Cardiac MRI of Transgenic Mice

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Introduction

With the advent of molecular techniques to produce targeted gene mutations, it has become possible to generate animal models for studying cardiovascular function and disease in relation to the physiologic and pathophysiologic role of almost any functional or regulatory protein in the intact animal. For technical and economic reasons the mouse is almost always the animal of choice for exploring genetic modifications. However, from the physiologist's point of view, the small size of the animal makes such murine model systems relatively difficult to study using the typical repertoire of physiological analyses used in the past to determine normal and abnormal cardiovascular function. Similarly, the adaptation of adequate imaging methods for the noninvasive characterization of the cardiac phenotype is not trivial. The mouse heart is quite small (mass: ca. 0.1 g) and in rapid motion (ca. 600 beats/min or 10 beats/s). Therefore, extraordinarily high spatial and temporal resolution are fundamental requirements for murine cardiac imaging.

Several imaging techniques are being adapted for mouse studies, including x-ray computed tomography, positron emission tomography, echocardiography, and magnetic resonance imaging (MRI). Echocardiography is presently the most commonly used technology for cardiovascular mouse imaging. Two-dimensionally directed M-mode echocardiography is available in many laboratories and has become a relatively inexpensive, portable imaging technique for rapid phenotype analysis [*1*]. However, echocardiographic measurements are based on geometric assumptions which may no longer be valid when the ventricle undergoes changes in shape [*2*].

MRI has proven to have the high accuracy and reproducibility required for the evaluation of ventricular function and myocardial mass in studies of both animals [*3,4*] and humans [*5,6*]. There are several clear and established strengths of MRI for murine cardiovascular studies, including an intrinsically high tissue contrast, tomographic (geometric, morphologic) data acquisition, and the ability to study and visualize many physiologic parameters. High tissue contrast in the MR images greatly enhances the delineation of cardiac and vascular structures, usually without the need for contrast agents. Unlike echocardiography, the fundamental tomographic nature of MRI eliminates the need for geometric assumptions when calculating ventricular mass and function. MRI is presently the most accurate and reliable method for quantifying left ventricular mass in mice in vivo [*7-10*]. MRI is probably also the leading noninvasive imaging technique for characterizing the structure and function of the irregularly shaped right ventricle in mice, where echocardiographic techniques may require the use of transesophageal probes. The high spatial resolution and tissue contrast of MRI have also been exploited for vascular imaging in mice, and the identification of aortic lesions and coronary arteries has been recently described [*11*].

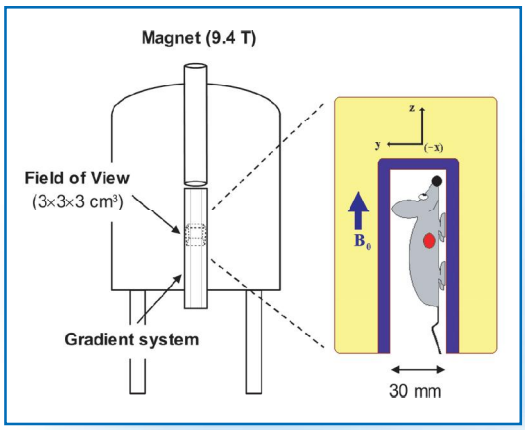

Fig. 1: Positioning of the anesthetized mouse within the vertical magnet.

This report presents methodologies and examples for the physiologic analysis of the cardiovascular phenotype of transgenic mice and outlines possible avenues and approaches for improving the quality and information content of cardiac MRI of mice in the near future.

