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Original article

Transmural dispersion of repolarization and cardiac remodeling in ventricles of rabbit with right ventricular hypertrophy

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ABSTRACT

Introduction: Recent publications demonstrated that rabbits with right ventricular hypertrophy (RVH) possess high sensitivity and specificity for drug-induced arrhythmias. However, the underlying mechanism has not been elucidated. This study aimed to evaluate RVH induced changes in cardiac remodeling especially the transmural dispersion of repolarization (TDR), epicardial monophasic action potentials (MAP), and hERG mRNA expression in rabbits. **Methods**: New Zealand White rabbits (n = 13) were divided into 2 groups: sham operated (SHAM, n = 6) and pulmonary artery banding (PAB, n = 7). PAB was induced by narrowing the pulmonary artery. Twenty weeks after surgery, hemodynamic, cardiac function, electrocardiograms, and MAP were obtained from PAB compared with SHAM. After measurement, rabbits were sacrificed to collect ventricular myocardium for histopathological analysis and measurement of hERG mRNA expression by real time PCR. **Results**: After 20 weeks, the % HW to BW ratio of whole heart and right ventricle (RV) and left and right ventricular free wall thickness was significantly increased in PAB when compared with those in SHAM. PAB has a significant electrical remodeling as demonstrated by lengthening of QT, QTc intervals, and increased Tp–Te duration. PAB also has a significant functional remodeling verified by decreased contractility index of RV and lengthened time constant of relaxation of LV. MAP of RV epicardium was significantly shortened in PAB consistently with increased hERG mRNA expression at the epicardium of RV. **Discussion**: The rabbit with PAB demonstrates cardiac remodeling diastolic and systolic dysfunctions. These rabbits also demonstrate increased TDR and electrical remodeling related to the change of hERG mRNA expression which may be prone to develop arrhythmias.

1. Introduction

Right ventricular hypertrophy (RVH) is an increase in size of the right ventricular muscle or an enlargement of the right ventricular chamber of the heart. This pathological condition may result from pulmonary artery stenosis (PS), tetralogy of Fallot, pulmonary artery hypertension (PAH), essential hypertension or pulmonary diseases (Haddad, Hunt, & Rosenthal, 2008). Several clinical studies demonstrated that RVH prevalence in systemic hypertension patients varied from 17 to 80% (Cuspidi, Sala, Muiesan, Luca, & Schillac, 2013).

A consequence of an increase in ventricular mass is the development of diastolic and systolic dysfunctions, right heart failure, and sudden cardiac death (Gan et al., 2007; Piao et al., 2010). Recently, Humbert, Sitbon, and Chaouat (2010) reported that the overall mortality rate of patients with RVH due to PAH was 30–50% in which 17–28% of that mortality rate was sudden cardiac death. The major cause of the sudden cardiac death may result from spontaneous ventricular fibrillation (Umar et al., 2012). This arrhythmia has been demonstrated to associate with electrical and structural remodeling of the right ventricle (Lee, Kodoma, Anno, Kamiya, & Toyama, 1997; Umar et al., 2012).

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 (Henkens, Scherptong, & Kralingen, 2008). In animal model of ventricular hypertrophy, Panyasing, Kijtawornrat, Rio, Carnes, and Hamlin (2010) have shown that rabbits with RVH tended to develop long QT syndrome and torsades de pointes in response to delayed rectifier potassium channel blocker infusion when compared to left ventricular hypertrophy and biventricular hypertrophy. Furthermore, QTc interval dispersion was also seen in both humans and animals with RVH (Panyasing et al., 2010; Tuncer, Gunes, & Guntekin, 2008). However, to date a systemic study of cardiac remodeling (i.e., electrophysiology, hemodynamic, ventricular function, and anatomical study) of the right ventricular hypertrophy due to

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pulmonary artery stenosis and the role of human *ether-a-go-go* related gene (hERG) in the rabbit model of RVH is still lacking. The present study was designed to evaluate RVH induced changes in cardiac remodeling especially the transmural dispersion of repolarization, epicardial monophasic action potentials, and hERG mRNA expression in rabbits.

2. Methods

2.1. Animals

Adult male New Zealand White rabbits, weighing between 2.3 and 2.9 kg, were used in this study. All animals were housed individually in a standard rabbit cage and all procedures were approved by the Faculty of Veterinary Science Animal Care and Use Committee, Chulalongkorn University. The rabbits were randomly divided into two groups: sham-operated group (SHAM, n = 6) or pulmonary artery banding group (PAB, n = 7). On the operating day, the rabbits were anesthetized with a combination of tiletamine hydrochloride and zolazepam hydrochloride (15 mg/kg, I.M.) and further maintained with 2–3% isoflurane via a customized facemask designed for small animals throughout the surgical procedures. Detail of surgical procedure was previously described by Panyasing et al. (2010). Briefly, the rabbits were placed in a dorsal recumbency position and a midline incision of approximately 1.5 in. in length was made after 2% lidocaine infiltration. The sternum was then cut and retracted so that the pericardial sac could be seen. The longitudinal cut was performed on the pericardium to reveal the pulmonary artery. The diameter of this vessel is approximately 6.0–6.4 mm for all rabbits used in this study. The pulmonary artery was constricted to approximately 3.2 mm at the origin of the vessel. Therefore, the vessel was about 50% narrowed. The pericardial sac was left open, the sternum, muscle layers, and subcutaneous were closed. All rabbits were given carprofen 4 mg/kg, I.M., SID for 3 days and 25 mg/kg enrofloxacin, I.M., SID for 7 days post-operatively. Rabbits in SHAM group were anesthetized and operated as in PAB group except for the banding of pulmonary artery.

