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Functional Assessment of Isolated Right Heart Failure by High Resolution *In-Vivo* Cardiovascular Magnetic Resonance in Mice

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ABSTRACT

Precise and noninvasive characterization of the development of the cardiac phenotype in murine models of heart failure has been widely demanded in modern cardiovascular research. High-resolution cardiovascular magnetic resonance (CMR) has been proven to be a powerful tool for the accurate and reproducible assessment of LV and RV parameters in healthy mice. Whereas changes in LV parameters in models of heart failure have been thoroughly evaluated, RV dysfunction has not. Purpose of this study was to characterize a model of isolated RV failure induced by pulmonal banding by *in vivo* CMR at 7T. RV parameters differed significantly from those of normal mice in terms of RV end-diastolic volume (EDV: 85 $\pm$ 14 μL vs. control 36 $\pm$ 3 μL, p $<$ 0.0001), RV end-systolic volume (ESV: 121 $\pm$ 10 μL vs. control 84 $\pm$ 4 μL, p $<$ 0.005) and RV ejection fraction (EF: 31 $\pm$ 6 % vs. control 57 $\pm$ 2 %, p $<$ 0.001). With regard to EDV, ESV, SV and EF LV parameters, there were no significant differences between pulmonary banded and control mice indicating overt isolated RV failure.

INTRODUCTION

Results obtained from patients with ischemic heart disease including right ventricular (RV) ischemia indicated that the importance of the RV during the development of congestive heart failure may have been underestimated (1). The assumption that the right ventricular response to pathological conditions such as increased afterload is identical or comparable to the LV is rather presumptuous, especially in light of the difficulties in assessing the anticipated complex geometrical and functional changes (2).

A set of early experiments in the 1970's including right ventricular cauterization were summarized by Sade and Castenada in stating that the right ventricle is "dispensable" (3). Eight years later, in 1984, Furey postulated an "essential function of the right ventricle" (4). However, despite more sophisticated experimental setups, no evidence for an essential right ventricular function was forthcoming. Another 10 years later, studies in patients with myocardial infarction indicated that the involvement of the right ventricle is a strong and independent prognostic factor (1) in ischemic heart failure.

With the advent of new transgenic mouse models, the functional consequences of various genetic manipulations for the LV and the RV require systematic investigation (2). We and others have already addressed this issue for LV analysis by using high-resolution cine cardiovascular magnetic resonance (CMR) which has proven highly accurate for quantifying LV function under both physiological and pathophysiological conditions (5–9). Despite the RV's complex geometry, we recently demonstrated that this also holds true for the accurate assessment of RV

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volumes and RV function in healthy mice (10). Taken together, CMR shows promise in its capability of evaluating significant changes in cardiopulmonary function in murine cardiovascular phenotype characterization (11). However, no experimental murine model of RV disease has yet been systematically evaluated or validated with regard to its capability of depicting functional consequences of isolated right heart failure.

The purpose of the present study was thus to evaluate the model of isolated RV failure induced by banding of the pulmonal artery (12). High field *in-vivo* cine-CMR was employed to noninvasively evaluate the extent of murine RV failure and to define a basis for further serial RV assessment and providing the opportunity to assess the time cause of therapy. The evaluation of pulmonal artery banding via x-ray contrast microangiograpy (12) has not been investigated further or used in later comparative studies probably due to its highly demanding technical requirements. Moreover, the technical setup does not permit serial evaluations of the mice. This model was recently validated histologically and compared to sham-operated mice (13). However, no precise and noninvasive imaging modality has been applied to this uniquely elegant model of isolated RV dysfunction.

