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A neonatal rat model of increased right ventricular afterload by pulmonary artery banding

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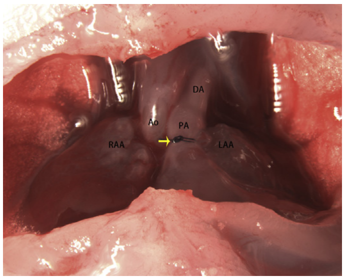
ABSTRACT

Objective: To construct a neonatal rat model of increased right ventricular (RV) afterload for studying the pathophysiological remodeling of the right ventricle in patients with congenital heart disease with increased RV afterload.

Methods: Surgery was performed within 6 hours after birth. Horizontal thoracotomy was performed by dissecting the intercostal muscles and splitting the sternum. The PA was then banded with 11-0 nylon thread. At postnatal day 7 (P7), constriction of PA was confirmed by echocardiography. The RV systolic and diastolic pressures were measured by cardiac catheterization. The RV end-systolic volume, end-diastolic volume, end-diastolic diameter, and free wall thickness were assessed by magnetic resonance imaging. The histological changes in sham-operated and PA-banding (PAB) hearts were evaluated by hematoxylin and eosin staining.

Results: Increased RV afterload was established by constriction of the PA in neonatal rats within 6 hours after birth. The survival rate was 75% at P7. Relative to the sham group, the peak pressure gradient across the PA constriction and RV systolic and diastolic pressures, end-systolic volume, end-diastolic volume, end-diastolic diameter, and free wall thickness were significantly increased in the PAB group at P7 ($P < .01$). Consistently, histological examination showed that the RV free wall was significantly hypertrophic in the PAB group.

Conclusions: We successfully established a neonatal RV afterload increase model through PAB within 6 hours after birth, which can be used to study the pathophysiological changes in congenital heart diseases with increased RV afterload. (J Thorac Cardiovasc Surg 2017;154:1734-9)



Constriction of the pulmonary artery in rats within 6 hours after birth.

Central Message

A neonatal increased right ventricular afterload model was established by banding the pulmonary artery within 6 hours after birth.

Perspective

Immature myocardium is different from mature myocardium in many aspects. However, our current understanding of neonatal right ventricular (RV) remodeling relies on knowledge gained from adult models. Here we show that it is feasible to generate a neonatal increased RV afterload rat model by banding the pulmonary artery within 6 hours after birth. We believe that this new model will greatly facilitate neonatal RV remodeling research.

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Increased right ventricular (RV) afterload and subsequent dysfunction are often encountered in patients with 3 types of congenital heart disease: pulmonary artery (PA) hypertension associated with left-to-right shunt, RV outflow obstruction (as in, eg, tetralogy of Fallot), and systemic RV. Increased RV afterload initially results in compensated or adaptive hypertrophy, but persistent abnormal afterload will lead to decompensated or maladaptive RV hypertrophy

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Abbreviations and Acronyms

H&E	= hematoxylin and eosin
LVWT	= left ventricular free wall thickness
MRI	= magnetic resonance imaging
P	= postnatal day
PA	= pulmonary artery
PAB	= pulmonary artery banding
PPG	= peak pressure gradient
RV	= right ventricular
RVP	= right ventricular pressure
RVWT	= right ventricular free wall thickness

Scanning this QR code will take you to a supplemental video for the article.

and then RV dysfunction, a condition in which the right ventricle is symptomatically unable to fill or eject properly. Although RV remodeling under increased afterload has been studied extensively in adults,[1-4] the mechanisms of RV hypertrophy and dysfunction in childhood are poorly understood owing to the lack of an appropriate model.

