Am J Physiol Heart Circ Physiol 294: H2435–H2443, 2008.
First published March 28, 2008; doi:10.1152/ajpheart.01190.2007.

Characterization of the structural and functional changes in the myocardium following focal ischemia-reperfusion injury

Navdeep Ojha,[1] Sashwati Roy,[1] Jared Radtke,[1] Orlando Simonetti,[2] Surya Gnyawali,[1] Jay L. Zweier,[2] Periannan Kuppusamy,[2] and Chandan K. Sen[1]

Departments of [1]Surgery and [2]Internal Medicine, Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, Ohio

Submitted 12 October 2007; accepted in final form 24 March 2008

Ojha N, Roy S, Radtke J, Simonetti O, Gnyawali S, Zweier JL, Kuppusamy P, Sen CK. Characterization of the structural and functional changes in the myocardium following focal ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 294: H2435–H2443, 2008. First published March 28, 2008; doi:10.1152/ajpheart.01190.2007.—High-resolution (11.7 T) cardiac magnetic resonance imaging (MRI) and histological approaches have been employed in tandem to characterize the secondary damage suffered by the murine myocardium following the initial insult caused by ischemia-reperfusion (I/R). I/R-induced changes in the myocardium were examined in five separate groups at the following time points after I/R: 1 h, *day 1*, *day 3*, *day 7*, and *day 14*. The infarct volume increased from 1 h to *day 1* post-I/R. Over time, the loss of myocardial function was observed to be associated with increased infarct volume and worsened regional wall motion. In the infarct region, I/R caused a decrease in end-systolic thickness coupled with small changes in end-diastolic thickness, leading to massive wall thickening abnormalities. In addition, compromised wall thickening was also observed in left ventricular regions adjacent to the infarct region. A tight correlation ($r^2 = 0.85$) between measured MRI and triphenyltetrazolium chloride (TTC) infarct volumes was noted. Our observation that until *day 3* post-I/R the infarct size as measured by TTC staining and MRI was much larger than that of the myocyte-silent regions in trichrome- or hematoxylin-eosin-stained sections is consistent with the literature and leads to the conclusion that at such an early phase, the infarct site contains structurally intact myocytes that are functionally compromised. Over time, such affected myocytes were noted to structurally disappear, resulting in consistent infarct sizes obtained from MRI and TTC as well as trichrome and hematoxylin-eosin analyses on *day 7* following I/R. Myocardial remodeling following I/R includes secondary myocyte death followed by the loss of cardiac function over time.

redox

ACUTE MYOCARDIAL DAMAGE caused by a surge in reactive oxygen species following ischemia-reperfusion (I/R) has been demonstrated and extensively studied (25, 26, 39). The secondary loss of myocytes subsequent to the initial insult is a progressive phase reported by numerous laboratories (2, 3, 12, 15, 18, 31, 32) and remains poorly understood. In addition, the functional significance of this secondary death process remains to be characterized.

The current state of magnetic resonance (MR) imaging (MRI) represents a versatile noninvasive tool for measuring cardiac function and structure (7, 8, 24, 28, 34, 37). Cardiac MRI has been applied to study I/R injury (8–11, 23, 24, 35–37), left ventricular (LV) remodeling (13), and myocardial metabolism (5, 19, 33) in canine as well as rodent models. Contrast-enhanced MRI monitors infarct damage after myocardial infarction (9, 10, 21, 30, 34). Because these observations are obtained from rodent as well as canine models, it is important to recognize that the rodent and canine models do have some contrasting features, primarily because of a higher abundance of collateral circulation in dogs. Unlike in rodents where complete ischemia is achieved, most dog models represent an uncontrolled mixture of incomplete and complete ischemia, which gives rise to a delay in cell death in those areas that were never totally ischemic. Murine MRI has been hampered by the lack of widespread availability of high-resolution scanners that provide requisite spatial and temporal resolution. The high (400–600 beats/min) heart rate of the anesthetized mouse coupled with limited dimensions (5 mm in diameter and 8 mm in length) of the heart poses significant challenges for routine high-resolution cardiac MRI. The ability to noninvasively and accurately perform the repeated examination of cardiac parameters without operator bias over a period of time after a myocardial insult is a major advantage of MRI over other techniques including echocardiography, hemodynamic measurement techniques, and histology. In this study, we have simultaneously employed histological analyses and 11.7-T high-resolution cardiac MRI (2 to 3 time points/mouse) to study LV function, structure, and infarct volume in mice after I/R injury.

MATERIALS AND METHODS

Animal and experimental protocol. All animal procedures were approved by the Institutional Laboratory Animal Care and Use Committee of the Ohio State University. Thirty-one young adult (10–12 wk of age) male C57BL/6 mice (Harlan Technologies) were used in this study. The vast majority (94%) of the animals survived the study protocol. Baseline MR images were obtained 5–7 days before surgery to ensure the normalization of all cardiovascular parameters. The mice were divided into five groups ($n = 4$–9 animals) and were euthanized for harvesting heart tissue accordingly. The mice were euthanized either at 1 h, *day 1*, *day 3*, *day 7*, or *day 14* postreperfusion immediately after MRI.

Survival surgery for myocardial I/R. The mice were anesthetized, held on a warm tray (37°C), and intubated endotracheally. The mice were ventilated on room air-isoflurane at an appropriate rate and tidal volume. Cardiac electrophysiology was monitored throughout the surgery using a standard three-lead ECG setup, and changes were recorded using PC Powerlab software (AD Instruments). The heart was accessed via left thoracotomy. The left lung was retracted to

Address for reprint requests and other correspondence: C. K. Sen, 512 Heart and Lung Research Inst., 473 W. 12th Ave., Columbus, OH 43210 (e-mail: chandan.sen@osumc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

allow entrance to the pericardium. The left auricle was elevated to expose the left anterior descending coronary artery (LAD), which was isolated using a 7-0 silk suture on a taper needle. The suture was tightened over a piece of polyethylene-10 tubing to provide for reversible ischemia via the occlusion of the coronary artery. Ischemia, documented by laser-Doppler blood flow measurement, continued for 60 min after occlusion. After 60 min, the suture was released to allow for reperfusion of the injured myocardium. On successful reperfusion, the thorax was closed with interrupted sutures, and the skin incision was closed with surgical clips. A catheter was used to aspirate the left thorax to reestablish the negative thoracic pressure and facilitate lung reexpansion (17, 27).

