Original rat model of high kinetic unilateral pulmonary hypertension surgically induced by combined surgery

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Objectives: The characteristic morphologic lesions observed in the lungs of patients with congenital cardiac anomalies have not been closely modeled in rat shunt-related models, except for the reversible grade 1 changes. The present study reported an original rat model of unilateral pulmonary hypertension surgically induced by combined surgery to reproduce more advanced pulmonary vascular lesions.

Methods: The right pulmonary artery was ligated through a right posterolateral thoracotomy, and a cervical shunt was established 1 week later. The immediate and chronic effects on the pulmonary hemodynamics were evaluated through right heart catheterization immediately after and at 8 and 12 weeks postoperatively. The morphologic changes in pulmonary vasculature were analyzed after staining with hematoxylin-eosin and modified Weigert's method. The right ventricular hypertrophy index was calculated and artery blood gas analysis performed.

Results: A pulmonary hypertensive status was successfully induced immediately after cervical surgery and progressively aggravated into a borderline state with disease course advancing. Pulmonary vasculopathy demonstrated a transition from reversibility (muscularization, intimal proliferation of grade 1-2) at 8 weeks to irreversibility (intimal fibrosis, entirely luminal occlusion, grade 3) at 12 weeks postoperatively. Conspicuous right ventricular hypertrophy and decreasing partial arterial pressure of oxygen were also observed.

Conclusions: The present shunt-related model successfully simulated a hypertensive status in pulmonary circulation and reproduced the characteristic transition of pulmonary vasculopathy from reversibility to irreversibility within a relatively short period. Thus, this model could offer an alternative with low mortality and high reproducibility for investigations on the underling mechanisms of shunt-related pulmonary hypertension. (J Thorac Cardiovasc Surg 2013;146:1220-6)

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In patients with systemic-to-pulmonary shunts, the status of the pulmonary vascular structure and the extent of pulmonary hypertension (PH) are important determinants of the feasibility of corrective procedures, the clinical course, and long-term survival. Recently, drugs targeting the signaling pathways involved in the pathogenesis of PH have been confirmed to partially or completely reverse the hypoxia/monocrotaline-induced PH in rats. However, these targeted drugs only exhibited partial improvements in symptoms, exercise tolerance, and hemodynamic indexes, with slight efficacy on mortality in patients with severe PH.[1] This has largely attenuated the dogma that the hypoxia/monocrotaline-induced PH model should be used to delineate the potential mechanism of PH, including shunt-related PH[1-3] and thus would be the most scientific method for determining the underlying mechanism of hyperkinetic PH in experimentally validated shunt-related models.

Many shunt-related models have been described and studied. These have included peripheral shunts established in the cervical,[4] abdominal,[5] or femoral[6] unit and central shunts prepared between the left innominate artery,[7] left carotid artery,[8] or ascending aorta[9] and pulmonary artery trunk. However, peripheral shunts have only induced limited pulmonary vascular remodeling (reversible grade 1). The high-level surgical requirements, relatively greater mortality,[10] and inadequacy of biochemical reagents for large animals have impeded the adoption of central shunt models; thus, an acceptable shunt-related model is still lacking.

Therefore, we created an original, reproducible, shunt-related model in rats using a 2-stage surgical procedure.

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Abbreviations and Acronyms

CSG	= combined surgery group
LCCA	= left common carotid artery
LCSG	= left cervical shunt group
LEJV	= left external jugular vein
mPAP	= mean pulmonary arterial pressure
MT	= medial thickness
PASP	= pulmonary arterial systolic pressure
PH	= pulmonary hypertension
RPALG	= right pulmonary artery ligation group
RVHI	= right ventricular hypertrophy index
RVSP	= right ventricular systolic pressure
SD	= Sprague-Dawley
SOG	= sham operation group

Subsequently, we performed hemodynamics evaluation, morphologic analysis, and blood gas analysis to explore the extent of PH achieved with this model.

METHODS

Male Sprague-Dawley (SD) rats from Vital River Laboratory Animal (Beijing, China) were used (age, 8 weeks; weight, 280 ± 20 g). They were randomly allocated to the right pulmonary artery ligation group (RPALG, n = 16), left cervical shunt group (LCSG, n = 16), combined surgery group (CSG, n = 32), and sham operation group (SOG, n = 24).

All rats received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication no. 85-23, National Academy Press, Washington, DC, revised 1996). The ethics committee on animal study of Fuwai Hospital approved the experimental protocol.

Discrepancy in Bilateral Lungs of SD Rats

The right lung of SD rats consists of 4 lobes and the left lung of only 1 lobe. Although this unique arrangement has been attributed to the anatomic deflection of the heart, we still needed to ascertain its true implications in creating shunt-related PH.

