Cardiac MR Characterization of Left Ventricular Remodeling in a Swine Model of Infarct Followed by Reperfusion

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Background: Myocardial infarction (MI) survivors are at risk of complications including heart failure and malignant arrhythmias.

Purpose: We undertook serial imaging of swine following MI with the aim of characterizing the longitudinal left ventricular (LV) remodeling in a translational model of ischemia-reperfusion-mediated MI.

Animal Model: Eight Yorkshire swine underwent mid left anterior descending coronary artery balloon occlusion to create an ischemia-reperfusion experimental model of MI.

Field Strength/Sequences: 1.5T Philips Achieva scanner. Serial cardiac MRI was performed at 16, 33, and 62 days post-MI, including cine imaging, native and postcontrast T1, T2 and dark-blood late gadolinium enhanced (DB-LGE) scar imaging.

Assessment: Regions of interest were selected on the parametric maps to assess native T1 and T2 in the infarct and in remote tissue. Volume of enhanced tissue, nonenhanced tissue, and gray zone were assessed from DB-LGE imaging. Volumes, cardiac function, and strain were calculated from cine imaging.

Statistical Tests: Parameters estimated at more than two timepoints were compared with a one-way repeated measures analysis of variance. Parametric mapping data were analyzed using a generalized linear mixed model corrected for multiple observations. A result was considered statistically significant at $P < 0.05$.

Results: All animals developed anteroseptal akinesia and hyperenhancement on DB-LGE with a central core of nonenhancing tissue. Mean hyperenhancement volume did not change during the observation period, while the central core contracted from $2.2 \pm 1.8 \text{ml}$ at 16 days to $0.08 \pm 0.19 \text{ml}$ at 62 days ($P = 0.008$). Native T1 of ischemic myocardium increased from $1173 \pm 93 \text{msec}$ at 16 days to $1309 \pm 97 \text{msec}$ at 62 days ($P < 0.001$). Mean radial and circumferential strain rate magnitude in remote myocardium increased with time from the infarct ($P < 0.05$).

Date Conclusion: In this swine model of MI, serial quantitative cardiac MR exams allow characterization of LV remodeling and scar formation.

Level of Evidence: 2

Technical Efficacy: Stage 2

Contemporary treatment of myocardial infarction (MI) has increased the proportion of survivors, leading to an expanded population at risk of late complications including sudden cardiac death and heart failure.

Cardiac magnetic resonance imaging (MRI) is the noninvasive reference standard tool for cardiac structural and functional assessment, scar imaging using late gadolinium enhancement (LGE), and tissue characterization using myocardial tissue relaxometry (eg, T1, T2, and T2* mapping). Cardiac MR has been assessed extensively in animal models of acute and chronic myocardial ischemic injury, in which serial LGE imaging studies distinguished reversible and irreversible myocardial damage following MI.2 Serial MRI studies combined T2 and T2* mapping to...
characterize the progression of edema and hemorrhage in a porcine MI model and serial T2 mapping has been used to define the bimodal time course of edema in the week following MI.

Large animal models are critical for elucidating the processes responsible for post-MI arrhythmias. The ideal model in which to study scar-mediated arrhythmias is when the acute stage of inflammation has resolved. These observations prompted the recent development of porcine MI models with high incidence of scar-mediated reentrant ventricular tachycardia (VT) in the chronic phase following MI. Detailed characterization of the inducible VT in this model demonstrated reentrant mechanisms with circuits that are commonly localized to the endocardium, similar to the human phenotype. However, serial MR examinations of arrhythmogenic post-MI scar development and left ventricular (LV) remodeling in this model are needed.

Thus, we sought to use MR to characterize late-stage LV remodeling and scar formation in a swine model of MI that has been used extensively to study VT by performing serial MR scans over 9 weeks following MI.

