Dual Manganese-Enhanced and Delayed Gadolinium-Enhanced MRI Detects Myocardial Border Zone Injury in a Pig Ischemia-Reperfusion Model

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Background—Gadolinium (Gd)-based delayed-enhancement MRI (DEMRI) identifies nonviable myocardium but is nonspecific and may overestimate nonviable territory. Manganese (Mn2+)−enhanced MRI (MEMRI) denotes specific Mn2+ uptake into viable cardiomyocytes. We performed a dual-contrast myocardial assessment in a porcine ischemia-reperfusion (IR) model to test the hypothesis that combined DEMRI and MEMRI identifies viable infarct border zone (BZ) myocardium in vivo.

Methods and Results—Sixty-minute left anterior descending coronary artery IR injury was induced in 13 adult swine. Twenty-one days post-IR, 3-T cardiac MRI was performed. MEMRI was obtained after injection of 0.7 mL/kg Mn2+ contrast agent. DEMRI was then acquired after injection of 0.2 mmol/kg Gd. Left ventricular (LV) mass, infarct, and function were analyzed. Subtraction of MEMRI defect from DEMRI signal identified injured BZ myocardium. Explanted hearts were analyzed by 2,3,5-triphenyltetrazolium chloride stain and tissue electron microscopy to compare infarct, BZ, and remote myocardium. Average LV ejection fraction was reduced (30±7%). MEMRI and DEMRI infarct volumes correlated with 2,3,5-triphenyltetrazolium chloride stain analysis (MEMRI, $r=0.78$; DEMRI, $r=0.75$; $P<0.004$). MEMRI infarct volume percentage was significantly lower than that of DEMRI (14±4% versus 23±4%; $P<0.05$). BZ MEMRI signal-to-noise ratio (SNR) was intermediate to remote and core infarct SNR (7.5±2.8 versus 13.2±3.4 and 2.9±1.6; $P<0.0001$), and DEMRI BZ SNR tended to be intermediate to remote and core infarct SNR (8.4±5.4 versus 3.3±0.6 and 14.3±6.6; $P>0.05$). Tissue electron microscopy analysis exhibited preserved cell structure in BZ cardiomyocytes despite transmural DEMRI enhancement.

Conclusions—The dual-contrast MEMRI-DEMRI detects BZ viability within DEMRI infarct zones. This approach may identify injured, at-risk myocardium in ischemic cardiomyopathy. (Circ Cardiovasc Imaging. 2011;4:574-582.)

Key Words: ischemia-reperfusion injury ■ magnetic resonance imaging ■ gadolinium ■ manganese ■ cell viability

Clinical Perspective on p 582

Coronary artery disease and ischemic cardiomyopathy are leading causes of morbidity and mortality. The improved survival of ischemic cardiomyopathy in patients with coronary revascularization has been well documented4–6; however, the assessment of viable myocardium is crucial in determining which patients will benefit from revascularization. Delayed-enhancement MRI (DEMRI) is considered a gold standard for myocardial viability. This technique exploits the MRI T1-shortening effect of gadolinium (Gd), which distributes primarily within the extracellular space.5 Gd accumulates in acutely or chronically infarcted myocardium, and transmural late enhancement traditionally is believed to indicate irreversible myocardial injury. However, Gd-based DEMRI does not provide direct cell viability information because of its nonspecific distribution properties.

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However, DEMRI also may overestimate infarct areas because of the nonspecific distribution of Gd and the kinetics of its T1 signal in vivo.9–13 Recent studies have demonstrated that DEMRI may consistently overestimate the amount of nonviable myocardium by nearly 15%, predominantly within the heterogeneous border zone (BZ). There are limited data on whether viable and potentially salvageable cardiomyocytes exist within these nonviable DEMRI territories. Similarly, there is no established imaging strategy to identify these BZ areas, which could have a meaningful survival impact for patients who sustain acute myocardial infarction and develop subsequent ischemic cardiomyopathy. An alternative approach using additional contrast agents may complement Gd-based techniques in this capacity.