It is well known that immature and mature myocardium differ in quite a few biochemical and physiological aspects.[5] For example, immature myocardium is more dependent on glycolysis for ATP production due to abundant intracellular glycogen granules and more reliant on extracellular calcium for excitation-contraction coupling and intracellular calcium homeostasis.[6-9] Several recent studies have shown that mammalian heart can regenerate through cardiomyocyte proliferation within 1 week after birth in response to physical or ischemic lesions.[10,11] Given all these different biological characteristics of immature myocardium, the knowledge gained from adult models might not completely comply with the remodeling of immature myocardium. Therefore, a reliable model that can recapitulate the pathophysiological status of increased RV afterload from the beginning of postnatal life is desirable.

Current increased RV afterload models are established either by hypoxia/monocrotaline-induced pulmonary hypertension or by PA trunk constriction in young large animals.[12-16] Neither of these methods produces increased RV afterload immediately after birth, however. The former takes weeks to develop pulmonary hypertension, and the latter has never been reported in postnatal day 0 (P0) large animals. Even if PA banding (PAB) could be performed in P0 large animals, the cost and time for detailed molecular studies would make it a daunting task. Recently, several common cardiac surgeries, including coronary artery ligation and apical resection, were successfully performed in neonatal rodents.[10,11] Thus, we wonder whether the more complicated PAB procedure could be implemented in neonatal rodents.

In this study, we generated the first neonatal increased RV afterload rat model by constricting the PA within 6 hours after birth. Increased RV afterload and myocardial hypertrophy were confirmed by echocardiography, hemodynamic measurements, magnetic resonance imaging (MRI), and histology at P7. In doing so, we successfully simulated the pathophysiological status of increased RV afterload on immature myocardium, and the powerful genetic manipulation techniques available for rodents will further facilitate the molecular studies in RV remodeling.

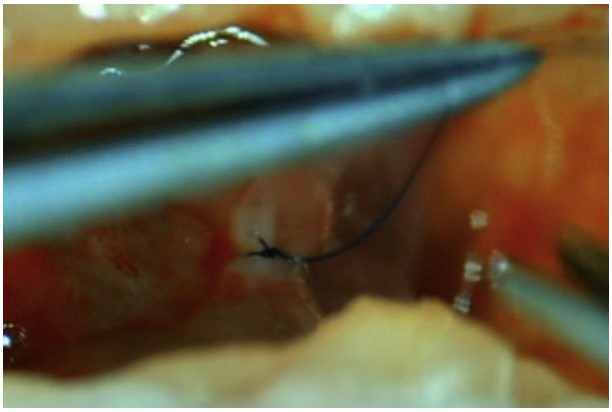
MATERIALS AND METHODS

Animals

The surgical protocols followed in this investigation were approved by the Animal Care and Use Committee of Shanghai Children's Medical Center. PAB and sham operations were performed on neonatal Sprague–Dawley rats within 6 hours after birth and in adherence with the guidelines from the Committee for the Care and Use of Laboratory Animals.

Pregnant Sprague–Dawley rats were purchased from Xipu'er-bikai Experimental Animal Co, Ltd (Shanghai, China). At 6 hours after birth, the rat neonates (both males and females) were randomized to 2 groups: the experimental group (PAB group), comprising 30 neonatal rats that underwent PAB surgery, and the control group (sham group), comprising 30 neonatal rats that underwent same procedure except for the banding step.

Postoperative measurements, such as echocardiography studies, MRI evaluation, and histology, were conducted blindly. Twelve rats (6 for the



VIDEO 1. Constriction of the PA in a 4-hour-old rat. Following a transverse skin incision, horizontal thoracotomy at the third intercostal space was performed by dissecting intercostal muscles and cutting the sternum. After the pericardium was opened, the PA was separated from the aorta with micro scissors. A 11-0 nylon thread was positioned under pulmonary artery and a 30-Gauge needle was placed upon the pulmonary artery. The pulmonary artery and needle were then tied together. When the needle was removed, a fixed constricted opening was created. The sternum and thoracic wall were sutured with a 9-0 nylon thread. Afterward, the neonates were placed under a heat plate to warm up. Finally, the natural movements and a red/pink complexion were achieved. Video available at: http://www.jtcvsonline.org/article/S0022-5223(17)31192-3/addons.