METHODS

Mice and pulmonal banding

Ten healthy C57bl/6 mice (Charles River WIGA, Sulzfeld, Germany) of both genders were anesthetized with isoflurane at 1.5 % and 1.5 L/min oxygen flow. A mixture of 16 mL 0.9% saline (Sigma-Aldrich Chemie GmbH, Munich, Germany), 4 mL 100% EtOH (Sigma-Aldrich Chemie GmbH, Munich, Germany) and 1.6 mL Nembutal (Pentobarbital, Bayer, Leverkusen, Germany) was then administered i.p. for deep anesthesia. The mice were intubated under a microscope with a steel tube having an outer diameter of 1.1 mm. They were immediately ventilated with positive airway pressure (stroke volume 1.0 to 1.5 mL; ventilation rate 60 to 80 per minute) by connecting the tube to a rodent ventilator. The mice underwent pulmonal banding according to Rockman et al. (12) and Tarnavski et al. (13). A thoracotomy of the left side was performed through the fourth or fifth intercostal space. After the dissected thoracic halves had been separated for better operating conditions, the pericardial sack was opened and the pulmonal arteries isolated. A 6-0 silk ligature was placed around the pulmonary artery before tightening against a 25 gauge needle placed directly beside the artery for reproducible stenosis. Prompt removal of the needle insured continuous flow to the lung.

At the end of the operation, the thorax was closed by suture. The tracheal tube was disconnected from the ventilator, allowing free breathing. The animals recovered from anesthesia and were extubated within 30 minutes after the end of the operation. They were given an 8 week period to recover from the operation and develop right heart failure.

All experimental animal procedures were in accordance with our institutional guidelines and approved by our local ethics committee.

In-vivo CMR

Experiments were performed on an experimental 7.05-T MR scanner (Bruker, Karlsruhe, Germany), equipped with a microscopy gradient system having a maximum gradient strength of 870 mT/m and a rise time of 280 μs at maximum gradient switching. For MR signal transmission and reception, we used a home-made birdcage probehead as previously described. ECG-triggering was done by a home-made ECG-unit capable of filtering and enhancing the QRS-signal from the equipment-generated noise (14).

CMR was performed as previously described (10) using an ECG-triggered fast gradient echo (FLASH) cine sequence with the parameters; TE 1.5 ms; TR 4.3 ms, field of view of 30 mm^2, in-plane resolution 117 μm^2, acquisition matrix of 256 $\times$ 256, and slice thickness of 1.0 mm. For image acquisition, mice were deeply sedated using isoflurane at 1.5% and 1.5 L/min oxygen flow with a nose cone throughout the experiment. The mice were kept normothermic on a warming pad during the experiment. After the end of the experiment anesthesia was quickly discontinued and recovery supported by a gentle heat lamp. All mice recovered from anesthesia within 30 minutes after the end of the experiment.

To assess RV and LV systolic and diastolic parameters, we acquired data in contiguous short-axis slices orthogonal to the interventricular septum ensuring that RV and LV were depicted (Fig. 1).

Data analysis

For LV and RV measurements, epi- and endocardial borders were manually delineated in all slices in end-systolic and end-diastolic frames as described before (14) (Fig. 2). To define the end-systolic frame, the frame with the smallest RV or LV cavity volume and maximum myocardial diameter was selected as representing maximum contraction. For end-diastolic frames, maximum cavity volume and smallest ventricle wall diameters were considered the point of total relaxation. Total LV and RV ESV and EDV were calculated according to Simpson as the sum of all slice volumes. Stroke volume, EF and CO were calculated in the standard manner.

Statistical analysis

Statistical analysis was made using StatView software (SAS Institute Corp., Cary, NC, USA). All results are given as mean $\pm$ SEM. Comparisons between control animals and pulmonally banded mice were made using the Student paired $t-$test. Differences were considered statistically significant at a value of $p \leq .05$.