Manganese (Mn$^{2+}$) is an essential metal divalent cation that can enter cells through voltage-gated calcium channels. Mn$^{2+}$-enhanced MRI (MEMRI) exploits the T1-shortening effect of Mn$^{2+}$, and its uptake depends on viable, functioning cells.18–20 Used widely in neuronal imaging, the uptake of Mn$^{2+}$ into viable myocardial cells also has been well documented.18,21–23 Recent studies attempted to characterize infarcted myocardium with MEMRI alone (MEMRI defect area) and found a correlation with histopathological infarct volumes.16,18,24 In the present study, we combined DEMRI and MEMRI techniques and identified overlapping MEMRI-positive (viable) and DEMRI-positive (nonviable) BZ regions from infarcted myocardium in a porcine ischemia-reperfusion (IR) model. This MRI-detectable, mismatched BZ contained live cardiomyocytes with ultrastructural preservation.

### Methods

#### Ischemia-Reperfusion

All animal studies were approved by the Stanford University Administrative Panel on Laboratory Animal Care. Thirteen Yorkshire swine (30 to 45 kg) were subjected to left anterior descending coronary artery (LAD) IR for 60 minutes, as previously described.19,26 The swine were anesthetized by inhaled isoflurane (1% to 2%). A bolus of heparin 300 IU/kg IV was administered. A 10-mm over-the-wire angioplasty balloon was placed in the LAD proximal to the first diagonal branch and inflated for 60 minutes.

To prevent arrhythmias, amiodarone 150 mg IV was administered before the induction of ischemia. If indicated, nonsynchronized direct current defibrillation was attempted at 360 J.

After the 60-minute occlusion, the balloon was deflated, and reperfusion of the LAD was documented by coronary angiography. The vessel sheath was removed, and the wound was closed. The animals were weaned from mechanical ventilation and transferred to the animal care facility.

#### MEMRI and DEMRI Image Analysis

Twenty-one days post-IR, 3-T cardiac MRI was performed (Signa 3T-HDx, General Electric) using an 8-channel chest coil (General Electric) and cardiac vector gating. Swine were anesthetized by inhaled isoflurane (1% to 2%). Following localization images, fast imaging employing steady-state acquisition (steady-state free procession: repetition time, 3.8 ms; echo time, min-full; flip angle, 45°; slice thickness, 10 mm; matrix, 224×224; field of view, 35) cine images were obtained in standard long- and short-axis image planes. MEMRI (fast gradient echo [FGRE]-IR: repetition time, 4.7 ms; echo time, 1.3 ms; inversion time, 200 ms; flip angle, 10°; slice thickness, 10 mm; matrix, 224×192) was obtained 25 to 40 minutes after an EVP1001-1 0.7 mL/kg IV bolus (Eagle Vision Pharmaceutical Corp; Downington, PA). After the MEMRI, a 30-minute washout period preceded infusion of Gd for DEMRI imaging. Three-dimensional DEMRI (3D myocardial delayed enhancement) (3D-FGRE-IR: repetition time, 4.6; echo time, min-full; flip angle, 15°; slice thickness, 1.4 mm; matrix, 256×256; field of view, 35) was acquired to 20 minutes after injection of Gd 0.2 mmol/kg (Magnevist; Bayer).

Images were analyzed using Osirix (Pixmeo Inc; Geneva, Switzerland) with manual left ventricular (LV) mass and infarct volume tracing. MEMRI defect areas and DEMRI enhanced areas were designated infarct areas. These areas were traced in short-axis slices and integrated to determine infarct volumes by MEMRI and DEMRI in matched swine hearts. Percent infarct volume was calculated as (infarct volume×100)/total LV mass volume. MEMRI defect volumes were not reliably traced using semiautomatic methods; therefore, both MEMRI and DEMRI infarct volumes were visually traced for consistency. To account for potential partial-volume effects with MEMRI tracing, visually traced DEMRI infarct volumes were compared to a subset of semiautomatic, full-width half-maximum (FWHM) tracings (Osirix plugin; courtesy of D. Murday), and the 2 methods were not statistically different (mean infarct volume: visual tracing, 22.7±4.4%; FWHM, 25±6.3%; n=6 hearts), exhibiting consistent agreement between individual measurements as well (Bland-Altman bias of visual versus FWHM method, −3.1±2.3%). Therefore, visual tracing was used for both MEMRI and DEMRI infarct volume assessment.