Table 1. Hemodynamic parameters for mice in horizontal or vertical positions.[a]

	MSP [mmHg]	**EDP** [mmHg]	**HR** [bpm]	**SV** [μl]	E_α [mmHg/ml]
baseline at rest	107.4 ± 0.3	2.4 ± 0.4	533.0 ± 9.2	21.8 ± 0.7	5.2 ± 0.3
vertical (60 min)	104.0 ± 1.2	1.1 ± 0.2	518.2 ± 2.6	20.1 ± 0.4	5.8 ± 0.3
horizontal	106.5 ± 0.7	2.0 ± 0.7	520.9 ± 2.1	22.2 ± 0.1	5.1 ± 0.4

[a] means ± SD, $n = 5$ are given for the following parameters: MSP = mean systolic pressure; EDP = end-diastolic pressure; HR = heart rate; SV = stroke volume; E_a = systemic peripheral resistance; pressure-volume measurements were made with a 1.4 F Millar Micro-Tip catheter; systole and diastole are the cardiac contraction and relaxation phases.

Experimental

Anesthesia

The choice of anesthesia for cardiac imaging of mice is crucial since many anesthetics affect the cardiorespiratory system. Anesthetic regimens are of two types, injection and inhalation. The various compounds used with mice have been extensively reviewed [*12*] and include the injectable agents avertin, pentobarbital, inactin (thiobutabarbital), chloralose-urethane, and ketamine (usually combined with other agents such as acepromazine, xylazine, and/or diazepam) as well as the fluorine-containing inhalation agents isoflurane, methoxyflurane, and halothane. Inhalation anesthesia permits excellent control of the depth and length of anesthesia. There are several advantages for isoflurane, which is commonly used in the clinic. In contrast to halothane, which undergoes metabolic conversion to toxic compounds, isoflurane is relatively nontoxic, preserves sympathetic vasomotor activity, causes minimal cardiac depression, and allows very careful, minute-to-minute control of the anesthetic plane. By way of comparison, the first derivative of left ventricular pressure development (dP/dt) averages ca. 13000 mmHg/s under isoflurane, and mean arterial pressure is typically 90-100 mmHg [*13*]. The anesthesia circuit consists of an isoflurane vaporizer, an oxygen and nitrogen mixing unit, and a scavenging system. Isoflurane anesthesia can be quickly induced by manually restraining the animal and placing its head in a home-build face mask through which a steady stream of 2.5% isoflurane in a 4:1 mixture of nitrogen/oxygen is introduced (note: nitrous oxide, N_2O, is not used). After deep anesthesia has been achieved, usually within 30 s, the isoflurane concentration is reduced to 1.5% and can be varied slightly to adjust the depth of anesthesia.

The vertical position

Typical wide-bore magnet systems used for high-field imaging have a ca. 9-cm vertical bore, which requires that the anesthetized mouse be studied in the unnatural vertical position (**Fig. 1**). Thus, it is important to ensure that a prolonged upright body position does not significantly alter hemodynamic and contractile parameters. Therefore, benchtop tests were performed outside the magnet by first placing a mouse in a horizontal (supine) position on an operation table under