Histological determination of infarct size. The mice were euthanized, and the hearts were excised and encased in 2% agarose solution. After the agarose was settled for 2 min, the heart was laterally cut just below the left auricle, and 1-mm-thick sections were made. The agarose was removed, and the sections were incubated with 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C for 20 min, with rotation every 2 min to allow uniform tissue staining. Each slice was photographed with a digital camera mounted on a dissecting microscope. Following photography, the sections were fixed in 10%-buffered Formalin solution for 2 wk after which they were processed for hematoxylin-eosin staining. ImageJ software (National Institutes of Health) was used for the planimetry of the LV border and infarct region on TTC images. Infarct volume was calculated as a percentage of total LV volume (including septum). The exact correlation between the TTC and MRI slice was not always possible because of slice thickness mismatch and partial voluming effects. Masson's trichrome and hematoxylin-eosin staining were done on the same heart sections used for TTC staining. Standard paraffin embedding and tissue processing protocols were used. Sections of 5-μm thickness obtained from each 1-mm-thick slice were mounted on glass slides and stained with hematoxylin-eosin and standard Masson's trichrome staining protocol. The stained sections were photographed using a digital camera (model D30; Hitachi) mounted on a microscope (Axiovert 200M; Zeiss). Manual planimetry was performed on the microscope using PALM RoboSoftware v2.2 to calculate infarct volume using hematoxylin-eosin-stained slices. The myocyte-silent space in hematoxylin-eosin sections was interpreted as the infarct area in this study.

MR image acquisition. In the animal preparation for MRI, the mice were anesthetized with 1.5% isoflurane mixed with 1.5 l/min carbogen. Subdermal ECG leads were fixed on the right forelimb and right leg of the animal. A respiratory sensor was placed under the abdomen and secured to the animal. Gadolinium-diethylenetriaminepentaacetic acid-bismethylamide (Gd-DTPA-BMA) contrast agent (Omniscan) was injected intraperitoneally at a dose of 0.6–0.9 mmol/kg of body wt just before placing the animal in the magnet. Core body temperature was maintained at 37.0 ± 0.5°C using circulating warm air. Core body temperature, respiration, and ECG were monitored with the SAII model 1025 monitoring and gating system (Small Animal Instruments, Stony Brook, NY). The MRI protocol included localizer FLASH scans, short-axis cine loops (6–8 slices), and contrast-enhanced infarct imaging (6–8 slices). MRI was performed on a Bruker 11.7-T vertical MR scanner with a maximum gradient strength of

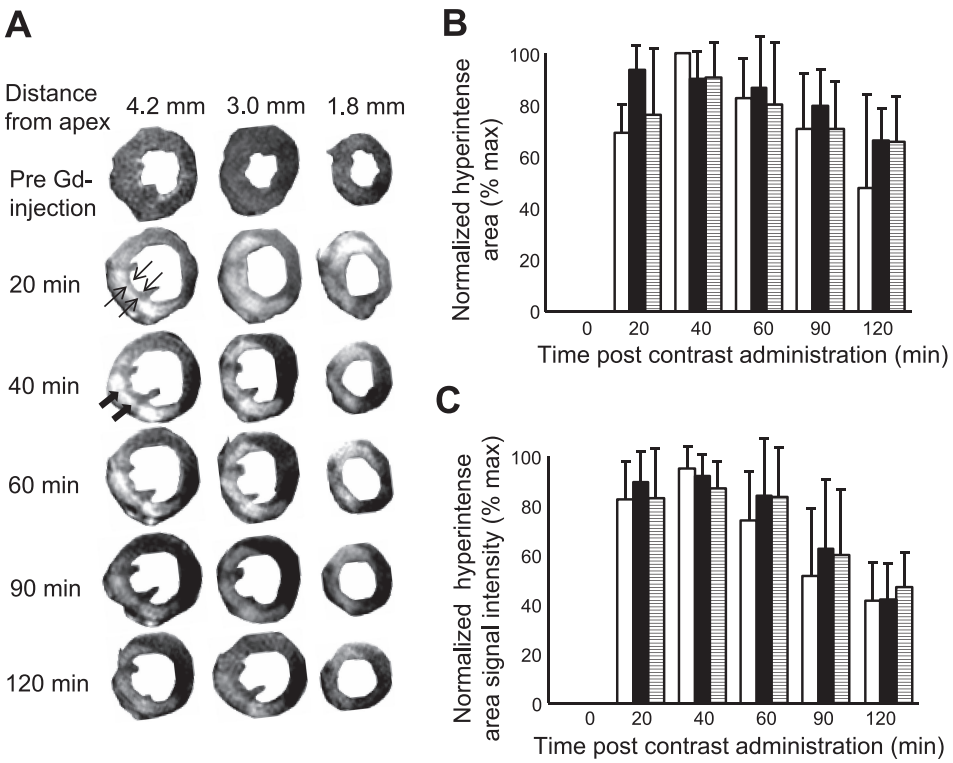

Fig. 1. Time course of signal enhancement in heart after contrast agent administration. Contrast agent gadolinium-diethylenetriaminepentaacetic acid-bismethylamide (Gd-DTPA-BMA) was injected intraperitoneally (0.6–0.9 mmol/kg body wt) in a mouse 3 days after 60 min transient left anterior descending coronary artery (LAD) occlusion surgery. The mouse was then immediately placed into the MRI, and data acquisition was started. Contrast-enhanced images were acquired with an inversion recovery pulse sequence with an inversion time of 150–200 ms. *A*: time course of signal enhancement in 3 heart slices is shown with contrast-enhanced regions, indicating myocardial tissue damage. Magnetic resonance (MR) images were segmented with Photoshop for better display of left ventricle (LV). Thick arrows indicate region of hyperintensity, which was interpreted as infarct. Thin arrows indicate a thin layer of viable tissue between the infarct region and LV lumen, which aided in infarct segmentation and planimetry. *B*: hyperintense area on each image remains constant from 40 to 60 min postcontrast administration. MR slices in left, middle, and right columns of *A* are represented by white, solid, and striped bars, respectively. *C*: signal intensity of hyperintense region was normalized to maximum level of each slice. Peak signal intensity was observed 40 min after contrast agent administration. Thus all contrast-enhanced images in this study were acquired 30–45 min after contrast agent administration.

