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Cardiovascular phenotype characterization in mice by high resolution magnetic resonance imaging

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1. Introduction

Transgenic mouse models offer the unique opportunity to investigate the pathophysiological consequences of augmented, deleted or mutated specific genes and their dependent gene products. To analyze the resulting phenotype of such models, reliable measurement tools are required to allow for in vivo studies of murine cardiac morphology and function. Hemodynamic measurements using high-fidelity left ventricular (LV) micromanometer catheters have been used in open chest preparations [1]. However, to avoid hemodynamic changes due to altered intrathoracic pressure in the open chest model, Micro-Tip manometers are now used in a closed-chest preparation to obtain accurate hemodynamic measurements under more physiologic conditions [2].

The main limitation of these invasive methods is the lack of repeatability of measurements in individual animals. Such follow-up of cardiac function, however, is necessary to gain information about morphologic and functional changes of the heart over time under defined conditions. With echocardiography, quantification of left ventricular mass (LVM) may be feasible in mice [3,4]. Detection of acute functional changes during pharmacologic stimulation has also been demonstrated [5,6]. However, volumetric measurements in echocardiography are based on geometric models, which may not apply when the ventricle undergoes asymmetrical shape changes [7].

Magnetic resonance imaging (MRI) as an intrinsically three-dimensional method allows for volumetric quantification without relying on geometrical models [8]. This renders the MR method uniquely suited for the assessment of volumetric and functional changes in hearts with shape distortions and asymmetric ventricular dilatation, as they occur in ventricular remodeling after myocardial infarction or in dilated cardiomyopathy.

The scope of this article is to give an overview of the current methodological state of art of high-resolution cardiac MRI in mice and to demonstrate potential applications of MR imaging methods for murine cardiovascular phenotyping.

2. Technical requirements

Although the feasibility of MRI to visualize the mouse heart on a clinical MR scanner at 1.5 T has been demonstrated, it is recommended to move to higher field strengths such as provided by experimental MR scanners ranging from 200 to 500 MHz (corresponding to 4.7–11.75 T) to gain sufficient signal and, hence, to allow for high spatial resolution. Furthermore, in the light of typical resting heart rates between 500 and 600 bpm in the mouse, sufficient temporal resolution is necessary to achieve time-resolved cardiac images with minimized motion-related artifacts. Therefore, high performance gradient systems with high maximal field strength and rapid slew rates are used. An important contribution to overall image quality can be made by optimization of RF coil design such as short birdcage design for high filling factor and sensitivity in the isocenter or quadrature coil operation for general in-

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crease in sensitivity. Reliable and robust ECG-triggering is crucial for cardiac MRI independent from the species studied, whereas correction for breathing motion is not needed for most MR sequences in the mouse due to the typical murine breathing pattern under anesthesia with long stillstand of breathing at end exspiration [9]. Furthermore, inhalative anesthetics such as isoflurane given via a nose cone exert little negatively chronotropic and inotropic effects than many other narcotics [10], and allow for short induction and recovery times.

3. Quantification of LV volumes and mass

We and others have recently been able to demonstrate the feasibility of in vivo MRI to allow a detailed visualization of the cardiovascular system. Due to the high contrast between non-saturated spins in the ventricular blood and saturated myocardium in cine-FLASH images, clear definition of endocardial and epicardial borders allows for semi-automated segmentation in left-ventricular short-axis images [11]. By compartimentation of LV cavity and myocardium in multiple contiguous slices covering the entire heart, total LV volumes and mass can be calculated with similar accuracy and reproducibility as known from human MR studies. Applying this technique, LV morphology can be accurately assessed and hypertrophic changes of the heart can be quantified [12,13].

By optimization of hardware components such as birdcage coils and modification of imaging parameters in order to achieve higher spatial resolution, non-invasive assessment of cardiac anatomy and function is even feasible in newborn mice. We have been able to demonstrate the feasibility of microscopic imaging with high spatial and temporal resolution (in plane pixel size 80×80 μm^2, slice thickness 500 μm, acquisition window per cine frame 8.6 ms) for cardiac phenotype characterization in mice starting 3 days after birth (Fig. 1) [14]. As transgenic mice may develop an early phenotype or even may die within the first week of life, MRI may give new insights into the underlying pathophysiology in these models. Furthermore, MRI as a non-invasive method allows to follow up changes of LV volumes and mass in the neonatal and juvenile period by serial investigation of individual animals.

