). The thread of the interrupted suture was pulled up to lift the heart, and the left atrium (LA) was half clipped under direct vision using Horizon Ligating Clips (Teleflex, Morrisville, NC) and a Horizon Manual-Load ligating Clip Applier (Teleflex). The thorax was then closed. The procedure is shown in Videos 1 and 2. When left ventricular (LV) inflow velocity was 2.0 m/s or higher on echocardiography at 2 weeks after surgery, the rats were subjected to further analysis. Because LV inflow velocity of 2.0 m/s or higher is considered significant in human mitral valve stenosis,[10] we used the LV inflow velocity of 2.0 m/s for a cutoff value to estimate the PH-LHD model rats. In the SOC group (n = 5), only thymectomy was performed and the thorax was then closed. We set the observation period for 10 weeks after surgery, because Wang and colleagues[11] previously reported that aortic constriction caused PH-LHD 9 weeks after surgery. All rats were maintained at 22°C ± 2°C under a 12-hour light/dark cycle following the National Institute of Health guidelines for animal experiments. This experiment was performed after approval by the Institutional Animal Care and Use Committee of The Jikei University (2015-118C1).

Echocardiography

Echocardiography was performed at 2, 4, 6, and 10 weeks after surgery in both groups, and the rats were sedated with 1.5% isoflurane using a

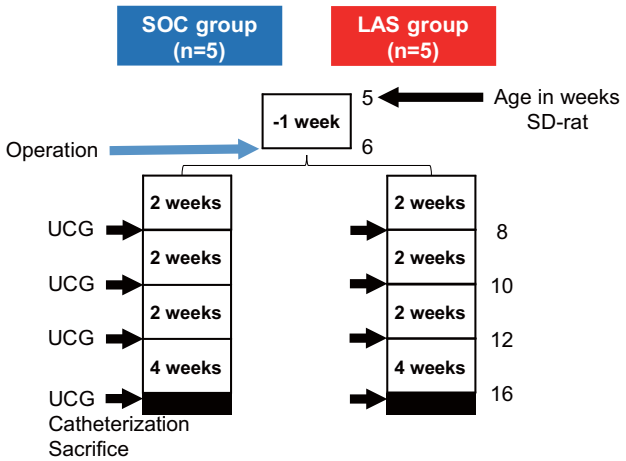

FIGURE 1. Study protocol of the SOC and LAS groups. Five-week-old Sprague–Dawley rats were randomly divided into the LAS and SOC groups. Ultrasound cardiography was performed 2, 4, 6, and 10 weeks after surgery, and cardiac catheterization and organ excision were performed 10 weeks after surgery. *SOC*, Sham-operated control; *LAS*, left atrium stenosis; *SD*, Sprague–Dawley; *UCG*, ultrasound cardiography.