Simultaneous Evaluation of Infarct Size and Cardiac Function in Intact Mice by Contrast-Enhanced Cardiac Magnetic Resonance Imaging Reveals Contractile Dysfunction in Noninfarcted Regions Early After Myocardial Infarction

Zequan Yang, MD, PhD; Stuart S. Berr, PhD; Wesley D. Gilson, MS; Marie-Claire Toufektsian, PhD; Brent A. French, PhD

Background—The objective of this study was to noninvasively determine the effects of reperfused myocardial infarction (MI) on regional and global left-ventricular (LV) function 24 hours after MI in intact mice with contrast-enhanced cardiac MRI and a single, gradient-echo pulse sequence.

Methods and Results—Twenty-three mice received baseline MRI scans followed by either 60 minutes of coronary occlusion (MI group, n=11) or thoracotomy without occlusion (sham group, n=8). Gadolinium-DTPA–enhanced magnetic resonance (MR) images were acquired 24 hours after surgery. Hearts were then excised for conventional infarct size determination via 2,3,5-triphenyl tetrazolium chloride (TTC) staining. In addition to infarct size, analysis of the MR images yielded left ventricular (LV) mass, LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV), LV ejection fraction (LVEF), cardiac output, and percent LV wall thickening (%WTh). Twenty-four hours after surgery, infarct size was 28.1±1.8% of LV mass by MRI and 27.5±1.7% by TTC (P=NS). Bland-Altman analysis revealed close agreement between the results obtained by the 2 methods. MI had little effect on LVEDV but caused a 98% increase in LVESV (from 11.3 to 22.4 μL, P<0.05), which resulted in a significant reduction in LVEF (from 70% to 37%, P<0.05). Compared with LV regional function at baseline, %WTh 24 hours after MI was significantly depressed, not only in infarcted myocardium but also in regions remote from the infarct zone. In contrast, sham-operated mice showed a small but significant increase in %WTh 24 hours after surgery (P<0.05).

Conclusions—MRI can accurately assess both infarct size and cardiac function in intact mice early after large, reperfused MI, revealing the existence of contractile dysfunction in noninfarcted regions of the heart. (Circulation. 2004;109:1161-1167.)

Key Words: myocardial infarction ■ magnetic resonance imaging ■ cardiac output ■ cardiac volume ■ myocardial contraction

The degree and extent of myocardial injury after an acute ischemic event are strong predictors of patient outcome, and interventions that reduce injury significantly improve prognosis after myocardial infarction (MI).1-3 Methods for distinguishing between reversibly damaged (stunned or hibernating) and irreversibly damaged (infarcted) myocardium after MI would thus aid greatly in patient management.1-3 The noninvasive technique of contrast-enhanced MRI can accurately determine the size of MI compared with histological standards4,5 and thus shows great promise for the noninvasive evaluation of MI in patients.6,7 The primary objective of the present study was to determine whether there exists a similar relationship between area of contrast enhancement and infarcted myocardium in mice after MI. In addition to determining infarct size in vivo, we describe methods by which one can simultaneously measure cardiac mass and left ventricular (LV) volumes and evaluate global and regional cardiac function in mice, all with a single MRI pulse sequence. The application of these MRI techniques in mice is significant because it reveals that contractile dysfunction affects the entire LV early after large MI in mice, just as it does in other species, including humans.8 Just as transgenic and gene-targeted mice have greatly improved our understanding of angiogenesis, arterial stenosis, atherosclerosis, and hemostasis,9 it is anticipated that genetically manipulated mice will yield similar insights into the pathophysiology of LV dysfunction after MI.

Methods

Animals and Experimental Protocol

This study conformed to the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 85-23, 1985) and was

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conducted under protocols approved by the Institutional Animal Care and Use Committee. Twenty-three male 129/Sv mice (12 to 16 weeks old) bred in-house were used. Baseline magnetic resonance (MR) images were acquired 3 to 5 days before surgery to ensure that all cardiovascular parameters were fully normalized before surgery. Animals underwent either 60 minutes of coronary occlusion (MI group, n=15) or 70 minutes of thoracotomy without coronary occlusion (sham group, n=8). Mice were imaged again 24 hours after surgery. Immediately after the 24-hour MRI follow-up, the mice were killed to enable myocardial infarct size determination with 2,3,5-triphenyl tetrazolium chloride (TTC). Of the 23 mice scheduled to undergo open-chest surgery and MR imaging, only 1 (in the sham group) died prematurely, and this was due to complications of anesthesia during MRI.