1,000 mT/m. A 30-mm birdcage radiofrequency coil was used. Shim currents were initialized to previously saved values from earlier imaging experiments, and automatic shimming was then done. The proton line width of <600 Hz (1.2 ppm) was routinely obtained in <30 s and yielded good signal to noise ratio (SNR) on the MR images. After localizer scans were completed, an ECG-triggered two-dimensional FLASH pulse sequence was used to generate cine loops of cardiac motion. For baseline images, a flip angle of 20° was used since it generated good contrast between blood and the myocardium at heart rates from 375 to 500 beats/min (R-wave-R-wave interval from 160 to 120 ms). The flip angle was increased to 60° (to increase T1 weighting) for postcontrast images in an attempt to obtain contrast-enhanced images concurrently with cine images (38). Sixteen frames were obtained in each cardiac cycle that led to a reception time between 7.5 and 10 ms. Echo time was kept constant at 1.43 ms. A spatial resolution of 117 μm in plane (slice thickness, 1 mm; field of view, 3 cm × 3 cm; and matrix size, 256 × 192) was obtained. To obtain contrast-enhanced images, an ECG-triggered inversion recovery (I/R) sequence with an inversion time of 150–200 ms was used. Double inversion preparation pulses were used as required to null blood signal and improve image quality. This sequence was used to obtain 6–8 1-mm-thick sections at 117 μm in-plane resolution. Contrast-enhanced images were acquired 30–45 min postcontrast administration to accurately measure infarct volume (21, 34). The contrast-enhanced images from cine loops were later correlated with inversion recovery images to provide additional frames of reference for the measurement of infarct area. The full imaging protocol was completed within 1 h after anesthetizing the animal.

Determination of cardiac functional parameters. The MR images were converted to DICOM format using Paravision 4.0 and processed with ImageJ software. After the end-diastolic (ED) and end-systolic (ES) phases were identified on a slice-by-slice basis, the endocardial and epicardial borders were traced and LV ES volume (ESV), LV ED volume (EDV), LV stroke volume (SV), cardiac output (CO), and LV ejection fraction (EF) were computed from the traced borders (24). LV mass was calculated by multiplying the LV myocardial volume with density (34).

Determination of infarct volume. The contrast-enhanced MR images in DICOM format were processed in ImageJ. Appropriate contrast enhancement of the images was done to maximize the signal from the hyperintense region and the null signal from the nonenhanced region. Manual planimetry was performed on images obtained from the inversion recovery imaging sequence. To help exclude artifacts during planimetry, contrast-enhanced images obtained with the FLASH cine sequence were examined as required to provide an additional reference. For each slice, the hyperenhanced region and total LV myocardial area were calculated. Slice hyperintense areas were then summated to generate infarct volume as a percentage of LV myocardial volume.

Determination of LV wall thickening. A slice from the midventricular section of the heart, 4.2 mm from apex, was chosen for the analysis of segmental wall thickness to determine abnormalities in LV wall motion compared with that of the control group. Typically, this slice had both infarcted and noninfarcted regions. Wall thickness analysis was conducted employing software developed in our laboratory using Matlab. After epicardial and endocardial borders in end systole and end diastole were contoured, the LV wall thickness was calculated at each 2° angular distance from the center of the LV. Papillary muscles were not included in the wall thickening analysis. Twenty-two contiguous wall thickness measurements were averaged to segment the LV into eight equiangular sectors of 45° each (Fig. 8*A*). The averaged wall thickness was indexed to the right ventricular insertion point in the anterior wall and was plotted against the corresponding angle at each time point after I/R injury. Fractional shortening (FS) was calculated as FS = (wall thickness at end systole/wall thickness at end diastole) − 1.

Statistical analysis. Planimetry and the analysis of segmental LV wall thickness were performed by a trained investigator blinded to the study groups. All values are reported as means ± SD. Paired and unpaired two-tailed Student's *t*-tests were used where appropriate. One-way ANOVA followed by Tukey's pairwise comparisons test was used to compare parameters between different groups. A value of $P < 0.05$ was considered statistically significant.