Materials and Methods

Animal Model

The study protocol is summarized in Fig. 1. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) and conformed to the position of the American Heart Association on Research Animal Use and the Declaration of Helsinki. Eight Yorkshire swine underwent a 180-minute balloon occlusion of the mid left anterior descending (LAD) coronary artery as previously described. In brief, after 4 days of amiodarone 800 mg twice daily (BID), each swine was premedicated with an intramuscular tiletamine/zolazepam combination. Anesthesia was induced and maintained with inhaled isoflurane. Under fluoroscopic guidance, an angioplasty balloon was inflated in the mid-LAD (Fig. 2) for 180 minutes. The balloon was then deflated and withdrawn to create an ischemia-reperfusion-mediated MI. Lidocaine and metoprolol were used to suppress arrhythmias. In the event of ventricular fibrillation (VF), external defibrillation was performed. Anticoagulation was maintained with unfractionated heparin. Postoperative analgesia was maintained with intramuscular buprenorphine (0.03 mg/kg) and meloxicam (0.3 mg/kg) followed by 3 days of 3 µg/kg/hr transcutaneous fentanyl. Oral amiodarone (800 mg BID) was continued for an additional 4–5 days after MI and then decreased to 400 mg once daily throughout the survival period, in order to minimize the risk of death from spontaneous ventricular arrhythmias. A control animal of comparable weight (n = 1) was treated with amiodarone and scanned at the same intervals.

Data Acquisition

All imaging was performed using a 1.5T Philips Achieva (Philips Healthcare, Best, The Netherlands) scanner with a 32-channel cardiac phased array receiver coil. Scanning was performed before and after intravenous 0.15–0.2 mmol/L gadobenate dimeglumine (Multithance; Bracco Diagnostic, Princeton, NJ) at 16, 33, and 62 days following MI.

Balanced steady-state free precession (bSSFP) cine imaging was acquired in the horizontal long axis, vertical long axis, and short axis (in-plane resolution = 1.25 × 1.25 mm²; slice thickness = 10 mm, repetition time [TR] / echo time [TE] / flip angle [α] = 3.2 msec / 1.6 msec / 60°). T1 mapping was performed prior to and at least 20 minutes following gadolinium administration, using a slice interleaved T1 (STONE) mapping sequence. T1 maps were acquired during free breathing using slice-tracking (five short-axis slices with in-plane resolution = 2 × 2 mm²; slice thickness = 10 mm; field-of-view [FOV] = 360 × 280 mm²; TR/TE/α = 3.1/1.5msec/70°; number of linear ramp up pulses = 10; SENSE-rate = 2; linear k-space ordering). Retrospective image registration was performed using a nonrigid registration algorithm to compensate for residual in-plane motion. Voxelwise T1 parametric maps were reconstructed using a two-parameter fit model. T2 mapping was performed prior to contrast administration using free breathing, slice interleaved T2 mapping (five short-axis slices with in-plane resolution = 2 × 2 mm²; slice thickness = 10 mm; FOV = 320 × 320 mm²; TR/TE/α = 2.8/1.4msec/30°; SENSE rate = 2; linear k-space ordering). Following retrospective image registration, voxelwise T2 maps were reconstructed using a three-parameter fit model.

Scar imaging was performed using a dark-blood LGE (DB-LGE) sequence that uses a combination of an inversion pulse followed by a T2 magnetization preparation pulse to simultaneously suppress signal of blood pool and healthy myocardium. All images...
were acquired during free breathing using a respiratory navigator with adaptive gating window (spatial resolution = 1.3 × 1.3 × 1.3 mm³; FOV = 320 × 335 × 90 mm³; gradient echo imaging readout with TR/TE/R = 2.6/1.3 msec/55°; SENSE rate = 2; centric phase-encoding order).

Data Analysis
Analysis was performed using CVI42 (v. 5.2.2, Circle Cardiovascular Imaging, Calgary, Canada) for chamber volumes, function, and strain and in-house dedicated software for parametric map analysis. Chamber volumes, systolic function, and LV mass were calculated from the short axis cine imaging from manually drawn endocardial and epicardial contours. Strain analysis was conducted using the tissue-tracking module within CVI42 applied to the cine imaging. After manual identification of right ventricle insertion points, the software automatically segments the LV according to the American Heart Association 16-segment model and calculates segmental strain parameters. The ischemic segments were defined as the mid anteroseptal and apical septal segments and the remote regions defined as the mid inferolateral and apical lateral segments, reflecting segments universally involved or remote from the infarction. Prior to strain analysis, DB-LGE imaging was reviewed to confirm that there was no enhancement in the segments classified as "remote." Peak radial and circumferential strain and peak systolic radial and circumferential strain rate were calculated.