Ischemia/Reperfusion and Histological Determination of Infarct Size
A standard myocardial ischemia/reperfusion protocol was used, as described previously.10 Mice were anesthetized with sodium pentobarbital (100 mg/kg IP) and intubated. Artificial respiration was maintained with a rodent ventilator. A parasternal incision was made by cutting the left third and fourth ribs and intercostal muscles with a cautery pen. A range of infarct sizes was obtained by passing a 7-0 silk suture beneath the left anterior descending artery at points 1 to 2 mm inferior to the left auricle, then tightening it over a length of PE-20 tubing (which was removed 60 minutes later to achieve reperfusion). On reperfusion, the chest was closed in layers. The endotracheal tube was removed once spontaneous breathing resumed.

After MR imaging, the mice were killed, and excised hearts were cannulated through the ascending aorta with a 23-gauge needle for sequential perfusion with 2 to 3 mL 37°C 0.9% sodium chloride and with 3 to 4 mL 37°C 1.0% TTC in phosphate buffer (pH 7.4). After TTC staining, the left anterior descending artery was reoccluded by tightening the suture left in the myocardium after MI. The hearts were then perfused with 2 to 3 mL of 10% Pthalo Blue (Heubach Ltd) to delineate nonischemic myocardium. The hearts were weighed, then frozen and trimmed free of right ventricle and atria. The LV was cut into 5 to 7 transverse slices, each approximately 1 mm thick. Each slice was weighed and photographed from both sides with a high-resolution digital camera on a dissecting microscope. Photoshop 5.0 (Adobe) was used to digitally planimeter the borders of the entire heart, the nonischemic area, and the infarcted area on both sides of each slice. The sizes of the nonischemic area, ischemic area (area at risk), and infarcted area for each slice were calculated as percentages of the total area multiplied by the total weight of that slice. Perfect coregistration between the 1-mm-thick tissue sections and the 1-mm-thick cardiac MRI planes was not always possible owing to partial-volume effects.

MR Image Acquisition

Sedation and Placement of Intravenous Line
In preparation for MRI 24 hours after the procedure, mice were sedated with diazepam (10 to 15 mg/kg IP). Both forelimbs were shaved, and ECG surface electrodes were attached (Blue Sensor, Medicotest). An indwelling intravenous line was placed by inserting PE-10 tubing into the right femoral vein under local anesthesia with bupivacaine. The mouse was then placed prone inside the radiofrequency coil, and the ECG leads were connected to ECG monitoring cables.

Imaging was performed on a Varian Inova 4.7T MR scanner with Magnex gradients. An ECG-triggered, 2D cine gradient echo pulse sequence was used with a slice thickness of 1 mm and a zero-filled, in-plane resolution of 100×100 μm². The time to echo was 5 ms. The R-R interval (110 to 160 ms for 375 to 545 bpm) was divided into 12 equally spaced time points that led to a repetition time of 9 to 13 ms. The same line of k-space was collected for each of the 12 phases during 1 heart cycle. This was repeated until all the phase-encoding steps were acquired. Although spatial resolution in the phase-encode direction could be increased by scanning for longer periods, the acquisition of 128 lines served to balance spatial and temporal resolutions. A flip angle of 20° was used for baseline imaging. However, the flip angle was increased to 60° to increase T1-weighting for contrast-enhanced imaging. Although a segmented inversion-recovery pulse sequence is commonly used for contrast-enhanced imaging in larger animals,7 the rapid heart rate of mice prevented its direct application here. During each session, the entire LV was imaged with 5 to 7 contiguous short-axis slices. For contrast-enhanced imaging, a 0.3- to 0.6-mmol/kg bolus of gadolinium DTPA (Gd-DTPA) was injected through the intravenous line while the mouse was inside the magnet. A complete set of contrast-enhanced cine images covering the heart from base to apex was obtained 15 to 30 minutes after contrast injection.

Image Processing

Determination of Cardiac Structure and Global Function
The MR images were converted to DICOM format for analysis with ARGUS (Siemens Med Systems). After the endocardial borders were traced, the end-diastolic and end-systolic phases during 1 heart cycle. This was repeated until all the phase-encoding steps were acquired. Although spatial resolution in the phase-encode direction could be increased by scanning for longer

Determination of Infarct Size by MRI
MR images were also converted to TIFF images and transferred to Adobe PhotoShop for further analysis. At each short-axis slice position, the end-diastolic phase was selected for infarct analysis. Image contrast and brightness were adjusted to null the noninfarcted myocardium while maximizing signal in the contrast-enhanced region. The endocardial and epicardial borders, as well as the borders of the signal-enhanced region, were then planimetered from the Gd-DTPA images. To help exclude artifacts during planimetry, adjacent phases at the same slice position and end-diastolic phases from adjacent slice positions were queried as necessary to provide additional frames of reference. At each slice position, the number of enhanced and none enhance pixels were determined, and the size of the contrast-enhanced region was then expressed as a percentage of the LV mass.