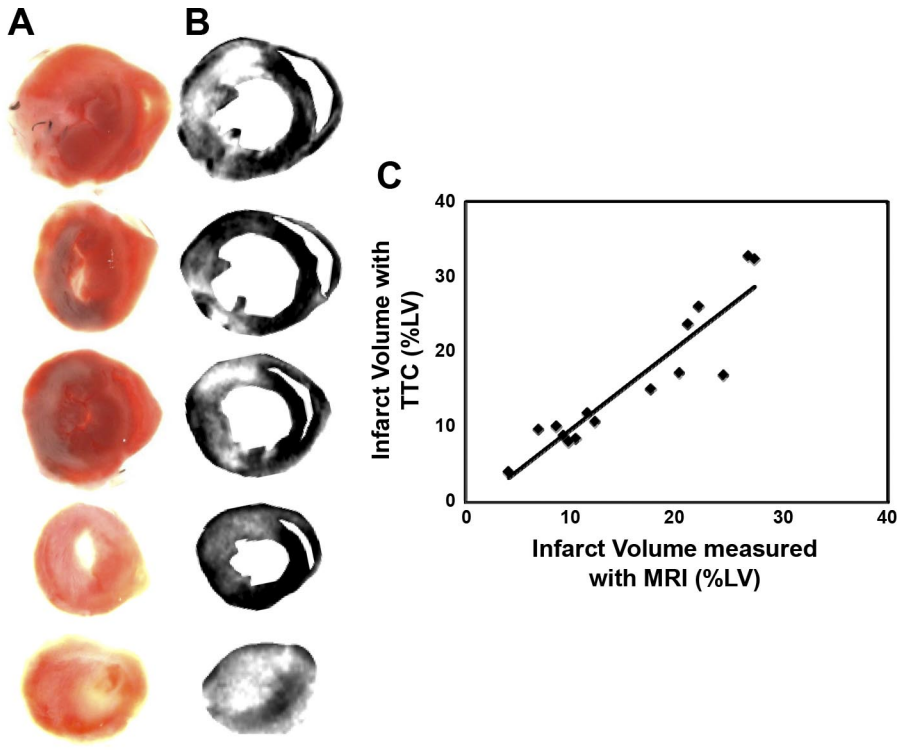

Fig. 2. Contrast-enhanced mouse heart MR images compared with corresponding tissue slices photographed postmortem. *A*: MR images were obtained 3 days after 60-min occlusion of LAD and 40 min after injection of Gd-DTPA-BMA contrast agent. *B*: color images obtained by digital photography of corresponding tissue sections stained with triphenyltetrazolium chloride (TTC). *C*: good correlation (r^2 = 0.85) was found between spatial location and extent of myocardial damage delineated by enhanced regions (white) in MR images and necrotic regions (white) not stained red by TTC. Best-fit linear trend line is drawn through the individual data points.

RESULTS

ECG recordings during surgery were helpful in objectively discriminating between successful and failed LAD occlusions (supplementary Fig. S1; note: all supplementary material can be found with the online version of this article). All mice that developed myocardial infarction, as later proved by MRI and histology, exhibited a strikingly pathological, infarct-typical ECG pattern during and after the surgical procedure. The pathological ECG pattern was observed for the complete duration of the study. Early manifestations of infarction included reduced R-wave amplitude, marked ST-segment elevation, and the development of a large Q-wave (supplementary Fig. S1). The MRI protocol used in the current study involved the intraperitoneal administration of a contrast agent outside the MR scanner. The time course of the hyperenhancement of the LV was followed to obtain a time window where MRI reflected true infarct size as later confirmed with TTC staining. The earliest contrast-enhanced images were obtained 20 min following contrast administration. A thin layer of viable tissue was observed between blood and a region of hyperintense myocardium, which allowed for an easy delineation of the infarct region (arrows in Fig. 1*A*). In a mouse subjected to serial analysis over the first 2 h after contrast injection, infarct size measured with MRI remained constant from 20 to 60 min postcontrast injection (Fig. 1*B*), thus providing a wide window of time for acquiring MR images. In some regions of the heart, hyperintensity was noted even up to 2 h after contrast injection. Sections of the myocardial tissue were stained with TTC after MRI (Fig. 2, *A* and *B*). Infarct volumes calculated from MRI obtained 30–45 min after contrast administration and TTC were plotted against each other (Fig. 2*C*). A tight correlation (r^2 = 0.85, slope = 1.07, and intercept = −0.87) between measured MRI and TTC infarct volumes was noted. The correlation was especially strong in infarcts smaller than 20% of the LV.

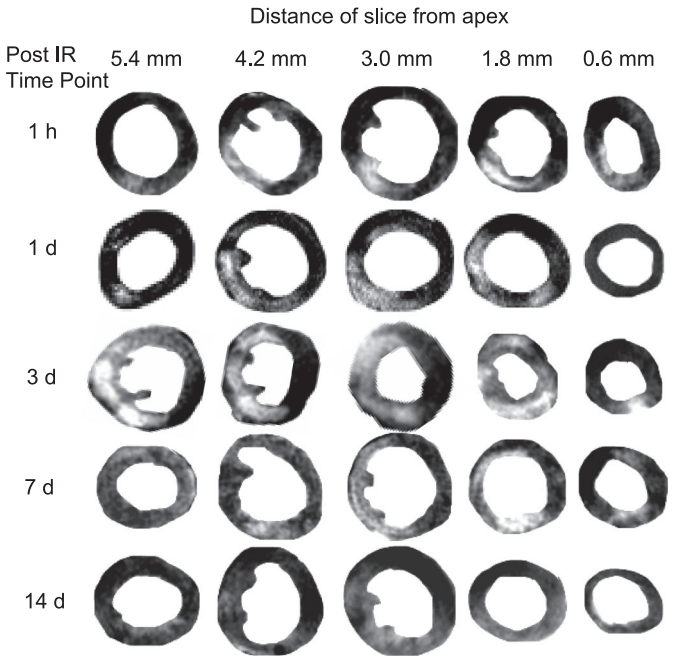

Fig. 3. MRI assessment of infarct size at different time points post-ischemia-reperfusion (I/R). Five groups of mice were imaged at 5 time points after 60-min transient LAD occlusion and reperfusion. MRI protocol was as described in *MR image acquisition*. Five sequential tissue slices from each animal are shown, where slice thickness is 1 mm and distance of center of slice from apex is given. Infarct volume was calculated with planimetry at each time point. Maximal infarct damage was observed 3 days after surgery [n = 5 animals in 1-h (1h) group, n = 6 in *day 1* (1d) group, n = 9 in *day 3* (3d) group, n = 7 in *day 7* (7d) group, and n = 4 in *day 14* (14d) group].

