Pressure Overload Creates Right Ventricular Diastolic Dysfunction in a Mouse Model: Assessment by Echocardiography

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Background: Noninvasive diagnostic tools for right ventricular (RV) dysfunction measurements are increasingly being used, although their association with the pathologic mechanisms of dysfunction is poorly understood. Although investigations have focused mainly on RV systolic function, RV diastolic function remains mostly neglected. The aim of this study was to test which echocardiographic parameters best reflect RV diastolic function in mice.

Methods: Pulmonary artery banding (PAB) was used to induce RV pressure overload in mice. Transthoracic echocardiography and invasive hemodynamic measurements were performed after 3 weeks in PAB and sham-operated mice. Subsequently, the hearts were investigated by histology and analyzed for gene expression.

Results: PAB-induced pressure overload (RV systolic pressure PAB 52.6 ± 11.8 mm Hg vs sham 27.0 ± 2.7 mm Hg) resulted in RV hypertrophy and remodeling, as reflected by increased Fulton index (PAB 0.37 ± 0.05 vs sham 0.25 ± 0.02, $P = .001$). Masson's trichrome staining revealed increased interstitial fibrosis (PAB 12.25 ± 3.12% vs sham 3.97 ± 1.58%, $P = .002$). This was associated with significant systolic RV dysfunction as demonstrated by reduced contractility index and diastolic dysfunction as demonstrated by end-diastolic pressure (PAB 2.66 ± 0.83 mm Hg vs sham 1.49 ± 0.50 mm Hg, $P < .001$) and τ (PAB 40.0 ± 16.1 msec vs sham 13.0 ± 3.5 msec, $P < .001$). Messenger ribonucleic acid expression of β-myosin heavy chain, atrial and brain natriuretic peptides, collagen family members was elevated, and the sarco/endoplasmic reticulum Ca^{2+}-ATPase was decreased. Echocardiography revealed significant increases in RV free wall thickness and isovolumic relaxation time and a decrease in left ventricular eccentricity index, E′, and tricuspid annular plane systolic excursion. Isovolumic relaxation time and E′ were significantly correlated with end-diastolic pressure ($rs = 0.511$ and -0.451) and τ ($rs = 0.739$ and -0.445, respectively). Moreover, E′ was negatively correlated with the degree of RV fibrosis ($rs = -0.717$).

Conclusions: Within 3 weeks, PAB causes pressure overload–induced RV hypertrophy and remodeling with compensated systolic and diastolic dysfunction in mice. RV free wall thickness, tricuspid annular plane systolic excursion, E′, E/E′ ratio, and isovolumic relaxation time appear to be the most reliable echocardiographic parameters for the assessment of RV dysfunction. (J Am Soc Echocardiogr 2015;28:828-43.)

Keywords: Right ventricle, Fibrosis, Echocardiography, Dysfunction, Diastolic dysfunction, Pressure overload

Right ventricular (RV) functional status predicts survival of patients with pulmonary arterial hypertension (PAH) and congenital heart diseases.[1] Echocardiography as a noninvasive tool is increasingly being used for the functional assessment of the right ventricle. However, the association of echocardiography-derived parameters with morphologic and histologic changes of the right ventricle is not well characterized. Previous investigations have focused on systolic function,[2] but little is known about diastolic function.

In the left ventricle, diastolic dysfunction is known to be an important factor contributing to mortality.[3,4] In patients with PAH, the presence of a delayed diastolic RV filling profile, suggestive of diastolic dysfunction, has been demonstrated by magnetic resonance imaging.[5] Elevated RV filling pressure, which may be caused by RV diastolic dysfunction, predicts the risk for cardiac events.[6-8] Accordingly, dilatation of the right atrium has been

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Abbreviations

DTI = Doppler tissue imaging

EDP = End-diastolic pressure

ET = Ejection time

IVRT = Isovolumic relaxation time

LV = Left ventricular

PAB = Pulmonary arterial banding

PAH = Pulmonary arterial hypertension

RV = Right ventricular

RVFW = Right ventricular free wall

RVSP = Right ventricular systolic pressure

TAPSE = Tricuspid annular plane systolic excursion

TCOT = Tricuspid closure-to-opening time

associated with adverse clinical outcomes.[9] However, the pathologic mechanisms underlying development and progression of RV diastolic dysfunction are poorly characterized, and the diagnostic tools are not well established.

Echocardiography is the technique of choice for the serial assessment of cardiac function. However, RV function is difficult to evaluate using echocardiography because of RV shape, position, and variation with respiration.[10] Investigations of left ventricular (LV) diastolic function are facilitated by the ellipsoid shape of the left ventricle and multiple clinical studies have demonstrated robust associations of echocardiographic parameters with LV structural changes.[11] For RV diastolic function, such tools are still missing.