Determination of LV Segmental Wall Thickening
Two slices were selected from the midventricular level of each heart for analysis of regional contractile function. The midventricular level was chosen because the infarct pattern in this region typically includes all 3 tissue types of interest (enhanced, adjacent, and remote). Slices closer to the base of the heart contain light infarcted tissue, whereas slices closer to the apex are nearly circumferentially infarcted. The circumference of each short-axis slice was divided into 8 equiangular radial segments indexed vertically to the apex and radially to the anterior right ventricular insertion point. Papillary muscles were excluded during the wall-thickening analysis by interpolating the circumferential arc from the adjacent endocardial borders during planimetry. The area thickness of each segment was computed, and absolute wall thickening and percent wall thickening were computed from the corresponding segment thicknesses at the end-diastolic and end-systolic phases. With post-Gd-DTPA images from the MI group acquired 24 hours after reperfusion, these segments were classified as belonging to the enhanced, adjacent, or remote zones (Figure 1). Segments were classified as enhanced if >50% of the LV wall in that segment was enhanced by the contrast agent. Segments immediately adjacent to enhanced segments were classified as adjacent, and the remaining segments were categorized as remote. Segments from the baseline study of the MI group that corresponded to the enhanced, adjacent, or remote zones were selected by first classifying the segments on the contrast-enhanced MR images acquired 24 hours after the MI and then mapping the
classification back onto the images acquired at baseline. Similarly, segments from the sham group that corresponded to the enhanced, adjacent, or remote zones of the MI group were selected by mapping sector classifications from 7 randomly selected mice in the MI group onto 7 mice in the sham group.

Statistical Analysis
All values are presented as mean±SEM. The paired, 2-tailed Student’s t test was used to test the difference of the mean value within the same group. A 1-tailed Student’s t test was used to test significance of the correlation between the parameters defined by MRI and postmortem analysis. Parameters from different groups were analyzed by 1-way ANOVA, followed by the Bonferroni method for comparison of different groups. Statistical significance was determined at the P<0.05 level.

Results
All mice with the exception of 1 in the sham group survived the surgical procedures and cardiac MR imaging. The baseline MRI sessions required 35±5 minutes per mouse. In the sham group, heart rate increased significantly between baseline and 24 hours after surgery (from 450±17 to 494±3 bpm; Table). Heart rate in the MI group also increased between baseline and 24 hours after surgery (from 456±25 to 472±14 bpm; Table), but the difference did not reach statistical significance.

Postmortem Determination of Heart Weight and Myocardial Infarct Size
In the MI group, both the heart and LV wet weights were significantly increased 24 hours after MI compared with the sham group (Table). In the MI group, 60 minutes of coronary occlusion at a variety of different points along the left anterior descending coronary artery (proximal to distal) produced a wide range of risk regions, which resulted in a corresponding range of MI sizes (from 16% to 38% of LV mass). The mean infarct size in this study was 28.1±1.8% of LV mass.

MRI Determination of LV Mass
MRI-derived LV mass data for the sham and MI groups were pooled and plotted against the corresponding postmortem results (Figure 2A). The correlation was found to be strong (r=0.98), the slope was close to unity (1.01), and the intercept was close to zero (−4.91). Bland-Altman analysis revealed a mean difference of only 3.5 mg between the 2 methods, with upper and lower 95% limits of agreement at 11.5 and −18.5 mg, respectively (Figure 2B). The sequential, noninvasive determinations of LV mass by MRI revealed that in the sham group, LV mass 1 day after surgery was no different from baseline (85±6 versus 86±6 mg, respectively, P=NS; Table). However, LV mass in the MI group on the day after MI was significantly increased compared with baseline (from 87±6 to 120±6 mg, P<0.05; Table).

MRI Determination of Myocardial Infarct Size
The sizes of the contrast-enhanced areas in the MR images were plotted against the corresponding areas obtained from TTC staining (Figure 3A). The areas of contrast enhancement ranged from 15% to 41% of LV mass, with a mean of 27.5±1.7%. A strong correlation was found between MRI and TTC areas, with r=0.96, the slope close to unity (1.06), and the intercept close to zero (−0.985). Bland-Altman analysis revealed a mean difference of only 0.6% of LV mass between the 2 methods, with upper and lower 95% limits of agreement at 4.5% and −3.3%, respectively (Figure 3B). On visual comparison, the contrast-enhanced regions in the MR images were found to correspond closely to infarcted regions as defined by TTC (Figure 4).