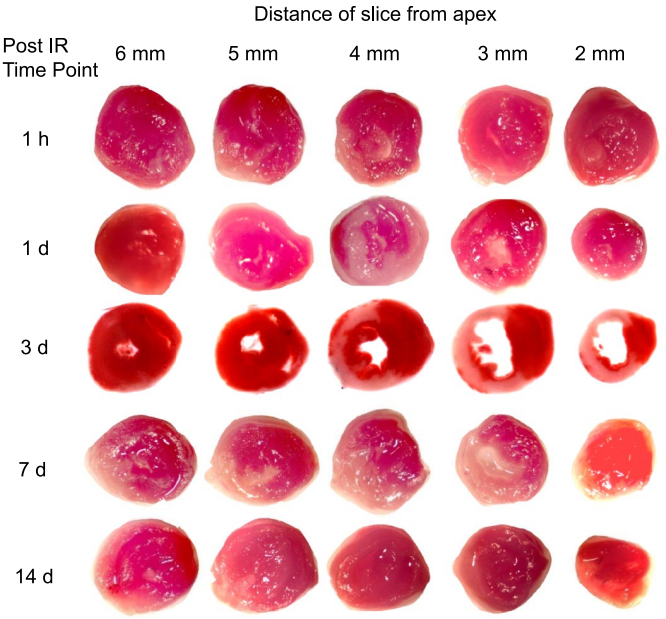

Fig. 4. TTC assessment of infarct size at different time points post-I/R. Myocardial TTC staining was performed on animals immediately after MRI experiment was completed on each of the 5 time points. One-millimeter-thick heart slices were incubated with 2% TTC for 20 min at 37°C and digitally photographed. Infarct volume was calculated from TTC images with manual delineation of necrotic region (white) using digital planimetry (n = 15 animals).

Time-dependent changes in I/R-induced myocardial infarction were examined in mice with MRI over 14 days after surgery (Fig. 3). Separate groups of mice were imaged at five time points after I/R: 1 h, *day 1*, *day 3*, *day 7* , and *day 14*. Infarct volumes at each time point were calculated as described in MATERIALS AND METHODS. Maximum infarct size was observed in the group assessed at *day 3* after I/R surgery, and infarct size in this group was significantly larger than that in the 1-h group and the group assessed at *day 14* post-I/R. TTC staining on heart sections was performed immediately after MRI (Fig. 4). Infarct sizes measured with TTC closely matched those measured with MRI. Both data sets consistently demonstrated infarction until *day 7* after I/R. Of note, the hematoxylin-eosin staining of tissue revealed very little structural loss of myocytes at 1 h (0.3% LV), *day 1* (1.03%), and *day 3* (2% LV) following I/R. Infarct size measured by hematoxylin-eosin staining on the same heart sections on which MRI and TTC staining were done increased to a maximum at *day 7* following I/R (Figs. 5 and 6). Infarct volume over time measured with the three different techniques is plotted in Fig. 6. An apparent disagreement among the techniques was observed at acute time points of 1 h, *day 1*, and *day 3* after I/R injury.

I/R insult caused a statistically significant worsening of all global LV functional parameters (Fig. 7). There was a significant increase in EDV between baseline and *day 7* and a significant increase in ESV between baseline and *day 1*. This

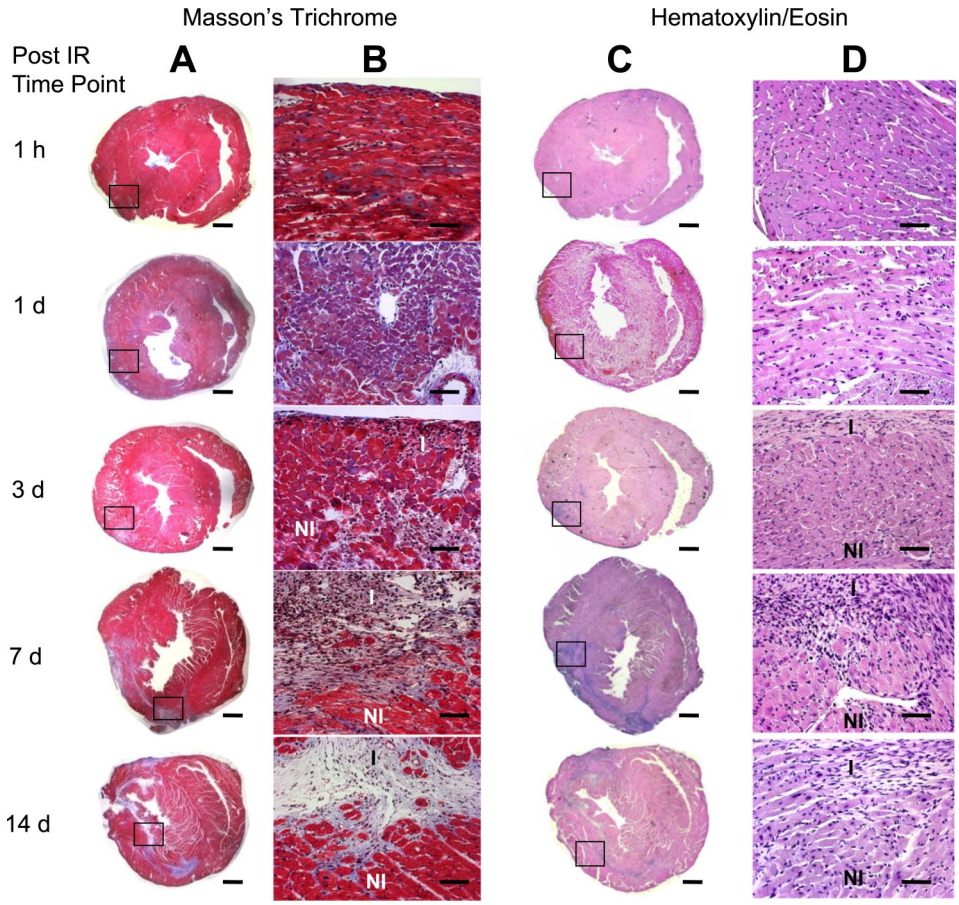

Fig. 5. Histological assessment of infarct size at different time points post-I/R. Tissue sections were obtained from Formalin-fixed heart slices that had been used for TTC staining. Hematoxylin-eosin and Masson's trichrome staining was done on 4 representative sections obtained from each 1-mm tissue slice. Infarct on hematoxylin-eosin-stained sections was defined as regions without myocytes and rich with atypical nuclei. Collagen accumulation in the 5 groups of mice was assessed by the blue color on Masson's trichrome staining. Infarct volume was calculated by manual delineation of infarct region on hematoxylin-eosin-stained sections using a digital camera mounted on a microscope. Images shown here represent heart sections from 4 mm above the apex. Images shown in *A* and *C* were taken at ×1.25 magnification (scale bar = 1 mm). *B* and *D*: magnified view at ×20 magnification of boxed region (scale bar = 50 μm). Maximum loss of myocytes and collagen accumulation were observed at 7 days after I/R injury (n = 4 animals at each time point). I, infarct; NI, noninfarct.