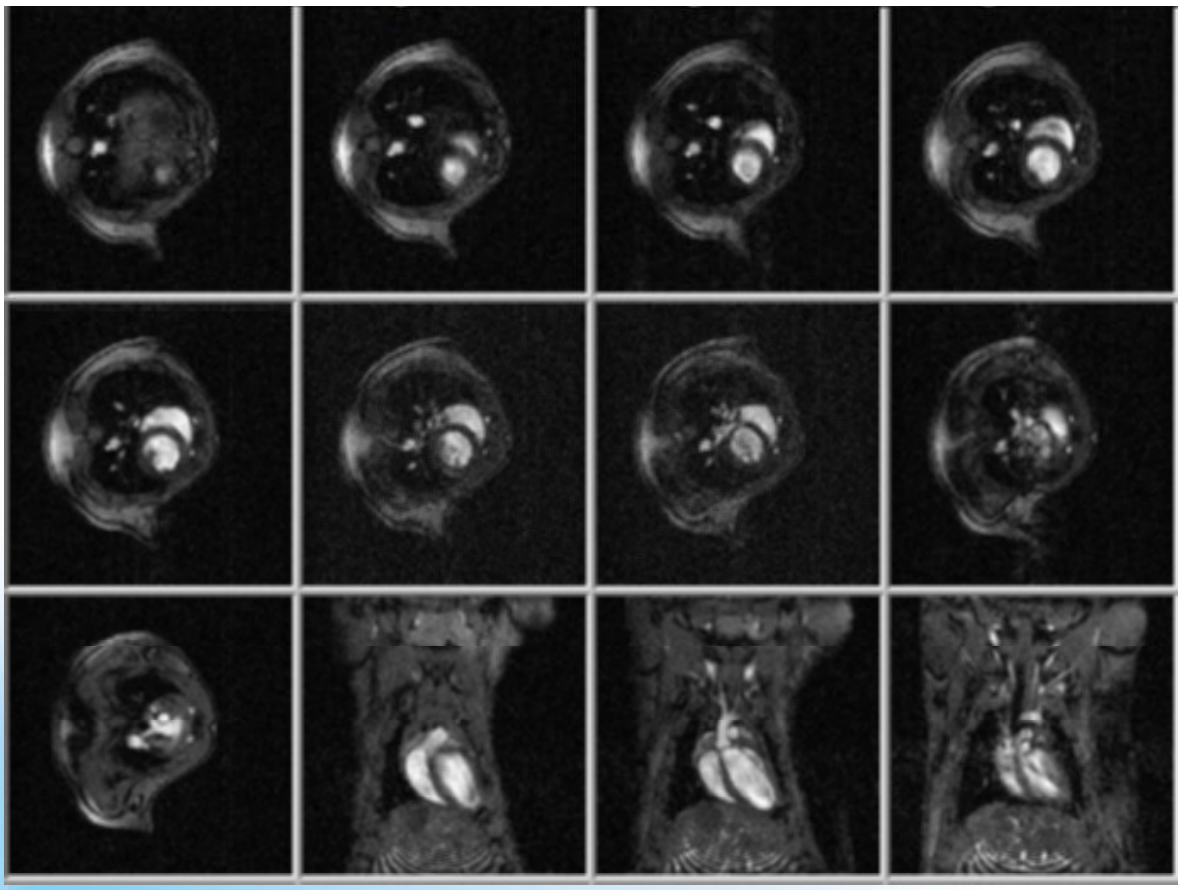

Fig. 2: Contiguous ventricular short axis slices as well as coronal slices (slice thickness: 1 mm) were acquired to cover the entire heart.

isoflurane anesthesia with body temperature held at 37°C via a warming pad. A 1.4 F Millar Micro-Tip catheter was advanced via the right carotid artery into the left ventricle (LV) and continuous registration of LV hemodynamics was performed with the animal supine at rest and after tilting the table by 90° to a vertical position. Immediately after the mouse was oriented vertically, a slight decrease in LV mean systolic and end-systolic pressures was observed, accompanied by a small decrease in heart rate and an increase in the systemic peripheral resistance (Table 1). These parameters remained stable over a follow-up period of 60 min in the vertical position, and the detected alterations were fully reversed after the mouse was returned to the horizontal position. In contrast to the normal orthostatic response in humans, anesthetized mice cannot fully compensate for the change in body position. This is most likely due to an impaired response of the sympathetic nervous system during anesthesia. However, the observed alterations in hemodynamic parameters are rather small, and a prolonged upright body position is easily tolerated by the mice. Hence, high-resolution MR studies for cardiovascular phenotype characterization of transgenic mice can be performed with a vertical-bore magnet under quasi-physiologic conditions.

