Advanced methods for quantification of infarct size in mice using three-dimensional high-field late gadolinium enhancement MRI

Steffen Bohl,1,2,6 Craig A. Lygate,3,6 Hannah Barnes,2 Debra Medway,2 Lee-Anne Stork,2 Jeanette Schulz-Menger,1 Stefan Neubauer,2,3 and Jurgen E. Schneider2,3

1Franz Volhard Klinik, Clinical and Molecular Cardiology, Charité Campus Buch, University Medicine Berlin, HELIOS Klinikum Berlin-Buch, Germany; 2British Heart Foundation Experimental Magnetic Resonance Unit, Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; and 3Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, United Kingdom

Submitted 15 December 2008; accepted in final form 9 February 2009

Bohl S, Lygate CA, Barnes H, Medway D, Stork L, Schulz-Menger J, Neubauer S, Schneider JE. Advanced methods for quantification of infarct size in mice using three-dimensional high-field late gadolinium enhancement MRI. Am J Physiol Heart Circ Physiol 296: H1200–H1208, 2009. First published February 13, 2009; doi:10.1152/ajpheart.01294.2008.—Conventional methods to quantify infarct size after myocardial infarction in mice are not ideal, requiring either tissue destruction for histology or relying on indirect measurements such as wall motion. We therefore implemented a fast, high-resolution method to directly measure infarct size in vivo using three-dimensional (3D) late gadolinium enhancement MRI (3D-LGE). Myocardial T1 relaxation was quantified at 9.4 Tesla in five mice, and reproducibility was tested by repeat imaging after 5 days. In a separate set of healthy and infarcted mice (n = 8 each), continuous T1 measurements were made following intravenous or intraperitoneal injection of a contrast agent (0.5 μmol/g gadolinium-diethylenetriamine pentaacetic acid). The time course of T1 contrast development between viable and nonviable myocardium was thereby determined, with optimal postinjection imaging windows and inversion times identified. Infarct sizes were quantified using 3D-LGE and compared with triphenyltetrazolium chloride histology on day 1 after infarction (n = 8). Baseline myocardial T1 was highly reproducible: the mean value was 952 ± 41 ms. T1 contrast peaked earlier after intravenous injection than with intraperitoneal injection; however, contrast between viable and nonviable myocardium was comparable for both routes (P = 0.31), with adequate contrast remaining for at least 60 min postinjection. Excellent correlation was obtained between infarct sizes derived from 3D-LGE and histology (r = 0.91, P = 0.002), and Bland-Altman analysis indicated good agreement free from systematic bias. We have validated an improved 3D MRI method to noninvasively quantify infarct size in mice with unsurpassed spatial resolution and tissue contrast. This method is particularly suited to studies requiring early quantification of initial infarct size, for example, to measure damage before intervention with stem cells.

murine cardiac magnetic resonance imaging; late enhancement imaging; viability; T1 mapping

* S. Bohl and C. A. Lygate contributed equally to this work.

Address for reprint requests and other correspondence: J. E. Schneider, BHF Experimental MR Unit, Wellcome Trust Centre for Human Genetics, Univ. of Oxford, Roosevelt Dr., Oxford, OX3 7BN, United Kingdom (e-mail: Jurgen.Schneider@cardiov.ox.ac.uk).

The overall accuracy of the LGE technique relies on both exact timing and Gd dosing. However, crucial timing parameters for LGE imaging, such as latency after intravenous or intraperitoneal injection and ensuing inversion times (TI) for IR acquisitions, have not been characterized in the mouse.
Intravenous injection should be the optimal route of delivery but is technically challenging in mice, and multiple injections adversely affect the success rate. Intraperitoneal injection of Gd is an attractive alternative due to the ease of the administration and low failure rate, but absorption may be variable, particularly in situations where mesenteric blood flow could be compromised, such as after myocardial infarction in mice. As a result, inaccurate LGE imaging may be due to unsuitable dosing of the contrast agent, erroneous TI, incorrect timing of imaging after injection, or a combination of the three.