MRI Determination of Global LV Function
In the MI group at baseline, the mean LVEDV, LVESV, and cardiac output (CO) as determined by MRI were 33±2 mL, 11±2 mL, 70±3%, and 363±37 mL·g⁻¹·min⁻¹, respectively (Figure 5). When measured 24 hours after the

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Sample sizes: sham, n=7; MI at baseline, n=9; MI at day 1, n=15.

Figure 1. Segmentation methods used to determine regional wall thickening from contrast-enhanced MR images. LV wall was sectored into 8 equiangular segments and indexed to right ventricular insertion point in anterior wall, then classified as described in Methods.

Figure 2. MRI determination of LV mass and comparison with postmortem analysis. A, Scatter plot demonstrating strong correlation (r=0.98) between MRI-determined LV mass and the corresponding postmortem data in the sham group. B, Scatter plot demonstrating strong correlation (r=0.98) between MRI-determined LV mass and the corresponding postmortem data in the MI group.

Figure 3. Postmortem determination of myocardial infarct size. A, Scatter plot demonstrating strong correlation (r=0.98) between MRI-determined infarct size and the corresponding postmortem data in the sham group. B, Scatter plot demonstrating strong correlation (r=0.98) between MRI-determined infarct size and the corresponding postmortem data in the MI group.

Figure 4. Visual comparison of contrast-enhanced regions in the MR images with TTC staining. A, Representative transverse MR image acquired 24 hours after MI and TTC-stained histological section from the same mouse showing good correspondence between contrast enhancement and TTC staining. B, Representative transverse MR image acquired 24 hours after MI and TTC-stained histological section from the same mouse showing good correspondence between contrast enhancement and TTC staining.

Figure 5. Cardiac function measured by MRI in sham and MI groups at baseline and 24 hours after MI. A, Representative transverse MR image acquired 24 hours after MI and TTC-stained histological section from the same mouse showing good correspondence between contrast enhancement and TTC staining. B, Representative transverse MR image acquired 24 hours after MI and TTC-stained histological section from the same mouse showing good correspondence between contrast enhancement and TTC staining.
procedure, sham surgery significantly increased heart rate (Table), but the concomitant increase in CO did not reach statistical significance (Figure 5). MI did not cause a significant change in LVEDV at 24 hours compared with baseline, but it did cause a 98% increase in LVESV (P<0.05), a 47% decrease in LVEF (P<0.05), and a 45% decrease in CO (P<0.05).

MRI Determination of Regional Function as Segmental Wall Thickening
The results of regional wall-thickening analysis performed 24 hours after infarction in the MI group were compared with the same group at baseline and with the sham group (Figure 6). In the sham group 24 hours after surgery, modest decreases in wall thickness at end diastole (Figure 6A) combined with significant decreases in wall thickness at end systole (Figure 6B) to yield dramatic decreases in both absolute wall thickening (Figure 6C) and percent wall thickening (Figure 6D) in all 3 zones. The loss of percent wall thickening (Figure 6D) in the infarcted zone was significantly greater than in the adjacent zone. Similarly, the loss of percent wall thickening in the adjacent zone was significantly greater than in the remote zone. The losses in absolute and percentage wall thickening in the remote zone were nevertheless substantial, being reduced by >50% compared with the corresponding baseline values (Figures 6C and D).

Discussion

In Vivo Sizing of MI by Gd-DTPA-Enhanced MRI
In the postcontrast MR images, the enhanced blood pool in the LV chamber and enhanced MI zone in the LV wall were clearly separated by a thin layer of nonenhanced (viable)
endomyocardium (Figures 1 and 4). This facilitated the
digital planimetry necessary to calculate infarct size and LV
volumes. The results yielded a strong correlation between
contrast-enhanced regions detected by MRI and areas of
infarction detected by TTC staining (Figure 3). Excellent
agreement was also found between in vivo MRI and post-
mortem measurements of LV mass (Figure 2). These results
are consistent with previous studies conducted in larger
animals, including humans,4–7 and demonstrate that contrast-
enhanced cardiac MRI can be used to accurately determine
infarct size 1 day after MI in intact mice. Using the same cine
images used for infarct size determination, we were able to
accurately measure LV chamber volumes at end diastole and
end systole, which in turn yielded LVEF and CO (Figure 5).
The use of a single MRI pulse sequence to determine both
infarct size and LV function shortened both image acquisition
time and image processing time.