2.2. Assessments of cardiac function and electrophysiology

After 20 weeks, animals were anesthetized as previously described. The bipolar transthoracic electrocardiogram (ECG) was obtained. The high and low pass filters were set at 0.01 Hz and 1 kHz, respectively. Signals were digitally sampled at a frequency of 2 kHz. 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 (Millar instrument, Texas, USA) was inserted and placed at the level of aortic arch to measure aortic pressure (AoP). The left ventricular pressure (LVP) was recorded by advancing the catheter into the left ventricle. 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. The monophasic action potential (MAP) was recorded for both right and left ventricular epicardia by placing MAP electrode catheter (EP technologies, Boston Scientific, MN, USA) directly on the epicardium for 1 min each following a midline incision over the sternum. The MAP was recorded for 1 min each. All data were recorded with an IOX program (IOX version 1.8.5, EMKA Technologies, Fall Church, VA, USA).

2.3. 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 dried and weighed. A cross-section of the left and right ventricles was made just below the coronary grove. Left and right ventricular free wall thickness was measured at the level of the head of the papillary muscle. Weights of atria, and left and right ventricles were also collected. Samples of the left and right ventricular endocardium and epicardium were collected from the strip of myocardium sectioned from the free-wall at the level of the head of the papillary muscle. These muscle strips were immersed in RNAlater and kept at −80 °C for further analysis of mRNA expression. The rest of the tissue samples were collected in 10% formalin for histopathological analysis. The normalized heart weight was calculated using the following formula: ((whole heart weight (g) / body weight (kg)) * 100) and presented as %HW/BW.

2.4. Data analysis

The ECG was analyzed automatically by using ECG auto program (ECG auto version 3.3.0.15, EMKA Technologies, Falls Church, VA, USA). Electrocardiographic parameters included were RR, PQ, QRS, QT, and the duration from the peak of T wave to the end of T wave (Tp–Te). QT interval was measured from the beginning of Q wave to the end of T wave. The QT interval corrected for changes of heart rate, QTc interval, was calculated by using the Carlsson equation (Carlsson, Abrahamsson, Andersson, Duker, & Schiller-Linhardt, 1993). Tp–Te duration was measured from the peak of T wave to the end of T wave. MAPs of right and left ventricular epicardia were analyzed for action potential duration (APD) after it was repolarized for 90% (APD_{90}). Recordings of AoP and RAP were analyzed for mean arterial pressure (MBP) and mean right atrial pressure, respectively. Recordings of left and right ventricular pressures were analyzed for indices of inotrope (contractility index, the dP/dt_{max} divided by the ventricular pressure at that point) and lusitrope (tau, ventricular relaxation time constant). Tau was calculated by Glantz method, $P(t) = P_0 e^{-t/\tau_E} + P_\alpha$, where P = pressure at time t, P_0 = amplitude constant, τ_E = Glantz relaxation constant, and P_α = non zero asymptote due to pleural and pericardial pressures (Raff & Glantz, 1981).

2.5. hERG mRNA expression analysis

Total RNA was extracted from the myocardium of the left and right ventricles from all groups with Aurum™ Total RNA Fatty and Fibrous Kit (BioRad, Hercules, CA, USA) in accordance with the manufacturer's instruction. Subsequently, the total RNA (1 μg) was reverse-transcribed into complementary DNA (cDNA) using the iScript™ Reverse Transcription Supermix for RT-qPCR kit (Bio-Rad Laboratories Inc, Hercules, CA, USA). The synthesized cDNA was quantified with spectrophotometer and stored at −20 °C for later analysis.

The real time PCR assays were performed with an ABI 7300 instrument (Applied Biosystem, Foster City, California, USA) using the Real-time-PCR Master Mix E4 (GeneOn, Ludwingshanfen am Rhein, Germany) in accordance with the manufacturer's protocol. For each PCR reaction, it composed of cDNA (1 μg), PCR master Mix, the forward and reverse primers (20 μM) and adjusted volume of nuclease-free water. All reactions were performed in duplicate. The PCR was performed under the following conditions: 95 °C for 3 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing and extension at 58 °C for 1 min. Fluorescent signals were detected at the end of the extension step of each cycle. A dissociation step, consisting of 94 °C for 3 min, 58 °C for 30 s and 72 °C for 1 min was performed at the end to confirm product specificity. The primer sequences used in this study were: hERG, forward primer, 5′-CAGGC ACCACGCATC CA-3′, reversed primer, 5′-GTCAGGGTGT GTCGGAACTT-3′;18 s rRNA, forward primer, 5′-CCG CGG TTC TAT TTT GTT GGT TTT-3′, reversed primer, 5′-CGG GCC GGG TGA GGT TTC-3′. The accession numbers were as followed, hERG, OCU97513 and 18sRNA, AF102857.

The quantification of hERG mRNA was calculated using the comparative threshold cycle method. The results are reported as relative gene expression. The 18S rRNA was used as an internal control, against which each target signal was normalized, this was referred as the ΔCt.