A means to address the uncertainties associated with post-contrast imaging is to measure myocardial magnetic relaxation properties directly. This is an elegant way of characterizing biological tissues in general and could at the same time be used for determining Gd pharmacokinetics quantitatively (9). Widespread application of this technique in the cardiovascular field has been impeded by the need for extensive motion correction in the rapidly beating mouse heart. Typically, T1 relaxation is an order of magnitude longer than a cardiac cycle and in the order of a respiratory cycle in the anesthetized mouse. Reliable T1 values, however, are obtained only after complete relaxation (i.e., after $5 \times T1$). Furthermore, cardiac motion itself restricts data acquisition to end diastole. To overcome these challenges, rapid SNAPSHOT-FLASH IR imaging (SNAP-IR) has been implemented by single laboratories (18, 19, 22). In this technique, each voxel is filled with the T1 of the underlying tissue (6) and, therefore, directly and inversely represents the actual Gd concentration.

In this study we have used SNAP-IR to identify the time course of Gd washin and washout in viable and nonviable myocardium. Simultaneously, TIs suited to suppress the signal of healthy myocardium in IR acquisitions could be derived from these measurements and were used to infer the maximum achievable T1 contrast between these two compartments. This information was used to implement a high-resolution 3D IR-LGE imaging sequence for the measurement of infarct size at early time points after myocardial infarction and to validate it against histology.

MATERIALS AND METHODS

Animal Preparation

Male C57BL/6 mice were obtained from a commercial breeder (Harlan) at 20–25 g body wt and kept under controlled conditions for temperature, humidity, and light. All procedures complied with the Home Office Guidance on the Operation of Animals (Scientific Procedures) Act, 1986 (HMSO) and with institutional guidelines and were approved by the Animal Ethics Review Committee.

In vivo MRI set-up. Anesthesia was induced in an induction chamber using 4% isoflurane in 100% $O_2$. Animals were then intubated and ventilated at 150 strokes/min and 250 $\mu l$ stroke volume of 2% isoflurane in 100% $O_2$ (flow rate, 0.5 l/min) using a rodent ventilator (Hugo-Sachs MiniVent; HARVARD Apparatus, Holliston, MA). The surgical procedure to induce permanent occlusion of the left coronary artery (LCA) was performed according to the method described by Lygate et al. (11) and lasted 12–15 min from skin incision to chest closure.

Histological infarct assessment. Mice were euthanized by cervical dislocation. Hearts were then rapidly excised, trimmed of extracardiac tissue, mounted on a Langendorff apparatus, and perfused with saline to wash out remaining blood. Hearts were then stored at −20°C for 15 min and then sliced in 6 to 7 short-axis sections by hand on a cold-plate using a razorblade. After hearts were thawed, they were immersed in 1% triphenyltetrazolium chloride (TTC) at 37°C for 25 min under constant agitation. Afterward, slices were incubated in 10% neutral-buffered Formalin for 90 min to enhance contrast between viable and nonviable tissue. Each slice was digitally photographed on both sides, and manual planimetry of viable (brick red) and nonviable (pale) left ventricular (LV) areas within each slice was performed offline (ImageJ; National Institutes of Health). After the right ventricle was clipped, the results were corrected for slice weight, and infarct size was expressed as a percentage of total LV weight (%LV).

Magnetic Resonance Imaging

Set-up. All experiments were carried out on a horizontal bore 9.4 Tesla MR system (Varian) with a shielded gradient insert (1,000 mT/m; rise time, 130 μs; Magnevix Scientific, Oxn, OK). Quadrature birdcage coils (Rapid Biomedical, Würzburg, Germany) were used to transmit/ receive the magnetic resonance signals (phantom experiments: 28 and 39 mm inner diameter; animal experiments: 33 mm inner diameter). After localizer scans (segmented double-gated FLASH imaging) were run to ensure correct positioning, tuning, and matching the probe, slice-selective shimming and flip angle calibration were performed manually before each experiment.

The detailed parameters of all MRI pulse-sequences as well as the postprocessing and analysis of the image data are described in the Appendix.