RESULTS

LV and RV cine-CMR was successfully performed on 10 mice, 6 of which survived the pulmonal banding and 8 weeks postoperatively. Due to severe arrhythmia and consecutive, substantially impaired image quality, one mouse could not be included in the assessment of RV and LV parameters. The

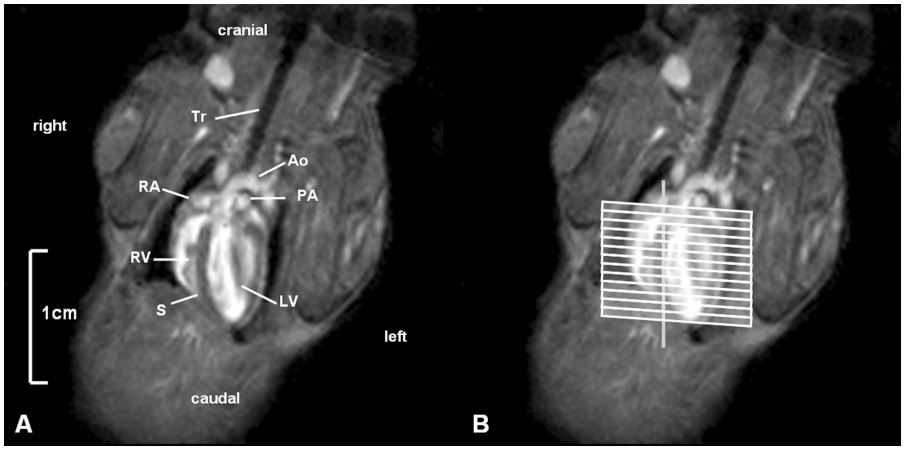

Figure 1. Anatomical overview (A) and orientation of slices (B) orthogonal to the interventricular septum. Tr = trachea, Ao = Aorta, RA = right atrium, RV = right ventricle, PA = pulmonal artery, LV = left ventricle, S = septum.

remaining 5 mice with a mean body weight of 31.7 ± 2.8 g and a mean heart rate of 453 ± 29 bpm provided excellent image quality and were included in this study. Due to the local ethics committee's restrictions, we had to use 13 previously examined and published age and body weight matched mice of both genders as controls (10).

LV parameters were indistinguishable between controls and pulmonally banded mice: LV end-diastolic (LV EDV 73 ± 13 μL vs. control 74 ± 4 μL, p = n.s.) and LV end-systolic volume (LV ESV 33 ± 8 μL vs. control 27 ± 3 μL, p = n.s.), LV stroke volume (LV SV 40 ± 8 μL vs. control 47 ± 2 μL, p = n.s.) and ejection fraction (LV EF 56 ± 8% vs. control 64 ± 3%, p = n.s.) as well as cardiac output (LV CO 18.2 ± 3.9 mL/min vs. control 21.8 ± 1.2 mL/min, p = n.s.) differed only slightly (Fig. 3).

RV parameters and function 8 weeks after pulmonal banding showed significant differences: RV ESV had increased more than two-fold (85 ± 14 μL vs. control 36 ± 3 μL, $p < 0.0001$), and the RV EDV measured 121 ± 10 μL vs. control 84 ± 4 μL ($p < 0.005$). As a consequence, RV EF dropped significantly (31 ± 6% vs. control 57 ± 2, $p < 0.001$), indicating RV failure. There was a trend toward lower RV SV and CO in the pulmonally banded compared to the control mice (RV SV: 37 ± 5 μL vs. control 47 ± 2 μL and RV CO: 16.8 ± 2.7 mL/min vs. control 21.9 ± 1.3 mL/min) (Fig. 4). However, this difference did not attain statistical significance indicating a compensated stage of RV failure. This concurs with the absence of clinical signs of RV failure such as ascites in these mice.

DISCUSSION

The RV's functional significance is not in doubt. Yet, models of isolated RV failure have rarely been described. And although advances in human RV evaluation have been made (15, 16), progress in the description of suitable animal models has lagged. Changes in RV function in global heart failure have been evaluated in rats after LAD banding (17). Isolated RV failure in a