Bilateral lungs of age-, gender-, and weight-matched SD rats (n = 36) were collected and individually weighed before and after oven drying at 50° for 72 hours. Next, the relationship between the bilateral lungs was assessed by plotting the wet or dry weight of the right lung to that of the left lung (Figure 1). The mean wet ratio was 1.96 ± 0.11 and the mean dry weight ratio was 1.95 ± 0.11. Using this anatomic discrepancy and the extant surgical procedure (ligation of the pulmonary artery),[11] we ligated the right pulmonary artery to increase the pulmonary blood flow in the left lung.

Surgical Protocol

The rats were anesthetized with 10% chloral hydrate and atropine sulfate (3 mL/kg and 0.1 mg, respectively, intraperitoneally), orotracheally intubated, and immobilized in a left lateral decubitus position. Ventilation was maintained with a Harvard Rodent Ventilator (Inspira ASVP, Harvard Apparatus, Holliston, Mass) for the whole procedure.

Right Pulmonary Artery Ligation Group

Under sterile conditions, the right pulmonary artery was approached through a posterolateral thoracotomy in the third intercostal space and then ligated (10-0 nylon suture) after dissecting the interspace between right superior vena cava and right primary bronchus (Figure 2, *A*). Next, the chest was closed in layers (3-0 silk suture), and all the air in the right pleural space was evacuated through a tube of polyethylene 50, serving as a chest drainage tube. Finally, the rats were weaned from mechanical ventilation and allowed to recover with continuous supplemental oxygen. However, the endotracheal tube was not extubated until their respiration had stabilized.

Left Cervical Shunt Group

Anesthesia was induced as described for the previous group, and systemic heparinization was implemented by heparin sodium (100 IU, intraperitoneally). During spontaneous breathing, the rats were fixed in a supine position, and the left common carotid artery (LCCA) and left external jugular vein (LEJV) were exposed. Next, the proximal LCCA was clamped with microvascular clips and the distal portion was ligated at the bifurcation point. The proximal LEJV was suspended to the adjacent jugular muscle by microscopic suture, and the distal portion was ligated at the confluence site. Subsequently, the LCCA was amputated before the bifurcation and immediately flushed with heparin (50 U/mL). Next, the proximal stump was brought through and everted over a 2-mm-long, 18-gauge, intravenous catheter and circumferentially ligated in the middle of the cuff body. Then, the proximal stump of the LCCA was rotated and inserted into the LEJV lumen through a transverse incision in the anterior wall of the LEJV and the anastomosis was secured by ligatures (Figure 2, *B*). Finally, the suspended suture and the vascular clips were sequentially removed, and the patency of the shunt was confirmed visually by the presence of a continuous thrill, apparent dilation, and contradictory color change of the proximal part of the LEJV.

Combined Surgery Group

The combined surgery was performed as a 2-stage procedure. In brief, the right pulmonary artery was ligated, and, 1 week later, the left cervical shunt was established (Figure 2).

Sham Operation Group

The rats in the SOG underwent the 2-stage procedure, except for ligation of the right pulmonary artery and generation of the cervical communication.

Postoperative Care

Postoperatively, the rats were kept in a constant-temperature room (21°C; relative humidity, 50%-70%), exposed to a normal light/dark cycle, and provided standard laboratory chow and water ad libitum. Aspirin (75 mg/kg/d) and hydrochlorothiazide (25 mg/d) were administered by hydroposia to prevent anastomosis occlusion and lung edema.

Hemodynamics

The pulmonary pressure changes were measured in the closed-chest rats with a technique routinely used in our laboratory.[12] In brief, a 13-cm-long, heparin-priming polyethylene catheter (outer diameter, 0.9 mm) connected to PowerLab 16/30 (ADInstruments, Dunedin, New Zealand) through a pressure transducer was introduced into the right external jugular vein and advanced into the right ventricle and the main pulmonary artery. The right ventricular systolic pressure (RVSP), pulmonary arterial systolic pressure (PASP), and mean pulmonary arterial pressure (mPAP) were recorded.

Immediate effect test. The acute effects of the 4 different surgical interventions on the pulmonary circulation were evaluated immediately after surgery. The shunt clamping test was performed to evaluate the reversibility of PH in the LCSG, CSG, and SOG.

Chronic effect test. The chronic effects of the 4 different surgical procedures on the pulmonary vasculature were investigated at 8 and 12 weeks after the surgical intervention. The baseline data of the hemodynamic variables before and after occlusion of the cervical shunt was measured in the LCSG, CSG, and SOG.

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