resulted in significant decreases in SV, CO, and EF at every time point relative to baseline. The worst cardiac function was measured at *day 7*, where I/R caused a 46% increase in EDV, 340% increase in ESV, 25% decrease in SV, 32% decrease in CO, 47% decrease in EF, and 38% increase in LV mass. The analysis of regional wall thickening indicated a trend toward worsening wall motion in the groups assessed at later time points after I/R (Fig. 8). Statistically significant differences in LV wall thickness were observed across the infarct zone and adjacent regions at end systole and end diastole, which led to severe differences in FS during the cardiac cycle. A small improvement in FS was observed in remote LV regions 180° away from the infarct zone. The differences in wall thickening that reached statistical significance are indicated in Table 1. LV ES thickness, ED thickness, and FS were compared at the following five time points: baseline, *day 1*, *day 3*, *day 7*, and *day 14* following I/R. For each myocardial sector, these wall motion parameters were serially compared with a different group of mice assessed at the previous time point. Such analysis revealed significantly worsened wall motion post-I/R in several myocardial segments. This observation is consistent with the noted loss of cardiac function following I/R over time.

DISCUSSION

Timely coronary reperfusion of the ischemic myocardium is aimed at reducing myocardial dysfunction and improving tissue survival. Reperfusion, however, also poses the threat of reperfusion injury. Reperfusion injury may be broadly viewed as having two major components: oxidant-induced myocardial tissue damage during the reperfusion process and progressive tissue damage secondary to the initial insult (3, 32). When compared with the first component, the process of progressive tissue damage after reperfusion remains poorly understood. Acute myocardial infarction in humans is associated with the activation of myocyte death in the surviving peri-infarct portion of the heart (20). It has been proposed that the secondary progressive myocardial tissue damage is aimed at clearing nonsalvageable cells (12). Others have proposed that excessive mechanical forces associated with increases in ventricular loading following I/R cause the secondary progressive tissue damage (15). Yet others have implicated post-I/R inflammation to cause the secondary progressive myocardial tissue damage (14, 31). In contrast, products of post-I/R inflammation, e.g., TNF-α, have been shown to induce cytoprotective signals that prevent and/or delay the development of cardiac myocyte death secondary to acute ischemic injury (18). In this study, we employed MRI and histological approaches to characterize post-I/R myocardial tissue damage from both functional as well as structural standpoints. High-resolution imaging performed on the 11.7 T MRI scanner with large gradient strength and fast slew rate allowed a superior SNR ratio and contrast between hyperintense and nonischemic regions. Voxel volumes of ~0.01 mm^3 and a temporal resolution of 6 ms were routinely obtained to generate cine loops, which were utilized to analyze cardiac parameters. The use of a double inversion preparation pulse to null blood signal proved critical to en-

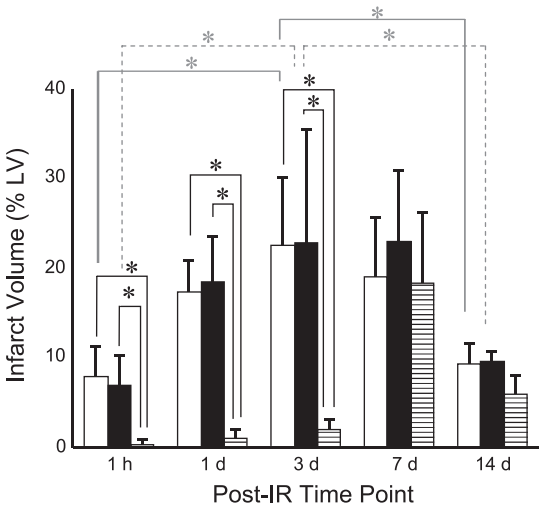

Fig. 6. Infarct volume as measured by 3 different techniques at different time points post-I/R. Infarct volume was calculated using MRI (white bars), TTC (solid bars), and hematoxylin-eosin histology (striped bars) using digital planimetry at 1h, 1d, 3d, 7d, and 14d after I/R surgery. Infarct volume was calculated as a percentage of LV myocardial volume. MRI and TTC data were closely associated with each other at all time points but not with histologically defined infarct volume. A statistically significant change in TTC and MRI infarct volume was observed between time points 1h and 3d and between 3d and 14d post-I/R ($n = 4$ animals in each group at each time point). *$P < 0.05$.

hance contrast with the noise ratio in certain cases. A tight positive correlation was found between infarct volumes measured with MRI and TTC, consistent with previous studies (9, 10, 16, 22, 23). It has been previously suggested that contrast-enhanced MRI overestimates the size of myocardial infarcts when images are acquired much less than 20 min after contrast injection (11, 21). We observed that infarct volume measured with MRI remains practically constant from 20 to 60 min after contrast administration, thus providing a wide window of time where MR images could be acquired.