In animal models, validation of echocardiography-derived parameters is possible via direct comparison with invasive measurements and morphological analysis. The feasibility of assessing RV function using echocardiography has been demonstrated in mice[12,13] and rats.[14,15] In mice, Doppler imaging has been used to assess RV function.[16,17] In rat models of PAH, echocardiography has been applied to evaluate RV systolic function, while diastolic function has not been addressed.[14,18] Thus, the validation of the described Doppler parameters for the assessment of RV diastolic function in mice has not been performed. We hypothesized that by comparing echocardiography-derived parameters with invasive hemodynamic measurements, gene expression, and morphologic analysis, the echocardiographic parameters that best reflect diastolic RV function could be identified. To this end, RV remodeling was induced in an experimental mouse model of chronic RV pressure overload via pulmonary arterial banding (PAB).[19,20]

METHODS

Animal experiments were approved by the Austrian Ministry of Education, Science and Culture according to the regulations for animal experimentation (BMWF-66.010/0057-II/3 b/2012 and BMWFW-66.010/0074-WF/II/3 b/2014). The study conformed to the directive of the European Commission on the protection of animals used for scientific purposes (EU Directive 2010/63/EU). C57BL/6J male mice 11 to 12 weeks of age were obtained from Charles River, Germany. Mice were housed with a 12 h/12 h light/dark cycle, at a constant room temperature of 22°C, with access to standard laboratory chow and water ad libitum.

Animal Model

The PAB surgery was performed as previously described[21] and in detail in the Appendix. Three weeks after surgery, mice underwent echocardiographic investigation followed by invasive hemodynamic measurements. After completion of the study, heart tissue samples were collected for histologic and molecular analysis. From a subgroup of mice, hearts were separated into the right and left ventricles and used for RV/(LV + septum) ratio measurements. A detailed description of the experimental plan is provided in Supplemental Figure 1S.

Echocardiography

A Vevo 770 High Resolution Imaging System with a 30-MHz RMV-707B scan head (VisualSonics, Toronto, ON, Canada) was used for transthoracic echocardiographic measurements. Investigations were performed under mild anesthesia with isoflurane (0.8%–1.2%) as previously described[22,23] and wherever possible in accordance with the guidelines for echocardiographic assessment of the right hearts.[24] A detailed description is provided in the Appendix.

Hemodynamic Measurements

RV systolic pressure (RVSP), blood pressure, and LV systolic pressure were measured under isoflurane anesthesia (2%–4%) using a closed-chest technique, as described previously.[25] Briefly, a 1.4-F pressure catheter (SPR-671; Millar Instruments, Houston, TX) was inserted into the right jugular vein and directed to the right ventricle to measure RVSP. Systemic measurements were obtained via catheterization of the carotid artery. The catheter was then directed into the left ventricle to obtain LV systolic pressure. Measurements were performed and recorded for ≥5 min. Analysis was performed only for the pressure recordings with stable hemodynamics. Care was taken to ensure that the measurements for hemodynamics and echocardiography were made at similar heart rates. Online tracings and analysis were made using LabChart 7 software (ADInstruments, Spechbach, Germany). We calculated +dP/dt, −dP/dt, end-diastolic pressure (EDP), pressure-time index, and τ from pressure tracings using software analysis module. We calculated (+dP/dt)/Pmax and (−dP/dt)/P as described previously.[26] Hemodynamic measurements were performed by experienced personnel blinded to the experimental group.

Tissue Collection and Analysis

After invasive hemodynamic measurements, animals were immediately sacrificed, and tissue samples were collected for gene expression and histologic analysis. Briefly, the chest was opened and the heart and lungs were flushed with ice-cold phosphate-buffered saline. The lungs and heart were excised, the atria were removed, and the right ventricle was separated from the left ventricle and septum. Tissue pieces were weighed and then snap-frozen in liquid nitrogen and stored at −80°C until analysis. For immunohistochemical analysis, the hearts were removed, fixed in formalin, and embedded in paraffin. For determination of congestion, internal organs (lungs, kidneys, and livers) were removed and weighed immediately. Values are presented after normalization to tibia length. No difference in tibia length was observed. For wet-to-dry ratio measurements, pieces of organs were weighed and then kept at 40°C for 2 to 3 weeks and reweighed until stable values were obtained.

Morphologic Analysis

For assessment of fibrosis, formalin-fixed, paraffin-embedded heart tissue was cut in 2-μm-thick sections and stained for collagen