Mechanism of Pressure-Overload Right Ventricular Hypertrophy in Infant Rabbits
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Summary
Although pressure-overload right ventricular hypertrophy is a long-term risk in some congenital heart diseases such as tetralogy of Fallot, how it develops is unclear. The aim of this study was to investigate the mechanism of development of this right ventricular heart failure.

Pulmonary artery banding in 10-day-old rabbits induced pressure-overload right ventricular hypertrophy as they grew. Comparisons were made with age-matched sham controls (n = 24 per group). In weekly serial echocardiography, the right ventricular contraction and diastolic function decreased from 3 weeks after surgery (P < 0.01), and the right ventricle became hypertrophic from 4 weeks after (P < 0.05). Pressure-overload increased cardiomyocyte apoptosis from 4 weeks postoperatively (TUNEL staining and Western blotting analysis, P < 0.05); and fibrosis occurred in the right ventricular cardiomyocytes at 8 weeks after operation (Masson’s trichrome stain, P < 0.01).

In our model, pressure-overload to the right ventricle caused the right ventricular disorder, hypertrophy, and fibrosis. Apoptosis of right ventricular cardiomyocytes was involved in progression. We have shown for the first time the mechanism whereby pressure-overload right ventricular hypertrophy develops in an infant rabbit model.

Key words: Apoptosis, Congenital heart disease, Heart failure, Hypertrophy, Right ventricular pressure overload

The prognosis of some congenital heart diseases such as tetralogy of Fallot has improved significantly with the advancement of medical science. Almost all babies born with tetralogy of Fallot can now expect to survive to adulthood. Consequently, long-term complications that did not exist before, such as right ventricular dysfunction and arrhythmia, have emerged. Right ventricular (RV) function is a major determinant in cases of tetralogy of Fallot. The long-term survival and quality of life of these patients depend on our ability to preserve long-term RV function. Although there are many studies concerning left heart failure, only a few reports have considered the mechanisms of right heart failure, especially in congenital heart disease.

The aim of our study was to clarify the mechanism of progressive pressure-overload right ventricular hypertrophy in an infant rabbit model.

One potential cellular mechanism of myocardial dysfunction is apoptosis, which is a tightly regulated and enzymatically driven process in which cells die in response to various stimuli. Recent studies have demonstrated that activation of cardiomyocyte apoptosis was recognized to contribute to cardiac dysfunction in several situations, such as hypertension, ischemia-reperfusion injury, and pressure-overload.

Moreover, these apoptotic pathways increase myocardial fibrosis, and later lead to compromised cardiac function.

We examined these mechanisms in pressure-overload right ventricular hypertrophy in an infant rabbit model.

Methods
Animals: New Zealand White rabbits in a late stage of pregnancy were purchased from Saitama Experimental Animals Supply Co., Ltd. (Saitama, Japan). The infant rabbits produced from those pregnancies were used. The Animal Care and Use Committee of the University of Tokyo approved this study. All animals were acclimatized in the Animal Research Section of the Center for Disease Biology and Integrative Medicine. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996).

Models: Ten-day-old rabbits (mean body weight, 146 ± 36 g) were sedated by intramuscular injection of a mixture of ketamine hydrochloride (Daiichi Sankyo Co., Ltd., Tokyo) at 35 mg/kg and xylazine hydrochloride (Bayer HealthCare, Osaka, Japan) at 2 mg/kg. RV heart failure was induced by pulmonary artery banding (PAB) according to methods previously described by several groups. The rabbits were randomly separated into 2 groups: (1) a SHAM group (n = 24), in which, after left thoracotomy, the pulmonary artery (PA) was cleaned of loose tissue; (2) a PAB group (n = 24), in which, after left thoracotomy, the PA was cleaned of loose tissue, and then banded to the same size with 2-0 silk. The PA was constricted gradually as the rabbits grew, and a pressure-overload RV hypertrophy model was obtained. Various examinations were performed as presented in Figure 1A.

Echocardiography: The echocardiographic examinations were performed as presented in Figure 1B.
performed every week until 8 weeks after surgery (Figure 1A). Rabbits were sedated in the same way as for surgery. All ultrasound data were acquired with a ProSound 5500 (Aloka Co., Ltd, Tokyo) and a 15 MHz transducer.

To estimate the peak velocity across the banding site and the peak pressure gradient of the tricuspid valve regurgitation, spectral continuous-wave Doppler mode was used. Right ventricular overall systolic function was assessed as a percentage of the change in cavity area from end-diastole to end-systole. End-diastole was identified by the onset of the R wave from a simultaneously recorded electrocardiogram, while end-systole was regarded as the smallest right ventricular cavity size before tricuspid valve opening. Using the apical 4-chamber view, the endocardial borders of the right ventricular free wall and septum were traced from base to apex. Right ventricular fractional area change (RVFAC) was defined using the formula (end-diastolic area - end-systolic area)/end-diastolic area.

Increased.

**Figure 1.** A: Schematic of pressure-overload right ventricular hypertrophy modeling studies. B: Hematoxylin-eosin-stained sections at midventricle level of whole rabbit hearts. The upper row images show the representative SHAM group, and the lower row shows those from the PAB group. The right ventricular wall thickened and the chamber size increased.

**Statistical analysis:** Values are expressed as the mean ± SD. Statistical analyses were performed using the JMP Statistical Discovery Software (version 7, SAS Institute, Cary, NC, USA). The pairs of values were compared between the two experimental groups using Student’s t test after normal distribution was confirmed and equal testing was done. Non-parametric testing was employed when data were not normally distributed.
RESULTS

Morphology: The body weight gain of the PAB group decreased from 7 weeks after surgery (Figure 2A). The body weight of the PAB group at 8 weeks after operation was 1483 ± 244 g, and that of the SHAM group was 1826 ± 249 g.