TTC staining of tissue is coupled with the state of mitochondrial metabolism. Tissue with dysfunctional mitochondria does not stain with TTC, resulting in a pale appearance (4). In this study, infarct volumes measured in separate groups of mice showed significant differences from 1 h to *day 1* but no statistical difference in size from *day 1* to *day 7* post-I/R. With the assumption that infarct volumes were similar between groups, these data suggest a progressive increase in infarct volume between 1 h and *day 1* post-I/R. Moreover, this observation must be tempered by the fact that no statistical difference in infarct size was found between the *day 1* compared with the *day 3* and *day 7* post-I/R results. The significant decrease in infarct size observed between *day 3* and *day 14* post-I/R can be attributed to the wound contracture that accompanies scar maturation. Myocyte-deficient regions of the infarct site are known to be richly populated with fibroblasts that differentiate to myofibroblasts over time (17, 27, 29). Such myofibroblasts may account for the wound contraction noted between *day 7* and *day 14* after I/R. I/R injury compromises LV function (8, 24, 35, 37). Concurrent with the changes in infarct volume observed using all three techniques after I/R, a time-dependent change in LV function was also noted in this study. Increased LV, EDV, and ESV and reduced CO and EF were consistently observed until *day 7* following I/R. A marginal gain of LV function was observed on *day 14*, but it was still lower than baseline values, suggesting a more permanent change in cardiac function because of the injury. The segmental LV wall motion analysis performed in the current study points to a time-dependent worsening of wall motion after I/R. In the infarct region (20–200° on most slices), I/R caused a decrease in ES thickness coupled with small changes in ED thickness, leading to massive wall thickening abnormalities. This observation must be tempered by the fact that this was not

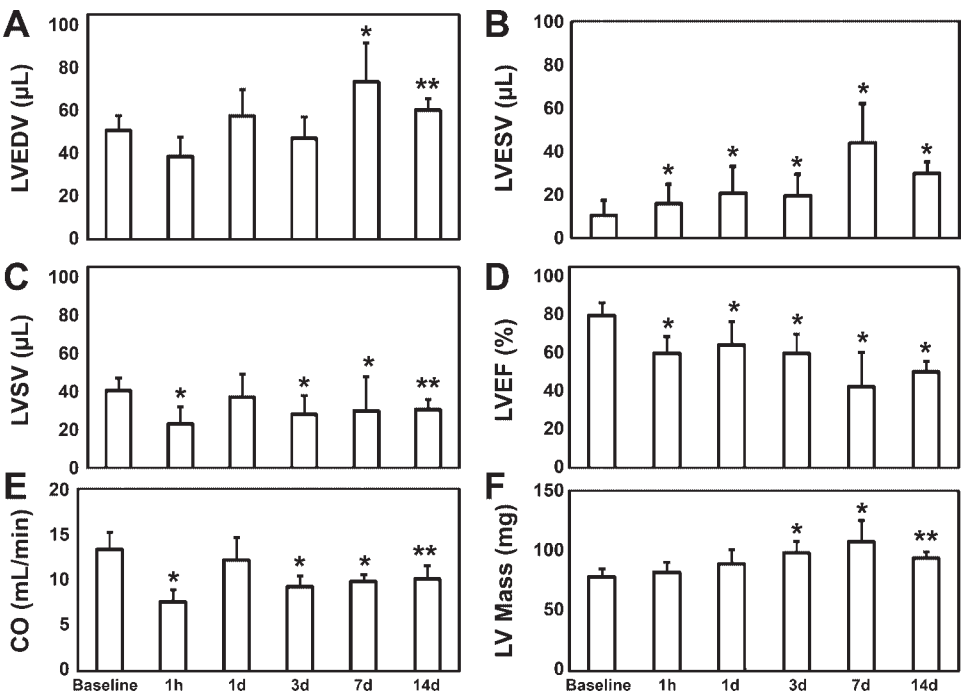

Fig. 7. MRI analysis of cardiac function after I/R injury. Global parameters of LV volume and function were measured with MRI in 5 groups at different time points post-I/R. I/R caused a significant increase in LV end-diastolic (LVED) volume (LVEDV; *A*) and LV end-systolic (LVES) volume (LVESV; *B*), whereas it caused a significant decrease in LV stoke volume (LVSV; *C*), cardiac output (CO; *D*), and LV ejection fraction (LVEF; *E*). A significant increase in LV myocardial mass (*F*) was also observed after I/R. *$P < 0.01$ and **$P < 0.05$ compared with baseline values ($n = 9$ animals in baseline group, $n = 5$ in 1h group, $n = 6$ in 1d group, $n = 9$ in 3d group, $n = 7$ in 7d group, and $n = 4$ in 14d group).

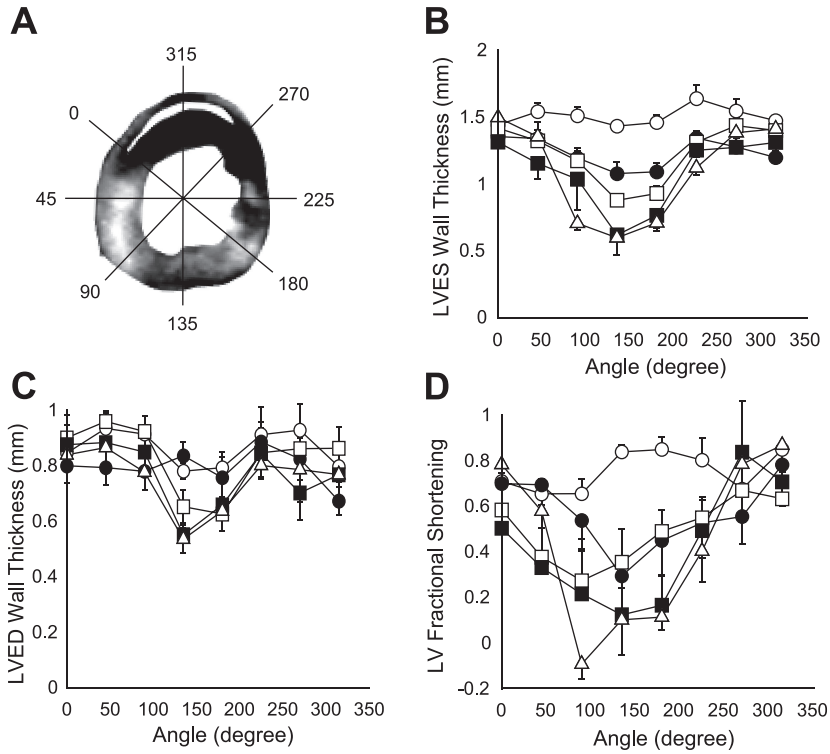

Fig. 8. Segmental LV wall thickening analysis before and after I/R. *A*: LV wall was segmented into 8 equiangular 45° sectors using custom-written software in Matlab. Wall thickness was calculated radially at 2° increments and was averaged for each sector. Thickness data were indexed to right-ventricular insertion point in the anterior wall. *B* and *C*: segmental LV wall thickness at end systole and end diastole, respectively. *D*: fractional shortening of LV wall in 1 cardiac cycle. Baseline data are represented by ○, 1d by ●, 3d by □, 7d by ■, and 14d by △ ($n = 4$ animals in each group).