In vivo study protocol. A total of 29 mice were used for this study. As a first step, the stability of myocardial T1 under isoflurane anesthesia was determined: five healthy mice underwent longitudinal myocardial T1 mapping over a period of 90 min (temporal resolution for 3 slices 5–8 min depending on heart and respiratory rate). To assess reproducibility of the technique, these measurements were repeated after 5 days in three out of the five mice. Two groups of healthy mice ($n = 4$ each) were then used to investigate the pharmacokinetics after intravenous or intraperitoneal injection of 0.5 $\mu l/m$ body wt Gd. This experiment was repeated in two groups of infarcted mice ($5 \times$ intravenous and $3 \times$ intraperitoneal injection) on day 1 after infarction to determine characteristics of either injection method and to optimize timing parameters (TI and latency after Gd injection) for the LGE imaging protocol. Finally, eight infarcted animals underwent cine-MRI of LV function/volumes and 3D-LGE imaging with full coverage of the LV on day 1 after infarction ($5 \times$ intravenous and $3 \times$ intraperitoneal injection). Infarct size was determined histologically and compared with magnetic resonance findings.

Cine imaging. Contiguous slices (7–10) were acquired in short-axis orientation covering the entire LV, using cardiac and respiratory lower abdominal quadrant. For the intravenous injections, the animals were prewarmed at 36°C for 10 min to maximally dilate the tail vein. After the induction of anesthesia, a 26-gauge indwelling catheter was placed in a tail vein and secured. The animal cradle was briefly removed from the magnet for the injections, which were followed by a warm saline flush of 50 $\mu l$.

Infarct model. Mice received 0.015 mg buprenorphine subcutaneously immediately before surgery. Anesthesia was induced in an induction chamber using 4% isoflurane in 100% $O_2$. Animals were then intubated and ventilated at 150 strokes/min and 250 $\mu l$ stroke volume of 2% isoflurane in 100% $O_2$ (flow rate, 0.5 l/min) using a rodent ventilator (Hugo-Sachs MiniVent; HARVARD Apparatus, Holliston, MA). The surgical procedure to induce permanent occlusion of the left coronary artery (LCA) was performed according to the method described by Lygate et al. (11) and lasted 12–15 min from skin incision to chest closure.
SNAP-IR pulse-sequence was validated against nonsegmented standard IR gradient-echo T1 measurements in phantom experiments and then used for in vivo T1 measurements. The TIs were recorded on a PowerLab data acquisition system (AD Instruments), and mean TIs were calculated. After detection of a respiratory event, 16–24 image segments (8 lines/cardiac cycle) were acquired on the inversion curve (i.e., covering 2 to 3 s of the recovery process). Images on the IR curve showing motion artifacts were excluded before fitting. T1 was calculated according to the method outlined in Ref. 6, and T1 maps were computed from relaxation time measurements derived from a train of images acquired at different time points after the initial inversion of longitudinal magnetization. In an IR acquisition, the signal of a given tissue will be zero when the TI is 0.69/\times T1_{tissue}. This information was used to determine suitable TIs to suppress signal from remote myocardium and to thereby increase contrast of infarcted regions at varying latencies after Gd injection. For the in vivo experiments, slices were acquired in standard double-oblique short-axis orientation using a field of view (FOV) of 25.6 × 25.6 mm; a minimum of three slices were acquired in each experiment (basal, midventricular, and apical). Acquisition time per slice was ~2 min depending on heart/respiratory rate.

3D IR imaging. A segmented, double-gated 3D IR gradient-echo pulse sequence was used. Extending the 3D slab from the level of the mitral valve to just outside the apex ensured full coverage of the LV (typically 9–12 mm). Acquisitions were started 12 and 25 min after intravenous and intraperitoneal Gd injections, respectively. Typically, the acquisition time for a 3D data set was 15–20 min depending on heart and respiratory rate. Care was taken to ensure stable heart and respiration rates by gradually adjusting the anesthetic dose as necessary.

Statistics

Normal distribution of the infarct size data was confirmed using Kolmogorov-Smirnov analysis. For comparison within groups, the paired t-test was used and for comparisons of independent groups the unpaired t-test was used (Excel, Office 2004 for Mac; Microsoft). The correlation between infarct quantification methods was determined (Pearson product-moment correlation coefficient, r) using Statistical Package for the Social Sciences 16.0 for Mac (SPSS). Values are presented as means with SD. A P value of <0.05 was considered statistically significant.