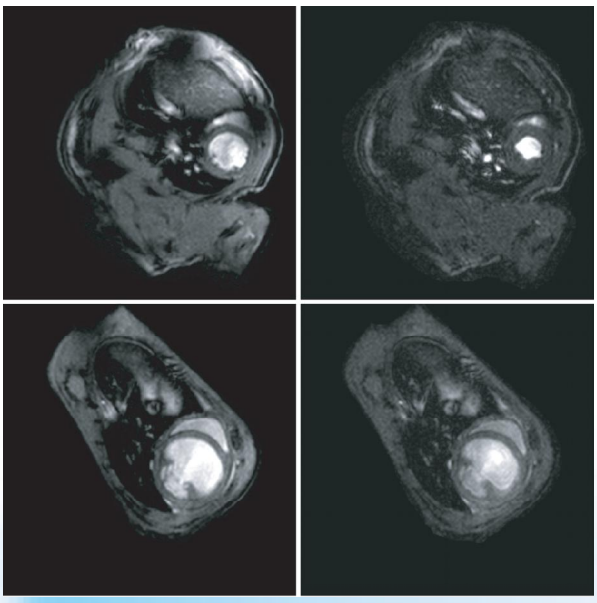

Fig. 3: End-diastolic (left) and end-systolic (right) axial images from a mouse overexpressing the cardiospecific EP3 receptor (bottom) and a normal wild-type mouse (top).

MRI

Under anesthesia vital cardiac function must be continuously assessed by three ECG lead wires, two attached to the front and one to the hind paws of the mouse. The ECG signal is also used to gate the MRI acquisition sequence to begin at defined points within the cardiac cycle. The respiratory cycle must also be monitored during the experiments, and this was accomplished with a home-built balloon sensor connected to a pressure transducer. The electrical ECG and respiratory signals were processed by a Bruker *Physiogard* monitor which generated the required trigger pulses for controlling data acquisition, e.g., when a cardiac QRS complex (*i.e.* the beginning of the cardiac systole) and an exhalation event were simultaneously detected.

MRI was performed at 9.4 T with a Bruker DRX 400 wide-bore NMR Spectrometer equipped with an actively shielded 40-mm gradient set (max. gradient 1 T/m; rise time: 110 μs for 100% gradient amplitude) and a 30-mm birdcage resonator. High-resolution images of the mouse heart were acquired with an ECG- and respiratory-triggered fast gradient-echo cine sequence (GEFI, FLASH) with an excitation flip angle $\alpha < 30°$. To obtain a maximum number of images over the entire cardiac cycle (ca. 100 ms at a heart frequency of 600 bpm), an echo time of about 2 ms was used. The repetition time TR was individually adapted according to the heart rate, and 20 images were acquired per heart cycle. Thus, the temporal resolution was ca. 5 ms, which was sufficient for an accurate determination of the end-diastolic and end-systolic phases within the cardiac cycle. A multislice 2D acquisition mode was used with six to eight contiguous ventricular short-axis slices (slice thickness: 1 mm) covering the entire heart

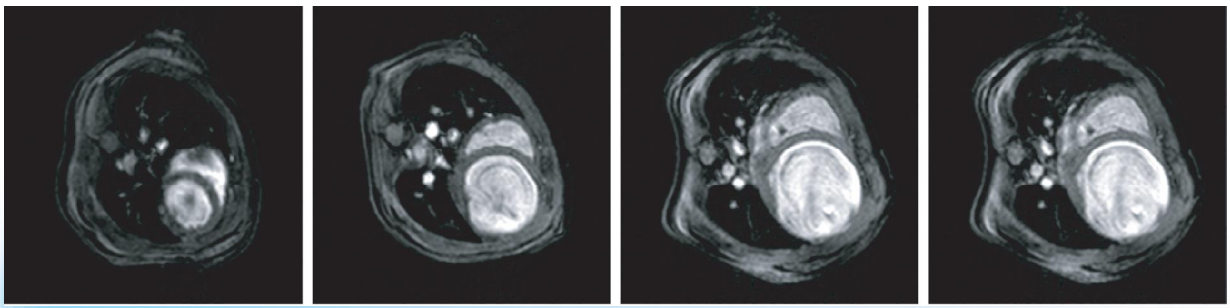

Fig. 4: End-diastolic images (axial slice) from a mouse were obtained before (left) and one, two, and four weeks after (right) induction of myocardial infarction by occlusion of the left anterior descending coronary artery (LAD).