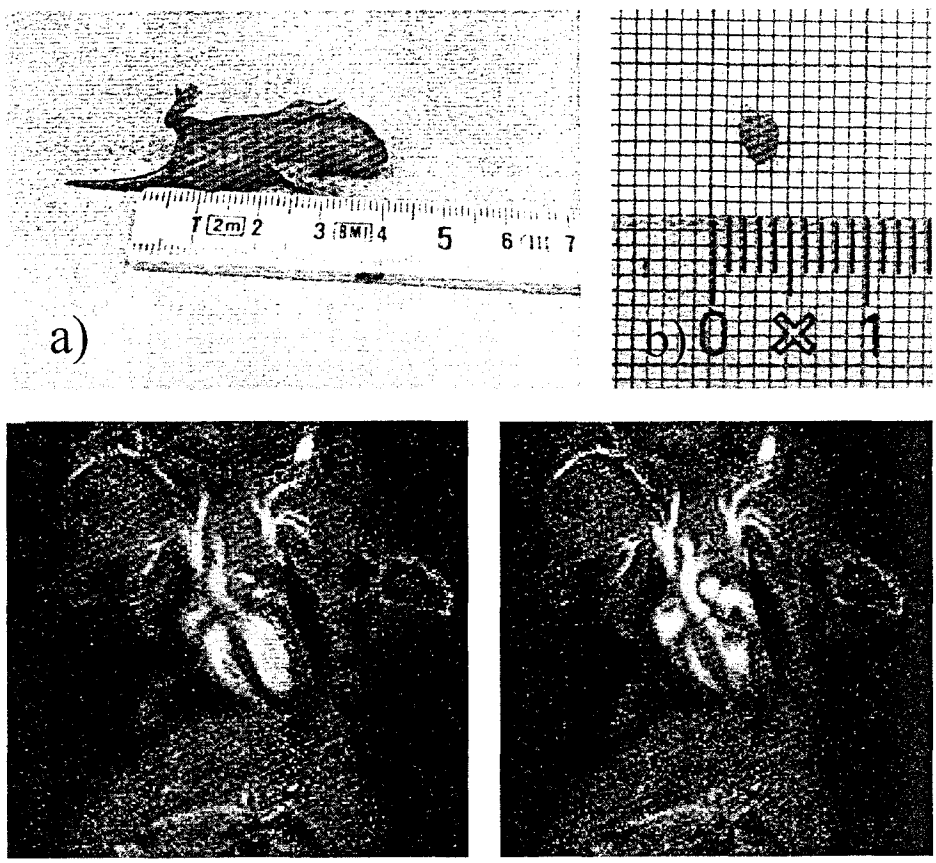

Fig. 1. (a) Newborn mouse 3 days after birth (body weight 1.9 g). (b) Corresponding left ventricle at autopsy (LV wet weight 10 mg). Coronal MR images acquired at (c) diastole and (d) systole giving a detailed view of the newborn cardiovascular system (in plane resolution 80 μm, slice thickeness 500 μm).

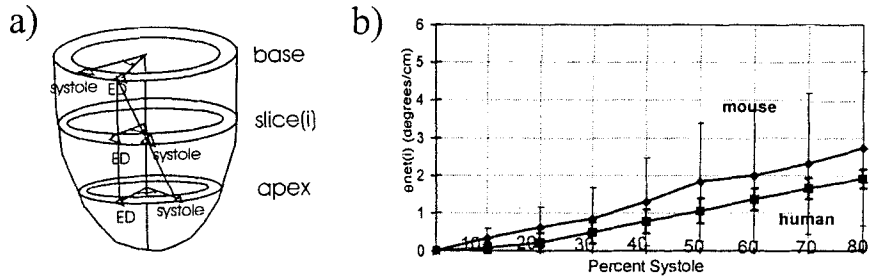

Fig. 2. (a) Definition of ventricular twist angle. Systolic twist angle of any point in myocardium is defined from its position at end diastole (ED) to any point during systole. (b) Net twist normalized to ventricular length (torsion angle in degrees/cm) for mice and humans vs. percent systole. Error bars shown are S.D. There is no statistically significant difference at any time point between human and mouse torsion angles (from [16], with permission of the authors).

4. Assessment of myocardial torsion and strain by MR tagging

The introduction of tagging into the palette of MR imaging methods allowed to gain new information about the different components of LV myocardial motion [15]. After placing 'tag' marks on the myocardium by distinct prepulses, the change in shape and orientation of these tags can be followed over the cardiac cycle. This allows to quantify radial and circumferential motion as well as torsion of the ventricle. This method proved useful in the understanding of LV mechanical response to different pathophysiologic conditions and is an important way to assess regional wall motion abnormality.