On all parametric maps regions of interest (ROI) were selected by a single observer (J.W.) with 3 years experience with cardiac MR analysis. Chamber volumes, myocardial wall thickness, and LV mass were calculated from manual endocardial and epicardial contours. Strain analysis was conducted using the tissue-tracking module within CVI42 applied to the cine imaging. After manual identification of right ventricle insertion points, the software automatically segments the LV according to the American Heart Association 16-segment model and calculates segmental strain parameters. The ischemic segments were defined as the mid anteroseptal and apical septal segments and the remote regions defined as the mid inferolateral and apical lateral segments, reflecting segments universally involved or remote from the infarction. Prior to strain analysis, DB-LGE imaging was reviewed to confirm that there was no enhancement in the segments classified as "remote." Peak radial and circumferential strain and peak systolic radial and circumferential strain rate were calculated.

On all parametric maps regions of interest (ROI) were selected by a single observer (J.W.) with 3 years experience with cardiac MR analysis, which were conservatively selected within the myocardial wall from each precontrast T₁ and T₂ map. A single ROI was selected on each parametric map to identify an area defined as “infarct tissue” (if present on the slice, comprising both enhanced tissue and “infarct core” according to the definition below) and an area defined as “normal tissue.” This was achieved by comparison of the parametric map alongside a multiplanar reconstruction from a DB-LGE image of the same volume as the parametric map. The mean parameter value within each ROI was automatically calculated.

The endocardial and epicardial borders were manually segmented from each DB-LGE scan. ROI were conservatively selected in nonenhancing myocardium remote from the vascular territory involved in the infarct (“normal myocardium”), enhanced areas within the vascular territory of the infarct (“ gadolinium enhanced tissue”), and nonenhanced areas within the vascular territory of the infarct and surrounded by enhancing tissue (“infarct core”). Voxel intensity histograms from each region were used to calibrate the threshold selected for automatic assignment of voxels to tissue compartments. Hyperenhanced tissue was defined as voxels with intensity higher than 3 standard deviations (SDs) above the mean of remote normal myocardium. Gray zone was defined as voxels with intensity of 2–3 SD above the mean intensity of remote normal myocardium. Volume of enhancing tissue and volume of infarct core was automatically calculated.

At the final cardiac MR examination the extracellular volume (ECV) fraction was estimated using pre- and postcontrast STONE T₁ maps and hematocrit, measured at the time of cardiac MR from a venous blood sample using a point of care analyzer (i-STAT, Abbott, Abbott Park, IL). ROIs were selected on the calculated ECV maps according to the same definitions used for analyzing the T₁ and T₂ maps.

Statistical Analysis
Normality of distribution of variables was assessed using the Shapiro–Wilk’s test. Normally distributed continuous variables are expressed as mean ± SD. Parameters estimated at more than two timepoints were compared with a one-way repeated measures analysis of variance (RM-ANOVA). Parametric mapping data were analyzed using a generalized linear mixed model corrected for multiple observations at each timepoint per subject. Estimated means and 95% confidence intervals (CIs) are reported for these data. Intraobserver and interobserver reliability was assessed in a subset of the parametric maps, comprising 20 T₁ and 20 T₂ maps, all of which...
included scar and healthy tissue. The analyzed slices were selected to ensure that at least one mid-ventricular slice from each animal was included, and that maps from at least two timepoints for each animal were included (with the exception of the animal that died after the first cardiac MRI scan). One observer (J.W.) selected ROIs in scar and healthy tissue at two timepoints, separated by at least 3 months. A second observer (J.J.) selected ROIs in scar and healthy tissue from the same maps. The presence of proportional bias between observations was tested by performing linear regression analysis between the mean value and difference between values for paired observations. The intraclass correlation coefficient (ICC) was calculated between observations from the same observer and between measurements from different observers using a two-way mixed model assessing absolute agreement between observations. A result was considered statistically significant at $P < 0.05$. Statistical analysis was carried out in SPSS (v. 23, IBM, Armonk, NY).