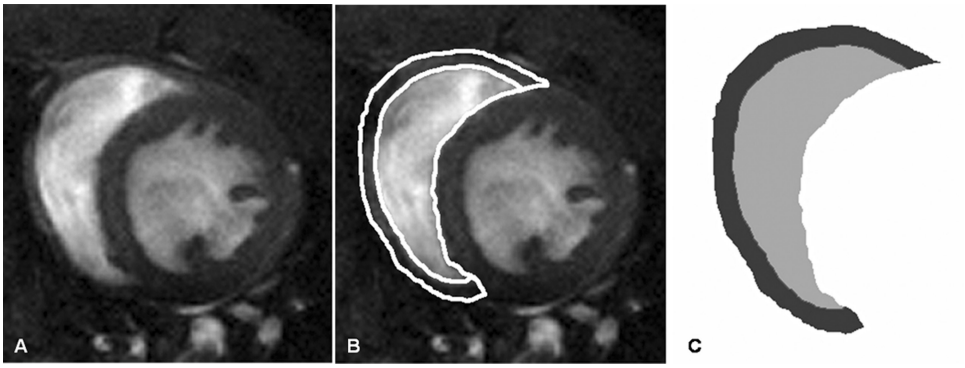

Figure 2. Example of RV delineation: A) raw data, B) manual delineation of epi- and endocardial borders and C) segmented volume.

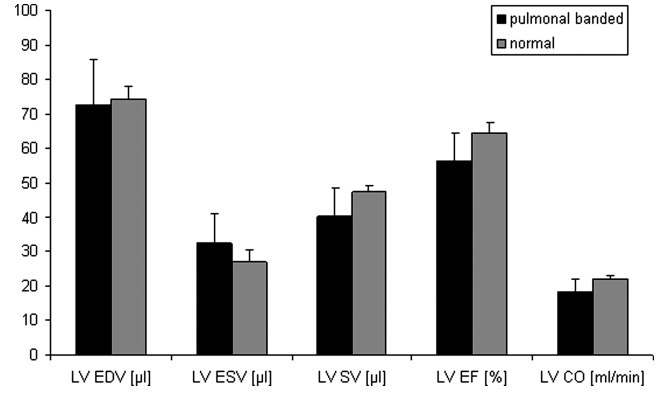

Figure 3. Changes in LV parameters 8 weeks after pulmonal banding. For all p = n.s.

widely-accepted animal model has not been morphometrically evaluated.

There are only two descriptions of morphologic changes in the cardiac phenotype after pulmonal banding in mice. Rockman et al. (12), the first group to introduce this technique of RV challenge, used digital x-ray contrast microangiography to assess the extent of changes in RV volumes. They also compared changes in RV wet weight and further genetical analyses to sham-operated mice, emphasizing this model's efficiency in generating isolated heart rate failure. Tarnavski et al. (13) compared histological sections of the RV and LV after pulmonary banding to sham-operated hearts reviewing operative murine cardiac models. To the best of our knowledge, ours is the first study to evaluate isolated right heart failure in mice via accurate CMR-based noninvasive cardiac phenotyping.

Compared to other models of RV challenge such as the effects of drug side effects (Crotaline, Bleomycin) or chronic hypoxia, the microsurgical approach to induce right heart failure has the advantage of being capable of inducing a reproducible stenosis. Unpublished results of our group indicate that induction of pulmonary hypertension and isolated RV failure in mice by repeated injections of Crotaline is not nearly as effective reported in previous rat studies (18, 19) or as those proposed for murine RV failure (20, 21).

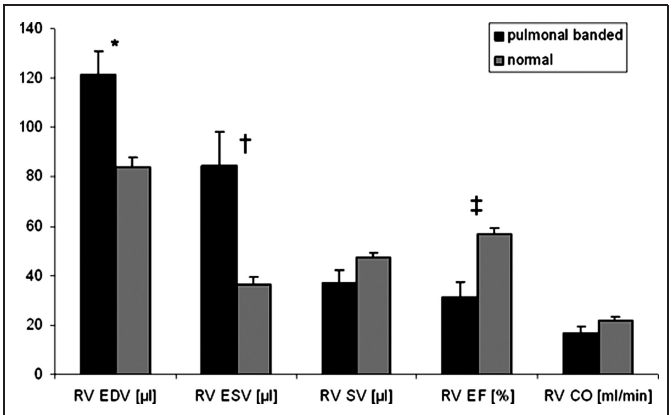

Figure 4. Changes in RV parameters after pulmonal banding. For *p < 0.005, †p < 0.0001, ‡p < 0.001, RV SV and CO remain p = n.s.