RESULTS

SNAP-IR was optimized in phantom experiments, and T1 measurements deviated from the standard method <5% for T1 values ranging from 100 to 3,000 ms. A pooled analysis of all myocardial T1 measurements performed in normal animals (14 studies without contrast agent in 6 mice) found an average T1 of 952 ± 41 ms at 9.4 Tesla. Reproducibility of these measurements was demonstrated in three mice that underwent MRI with identical settings at an interval of 5 days (difference of mean myocardial T1 first vs. second session was <2%; P = 0.43). T1 stability under isoflurane anesthesia was tested in five mice that underwent continuous T1 mapping over 90 min. A slight but significant decrease of myocardial T1 was observed when the first and last 20 min of each imaging session were compared (929 ± 31 ms vs. 895 ± 73 ms; P = 0.001). Figure 1 shows representative myocardial T1 maps of a normal and infarcted heart.

Gd Kinetics/T1 Dynamics in Healthy Mice

Figure 2 shows the time dependence of T1 in normal myocardium pre- and post-Gd injection. After intraperitoneal injection, myocardial T1 gradually decreased, reaching a minimum after 20–30 min. Within the 60 min observation period myocardial T1 values remained low but returned to normal by 24 h (P = 0.79 for baseline T1 vs. 24 h). After intravenous injection, the minimum myocardial T1 value was obtained within 1 min of administration and then gradually increased but did not return to precontrast values over the period of 60 min. Again, myocardial T1 returned to normal by 24 h after injection (P = 0.62). Lower myocardial T1 values were obtained from intravenous compared with intraperitoneal injection.

Gd Kinetics/T1 Dynamics in Infarcted Mice

In infarced mice myocardial T1 was measured in remote as well as in infarcted regions. For both injection methods, the pharmacokinetics in remote myocardial regions was comparable with findings in healthy mice. The injection success rate was 100% for both methods. The maximum T1 contrast was reached earlier and was higher after intravenous injection (Fig. 3, top) compared with intraperitoneal injection (Fig. 3, bottom). For example, T1 contrast greater than twofold was reached 6 min after intravenous injection versus 30 min after intraperitoneal injection. Both injection methods, however, retained sufficiently high contrast for clear delineation of infarcted myocardium for the duration of the 60-min observation period. Precontrast T1 was significantly higher in infarcted myocardium compared with unaffected regions (1,410 ± 137 ms vs. 1,050 ± 80 ms; P = 0.00001).

Cardiac Functional Parameters in Infarcted Mice

LV mass (LVM) was 84.9 ± 17.5 mg at autopsy, and mean body weight was 21.3 ± 3.3 g. Cine-MRI was used to determine cardiac function and volumes in infarcted mice. One animal had to be excluded due to technical problems, leaving n = 7 for analysis of cine data: LV end-diastolic volume (EDV) was 63 ± 11.9 μL and LV ejection fraction was 35.1 ± 5.3%. LV functional parameters were not different between the intraperitoneal and intravenous groups of infarcted mice who underwent LGE imaging. Infarct sizes by 3D-LGE were not different between the groups (intraperitoneal group, 39 ± 5%LV vs. intravenous group, 35 ± 10%LV; P = 0.5).

Infarct Size: MRI Versus Histology

Mean heart rate during 3D-LGE imaging was 395 ± 21 beats/min, and average repetition time (TR) per segment (seg) was 511 ± 72 ms. 3D-LGE clearly and easily differentiated between infarcted and unaffected areas of myocardium. Contrast was sufficient to allow infarct delineation using a semi-automated thresholding tool (Fig. 4). Figure 5 shows an example for 3D-LGE and corresponding histology. Infarcts were generally transmural. Average infarct size from histology was 35 ± 9%LV (range, 22–45%LV), and there was good correlation with 3D-LGE (36 ± 9%LV; r = 0.91; y = 0.948x + 0.0088; P = 0.002) and cine-MRI (35 ± 9%LV; r = 0.898; y = 1.0346x + 0.0069; P = 0.009). Infarct sizes derived from 3D-LGE and cine-MRI were not significantly different (P = 0.85). With the use of Bland-Altman analysis, the agreement...
was slightly better between histology and 3D-LGE; the mean of differences between 3D-LGE and histology was \( \pm 3.7\% \text{LV} \) compared with the mean of differences between cine-MRI and the histology of \( \pm 4.4\% \text{LV} \) (Fig. 6). Contrast resolution (CR) in LGE images (a normalized indicator of image contrast between infarct and remote myocardium) did not differ between the intraperitoneal and intravenous groups (CR = 0.608 ± 0.083 vs. 0.688 ± 0.078; \( P = 0.31 \)).