Results

Five male and three female Yorkshire pigs underwent 180-minute mid-LAD occlusion following which one female pig died at 15 days post-MI during their first cardiac MR scan from an anesthetic-related bradycardic arrest. Cardiac MR was performed at a median (range) of 16 (14–18), 33 (29–35), and 62 (58–64) days post-MI. All data assessed using the RM-ANOVA test were normally distributed at each timepoint, as assessed by the Shapiro–Wilk's test ($P > 0.05$).

From day 16 to day 62, LV ejection fraction (LVEF) progressively increased from $29.8 \pm 7.3\%$ to $43.7 \pm 6.7\%$ ($P < 0.001$) (Fig. 3A) and LV end diastolic volume (LVEDV) increased from $111.4 \pm 14.4\text{ ml}$ to $186.3 \pm 31.6\text{ ml}$ ($P < 0.001$). Between 16 and 62 days post-MI, LV mass increased from $64.1 \pm 7.4\text{ g}$ to $103.5 \pm 14.3\text{ g}$ ($P < 0.001$), during which time the mean animal weight increased from $43.9 \pm 3.8\text{ kg}$ to $82.8 \pm 6.5\text{ kg}$ ($P < 0.001$) (Fig. 3B). Mean end-diastolic LV mass/volume ratio (LVMVR) did not change significantly between day 16 (0.42) and day 62 (0.59, $P = 0.529$). All animals in the MI group demonstrated a wall motion abnormality in the mid to apical septum and apical anterior wall. At each timepoint, peak radial strain, peak circumferential strain magnitude, peak systolic radial strain rate, and peak circumferential strain rate magnitude was lower in infarcted segments than remote segments (Fig. 4, $P < 0.05$).

In remote segments, between 16 days and 62 days, there was an increase in peak systolic radial (from $184.9\%/\text{s}$ to $251.7\%/\text{s}$, $P = 0.005$) and circumferential (from $108.8\%/\text{s}$ to $167.1\%/\text{s}$, $P = 0.043$) strain rate magnitude. Among the three cardiac MR scans, there was no significant change in the peak systolic radial or circumferential strain rate magnitude in infarcted segments.

Over the three MR studies, there was no significant change in the LGE volume or gray zone (LGE: $P = 0.464$, gray zone: $P = 0.173$). The central nonenhancing core contracted from $2.2 \pm 1.8\text{ ml}$ at 16 days to $0.08 \pm 0.19\text{ ml}$ at 62 days ($P = 0.008$). At the final cardiac MRI, a nonenhancing core was present in only one animal (Fig. 5).

Infarcted tissue was identified on between three and five short axis slices from each parametric mapping sequence, reflecting differences in the extent of the infarct and the coverage of the maps. Slices with notable artifact
were excluded from subsequent analysis. A total of 148 ROIs were analyzed from T1 and T2 maps from the MI group (62 infarcted regions, 86 remote regions) and 26 from the control animal. Parametric mapping data from infarcted and remote myocardium at each timepoint was normally distributed (Shapiro–Wilk’s test, $P > 0.05$) in all cases with the exception of T2 in infarcted segments at 16 and 33 days. For interobserver and intraobserver reliability, there was no evidence of proportional bias between the observations (linear regression did not indicate a significant linear relationship between mean value and the difference between paired observations in any of the comparisons: T1 intraobserver $P = 0.837$; T1 interobserver $P = 0.201$; T2 intraobserver $P = 0.815$; T2 intraobserver $P = 0.390$). For single measures, the ICC between observations from the same observer and between observers demonstrated excellent agreement in each case (T1 intraobserver ICC = 0.980, $P < 0.0001$; T2 intraobserver ICC = 0.941, $P < 0.0001$; T2 intraobserver ICC = 0.942, $P < 0.0001$; T2 intraobserver ICC = 0.880, $P < 0.0001$). These results indicate that the method used to estimate T1 and T2 from the acquired parametric maps is a reproducible measure. Infarcted tissue T1 was 1173 msec at day 16 (95% CI 1123–1223 msec), 1242 msec at day 33 (95% CI 1183–1301 msec), and 1309 msec at day 62 (95% CI 1254–1363 msec). The generalized linear mixed model estimated an increase of 69 msec between day 16 and day 33 ($P = 0.006$) and an increase of 136 msec between day 16 and day 62 ($P < 0.001$; Fig. 6). At each timepoint, infarcted tissue T1 was higher than remote tissue ($P < 0.05$). There was no significant change in remote tissue (day 33, $P = 0.798$; day 62, $P = 0.535$, Fig. 6).