Henson et al. [16] compared the direction and degree of murine LV torsion during the cardiac cycle at different ventricular levels with healthy humans. Therefore, tagged cine-MRI with a grid spacing of 1.2 mm and an acquisition window of 9.4 ms was performed in mice at 4.7 T (Fig. 2a). Twist analysis could be achieved over the entire cardiac cycle due to the lack of tag fading over the rapid murine heart rate. There was no difference in magnitude and direction of ventricular torsion between mice and humans when normalized to ventricular length (Fig. 2b). They concluded that assessment of ventricular torsion may be a uniform measure of systolic ejection, which is independent of species and heart size.

Circumferential shortening in the mouse heart has recently been analyzed by Kolandaivelu and Balaban [17] using tagging at 4.7 T with in plane resolution of 120×230 μm and a tag spacing of 650 μm. As two sets of images with switched read and phase direction for orthogonal tags had to be acquired, imaging time amounted to approximately 30 min for two image sets.

5. Vascular morphology

The feasibility of visualization of atherosclerotic lesions by high resolution MRI in genetically engineered mice has recently been demonstrated [18]. Here, a T_1-weighted multislice spin echo sequence with TR/TE 1000/13 ms and voxel size of $97 \times 97 \times 500$ μm^3 was used for imaging of abdominal aorta and common iliac arteries at 9.4 T. In mice with apolioprotein E knockout, MR accurately determined plaque wall area in comparison to histopathology ($r = 0.86$). Furthermore, lesion shape and type were characterized by MRI (according to the most recent American Heart Association (AHA) classification). There was excellent agreement between MR and histopathology for grading of both lesion type ($r = 0.97$) and lesion shape ($r = 0.90$).

We recently presented another example of microscopic MRI by performing for the first time three-dimensional MR coronary angiography in the mouse in vivo [19]. Using an ECG-triggered segmented FLASH sequence at 7 T with acquisition of eight phase steps per cardiac cycle (TR/TE 4.3/1.6 ms), 3D image slabs in the LV short-axis orientation were obtained to preserve inflow contrast. With zero-filling in the third dimension, an isotropic resolution of 100 μm was achieved. The origin of the coronary arteries was clearly visible in all mice studied, and the course of the coronaries could be followed far to the periphery. With postprocessing such as maximal intensity projection and surface reconstruction, an overview of the murine coronary artery system was achievable.

6. Changes of murine LV function during pharmacologic stress

Due to the high temporal resolution of MRI, acute changes of LV volumes and function under β-adrener-

gic stress can be detected and quantified. We performed ECG-triggered cine FLASH MRI in 15 mice at baseline conditions and after intraperitoneal bolus injection of dobutamine (1.5 μg/g body weight) [20]. MR measurements during stress showed a 33% increase in heart rate (554 ± 28 vs. rest 417 ± 16 bpm) and a significant increase of cardiac output (16.7 ± 1.6 vs. rest 13.5 ± 1.0 ml/min) and ejection fraction (82 ± 2 vs. rest $69 \pm 2\%$) ($P < 0.01$ each). Whereas LV end-diastolic volume (39.0 ± 4.4 vs. rest 52.7 ± 5.7 μl) and end-systolic volume (7.7 ± 1.2 vs. rest 21.8 ± 5.8 μl) were significantly smaller under dobutamine compared to baseline, LV stroke volume was unchanged. Midventricular LV wall thickness significantly increased during stress both in diastole (0.99 ± 0.09 vs. rest 0.76 ± 0.04 mm) and systole (1.59 ± 0.06 vs. rest 1.31 ± 0.09 mm) ($P = 0.03$ each).

A different way to assess cardiac changes during dobutamine was followed by Hu et al. by focusing on signal enhancement due to Manganese (Mn^{2+}) infusion [21]. The underlying hypothesis was that Mn^{2+} can enter excitable cells through calcium channels. Once within the cardiomyocyte, it exerts its T_1-shortening properties and acts as an contrast agent, enhancing cardiomyocytes with higher work state and hence higher Ca^{2+} influx. Whether this technique provides reliable information on Ca^{2+} flux in the in vivo setting has to be validated.

7. Chronic cardiac changes in models of heart failure

The experimental heart failure models of aortic banding with pressure induced LV hypertrophy and LAD ligation with myocardial infarction and LV remodeling have successfully been transferred to the small mouse heart. This builds the base to study the structural and functional effects of murine gene targeting on chronically damaged hearts. The time frame of the transition from compensatory states to decompensation is much faster in the mouse compared to other rodents and particularly to humans. In order to address this point, we performed a MR study in mice either 2 weeks after myocardial infarction (MI) or aortic banding (AoB) and compared the changes detected to sham operated reference groups [22]. In mice with MI, there was gross dilatation of the LV with formation of an apical aneurysm. Whereas stroke volume was maintained, ejection fraction was decreased to a mean of 25%. Mean LV infarction size determined by MRI was 53%. MRI in mice with AoB revealed a 59% increase of LV mass and a 53% increase of LV ED wall thickness with preserved EF as an indicator for compensatory LV hypertrophy.