In swine with microvascular obstruction on DEMRI, these hypointense areas were included as infarct area. The core infarct was defined as the central portion of DEMRI signal, which displayed >50% of the maximal signal-to-noise ratio (SNR) on DEMRI images.27 The BZ was defined as the regions of overlap between positive MEMRI and positive DEMRI signal. The remote zone was defined as any region that did not have a positive DEMRI signal. MEMRI and DEMRI images were analyzed for SNR variation among the remote, border, and infarct zones. Average SNR was computed from each zone by averaging 3 regions of interest per zone for each swine heart and averaging these values across 13 swine hearts. SNR was calculated as follows: average SI of tissue/SD of air, where SI indicates signal intensity, and SD indicates the standard deviation.

#### Double Staining to Measure Infarct Size and Areas at Risk for Ischemia

After the MRI, in situ double staining with 1% Evans blue dye and a 1% solution of 2,3,5-triphenyltetrazolium chloride stain (TTC) was performed to delineate areas at risk for ischemia versus infarction as previously described.26 Evans blue dye binds plasma albumin and stains the vascular distribution of injected vessels. In viable myocardium, TTC is converted by mitochondrial dehydrogenase enzymes to a red formazan pigment that stains the myocardium red, leaving necrotic myocardium white because of lost dehydrogenase activity. Under anesthesia, the left and right coronary arteries were cannulated simultaneously, and a 0.014-inch guidewire was placed in the LAD. The LAD then was occluded with an over-the-wire balloon catheter at the site of the previous occlusion, guided by cine and still angiographic images from the original occlusion. In the pig coronary circulation, there is minimal natural collateral growth, providing a reliable and consistent staining method for the evaluation of the myocardial infarction. After confirmation of complete occlusion of the LAD by coronary angiography, 1% Evans blue dye was injected into the left (60 mL) and right (30 mL) coronary arteries through the guiding catheters. In addition, 20 to 30 mL of a 1% TTC solution was injected distal to the occluded LAD territory using the guidewire lumen of the over-the-wire balloon catheter. During this double-staining procedure, animals were anesthetized with 5% isoflurane and then euthanized with an intravenous potassium chloride injection. The myocardium supplied by the previously occluded LAD, defined as the area-at-risk (AAR) for ischemia,
was Evans blue dye negative. The swine hearts were then excised and LVs sectioned into 10-mm cross-sectional myocardial slices parallel to the atrioventricular groove from apex to base. All slices were weighed, photographed, and fixed in 10% formaldehyde. TTC-positive areas (white) were defined as core infarct, TTC-negative areas (red) that also were Evans blue negative were defined as the AAR. Infarct sizes were manually measured using Adobe Photoshop Elements 8 (Adobe Inc; San Jose, CA).

**Tissue Electron Microscopy**

Myocardial tissue was excised from DEMRI-negative (remote) and DEMRI-positive (infarct) regions by visually correlating the midventricular, cross-sectional gross specimen with midventricular in vivo short-axis DEMRI images. Samples also were excised from myocardial regions with overlapping positive MEMRI and DEMRI signal. The samples were cut into 2-mm³ pieces and fixed with 2% glutaraldehyde and 4% paraformaldehyde in sodium cacodylate buffer. Postfixation was performed with 1% osmium tetroxide for 1 hour at 4°C. After dehydration and embedding, sections were analyzed by a JEOL 1230 tissue electron microscope (JEOL Ltd; Tokyo, Japan) at 80 keV. Photos were taken using a Gatan Multiscan 791 digital camera (Gatan Inc; Pleasanton, CA).

Two to 3 nuclei were identified per cardiomyocyte, with 10 to 15 myocytes per zone (remote, border, and core) analyzed by a JEOL 1230 tissue electron microscope (JEOL Ltd; Tokyo, Japan) at 80 keV. Photos were taken using a Gatan Multiscan 791 digital camera (Gatan Inc; Pleasanton, CA).

