Rabbit Model of Right Ventricular Hypertrophy Induced by Pulmonary Artery Banding

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Objective - The aim of this study was to characterize a rabbit model of RVH induced by pressure overload due to narrowing of the pulmonary artery.

Materials and Methods - New Zealand White rabbits (n=10) were divided into 2 groups: sham operated (SHAM, n=5) and pulmonary artery banding (PAB, n=5). PAB was induced by narrowing the pulmonary artery. Twenty weeks after surgery, hemodynamic, cardiac function, and electrocardiograms were obtained from PAB compared with SHAM. After measurement, rabbits were sacrificed to collect ventricular myocardium for histopathological analysis.

Results - After 20 weeks, the % HW to BW ratio of whole heart and right ventricle (RV) and right ventricular free wall thickness were significantly increased in PAB when compared with those in SHAM. PAB had a significant electrical remodeling as demonstrated by lengthening of QT and QTc(F) intervals. PAB also had a significant functional remodeling verified by a decreased contractility index and lengthened time constant of relaxation of RV.

Conclusion - The rabbit with PAB demonstrates cardiac remodelling as well as diastolic and systolic dysfunction.

Keywords: Cardiac, Hypertrophy, Rabbit, Remodelling, Right ventricle
Introduction
Animal models are useful for several aspects including investigation of disease mechanisms, new therapeutic paradigms, and vaccine development. The rabbit model of right ventricular hypertrophy (RVH) is one of the animal models that has been used extensively for studying the pathophysiology of right ventricular hypertrophy in humans as well as model for arrhythmia screening in drug safety evaluation [1,2]. The pathological condition of RVH may result from pulmonary artery stenosis (PS), tetralogy of Fallot, pulmonary artery hypertension (PAH), essential hypertension or pulmonary diseases [3]. A consequence of an increase in ventricular mass is the development of diastolic and systolic dysfunction, right heart failure, and sudden cardiac death [4,5]. In patients with RVH due to PAH, remodeling of right ventricle resulted in increased heterogeneity of ventricular action potential duration, a substrate for reentry arrhythmia [6]. In animal models of ventricular hypertrophy, Panyasing and colleagues [1] have shown that rabbits with RVH tended to develop the long QT syndrome and torsade de pointes in response to delayed rectifier potassium channel blockers infusion when compared to left ventricular hypertrophy and biventricular hypertrophy. The present study was designed to characterize a rabbit model of RVH induced by pressure overload due to narrowing of the pulmonary artery.

Materials and Methods
Animals:
Adult male New Zealand White rabbits, weighing between 2.3 and 2.9 kg, were used in this study. All procedures were approved by Faculty of Veterinary Science Animal Care and Use Committee, Chulalongkorn University. The rabbits were randomly divided into two groups: sham-operated group (SHAM, n = 5) or pulmonary artery banding group (PAB, n = 5). Details of the surgical procedure were previously described by Panyasing and colleagues (2010). The pulmonary artery was constricted to approximately 3.2 mm at the origin of the vessel so that it was about 50% narrowed. Rabbits in the SHAM group were anesthetized and operated as in PAB group except for the banding of pulmonary artery.

Assessments of cardiac function and electrophysiology:
After 20 weeks, animals were anesthetized as previously described. The bipolar transthoracic electrocardiograms (ECG) were obtained. A cut-down technique was performed on the right internal carotid artery and the right jugular vein. For the right internal carotid artery, a 2 Fr micromanometer catheter was inserted and placed at the level of aortic arch to measure aortic pressure (AoP). For the right jugular vein, another 2-Fr Millar catheter was inserted into the right atrium to obtain
right atrial pressure (RAP). The right ventricular pressure (RVP) was recorded by advancing the catheter into the right ventricle.

**Measurement of heart weight and ventricular free wall thickness:**
At the end of the experiment, all rabbits were euthanized with 200 mg/kg pentobarbital sodium while they were under general anesthesia. The heart was collected, rinsed with physiologic normal saline, patted dry and weighed. The right ventricular free wall thickness was measured at the level of the head of papillary muscle. Weights of atria, left and right ventricle were also collected.