We therefore performed a follow-up study in mice with AoB with serial MR investigation at six time points (ranging from 1 to 27 weeks after operation) [23]. Here, there was a progressive increase of LV mass from 1 week post-op with preserved LV function until 7 weeks after operation. At the following time points, left ventricles started to significantly dilate and ejection fraction continuously decreased to 38% at 27 weeks post-op.

A similar approach was chosen by Berr et al. [24] who performed serial MRI in mice with MI to evaluate progression of heart failure. They found a progressive increase of LV EDV starting 1 week after MI until their last study point (8 weeks post-op). LV EF was significantly decreased 1 day after MI with no significant changes within the following 8 weeks.

8. Application of MRI in transgenic mouse models

High-resolution MRI has been successfully applied to a limited number of transgenic mouse models. This has allowed to gain insight into the consequences of gene-modulation on cardiac geometry and function in vivo. Using a spin-echo multislice MR sequence, Kubota et al. demonstrated the deleterious effects of TNF α on basal cardiac function [25]. Aged 6 months, TNF α overexpressing mice showed significant dilatation and reduction in left ventricular ejection fraction ($51 \pm 10\%$ vs. WT $71 \pm 2\%$; $P < 0.01$).

LV hypertrophic changes quantified by cine MRI were found in other transgenic mouse models, such as in mice with a dysfunctional guanylyl cyclase A gene (GCA $-/-$) [26]. These mice lack the ability to transduce the signals from atrial natruretic peptide and consecutively develop hypertension and LV hypertrophy. ECG-gated MRI at 1.5 T performed with a small surface coil revealed a significant increase in LV mass of the GCA $-/-$ (226 ± 43 mg) compared to wild type (156 ± 14 mg) without changes of LV volumes and ejection fraction.

We have recently been able to investigate the morphologic and functional consequences of chronic β-adrenergic receptor overdrive on the heart [27]. In a transgenic mouse model with heart specific overexpression of the β_1-adrenergic receptor, MRI revealed diastolic dysfunction at early age and severe reduction of LV ventricular global function in the presence of propranolol with ejection fraction of only 21% compared to EF of 67% in wild type littermates (Fig. 3). These findings may be of importance for a fundamental understanding of the role of the β-adrenoceptor system in chronic heart failure.

9. Conclusion

Due to its high temporal and spatial resolution, magnetic resonance imaging meets the requirements for

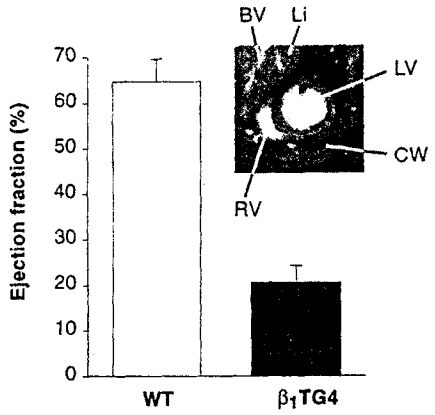

Fig. 3. Left ventricular ejection fraction in 35-week-old mice with overexpression of the β1-receptor (β1TG4) compared with wild type (WT). The insert shows a diastolic MR image acquired in mid-ventricular short-axis orientation (LV left ventricle, RV right ventricle, CW chest wall, Li liver, BV liver veins).

accurate and robust in vivo visualization of the murine cardiovascular system. As an intrinsically three-dimensional imaging technique, it allows for quantification of LV volumes without relying on geometric models. Therefore, MRI is uniquely suited for the investigation of morphologic and functional changes in models of heart failure.

The potential application of MRI in the mouse comprises visualization of cardiovascular anatomy and pathology in newborn and adult mice, detection of LV geometric and functional changes both acutely and chronically, visualization of cardiac microstructures such as cardiac valves and coronary arteries, and characterization and quantification of arteriosclerotic plaques in major murine arteries. Furthermore, MR spectroscopy applied to the mouse heart can give important information on in vivo myocardial metabolism. Thus, we feel confident that high resolution MRI may substantially contribute to the understanding of the basic mechanisms of a variety of cardiovascular diseases.

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