### Table. Cardiomyocyte Ultrastructure Score by Tissue Electron Microscopy

<table>
<thead>
<tr>
<th>Cell Structure</th>
<th>Characteristic Feature</th>
<th>Remote Zone</th>
<th>Border Zone</th>
<th>Infarct Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>Notched/furrowed membrane</td>
<td>4.8±0.4</td>
<td>3.9±0.9</td>
<td>1.6±0.7*</td>
</tr>
<tr>
<td></td>
<td>Homogeneous chromatin granules</td>
<td>4.8±0.4</td>
<td>3.9±0.9</td>
<td>1.5±0.5*</td>
</tr>
<tr>
<td></td>
<td>Chromatin accumulated along nuclear membrane</td>
<td>4.8±0.4</td>
<td>3.8±0.8</td>
<td>2±0.9*</td>
</tr>
<tr>
<td></td>
<td>Chromatin clots within nucleus</td>
<td>5±0</td>
<td>4.7±0.5</td>
<td>1.3±0.7*</td>
</tr>
<tr>
<td></td>
<td>Dense chromatin</td>
<td>5±0</td>
<td>4.7±0.5</td>
<td>1.3±0.7*</td>
</tr>
<tr>
<td></td>
<td>Dark chromatin finely structured</td>
<td>5±0</td>
<td>4.7±0.5</td>
<td>1.7±0.7*</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Dense perinuclear accumulation</td>
<td>4.6±0.8</td>
<td>4±0.9</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td></td>
<td>Fine filaments/glycogen granules between nucleus and mitochondria</td>
<td>4.8±0.4</td>
<td>3.7±1.1</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td></td>
<td>Destroyed cristae</td>
<td>5±0</td>
<td>4.6±0.5</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td></td>
<td>Few mitochondria near nucleus</td>
<td>5±0</td>
<td>4.6±0.5</td>
<td>1.3±0.5*</td>
</tr>
<tr>
<td></td>
<td>Mitochondria isolated in niche</td>
<td>5±0</td>
<td>4.9±0.3</td>
<td>1.2±0.4*</td>
</tr>
<tr>
<td>Myofibrils</td>
<td>Myofibrils aligned in 1 row</td>
<td>5±0</td>
<td>4.3±1.1</td>
<td>1.1±0.4*</td>
</tr>
<tr>
<td></td>
<td>T-tubules contain basal lamina</td>
<td>5±0</td>
<td>4.6±0.7</td>
<td>1±0*</td>
</tr>
<tr>
<td></td>
<td>Myofibrils are contracted</td>
<td>4.8±0.4</td>
<td>4.6±0.8</td>
<td>1±0*</td>
</tr>
<tr>
<td></td>
<td>Z-line disruption</td>
<td>4.8±0.4</td>
<td>4.9±0.3</td>
<td>1±0*</td>
</tr>
<tr>
<td></td>
<td>Lipid droplets between ruptured myofibrils</td>
<td>5±0</td>
<td>4.9±0.3</td>
<td>1±0*</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. Quantitative tissue electron microscopy data were obtained from evaluation of 10 to 15 cardiomyocytes per zone (infarct, border, or remote zone) from an explanted swine heart. Sixteen specific criteria of cardiomyocyte ultrastructure were evaluated and compared among regions.29 A score of 5 indicates a high abundance of a favorable characteristic or the complete absence of an unfavorable (italics) characteristic, as opposed to a 1, which denotes the absence of a favorable feature or a high abundance of an unfavorable feature. *P<0.05 compared to remote zone.

**Statistical Analyses**

Results in TEM score (range, 1 to 5) are shown as mean±SD. Significant differences (P<0.05) were tested using the Kruskal-Wallis test for the composite scores of the 3 myocardial zones and a Bonferroni posttest ANOVA for SNR comparison. DEMRI, MEMRI, and TTC methods were compared using a Wilcoxon signed rank test (2-tailed) on the related measures of infarct volumes among the groups. Spearman correlation tested for association between MRI measurements of infarct volume (dependent variable) and TTC infarct volume (independent variable).