**Histomorphological study:**
The heart tissues were fixed with 10% formaldehyde buffer for at least 48 hours. A 3 µm serial section was taken from each ventricle and stained with hematoxylin and eosin (H&E stain), Masson trichrome stain, or Periodic acid-Schiff (PAS) stain. All sections from both groups of rabbits were evaluated by an experienced pathologist.

**Statistical analysis:**
All data are presented as mean ± SEM. Parameters of electrophysiology, hemodynamics, and cardiac function were compared between groups at 20 weeks after surgery by using the Student’s unpaired t-test. A probability value of p < 0.05 was considered to be significant.

**Results**
In general, a total of 11 rabbits in the banding group were anesthetized and underwent open chest surgery. One rabbit in the pulmonary artery banding group died at the end of procedure due to pneumothorax. Therefore, the success rate of this procedure was 90.9 percent. All surviving rabbits did not show any sign of congestive heart failure during the study period (20 weeks).

The parameters of ECG were compared between the SHAM and PAB groups after 20 weeks of surgery (Table 1). In response to chronic pressure overload produced by narrowing of the pulmonary artery, the QT and QTc intervals were significantly lengthened (p<0.05). Chronic pressure overload did not alter RR, PQ, or QRS intervals.

The mean arterial blood pressure (MBP) in the PAB group was increased significantly 22.5% (p<0.05) whereas the mean RAP in PAB rabbits tended to increase when compared to SHAM (Table 2). End diastolic pressure (EDP) of the RV was 2.37 times higher in PAB than that in SHAM. End systolic pressure (ESP) of RV was 1.67 times
significantly higher in PAB than those in SHAM. The contractility index of the RV was significantly decreased (-39.30%) in PAB rabbits when compared to SHAM rabbits (p<0.05). The time constant of RV isovolumetric relaxation or Tau of the right ventricle for PAB rabbits tended to increase (26.04%, p = 0.1).

Anatomical remodeling was determined by assessment of the heart weight/body weight ratio, ventricular free wall thickness, and morphological changes. The %HW/BW in PAB rabbits were increased significantly (p<0.05) for the whole heart (16.66%) and for right ventricle (74.19%) when compared to SHAM rabbits. The right ventricular free wall thickness in PAB were increased significantly (p<0.01) 31.23% when compared with SHAM. The cardiac myocytes of right ventricle appeared to be similar in size in both groups as observed with H&E stains. A Periodic acid-Schiff (PAS) stain, however, demonstrated a marked positive glycogen-magenta in color, (Fig. 1A-B) in rabbits with PAB compared to SHAM rabbits. Moreover, a Masson’s trichrome stain showed marked fibroblast proliferation (blue color, Fig. 1C-D) in PAB rabbits compared with SHAM rabbits.

Discussion and conclusion
In the present study, PAB induced RVH and causes repolarization remodeling (increased QT and QTc intervals). It has been demonstrated previously that electrical remodeling especially in the right ventricle is the proximate cause of some arrhythmias [7-9].

At 20 weeks after surgery, the contraction of the right ventricle was significantly decreased as suggested by the contractility index. Recent studies in anesthetized guinea pigs and dogs demonstrated that contractility index is reliable for assessment of inotropic state of the heart than dP/dt_max [10]. The relaxation of the right ventricle of rabbit with RVH also decreased significantly as assessed by Tau. The decrease in both myocardial contraction and relaxation after pulmonary banding suggested that RVH in rabbits possesses systolic and diastolic dysfunction. These results are in accordance with previous study in infant rabbits in which the cardiac function was impaired beginning at 3 weeks after surgery [11].