Hematoxylin-eosin-stained short axis sections are shown in Figure 1B. The size of the left ventricle in the age-matched SHAM group was almost equal to that in the PAB group. However, the right ventricle of the PAB group became dilated as its wall thickened from 4 weeks postoperatively.

Echocardiography: The pressure-overload in the right ventricle occurred gradually as the rabbits grew (Figures 2A and 2B). The velocity of the main pulmonary artery finally rose to about 2.5 m/s in the PAB group (Figure 2B).

When pressure-overload took place, tricuspid valve regurgitation occurred. Estimated right ventricular systolic pressure increased to 32.5 ± 4.3 mmHg at 8 weeks postoperatively, when the pressure of the inferior vena cava was assumed to be 10 mmHg (Figure 2C).

Left ventricular contractile function is usually assessed by the ejection fraction (EF), but due to both RV shape and location and to the load dependence of its EF, accurate evaluation of its function is still a challenge. Anavekar, et al reported the usefulness of RVFAC to assess its contractile function.

The RV MPI is also used as an indicator of global RV function.

In this study, RVFAC in the PAB group decreased from 3 weeks postoperatively (Figure 2D), as RVMPI increased during the same period after surgery (Figure 2E). The RV free wall in diastole thickened from 4 weeks after operation (Figure 2F).

Echocardiography revealed that pressure-overload to the right ventricle caused right ventricular disorder from 3 weeks after surgery and right ventricular hypertrophy from 4 weeks postoperatively in the PAB group.

TUNEL staining: More TUNEL-positive nuclei were found in the PAB group than in the SHAM group (Figure 3A). In each myocardial portion of the SHAM group, the percentage of the TUNEL-positive nuclei was at a basal level (0.1-0.2%), but the percentage of TUNEL-positive nuclei increased from 4 weeks postoperatively in the PAB group (Figure 3B).

Western blot analysis: Caspase-3 is a key protease of the apoptotic machinery and exists as an inactive 32 kDa precursor which is converted into the active 17 kDa fragment upon activation with apoptosis-inducing stimuli. RV hypertrophy due to PAB was associated with a significantly increased labeling of active caspase-3 protein from 4 weeks after surgery (Figure 3C).

These results demonstrated that RV pressure-overload hypertrophy exacerbates apoptosis.

Fibrosis: Representative areas of fibrosis and myocardium are shown in blue and red, respectively (Figure 4A). The degree of fibrosis was calculated as the ratio between blue and red areas.
in the myocardium (Figure 4B). Late fibrotic replacement of dead cardiac myocytes occurred from 8 weeks after surgery (Figure 4A and 4B).

This result revealed that RV pressure-overload hypertrophy caused late fibrosis.

**Discussion**

The present study demonstrates for the first time that PAB-induced right ventricular hypertrophy in infant rabbits is associated with apoptosis and fibrosis. These data are in good agreement with the earlier study of Braun, et al which described RV hypertrophy in an adult rat model.20

Pressure-overload has been reported to play an important role in the development of cardiac hypertrophy.21 It also induces apoptosis and late fibrosis.11,22 Clinically, long-term RV hypertension causes various complications in some congenital diseases such as surgically repaired tetralogy of Fallot.56–61 The increased stress on the RV limits long-term survival.22–24 As surgical techniques for the primary repair of complex forms of congenital heart disease improve, long-term survival and quality of life will depend on right ventricular function.22 In contrast to the many studies on LV hypertrophy, there are very few investigations related to the RV, especially in infants.

Cardiac apoptosis in response to pressure overload has been reported to occur in LV hypertrophy induced by aortic banding or systemic hypertension.24–26 There are very few reports which evaluate myocardial apoptosis in pressure-overload right ventricular hypertrophy in adult rats. These studies mention that apoptosis is one of the potential mechanisms of myocardial dysfunction,24–26 and that PAB-induced RV hypertrophy causes RV apoptosis and fibrosis in adult rats.20 The present study examined progressive pressure-overload RV hypertrophy in infant animals for the first time.

In our model, pressure-overload to the right ventricle occurred gradually, as the rabbits grew (Figures 2A and 2B). After pressure-overload occurred, contraction and relaxation of the right ventricle significantly decreased in the PAB group from 3 weeks after surgery (Figures 2D and 2E). At least temporally, TUNEL and Western blot demonstrated that this pressure-overload caused apoptosis of the right ventricular cardiomyocytes from 4 weeks postoperatively (Figures 3A, 3B and 3C). On the basis of our results, we speculate that pressure-overload also resulted in right ventricular hypertrophy and fibrosis (Figures 1B, 2F, 4A and 4B).

Considering the results of this study, it would appear that right ventricular pressure-overload in infant rabbits is poorly tolerated and needs to be relieved early to prevent deterioration of RV function. In addition, this experimental model has the potential to aid in setting the ideal time for surgical interventions. Examination of this mechanism will improve prognosis of patients with such RV pressure-overload.

**Conclusions:** In our model, pressure-overload to the right ventricle caused right ventricular disorder, hypertrophy, and fibrosis. Apoptosis of the right ventricular cardiomyocytes was involved in this progression. We have shown for the first time, in an infant rabbit model, the mechanism whereby the pressure-overload right ventricular hypertrophy develops.

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**References**