**DISCUSSION**

This study utilized advanced methodology to optimize noninvasive assessment of myocardial infarction in mice. For the first time, parametric T1 maps were used to determine the T1 contrast over time after Gd administration between remote and infarcted myocardium and to predict suitable timing parameters for imaging of myocardial viability in mice. This fully

---

**Fig. 1.** T1 maps pre- and post-gadolinium (Gd) injection. *Left:* healthy myocardium [precontrast T1 (T1pre), 967 ± 58 ms vs. postcontrast T1 (T1post), 588 ± 65 ms] acquired 30 min after intraperitoneal (ip) injection of 0.5 \( \mu \text{mol Gd/g. Right:} \) infarcted myocardium. T1pre was 1,440 ± 154 ms in the infarcted anterior region and 1,070 ± 164 ms in the septal myocardium (T1 contrast, 0.74). T1post was 172 ± 35 ms in the infarcted region and 541 ± 63 ms in the septal myocardium (T1 contrast, 3.1). Data were acquired on day 1 after permanent coronary artery ligation and 12 min after intravenous (iv) injection of 0.5 \( \mu \text{mol Gd/g. T1 was measured in regions of interest containing typically 40–80 voxels.} \)

**Fig. 2.** Pharmacokinetics over 60 min after intraperitoneal (black columns) and intravenous (white columns) injection of 0.5 \( \mu \text{mol/g Gd-diethylenetriamine pentaacetic acid (DTPA) in healthy mice (n = 4/group). Peak Gd concentration is reached earlier after intravenous than after intraperitoneal Gd administration. Acquisition time for 3 slices/animal was 6–8 min, depending on variable heart and respiration rates. The average start time of consecutive scans is indicated on the x-axis. }\)
A quantitative approach found typical Gd pharmacokinetics for both the intraperitoneal and intravenous injection. Thereby, optimal contrast between viable and nonviable myocardium was ensured for LGE imaging. The proposed 3D-LGE approach enabled much smaller voxel sizes, e.g., 12- to 54-fold reductions compared with those achieved with high-resolution 2D multislice acquisitions (3, 14, 23), making rapid, accurate infarct measurements with high spatial resolution and remarkable tissue contrast possible.

Longitudinal (T1) and transverse (T2) relaxation times are characteristic for a given tissue and magnetic field strength. The mean T1 of healthy myocardium in this study using slice
selective spin-inversion was, as predicted, lower than in previous experiments on an 11.7-Tesla magnetic resonance system [1,100 ± 270 ms (18)] and in the range of human myocardium at 1.5 Tesla (12). In a study performed on rats, Kober et al. (10) report myocardial T1 values for global (1,310 ± 60 ms) and slice-selective (1,100 ± 60 ms) inversion pulses at 4.7 Tesla (10). Although the latter are, despite lower field strength, somewhat closer to our values, the remaining difference is likely due to the distinct methodology and the higher myocardial blood flow in mice compared with rats.

Generally, relaxation properties may deviate from normal in a disease state such as acute myocardial infarction. This was recently demonstrated by Messroghli et al. (13) in a study on patients with acute and chronic infarcts. Confirming this report in mice at a higher field strength, we found a ~35% deceleration of local T1 on day 1 after infarct surgery. This finding is...
most likely attributable to infarct-related oedema, where the abundant water molecules can move more freely, thereby prolonging T1 (and T2) relaxation. These alterations can subsequently be exploited for imaging purposes in weighted acquisitions and, if necessary, can be amplified by use of a contrast agent (as demonstrated in Fig. 1 before and after Gd injection). Certain Gd compounds accumulate in areas of expanded extracellular space, such as necrotic myocardium, thereby drastically reducing local T1, whereas the contrast agent is more rapidly washed out of neighboring but unaffected myocardium. As a result, there are concurrent washin and washout processes of the contrast agent, making it difficult to determine the window of opportunity that provides ideal contrast between the two compartments. In addition, true infarct size may be overestimated when images are acquired too soon after injection; however, there are conflicting results depending on the species/models investigated (14–16, 21). The proposed approach of continuous myocardial T1 measurements in a set of mice with nonreperfused infarctions readily identifies a suitable time window. Along the way, information on appropriate inversion times (TI) to suppress the signal originating from various tissues is collected. The use of a magnetic resonance signal only from the tissue of interest in an IR-prepared acquisition, such as the IR-LGE method described here, strongly enhances the image contrast between, e.g., infarct (= tissue of interest) and neighboring healthy myocardium (= to be suppressed) over and above the actual T1 differences and, thus, facilitates image interpretation. As a rule of thumb, a TI = 2/3 of the present T1 in a given tissue will suppress its magnetic resonance signal. Suitable TIs for different latencies after injection can be read from a T1/time chart (see Fig. 3).