**Results**

**LV Systolic Function and Morphology**

At 3 weeks post-IR, LV ejection fraction was markedly reduced (30±7%, n=13) compared with age-matched control swine (66±3%, n=3). LV volumes were dilated (end-diastolic volume, 141±23 mL; end-systolic volume, 100±23 mL), and all swine showed significant thinning and hypokinesis/akinesis of the anterolateral and septal walls.

**Quantitative Correlation of MEMRI-DEMRI Infarct Volume and Histopathology**

MEMRI from the IR swine showed reproducible, homogeneous myocardial Mn²⁺ uptake in remote (normal) areas of...
myocardium (Figure 1). Within the anteroseptal, anterior, and anterolateral walls, MEMRI signal defect (infarct) was consistently observed and corresponded to the core infarct zone of DEMRI images. Because of higher signal intensity, DEMRI-positive myocardium was easily demarcated against any residual MEMRI signal. MEMRI infarct volume correlated highly with TTC infarct volume ($r=0.78$, $P=0.002$), and DEMRI infarct volume correlated closely with TTC infarct volume ($r=0.75$, $P=0.003$).

**MEMRI Versus DEMRI Infarct Volume**

Quantitative comparison revealed that the MEMRI infarct volume percentage (14±4%, $n=13$) was significantly ($P<0.001$) lower than both DEMRI infarct volume percentage (23±4%, $n=13$) and TTC infarct volume percentage (19±3%). In addition, an overlapping BZ that showed positive MEMRI and DEMRI signals was consistently observed (Figure 1A). To determine whether the high correlation between MEMRI/DEMRI and TTC measures also exhibited agreement, Bland-Altman plots of infarct volumes were created to show the differences in agreement between TTC and DEMRI or TTC and MEMRI (Figure 1B). The measurement differences between TTC and DEMRI were all negative, and the limits of agreement did not include 0, indicating that DEMRI consistently overestimated the infarct volumes. Conversely, MEMRI measures were consistently lower than TTC measures, showing positive measurement differences between TTC and MEMRI. Moreover, the differences were proportional to the mean (larger differences for larger infarct volume). However, the Bland-Altman plots of infarct volume differences between TTC and MEMRI (all positive values) and TTC and DEMRI (all negative values) were within 2 SDs of the mean differences for each contrast agent (Figure 1B), indicating that the reduced infarct volume percentage of MEMRI compared to DEMRI was a consistent finding within the group.

**Figure 1.** DEMRI versus MEMRI versus TTC. **A**, Representative slice progressing from the base to the apex of the heart (top row to bottom row), showing short-axis in vivo MRI images and matching TTC sections from an explanted swine heart 21 days after ischemia-reperfusion. Note that the MEMRI signal defect area (middle) is visibly smaller than the corresponding DEMRI signal area (left). Tracings of MEMRI defect and DEMRI signal areas are shown in each slice. **B**, Bland-Altman plots showing the differences between TTC and DEMRI and TTC and MEMRI infarct volumes plotted against the average infarct volume (between TTC and DEMRI or MEMRI) for each swine heart. The average difference (bias) between the measurements is shown (dotted line) ±2 SD (solid lines) for both DEMRI and MEMRI. Note that all TTC-MEMRI differences were >0, all TTC-DEMRI differences were <0, and all values fell within 2 SD of their respective average differences ($n=13$). DEMRI indicates delayed-enhancement MRI; MEMRI, manganese-enhanced MRI; TTC, 2,3,5-triphenyltetrazolium chloride stain.
for both MEMRI and DEMRI. By MEMRI, the BZ SNR (7.5 ± 2.8, n = 13) was significantly lower than the remote zone SNR (13.2 ± 3.4), yet significantly higher than the MEMRI SNR from the core infarct zone (2.9 ± 1.6, P < 0.05 versus remote, P < 0.05 versus core infarct) (Figure 2). Similarly, by DEMRI, the BZ SNR tended to be lower than the core infarct SNR but higher than the remote zone SNR. These intermediate SNR levels further distinguished the BZ from both the remote and core infarct zones, indicating potentially heterogeneous cell populations within the BZ.