In concordance with known LV changes, increased RV afterload should trigger compensatory mechanisms such as myocardial hypertrophy. This temporary compensation for the increased RV pressure helps to overcome the pulmonal stenosis but ultimately leads to dilatation and RV failure, expectedly without impairment of LV function. However, we cannot ascertain whether there is a temporal evolution of the changes in RV function and whether this may have influenced the results of RV function.

However, our results clearly demonstrate isolated right heart failure and concur in this respect with the assessments of Rockman (12) and Tarnavski (13). As the LV parameters did not change during the 8 weeks after pulmonary banding, our assumption of the induction of isolated RV failure is reinforced. If and when LV function may deteriorate is pure conjecture. One must keep in mind that the pulmonally banded animals' LV values revealed a greater variance than those of control mice which may indicate associated changes within the LV during RV alterations.

RV changes after 8 weeks, however, revealed RV failure in a marked reduction in RVEF. As RVEDV and ESV increased drastically, we assume that compensatory mechanisms had already led to RV dilatation. As calculations of RV wall diameters or wet weight vary greatly due to the limitations inherent in spatial resolution at 7.05T imaging, we cannot prove this hypothesis. It will be a subject for future investigations with higher magnetic fields. Moreover, the fact that our results only reveal a tendency toward changes in RVSV and CO might have to do with the progression of RV compensatory mechanisms. RVSV and CO should be significantly decreased, corresponding to mechanisms during LV failure. In that respect, our results can be interpreted as a compensatory stage of dilated RV failure.

Limitations

Major disadvantages of any operative method are the learning curve involved, especially during microsurgery, and the morbidity associated with the procedure itself especially since pulmonal artery separation from the aorta is often associated with complications.

All our mice were given 8 weeks to develop RV failure. This time point was arbitrarily chosen. We expected the mice to have definitively developed heart failure in that time frame and expected they would be able to cope well with the examination and the necessary sedation. At the time of CMR, we noted no clinical signs such as marked increase in body weight, or clinically provable ascites or edema. Serial *in-vivo* evaluation of the compensatory changes with high-field murine CMR will be essential to better understand and define the development of RV failure, taking both acute changes and long-term compensatory mechanisms into account.

Although all animals were operated in identical fashion and a 25 G needle was used to facilitate reproducibility, we do not know whether a homogenous and reproducible pressure gradient across the banding was achieved. As invasive quantification

of pressure curves in mice is usually lethal, we will have to find a control for this important variable. A synchronized evaluation of the severity of pulmonary pressure will be required for quantification. In this respect, indirect quantification of the diameter in the stenotic region by additional CMR or new protocols using phase contrast imaging on mice for direct quantification of flow parameters may enable us to deepen our understanding while employing this model and its accurate evaluation technique.

Ultimately, a combined evaluation of LV and RV myocardial mass and contraction is mandatory. However, at an actual in-plane resolution of 117 μm, we decided against RV myocardial measurements. RV myocardial and wall motion analysis will become feasible with technical improvements and higher magnetic field strengths e.g. of 9.4, 11.7, or 17.6T.

CONCLUSION

We have demonstrated that *in-vivo* high-field CMR of murine cardiac function is highly accurate in analyzing LV parameters. After having successfully demonstrated RV assessment in normal mice, we were able to display and evaluate a model of isolated right heart failure in mice. Although we only examined a few animals and there are several relevant limitations to this study, we feel confident that further evaluation of this model, especially considering the quantification of induced stenosis and serial investigation of RV, changes over time in murine RV failure will reinforce our promising results. It then might serve as a useful research model for therapeutic strategies in RV failure and their outcome.

ABBREVIATIONS

RV Right ventricle
LV Left ventricle
EF Ejection fraction
SV Stroke volume
ESV End systolic volume
EDV End diastolic volume
CO Cardiac Output
CMR Cardiovascular Magnetic Resonance

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