Postmortem histological analysis is considered to be the gold standard for measuring infarct sizes in rodents. However, there are a number of disadvantages to the technique, making a reliable noninvasive alternative highly desirable. First, histological methods leave no residual tissue for further analysis aimed at providing biochemical/mechanistic insights. Second, visual interpretation and planimetry of heart sections may be subjective in cases with poor viable/nonviable contrast due to hemoglobin residues within the necrotic regions (17) or with TTC-induced geometric distortion of the sample. These issues are particularly significant when working on the small-sized mouse heart. Finally, animals must be euthanized to measure injury, meaning longitudinal studies require separate groups of mice for each time point. Noninvasive MRI circumvents these downsides and would enable valuable additional scientific readouts from each mouse, thereby reducing group sizes.

Characteristic pharmacokinetics were determined for intravenous and intraperitoneal Gd injections. After intravenous injection, T1 contrast peaked earlier and was slightly higher than after intraperitoneal injection. However, the actual CR (infarcted vs. healthy myocardium) in LGE images was comparable after tailoring of latency and TI. The anticipated absorption-variability following intraperitoneal injections was lower than expected, making intraperitoneal injection the likely method of choice in a routine, high-throughput setting due to the ease of administration. Both methods, however, retain a sufficient T1 contrast for a broad time frame, and T1 contrast/time curves may be used to choose variable TI for nullying of remote myocardium and thereby creating high contrast between viable and nonviable myocardium.

We have previously shown that infarct measurements based on wall motion analysis correlate tightly with true infarct sizes in a mouse model of chronic infarction with significant wall thinning (5). The presented study shows that reliable results can be achieved on day 1 after infarction when LV thinning is negligible, while complete akinesia is present due to fully transmural necrosis after permanent coronary occlusion. However, whereas LGE-MRI actually characterizes cardiac tissue, cine-MRI infers myocardial damage from contractile function, which may fluctuate over time (especially after reperfused, nontransmural infarctions) and is difficult to assess in the infarct borders. A comprehensive study protocol should address both issues.

Our study has a number of limitations. First, a substantial influence of anesthetic depth on myocardial T1 has been reported, potentially due to the vasodilatory side effects of isoflurane (4). This could be a potential source of error in longitudinal studies. We investigated anesthesia-related effects on T1 in mice undergoing 90 min of continuous T1 mapping. The slight T1 decrease (~4%) at the end of the observation period appears negligible for the interpretation of pharmacokinetics data where T1 was substantially and unambiguously lowered (~50% and more). Nevertheless, the effects of anesthetics on T1 relaxation are of great importance for perfusion measurements. The inflow of fresh, noninverted blood pool protons from outside the detection slice causes a relative shortening of T1 within the detection slice. Therefore, IR T1 mapping can be used to quantify perfusion [spin labeling (1)], with T1 changes in the order of 10% having been related to a doubling of blood flow (10).

It is also worth pointing out that gas delivery in a high-field magnetic resonance environment is not ideal with delivery lines of ~3 meters length from the vaporizer (located outside the magnet room) to the center of the magnet making the actual alveolar concentration difficult to control. Varying experimental set-ups (anatomic seal of the nose cone, temperature, carrier gas, flow etc.) in different laboratories may require different concentrations to achieve comparable anesthetic depths. However, we have demonstrated T1 reproducibility in a paired analysis of only three animals.

Second, for future studies knowledge of the effective biological half-life of Gd in the mouse would be desirable. We did not obtain this data since this would necessitate excessively long imaging times (>2 h), which are poorly tolerated in mice with large infaracts. However, we were able to demonstrate that T1 in viable and nonviable myocardium is normalized at 24 h after administration. Third, whereas contrast between viable and nonviable myocardium was generally unambiguous, the blood infarct boundary was less well defined due to comparable signal intensities, particularly in apical slices where partial volume effects occur. Usually, however, a thin subendocardial rim of viable tissue was found both on MRI and histology, which facilitated border discrimination. Additionally, end-diastolic frames of corresponding cine slices may be used to correctly identify the endocardial edge.