The T2 of infarcted tissue was above remote ($P < 0.05$) and peaked at 33 days post-MI (Fig. 7), although the differences between the timepoints did not reach statistical significance ($P = 0.12$). There was no significant change in mean T2 time of remote tissue (day 33, $P = 0.630$; day 62, $P = 0.370$). Mean calculated ECV of infarcted tissue at 62 days was 0.53 ± 0.17, while mean ECV of remote myocardium at 62 days was 0.22 ± 0.03 ($P < 0.001$). Figure 8 shows mean segmental ECV at 62 days for the MI group.
animals. The limited spatial resolution of the mapping sequences did not allow reliable distinction between areas of infarct, core, and gray zone.

Discussion

In this swine infarct study, serial cardiac MR scans were used to characterize the evolution of LV parameters that occur in an LAD ischemia-reperfusion model of MI developed to study ventricular arrhythmia. These experiments demonstrated 1) the volumetric quantification of relevant tissue compartments at serial timepoints following MI including a prominent infarct core; 2) the use of parametric mapping to document LV remodeling following MI including a progressive increase in native T1 of infarcted tissue following MI; and 3) evolution of regional strain remote from the infarct.

The LV cavity was increased in the MI group in comparison with the control animal and the absolute magnitude of this difference was similar at each timepoint, indicating that LV dilatation occurs rapidly (first 15 days) following MI, as described in humans following ST elevation MI.18 LV and RV ejection fraction progressively improved over the observation period. LV dilatation and increased end-diastolic pressure are associated with a greater incidence of post-MI arrhythmias,19 an association thought to be multifactorial, involving chronic changes in cardiomyocyte ion channel expression and behavior. The identification of LV dilatation in this model resembles the LV remodeling process observed in MI survivors and may contribute to arrhythmogenesis.20

An improvement in LV systolic function may be achieved in a large proportion of patients.21 The magnitude of the improvement in LVEF is generally lower than observed in this study, however, and is in the context of targeting optimal medical therapy in these patients, which was not used in this experiment. An important confounding factor likely to contribute to the more extensive recovery of

FIGURE 5: DB-LGE from two animals (number 7 and number 5) at 16, 33, and 62 days post-MI with arrows indicating nonenhancing infarct core. In animal 7 the infarct core had resolved by day 62. In animal 5 the infarct core remained present on CMR#3. A: infarct; B: gray zone; and C: core display the mean volume of tissue in each compartment at each CMR examination. There was a significant decrease in the volume of the infarct core present at day 62 post-MI.
LVEF observed here is the growth of the pigs, whose body weight nearly doubled during the course of the study, with a parallel increase in LV mass occurring during the same timeframe, occurring as part of the normal process of growth in addition to eccentric hypertrophy, which may result from the infarct. Therefore, normal growth of the pigs is likely to have contributed to preservation of the LV mass/volume ratio, reducing persistence of neurohormonal...
activation and afterload excess that may be observed in human ischemic cardiomyopathy in which such growth does not occur. These processes are likely relevant to the greater than expected increase in LVEF during the experiment.

We found no change in the observed strain rate of the infarcted segments during the observed period, while there was a significant increase in peak systolic radial strain rate and peak circumferential strain rate magnitude in remote myocardium. The increased strain rate in segments remote from the infarct may reflect an improvement in myocardial contractility that occurred during the experimental period. However, strain rate is also sensitive to changes in loading conditions. The relative contributions of recovery of mechanical myocyte function, neurohormonal effects that follow MI, and changes in the LV loading conditions to the observed change in strain rate remote from the infarct cannot be determined.