One potential limitation of this study was that Mn²⁺ administration before Gd might affect the T1-shortening properties of Gd images. To explore this possibility, we examined several swine with a reverse order protocol (DEMRI before MEMRI or MEMRI alone) and did not observe any noticeable effect on DEMRI infarct percentage (21 ± 9%; ejection fraction, 29 ± 7%; n = 8). Intermediate BZ DEMRI SNR was similarly observed in the DEMRI-before-MEMRI hearts (6.9 ± 2.2, n = 8, P > 0.05) compared with DEMRI-after-MEMRI BZ SNR. Notably, MEMRI defect was not interpretable on images with the DEMRI-before-MEMRI reverse protocol because of the high-intensity T1 signal of DEMRI in the infarct zone. In summary, digital subtraction of MEMRI-negative infarct regions from DEMRI-positive infarct regions revealed an overlapping positive signal designated as BZ areas. This overlap suggested a significant number of viable myocytes in these transmural DEMRI-positive regions, which may contribute to the intermediate MEMRI and DEMRI SNR.

**TEM of Infarct, Border, and Remote Zone Myocardium**

To further characterize the ultrastructure of the contractile apparatus within these BZ regions, TEM was performed from the core infarct, border, and remote zone regions, using established characteristics of normal nuclear, chromatin, mitochondrial, and sarcomeric structure and organization. As noted previously, these outer edges of positive TTC and DEMRI (nonviable) signal, when superimposed on MEMRI images, overlapped significantly with the outer regions of positive MEMRI (viable) signal. The core infarct, border, and remote zones were quantitatively analyzed. BZ cells showed significant ultrastructural preservation (overall TEM score, 4.4 ± 0.4; n = 10) similar to remote zone cells (TEM, 4.9 ± 0.1; n = 10) and unlike infarct zone cells (TEM, 1.3 ± 0.3; n = 10; P < 0.05 versus remote zone score). These results are shown in the Table. Together, the intact cell structure and observed Mn²⁺ uptake of BZ cells suggest viable cardiomyocyte populations within the areas of transmural DEMRI.

**Discussion**

In the present study, we applied a novel, dual-contrast MEMRI-DEMRI strategy to determine whether viable myocardium was detectable within regions of positive, transmural DEMRI. MEMRI infarct volume was found to be significantly (39%) lower than DEMRI infarct volume in this 21-day swine IR model. This signal mismatch identified a BZ that was positive for both MEMRI signal (viable) and transmural DEMRI signal (nonviable) and showed an intermediate SNR by MEMRI compared with the core infarct and remote zones. TEM analysis also revealed preservation of cell architecture and contractile elements within this BZ region. Overall, these results suggest that dual-contrast MEMRI-DEMRI may allow accurate detection of viable myocardium within the infarct BZ that appears nonviable by DEMRI alone.

Although most patients who experience a myocardial infarction will survive the acute event, they often develop...
heart failure and a progressive decline in ventricular function that is believed to involve apoptosis and collagen deposition, particularly in BZ areas. Revascularization decisions for these patients frequently are made at intermediate and chronic time points after myocardial infarction and often are based on viability assessment. In the present study, a 21-day time point after IR was chosen to explore this intermediate time point, and DEMRI infarct volume measurements were significantly higher than either MEMRI or TTC infarct volumes. These data suggest that DEMRI may overestimate infarct volume at intermediate post-IR time points and that combination with MEMRI may accurately delineate these injured, but live populations of cardiomyocytes.

Intermediate MEMRI SNR measurements also reflected tissue heterogeneity within this mismatched BZ, which has not been previously reported, to our knowledge, using MEMRI in a myocardial IR model. Although prior studies have reported heterogeneous DEMRI signal in infarct BZs, Gd accumulation is nonspecific and provides limited information on actual cell viability. Because of specific Mn uptake into viable, functioning cells, the observed MEMRI SNR heterogeneity points to significant populations of cardiomyocytes that are alive with intact Ca channel function (MEMRI positive) in this region despite surrounding necrotic tissue (DEMRI positive). The intermediate degree of Mn uptake within the BZ demonstrates that this region is not only visually and quantitatively distinct from the core infarct zone by TEM analysis, but also biologically distinct. Prior MEMRI studies have imaged myocardial infarct areas, but they either focused on the acute ischemia phase or looked at chronic time points with ex vivo analysis. Intermediate MEMRI and DEMRI SNR, with intact cell structure, suggests that the DEMRI signal may still be evolving at 21 days post-IR and not yet reflective of true infarct size. Future studies may examine earlier and later time points to further characterize this mismatched region.