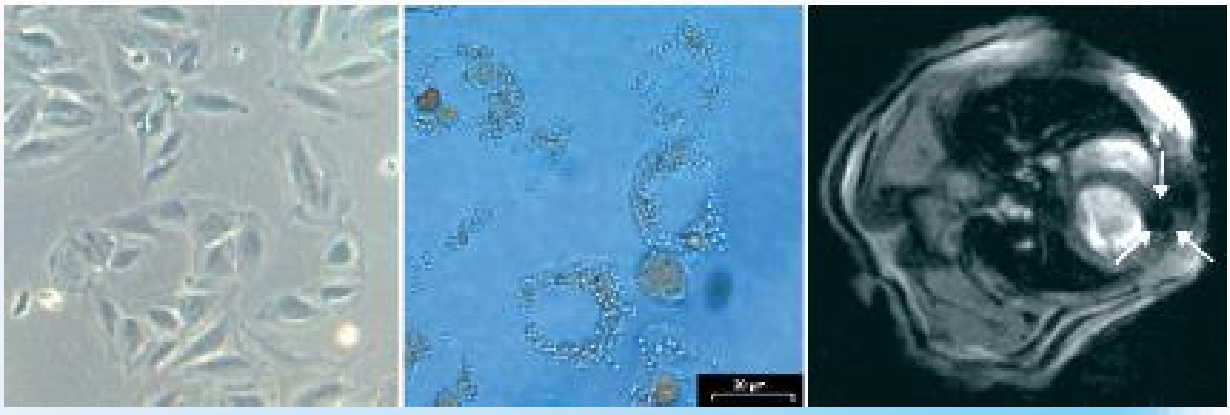

Fig. 5: Light microscopy of unloaded (left) and SPIO-loaded HUVEC (middle). Cells were incubated for 24 h with [Fe] = 2 mmol/l. MRI after intracardiac injection of 10^4 cells in 20 μl buffer shows pronounced hypointensity at the location of injection (white arrows).

(**Fig. 2**) with a field of view of 30 × 30 mm over a 128 × 128 data matrix (zero-filled to 256 × 256, resulting in pixel dimensions of 117 × 117 μm). The total acquisition time per slice for one cine sequence was 1-2 min, depending on the individual respiration rate. Cavity and myocardial volumes in each slice were obtained by multiplication of the measured component area by the slice thickness, which was corrected at the base and apex from additional coronal slices. Since contiguous slices were acquired without gaps, total volumes could be calculated as the sum over all slice volumes, without the need for additional volumetric assumptions.

MRI Analysis of Mouse Models

Our recently completed and ongoing projects include the cardiovascular phenotyping of mice with cardiac-specific overexpression of the inducible nitric oxide synthase (iNOS) or the EP3 prostaglandin receptor. Several knockout mice have been studied with gene defects resulting in a deficiency of a specific protein such as myoglobin, survivin, or taurine transporter, and a double transgenic mouse model has been examined with cardiac-specific overexpression of iNOS and concomitant myoglobin deficiency [*14*]. Within the scope of this report only one of these studies will be described as an example. Furthermore, an experimental approach is presented which allows the noninvasive tracking of magnetically labeled cells and can be applied to elucidate the therapeutic potential of intramyocardial delivery of cells for limiting infarct size and restoring cardiac function in transgenic mouse models of myocardial infarction.