LGE using an inversion recovery (IR) sequence has been the basis of most cardiac MR scar imaging. A nonenhancing central core in myocardium subjected to ischemia followed by reperfusion is well recognized and results from a combination of microvascular obstruction (MVO) and reperfusion-related intramyocardial hemorrhage (IMH). Both MVO and IMH are adverse prognostic features in human clinical studies with an incidence reported between 31–73% on LGE imaging within the first month of MI. This study was not designed to make the distinction between MVO and IMH, and so this area is referred to as the infarct core. We found the infarct core volume at 16 days post-MI was 20% of the LGE volume. By 33 days the infarct core had contracted, but was still present in all animals. At 62 days it was only observed in a single animal.

Myocardial T2 are elevated with increased tissue water content and T2-weighted imaging and T2 mapping identify myocardial edema. Pathologic human studies have demonstrated that myocardial edema following MI is generally resolved by 28 days. In a similar animal model, Fernandez-Jimenez et al demonstrated that following ischemia-reperfusion injury, myocardial edema follows a bimodal pattern and that the first wave of edema is mediated by reperfusion. In both the Fernandez-Jimenez’s study and our study, the hypointense infarct core was included in the ROI used to estimate T2. Fernandez-Jimenez found the T2 for ischemic myocardium at day 7 post-MI was 78 msec as compared with 65 msec at day 16 and 73 msec at day 33 in our study. The presence of deoxyhemoglobin secondary to IMH in the infarct core would have significant paramagnetic effects, reducing the estimated T2 in that segment. Maturation of hemorrhagic components and the decrease in volume of infarct core between 16 days and 33 days post-MI (by a factor of almost 3) would be expected to reduce the impact of the infarct core on estimated myocardial T2. This process is likely relevant to the change in T2 measurements observed between 16 and 33 days.

At 62 days post-MI, T2 of infarcted myocardium remained significantly higher than remote myocardium. While the elevated T2 at day 62 may reflect ongoing edema, T2 relaxation does not follow tissue water content exactly, as demonstrated in the Fernandez-Jimenez report. Given the expectation that the majority of edema secondary to MI would have been expected to be resolved before day 62, it is more likely that these results reflect differences between the T2 relaxation properties of mature scar and remote myocardium.

Native myocardial T1 is increased with intracellular and extracellular processes, including fibrosis. An increase in the native T1 in infarcted tissue is observed in the early months following MI in clinical studies and experimental models. Increased myocardial native T1 has also been shown in patients with nonischemic dilated cardiomyopathy with complex ventricular arrhythmia. In our study there was a progressive increase in the native T1 of infarcted
myocardium, with a gradual diminution in the infarct core size. It is also likely that during this period the acute edema that followed MI reduced and necrotic tissue was replaced with dense collagenous scar. Without resolving the gadolinium-enhanced tissue and infarct core, it is not possible to precisely identify the contribution of each component to the measured $T_1$ time at each stage. However, the observation can be made that following severe ischemia-reperfusion injury resulting in a large infarct core, a reduction in volume of the core and maturation of the scar was associated with a gradual increase in native $T_1$ in infarcted tissue.

This model of MI was created in adolescent pigs who continued to grow. A number of the potential differences that this may introduce between the model and the majority of patients affected by MI are discussed above. This experiment was completed at 62 days. While this allowed the opportunity to characterize this phase of postinfarct remodeling, further remodeling after 2 months is likely, in particular in view of the observation that hyperenhanced tissue volume did not decrease during the experiment.

In conclusion, in a swine MI model serially followed with cardiac MR, we demonstrated a progressive improvement in LVEF that occurs alongside an increase in strain with cardiac MR, we demonstrated a progressive improvement in LV volume did not decrease during the experiment.

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Conflict of Interest
The authors report no conflicts of interest.

Ethical Approval
The research protocol was approved by the Beth Israel Deaconess Institutional Animal Care and Use Committee and conformed to the position of the American Heart Association on Research Animal Use and the Declaration of Helsinki.

Availability of Data
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References