Finally, it is important to acknowledge that although relaxation time determination may be highly reproducible for a given tissue and field strength, the methods employed will
inevitably affect the absolute values. This means that these measurements may not be accurate, despite being precise.

In summary, this is the first study to validate 3D IR-LGE imaging of myocardial infarction in mice. This method offers superior contrast and resolution compared with previous 2D approaches, making even nontransmural infarctions readily assessable. A technique to measure myocardial T1 in vivo was validated and then used to comprehensively investigate myocardial gadolinium kinetics in healthy and infarcted mice after intra-peritoneal and intravenous injection. The results enabled the optimization of infarct imaging with individually tailored timing parameters for both delivery routes and should be useful for other laboratories planning serial, high-throughput studies.

We conclude that 3D-LGE is suitable for replacing histological assessment of infarct size in the mouse heart. This will be particularly advantageous when infarct measurements are required before intervention, e.g., in the emerging field of regenerative therapies (stem cell/cell cycle modification studies), when preservation of myocardial tissue is needed for further biochemical analysis or when longitudinal imaging in the same animal is desirable.

APPENDIX

**Pulse-sequence Parameters**

1) Cine-MRI: matrix, 256 × 256; FOV, 25.6 × 25.6 mm; echo time (TE)/TR, 1.8/4.7 ms; flip angle (FA), 15°; slice thickness (silt), 1 mm; number of averages (NA) = 2.

2) SNAPSHOT-FLASH T1-measurements: matrix, 128 × 128; FOV, 25.6 × 25.6 mm for in vivo experiments; segmented k-space acquisition (PE/seg = 8); TE/TR = 1.33/3 ms; TR/seg = depending on cardiac cycle length (typically 100–200 ms); FA = 8°, silt, 1 mm; NA = 1, slice-selective hyperbolic secant inversion pulse.

3) 3D IR imaging: matrix, 256 × 192 × 32; FOV, 25.6 × 25.6 × 16 mm; PE/seg = 8; TE/TR = 1.8/3.4 ms; TR/seg = T1 = 1 R-R cycle (typically 400–700 ms); NA = 1; FA = 10°; TI individually adjusted in multiples of cardiac cycle lengths (typically 250–500 ms) to minimize signal contribution from remote myocardium, hyperbolic secant inversion pulse.

**Postprocessing of Magnetic Resonance Data and Image Analysis**

Image reconstruction and analysis was performed using custom-made software programmed in IDL (ITT Visual Information Solutions, Boulder, CO) unless otherwise stated.

**Cine imaging.** Image data were exported into TIFF format and loaded into Amira 4.1.2 (Visage Imaging Europe, Berlin, Germany). End-diastolic and end-systolic frames were selected according to the maximal and minimal ventricular volume, and endo- and epicardial borders were traced manually. Myocardial and cavity cross-sectional areas were multiplied by slice thickness to derive EDV and endo-systolic volume. Ejection fraction was calculated as the ratio of stroke volume to EDV. Whereas LVM was calculated as the ratio of stroke volume to EDV, whereas LVM was calculated as the ratio of stroke volume to EDV. The resulting volume was multiplied by the specific density of myocardial tissue to provide an estimate of LVM. Hyperenhancing lesions were also quantified using the threshold tool. Their mass was calculated, and infarct size was expressed as percentage of total LVM (%LV), derived from the same data sets. CR was calculated from ROIs as a normalized indicator of image contrast between healthy and infarcted myocardium for intravenous and intraperitoneal delivery of the contrast agent (CR = SignalRemote/SignalInfarct).

**Grants**

This work was supported by grants from the German Cardiac Society (to S. Bohl), Else Kröner-Fresenius-Stiftung, Bad Homburg v.d.H, Germany (to S. Bohl and J. Schulz-Menger) and from the British Heart Foundation (BS/06/ 001; to K. E. Schneider). The British Heart Foundation Experimental Magnetic Resonance Unit is funded by the British Heart Foundation.

**References**


