Time course of the pulmonary artery banding model monitored by pulmonary artery flow velocity: a possible non-invasive biomarker for disease progression in pulmonary hypertension

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Introduction

The pulmonary artery (PA) banding model is commonly used to examine direct effects of substances on right ventricular function and morphology. The pathophysiological time course of maladaptive functional and structural processes however has not been fully elucidated. We aimed to characterize the model by measuring functional parameters and gene expression in the right ventricle, which also served as a first screening of putative biomarkers.

We sequentially investigated, whether the grade of PA occlusion can be non-invasively visualized by echocardiography and correlates with right ventricular systolic pressure (RVP).

Furthermore, it was of interest if plasma markers for remodeling processes and pressure overload also reflected the pathophysiology of this model and if there was a different regulation in the acute and chronic phase.

Methods

Study design
60 male Sprague Dawley rats (250 g body weight) underwent either sham surgery (Control), 1.7 mm-Banding, or 1.3 mm-Banding. The animals were subjected to four clusters, each consisting of a Control group (n=3), a 1.7 mm-Banding group (n=6) and a 1.3 mm-Banding group (n=6). Over a period of 4 weeks, echocardiographic and final hemodynamic measurements were performed and organ weights were determined in one cluster a week and blood and tissue were withdrawn for further analysis.

Parameters
• PA flow velocity was measured echocardiographically in the PW Mode with a high-resolution imaging system (Visual Sonics Vivos 7708). The pressure gradient (ΔP) was calculated according to the simplified Bernoulli equation ΔP = 4v² (v = velocity).
• HVP was invasively measured with a Millar® microtip catheter
• Right ventricle and left ventricle + septum weight ratio RVP/(LV+S) was measured in plasma via RIA (in pg/ml).
• Matrix Metalloproteinase 1 (TIMP-1) and Osteopontin (OPN) levels were determined in plasma via ELISA (in pg/ml).
• Relative gene expression of ANP, BNP, MMP-2, TIMP-1, OPN, Myosin Heavy Chain alpha and beta (MYHCA, MYHCB), Transient Potential Channel 6 (TRPC-6) and Samplastin / Endoplasmic Reticulum Calcium ATPase 2 (SERCA-2) in the right ventricle was determined via RT-PCR; data are shown in percentage of the respective control, which was set as 100%.

Statistics
Data are expressed as mean ± SD. Differences between the groups were evaluated by one-way ANOVA for each day, followed by Newman-Keuls post hoc analysis for multiple comparison. Pearson’s correlation coefficient was employed to correlate variables. Statistical significance was set at p<0.05.

Results

RVP and right ventricular hypertrophy shown as RVP/(LV+S) were dependent on the degree of the stenosis (Figure 1). Although ascos and pleural effusion occurred in some animals with 1.3 mm-Banding, no dilation of the right ventricle was observed. This led to the suggestion that the animals were in a state of compensated hypertrophy rather than in a state of insufficiency. Nevertheless, a shift towards a fetal gene expression pattern was observed as reflected by the absence of MYHCB / MYHCA, which was elevated in 1.3 mm-Banding compared to Control. An up-regulation of TRPC-6 and a down-regulation of SERCA-2 gene expression in the 1.3 mm-Banding group implicated a first pathophysiological development towards a state of insufficiency in the right ventricle (Figure 2).

RVP, one of the main characteristics of the PA banding model, was well represented by the non-invasively determined PA flow velocity, which is proved by the high correlation coefficient of r=0.879 (Figure 3).

Furthermore, it was of interest if plasma markers for remodeling processes and pressure overload also reflected the pathophysiology of this model and if there was a different regulation in the acute and chronic phase.

Conclusion

• RVP and right ventricular hypertrophy shown as RVP/(LV+S) were dependent on the degree of the stenosis.
• Pathophysiological hemodynamic alterations, which are the main characteristics of the PAB model, were well represented by echocardiographically determined PA flow velocity as well as ANP and BNP plasma levels.
• Plasma MMP-2, TIMP-1 and Osteopontin were not sufficient to monitor remodeling processes in the right ventricle.

Background Literature


Nagao N, Ninomiya T, Okano Y et al. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. JACC 1998. 31: 205-298


Echocardiography

Hemodynamics

Plasma markers

Relative gene expression

Figure 1 RVP and RV/(LV+S) as measure for right ventricular hypertrophy. *P<0.05; **P<0.01; ***P<0.001 Banding vs. Control; *P<0.05; **P<0.01; ***P<0.001 1.3 mm-Banding vs. 1.7 mm-Banding

Figure 2 Relative gene expression in the right ventricle of the pressure sensitive markers ANP and BNP, the remodeling markers MMP-2, TIMP-1 and OPN, the quotient of MYHCA and MYHCB as well as TRPC-6 and SERCA-2 which are involved in Calcium-handling. *P<0.05; **P<0.01; ***P<0.001 Banding vs. Control; *P<0.05; **P<0.01; ***P<0.001 1.3 mm-Banding vs. 1.7 mm-Banding

Figure 3 PA flow velocity and the relation of the pressure gradient, calculated from PA flow velocity via Bernoulli’s equation, and the RVP. *P<0.05; **P<0.01; ***P<0.001 Banding vs. Control; *P<0.05; **P<0.01; ***P<0.001 1.3 mm-Banding vs. 1.7 mm-Banding

Figure 4 Plasma levels of the pressure sensitive markers ANP and BNP and the relation of these markers and the RVP. *P<0.05; **P<0.01; ***P<0.001 1.3 mm-Banding vs. 1.7 mm-Banding

Figure 5 Plasma levels of the remodeling markers MMP-2, TIMP-1 and OPN