Viable myocardium that falls outside the core infarct zone has been imaged previously using a combination of agents, including radiolabeled and fluorescent microparticles. However, in the present study, MEMRI infarct volume was compared directly to DEMRI infarct volume and found to be significantly smaller, pointing to viable cardiomyocytes within areas of transmural DEMRI. A technical limitation of this study, and of MEMRI in general, is the potential for partial-volume effects during infarct volume quantification. The use of 3D DEMRI images mitigated the partial-volume effects by using a thinner slice thickness (1.4 mm) than standard 2D DEMRI images (10 mm). As described, a subset of DEMRI infarct volumes were analyzed with a semiautomatic FWHM method, and infarct sizes were not statistically different from visual tracing results. The results are consistent with 2 recent studies in which manual tracing of DEMRI volumes was equivalent to both FWHM- and SD-based semiautomatic methods. Although some partial-volume effect may contribute to BZ signal, the TEM analysis showed relative preservation of the cytoarchitecture in this region, lending support to the overlap of the positive MEMRI and DEMRI underscores the need for improved BZ territories. These findings are consistent with previous publications that document DEMRI overestimation of infarct volume. Absolute and relative degrees of DEMRI infarct volume also have been observed to decrease over time after a myocardial infarction, which has been attributed to cell debris removal and resolution of myocardial edema. This diminishing zone of positive DEMRI underscores the need for improved imaging approaches to detect viable myocardial territories at early and intermediate time points after myocardial infarction with high accuracy to assist decisions on revascularization. Specifically, patients in the periinfarct period may benefit from an imaging strategy that better delineates the amount of injured, yet viable myocardium that would be jeopardized if not revascularized.

Figure 3. Tissue electron microscopy (TEM) of infarct, border, and remote myocardium. TEM sections of explanted swine heart, showing a single representative cardiomyocyte from remote, infarcted, and border zone myocardium, 21 days after ischemia-reperfusion. In the infarct zone, note the nuclear (N) disarray and clumped chromatin as well as the near absence of intact mitochondria (M) and disorganized remnants of sarcomeric myofibrillar organization (S). Border zone cells, however, exhibited much less disruption of cell architecture and relative preservation of its contractile components. Detailed TEM findings are shown in the Table.

<table>
<thead>
<tr>
<th>Remote</th>
<th>Core Infarct</th>
<th>Border Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="remote.png" alt="Remote" /></td>
<td><img src="core_infarct.png" alt="Core Infarct" /></td>
<td><img src="border_zone.png" alt="Border Zone" /></td>
</tr>
</tbody>
</table>

**Figure 3.** Tissue electron microscopy (TEM) of infarct, border, and remote myocardium. TEM sections of explanted swine heart, showing a single representative cardiomyocyte from remote, infarcted, and border zone myocardium, 21 days after ischemia-reperfusion. In the infarct zone, note the nuclear (N) disarray and clumped chromatin as well as the near absence of intact mitochondria (M) and disorganized remnants of sarcomeric myofibrillar organization (S). Border zone cells, however, exhibited much less disruption of cell architecture and relative preservation of its contractile components. Detailed TEM findings are shown in the Table.
The use of Mn\(^{2+}\) as an MRI contrast agent has been limited by its potential for adverse cardiovascular effects, which is mainly attributed to its competition for the L-type Ca\(^{2+}\) channel on the sarcolemmal membrane.\(^{41}\) To mitigate these effects, the EVP-1011 used for MEMRI contains calcium as well, and no toxicity was observed in swine dosed at 0.7 mL/kg. Indeed, clinical MEMRI agents are already in use, namely mangafodipir trisodium, which is Food and Drug Administration approved for liver tumor imaging.\(^{52}\) Although no approval for cardiac imaging with Mn\(^{2+}\) exists at present, EVP-1011 currently is being evaluated for Food and Drug Administration approval.

