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A novel mouse model of high flow-induced pulmonary hypertension—surgically induced by right pulmonary artery ligation

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ABSTRACT

Background: This study sought to establish a new model of high-flow pulmonary hypertension (PH) in mice. This model may be useful for studies seeking to reduce the pulmonary vascular resistance and delay the development of PH caused by congenital heart disease.

Materials and methods: The right pulmonary artery was ligated via a right posterolateral thoracotomy. Pulmonary hemodynamics was evaluated by right heart catheterization immediately after ligation and at 2, 4, 8, and 12 wk postoperatively. The right ventricle (RV) and the left ventricle (LV) with septum (S) were weighed to calculate the RV/(LV + S) ratio as an index of right ventricular hypertrophy. Morphologic changes in the left lungs were analyzed, and percentages of muscularized pulmonary vessels were assessed by hematoxylin and eosin, elastica van Gieson and alpha-smooth muscle actin staining. All the study data were compared with data from a model of PH generated by hypoxic stimulation.

Results: A pulmonary hypertensive state was successfully induced by 2 wk after surgery. However, the morphologic analysis demonstrated that pulmonary vascular muscularization, as evaluated using right ventricular systolic pressure and RV/(LV + S), was not significantly increased until 4 wk postoperatively. When mice from the new model and the hypoxic model were compared, no significant differences were observed in any of the evaluated indices.

Conclusions: High-flow PH can be induced within 4 wk after ligation of the right pulmonary artery, which is easily performed in mice. Such mice can be used as a model of high-flow PH.

Many congenital heart diseases associated with left-to-right shunting may be complicated by the development pulmonary hypertension (PH). PH is a syndrome characterized by increased pulmonary vascular resistance that leads to right ventricular hypertrophy and failure.[1-3] The condition adversely affects the prognosis of patients and significantly complicates surgical repair.[4,5] The major pathologic changes associated with PH are characterized by medial hypertrophy

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and muscularization of the small arteries, finally leading to plexiform lesions and obliterative intimal fibrosis.[6]

Under abnormal high-flow physiologic conditions, most of the pulmonary vasculature is exposed to high shear stress, resulting in pulmonary vascular remodeling. Experimental attempts to reproduce high-flow PH in animals have been made, and various shunt-related models, such as cervical, abdominal, femoral, and central shunts,[7-11] have been described. However, all these models produced only modest degrees of PH. The induction of hypoxia or a left pneumonectomy combined monocrotaline [12,13] also induced PH, but the pathophysiological mechanism is different from that of shunt-related PH, especially high-flow PH. Therefore, most models of high-flow PH have used large animals, such as pig, dog, sheep, calf, and rabbit, to facilitate surgery.[10,14-17] However, a common problem associated with these models is the difficulty in standardizing the pulmonary blood flow, which is associated with the surgeon's skills, type of operation, and individual differences in animals. Moreover, there have been limited reports of high-flow PH in mice.

Therefore, we created a novel, reproducible, high-flow mouse model through the ligation of the right pulmonary artery. We subsequently compared this model with a classic hypoxia-induced model of PH to evaluate the extent of PH. The study included a hemodynamic evaluation, an index of right ventricular hypertrophy, and a morphologic analysis. More importantly, with the wide application of genetically engineered mice, the establishment of this model may facilitate explorations of the molecular mechanisms underlying high-flow PH.

Materials and methods

Mice

Adult male C57BL/6 (H-2b) mice were purchased from HFK Bioscience Co, Ltd (Beijing, China). Six- to eight-week-old mice (18-25 g) were used for the experiments after being randomly allocated to the right pulmonary artery ligation group (RPALG, $n = 48$), the sham operation group ($n = 48$), the hypoxia group ($n = 12$), or the normoxia group ($n = 12$). The animals were housed in a specific pathogen-free facility at Tongji Medical College (Wuhan, China). All animal experiments were approved by the Animal Care and Use Committee of Tongji Medical College.

Surgery

Mice were anesthetized with 1% pentobarbital (30 mg/kg). The mice were placed in the supine position, orotracheally intubated with a 25-gauge catheter and then placed in a left lateral decubitus position for surgery. Ventilation was maintained with a ventilator (tidal volume, 1.5-2.0 mL; respiratory rate, 100-110/min; inspiratory/expiratory ratio, 1:2) throughout the entire procedure. Under sterile conditions, the hilum of the right lung was displayed through an anterolateral thoracotomy in the third intercostal space. Then, the right pulmonary artery was ligated (5-0 silk suture) after blunt dissection of the interspace between the right pulmonary vein and bronchus (Fig. 1A). All the air in the chest was evacuated by squeezing the chest, and the chest was immediately closed in layers (using 8-0 silk sutures). The right pulmonary artery was dissected but not ligated in the sham mice. Finally, all the mice except those in the sham group were allowed to recover in a supplemental oxygen box for the first 24 hours and were subsequently maintained on room air until sacrifice.

Hypoxic stimulation

Hypoxia was generated using a chamber in which the oxygen concentration was maintained at 10% by controlling the flow rates of compressed nitrogen and oxygen. The oxygen concentration was monitored with a sensor (Servoflo, Lexington, MA). Soda lime and anhydrous $CaCl_2$ were used to maintain minimal NH_3 and CO_2 levels within the chamber. The chamber was opened two times per week to clean cages, replenish food and water, and obtain body weight data. Control mice were housed in room air.

Pulmonary angiography with the microfil cast technique

Mice were anesthetized and placed in the supine position, orotracheally intubated, and mechanically ventilated. After ligation of the right pulmonary artery, the chest of each mouse Q6 was removed, and a mixture of microfil (0.6 mL/mouse, Flow Tech) was injected slowly at 0.8 mL/min speed through a microsyringe pump (LSP02-1B; longerpump.com, China), whereas the ventilator was shut down.[18] The mice were maintained at room temperature for 45 min. Next, the heart and lungs were removed, cleaned in phosphate-buffered saline and dehydrated in a gradient ethanol series (25%, 50%, 80%, 95%, and 100%). Finally, the tissues were hyalinized in Q7 methyl salicylate (M6752; Sigma) for 24 h. All images were captured under a microscope.

Evaluation of the hemodynamics and right ventricular hypertrophy

Mice were anesthetized and placed in the supine position, orotracheally intubated, and mechanically ventilated during the entire procedure. The heart rate, systemic blood pressure (SBP), and right ventricular systolic pressure (RVSP) were measured in a closed-chest mouse at different time points. In brief, a Micro-tip SPR-1000 catheter transducer (Millar Q8 Instruments) was inserted into the right ventricle to record the RVSP via the right external jugular vein, and another self-made catheter was inserted into the left jugular artery to monitor the SBP. Data were recorded using a PowerLab data Q9 acquisition system (ADInstruments) and were analyzed using the Chart 5 Pro software (ADInstruments). RVSP measurements associated with the heart rate and outside the range of 300-500 bpm were excluded from the analysis.

After hemodynamic measurements, the animals were exsanguinated, and lung tissue was collected for the histologic analysis. The heart was removed. The free wall of the right ventricle (RV) was dissected from the left ventricular septum, and the weight of the RV to the left ventricle plus the septum (LV + S) was calculated as an index of right ventricular hypertrophy.