Narrowing of pulmonary artery as used in this study was adjusted to approximately 50 percent of the diameter of the pulmonary artery. This process gradually produces pressure-overload to the right ventricle as the rabbits grew. In the current study, the morphological remodeling was confirmed by a significant increase of %HW/BW ratio of the right ventricle and the whole heart as well as the thickening of right ventricular free wall. The histopathological results of right ventricle demonstrated a
tendency for an increase of fibrous tissue and glycogen replacement of the dead cardiac myocytes in PAB rabbits when compared with SHAM rabbits. These results are consistent with previous studies in infant rabbits and adult rats which demonstrated that pulmonary artery banding induced pressure-overload, right ventricular hypertrophy, and apoptosis of the myocytes beginning at 4 weeks after banding and suggested that the apoptosis may lead to myocardial dysfunction [11-14].

Conclusions

Overall results of this study indicated that rabbits with PAB for 20 weeks developed right ventricular hypertrophy as suggested by electrical, functional, and anatomical remodeling. The electrical remodeling included prolongation of repolarization while the functional remodeling included impaired right and left ventricular function. The anatomical remodeling included thickening of right ventricular free wall, an increased %HW/BW ratio, a marked positive PAS staining, and marked fibroblast proliferation.

Acknowledgement

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References


Table 1 A comparison of electrical remodelling of the right ventricle in sham operated rabbits (SHAM) and pulmonary artery banded (PAB) rabbits. Values are presented as mean ± SEM. Each point was calculated from 1 minute data recording from anesthetized rabbits. *p<0.05 compared with SHAM by using Student unpaired t-test. QTc = corrected QT interval for heart rate by Fridericia formula; ms = milliseconds.

<table>
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<th>Parameters</th>
<th>SHAM</th>
<th>PAB</th>
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<tr>
<td>RR (ms)</td>
<td>195.1 ± 2.8</td>
<td>227.9 ± 12.9</td>
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<tr>
<td>PQ (ms)</td>
<td>63.4 ± 2.6</td>
<td>66.8 ± 3.8</td>
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<tr>
<td>QRS (ms)</td>
<td>49.3 ± 2.5</td>
<td>54.6 ± 1.9</td>
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<tr>
<td>QT (ms)</td>
<td>128.8 ± 4.0</td>
<td>162.9 ± 5.8*</td>
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<tr>
<td>QTc (ms)</td>
<td>222.0 ± 6.4</td>
<td>267.2 ± 7.4*</td>
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Table 2 Hemodynamic and cardiac function in sham operated rabbits (SHAM) and pulmonary artery banded (PAB) rabbits. Values are presented as mean ± SEM. Each point was calculated from 1 minute data recording from anesthetized rabbits. *p<0.05 and **p<0.01 compared with SHAM by using Student unpaired t-test. MBP = mean arterial blood pressure; RAP = mean right atrial pressure; EDP = end diastolic pressure; ESP = end systolic pressure; CI = contractility index (the maximal rate of rise of the ventricular pressure divided by the ventricular pressure at that point); Tau = the relaxation time constant; RV = right ventricle; mmHg = millimeter of mercury; ms = milliseconds.

<table>
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<th>PAB</th>
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<tr>
<td>MBP (mmHg)</td>
<td>54.6 ± 3.6</td>
<td>66.9 ± 2.7*</td>
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<td>RAP (mmHg)</td>
<td>3.7 ± 1.6</td>
<td>6.3 ± 2.0</td>
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<td>EDP_{RV} (mmHg)</td>
<td>2.9 ± 1.8</td>
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<td>ESP_{RV} (mmHg)</td>
<td>21.1 ± 2.32</td>
<td>35.3 ± 0.5**</td>
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<tr>
<td>CI_{RV}</td>
<td>76.6 ± 17.7</td>
<td>46.5 ± 4.8*</td>
</tr>
<tr>
<td>Tau_{RV} (ms)</td>
<td>19.2 ± 4.8</td>
<td>24.2 ± 1.9</td>
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Figure 1. Histopathological section of right ventricular free wall stained with periodic acid-Schiff (A,B) and Masson’s trichrome (C,D) in sham operated (SHAM) and pulmonary artery banding (PAB) rabbits. 400x; scale bar = 100 µM. Notice that the right ventricular myocytes appeared to be similar in size for both PAB and SHAM. In the PAB group, the PAS and Masson’s trichrome stain demonstrated glycogen deposits (magenta color) and fibroblast proliferation (blue color).