T2-weighted imaging also has been validated for characterizing periinfarct zone biology, including AAR, in both acute and subacute settings.\(^{43,44}\) The presence of T2-weighted imaging edema is particularly useful in the acute coronary syndrome setting because it was recently demonstrated to independently predict the presence of obstructive coronary disease and even 6-month survival in patients with this condition.\(^{45}\) However, by 14 to 28 days postinfarct, myocardial edema may have dissipated completely,\(^{46–49}\) which lessens the utility of T2-weighted imaging at intermediate and chronic time points. MEMRI-DEMRI affords unique information about infarct zone biology that may be particularly effective at intermediate and chronic time points postinfarct. Moreover, in contrast to T2-weighted imaging, which assesses AAR lying primarily outside the DEMRI territory,\(^{50}\) the MEMRI-DEMRI strategy presented herein detects live cardiomyocyte populations within DEMRI-positive zones.

An important limitation of the present study is that no revascularization data are available to confirm the functional viability of overlapping DEMRI- and MEMRI-positive regions. Consequently, there is no evidence that these live cells are capable of contributing to overall ventricular function if they were to be salvaged with revascularization. Indeed, these populations of live cells may represent arrhythmogenic foci, as recent work has linked the BZ or gray zone areas of DEMRI, which shows SNR heterogeneity, with increased propensity for inducible ventricular tachycardia\(^{51}\) and cardiovascular events.\(^{27}\) Future revascularization studies would be required to understand how these pockets of viable myocardium may affect long-term outcome and whether aggressive restoration of coronary blood flow to these incompletely scarred regions is beneficial.

In summary, the infarct BZ represents a heterogeneous region comprising complex perifascicular fibrosis. To better characterize this dynamic process, contrast agents that enable both anatomic (DEMRI) and biological (MEMRI) information may be advantageous. This study demonstrates a noninvasive, dual-contrast MEMRI-DEMRI detection method for viable myocardium within the infarct BZ. Cells in the BZ display relatively intact cytarchitecture, which suggests salvageable myocardium and arrhythmogenic foci. Future studies are needed to determine how these pockets of viable tissue may affect revascularization strategy in ischemic cardiomyopathy.

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References


**CLINICAL PERSPECTIVE**

Improved survival of patients with ischemic cardiomyopathy with coronary revascularization has been well documented. Clinically, the assessment of viable myocardium is crucial for determining which patients will benefit from revascularization. Gadolinium-based delayed-enhancement MRI (DEMRI) is considered a gold standard for imaging myocardial viability. Transmural DEMRI signal is believed to indicate irreversible myocardial injury; however, DEMRI signal is not specific for cell viability and may overestimate true myocardial infarct volumes by nearly 15% in some studies. In the present study, we demonstrate a novel, dual-contrast MRI strategy to noninvasively characterize the injured border zone of the myocardium postinfarction. Manganese is an essential metal cation that is avidly taken up by live myocardial cells. Manganese-enhanced MRI (MEMRI) signal is, therefore, a specific marker for viable cardiomyocytes, producing a bright T1 signal in live myocardium and a signal defect in infarcted myocardium. Adult Yorkshire pigs underwent 1-hour ischemia-reperfusion injury and were imaged by cardiac MRI 21 days later with MEMRI and DEMRI sequences. Both contrast agents correlated well with histopathological scar volume; however, MEMRI infarct volume was significantly (39%) smaller than DEMRI infarct volume. There was significant overlap in positive signal from MEMRI and DEMRI, and these overlap regions showed intermediate signal-to-noise ratio and preserved cytoarchitecture by tissue electron microscopy, indicating that live cells may reside within regions of transmural DEMRI-positive myocardium. The potential clinical impact of this novel imaging strategy is significant because MEMRI-DEMRI dual contrast may provide complementary information on complex, heterogeneous border zone biology that could help to guide therapy for patients with ischemic heart disease.
Dual Manganese-Enhanced and Delayed Gadolinium-Enhanced MRI Detects Myocardial Border Zone Injury in a Pig Ischemia-Reperfusion Model

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