Cardiac-specific overexpression of the EP3 prostaglandin receptor

The prostaglandin EP3 receptor on porcine sarcolemma has been previously identified to mediate acute contractile, electrophysiologic, and anti-ischemic effects [*15*]. To verify the significance of cardiac EP3 receptors in vivo and to investigate whether endogenous prostaglandin synthesis achieves concentrations sufficient for EP3 receptor stimulation, mice were produced with cardiac-selective overexpression of the porcine EP3 receptor (EP3B isoform) under the control of the alpha-MHC promoter (EP3-TG). Receptor density was increased 20-fold over the wild-type (WT) level, and functional G protein coupling was confirmed by radioactive tracer binding studies. MRI of EP3-TG mice at the age of 5-7 weeks revealed severe ventricular dysfunction, as indicated by a threefold increase in end-diastolic left ventricular (LV) volume and a more than tenfold increase in end-systolic volume ($p < 0.01$ for each result, see **Fig. 3**). LV ejection fraction (EF) was decreased to 31 ± 11% in EP3-TG vs. 82 ± 3% in WT ($p < 0.01$). Oral treatment of EP3-TG mice with the nonselective cyclooxygenase inhibitor diclofenac (7 mg/kg body weight per day over 3 weeks) resulted in a partial normalization of myocardial function. These findings suggest that cardiac EP3 receptors exert an inhibitory effect on ventricular function and that endogenously formed prostaglandins such as PGE2 achieve concentrations which are sufficient for long-term EP3 receptor stimulation.

Progression of myocardial infarction

Myocardial infarction is, by nature, an irreversible injury. Regional systolic function and regional metabolism decrease within a few heartbeats after a sudden decrease in myocardial perfusion. Irreversible cardiomyocyte injury begins after 15 to 20 minutes of coronary artery occlusion. New approaches for the regeneration of normal cardiomyocytes require established animal models which allow the continuous monitoring of myocardial function after the insult and during various therapeutic protocols. For this purpose, myocardial infarction was carried out in C57BL/6 mice by ligation of the left anterior descending coronary artery (LAD) proximal to its main bifurcation. As demonstrated impressively in **Fig. 4**, the progression of cardiac dilation can be monitored by successive MRI measurements. In the example shown the EF was reduced from ca. 80% to less than 20% after infarct; however, the mouse easily tolerated the multiple MRI investigations under anesthesia.

Cell transplantation and monitoring

One possible method for repairing acute myocardial infarction is the transplantation or mobilization of cells into the area of infarction, which have the potential to differentiate into contracting cardiomyocytes. For several years it has been possible to label cells with an MRI contrast agent (paramagnetic species); therefore, it should be possible to track and quantify cell transplantation noninvasively with high spatial resolution. Human umbilical vein endothelial cells (HUVEC), which can be induced to transdifferentiate into cardiac muscle cells [*16*], were magnetically labeled with superparamagnetic iron oxide (SPIO) particles by incubation for 24 h with [Fe] = 2 mmol/l. The accumulation of SPIOs in HUVEC could be visualized under light microscopy (**Fig. 5**). MR images recorded after intracardiac administration of 10^4 SPIO-loaded cells (injection volume: 20 μl) show a pronounced hypointensity (darkening) at the location of the injection (Fig. 5). Therefore, in vivo MR detection of magnetically labeled HUVECs is feasible and allows the noninvasive tracking of intramyocardial delivery. The ability to perform serial assessment of infarct size and regional cardiac function by MRI while tracking SPIO-loaded HUVEC should be of great assistance in developing optimal protocols for the delivery of HUVEC for cardiac regeneration.

Conclusions

MRI is generally accepted as a "gold standard" method for evaluating cardiac anatomy and volumes since cine MRI provides excellent contrast between the myocardium and blood. The technology now scales well for subjects ranging in size from man to mouse. MRI is an accurate method for the noninvasive assessment of murine cardiac phenotypes and pathologies and may be useful in following the functional consequences of genetic modifications. It is also feasible to label cells with iron particles and detect their distribution in the body in vivo. Thus, it will be possible to combine MRI-based monitoring of cardiac function with noninvasive tagging methods for the visualization of the fate of cells injected for the treatment of myocardial infarction.

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