Global and Regional Myocardial Dysfunction
After Acute MI
In this study, sham-operated mice showed no significant
changes in global cardiac function or in cardiac mass over
baseline, with the exception of a small but significant increase
in heart rate. In contrast, MI severely reduced cardiac
function, as evidenced by a significant increase in LVESV
and significant decreases in both LVEF and CO ($P<0.05$).
Interestingly, MI size did not correlate strongly with either
LVEF or CO at 24 hours after MI ($R=-0.53$ and $-0.497$,
respectively). This finding may be due in part to the fact that

Figure 5. Global parameters of LV volume and function as
determined by MRI. When assessed by MRI 24 hours after
reperfusion, LVEDV in MI group was not statistically different
from baseline (A). However, MI did cause significant increase in
LVESV (B) and significant decreases in LVEF (C) and CO (D)
compared with either MI group at baseline or sham group at
either time point ($P<0.05$ vs sham or same group at baseline
by ANOVA).
regional function as measured by percent wall thickening was depressed not only in the infarct region but also in noninfarcted regions. The reduction in percent wall thickening in noninfarcted regions was not entirely homogeneous, with regions adjacent to the infarct zone showing a greater deficit in function than found in regions remote from the infarct zone. Thus, we found that the deficit in global cardiac function observed 24 hours after MI was attributable not only to the loss of viable myocardium in the infarct zone but also to contractile dysfunction in noninfarcted regions of the heart. One contributing factor may be decreased wall compliance due to increased thickness of noninfarcted regions at the end-diastolic phase (Figure 5). Significant increases in both LV and heart mass in the MI group further indicate that myocardial edema may contribute to regional contractile dysfunction in noninfarcted regions (Table).

Acquisition of contrast-enhanced MR images was performed at least 15 minutes after contrast injection because our previous study in rats indicated that contrast-enhanced MRI overestimates the size of recent myocardial infarcts when images are acquired much less than 20 minutes after contrast injection. Similarly, a recent study by Flacke et al performed in rats early after MI found that Gd-enhanced areas on MR images acquired 5 minutes after injection were 24±3% larger than infarct areas determined by histological methods. Although the present study in mice did not include a time course of contrast enhancement, imaging from 15 to 30 minutes after contrast injection yielded values that correlated well with myocardial infarct size as defined by TTC staining (Figures 3 and 4). Although the infarcts assessed herein were substantial in size (mean 28±2%, range 16% to 38% of LV mass), subsequent validation studies of smaller infarcts (mean 17±2%, range 7% to 24% of LV mass) have yielded similar correlations between MRI and TTC. As with any analysis of segmental wall thickening, results obtained from the infarcted and adjacent zones are limited by imperfect registration between the boundaries of the contrast-enhanced regions and the corresponding sectors. Wall thickening in these 2 zones may also be affected by tethering effects. However, results from the remote zone should be largely free of these potential sources of error.

In conclusion, we have shown that cardiac MRI can be used to noninvasively determine the size of MI in mice. The same set of MR images can be used to determine LV mass and volumes over the cardiac cycle, thus yielding important indices of cardiac function. The same image sets can be used to assess regional cardiac function. Regional wall-thickening analysis of mice 24 hours after MI reveals that contractile dysfunction occurs not only in the infarcted and adjacent zones but also in areas remote from the infarcted zone. Therapeutic strategies aimed at minimizing contractile dysfunction in noninfarcted regions of the heart may prove beneficial in preventing acute pump failure early after large, anterior MI.

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Figure 6. Segmental LV wall-thickening (WTh) analysis before and 24 hours after MI. A and B, Segmental wall thicknesses at end diastole (ED) and end systole (ES), respectively, in sham and MI groups at baseline and at 24 hours after procedure. C and D, Absolute and percent changes in segmental wall thicknesses, respectively, in sham and MI groups at baseline and 24 hours after procedure. No differences were found between sham and MI groups at baseline, so baseline results from the 2 groups were combined to simplify presentation. Absolute wall thickening (C) and percentage wall thickening (D) in MI group were significantly reduced in all 3 zones (infarct, adjacent, and remote) compared with corresponding zones at baseline or in sham group (*P<0.05 vs baseline).
to Dr French) and R21CA89633 (to Dr Berr). Dr French is an Established Investigator of the American Heart Association.

References

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