a serial study. Furthermore, no attempt was made to distinguish between infarcted and noninfarcted sectors in the current study. The loss of wall thickening was associated, albeit not significantly, with the loss of cardiac function and increased infarct volume. In addition, compromised wall thickening was also observed in LV regions adjacent to the infarct region consistent with previous findings (8, 24, 34). Interestingly, an improvement in FS was observed over baseline in remote (250–360°) regions of LV. The recovery of cardiac contractility in remote regions at *day 14* after I/R occurred simultaneously with morphological adaptations and was paralleled by a significant increase in ventricular weight. Part of this hypertrophic response may be attributed to normal physiological growth of the tissue. However, the enlargement of EDV and the relative thickening of remote regions of LV suggest an eccentric hypertrophic response to I/R injury. De Celle et al. (6) studied LV remodeling in mice until 8-wk post-I/R and also reported a positive correlation between infarct size and ventricular weight. This long-term remodeling response can be explained as a compensatory mechanism for reduced cardiac contractility caused by time-dependent functional and structural damage caused by I/R. Findings of the current study would be best interpreted in light of the practical limitations in execution. MRI was performed at two to three time points per group. All conclusions drawn regarding progressive changes in structure and function are based on the assumption that infarct size was similar between the five groups of mice. With respect to the segmental analysis of FS, it should be noted that any conclusions drawn regarding progressive changes in wall motion are based on the assumptions that both the size and spatial distribution of infarct were equivalent between the different groups of mice.

Table 1. *Statistical analysis of wall motion abnormalities*

	Angle							
	0°	45°	90°	135°	180°	225°	270°	315°
End systole								
Day 1 vs. baseline		*		*	*	*		
Day 3 vs. *day 1*		*	*	*				
Day 7 vs. *day 3*				*	*			
Day 14 vs. *day 7*	#		*					
End diastole								
Day 1 vs. baseline		*		*	*	*		
Day 3 vs. *day 1*	#	#	#			#		
Day 7 vs. *day 3*	#				*			
Day 14 vs. *day 7*	*		*					
Fractional shortening								
Day 1 vs. baseline		#		*	*	*	*	
Day 3 vs. *day 1*		*	*					
Day 7 vs. *day 3*					*		#	
Day 14 vs. *day 7*	#		*				#	

Left ventricle (LV), end-systolic thickness, end-diastolic thickness, and fractional shortening were compared at five time points: baseline, *day 1*, *day 3*, *day 7*, and *day 14* after ischemia-reperfusion (I/R); $n = 4$ animals in each group. These wall motion parameters were compared serially with the previous time point using ANOVA for each myocardial sector. A progressive worsening of LV wall motion is observed after I/R. *Significant decrease in each parameter at $P < 0.05$; #significant increase in each parameter at $P < 0.05$.

In summary, we present the first evidence from tandem high-resolution cardiac MRI and histological studies aimed at characterizing the temporal changes in LV function, infarct size, and regional wall motion in mice after I/R. A time-dependent loss of myocardial function was observed, which was associated with increased infarct volume from 1 h to *day 1* post-I/R and worsened regional wall motion. The histological determination of infarct size did not match with MRI and TTC at acute time points after I/R, demonstrating the presence of a small number of structurally intact but functionally compro-

mised myocytes at the infarct site after I/R injury. This observation is consistent with the literature (1). With the progression of time, such affected myocytes were noted to structurally disappear, resulting in consistent infarct sizes obtained from MRI and TTC as well as trichrome and hematoxylin-eosin analyses on *day 7* following I/R. Specific molecular mechanisms implicated in such myocyte death in the peri-infarct region following I/R remain to be characterized.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grant RO1-HL-073087 (to C. K. Sen).

REFERENCES

1. **Ambrosio G, Weisman HF, Mannisi JA, Becker LC.** Progressive impairment of regional myocardial perfusion after initial restoration of postischemic blood flow. *Circulation* 80: 1846–1861, 1989.
2. **Ambrosio G, Zweier JL, Becker LC.** Apoptosis is prevented by administration of superoxide dismutase in dogs with reperfused myocardial infarction. *Basic Res Cardiol* 93: 94–96, 1998.
3. **Bardales RH, Hailey LS, Xie SS, Schaefer RF, Hsu SM.** In situ apoptosis assay for the detection of early acute myocardial infarction. *Am J Pathol* 149: 821–829, 1996.
4. **Benedek A, Moricz K, Juranyi Z, Gigler G, Levay G, Harsing LG Jr, Matyus P, Szenasi G, Albert M.** Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. *Brain Res* 1116: 159–165, 2006.
5. **Chacko VP, Aresta F, Chacko SM, Weiss RG.** MRI/MRS assessment of in vivo murine cardiac metabolism, morphology, and function at physiological heart rates. *Am J Physiol Heart Circ Physiol* 279: H2218–H2224, 2000.
6. **De Celle T, Cleutjens JP, Blankesteijn WM, Debets JJ, Smits JF, Janssen BJ.** Long-term structural and functional consequences of cardiac ischaemia-reperfusion injury in vivo in mice. *Exp Physiol* 89: 605–615, 2004.
7. **Epstein FH.** MR in mouse models of cardiac disease. *NMR Biomed* 20: 238–255, 2007.
8. **Epstein FH, Yang Z, Gilson WD, Berr SS, Kramer CM, French BA.** MR tagging early after myocardial infarction in mice demonstrates contractile dysfunction in adjacent and remote regions. *Magn Reson Med* 48: 399–403, 2002.
9. **Fieno DS, Hillenbrand HB, Rehwald WG, Harris KR, Decker RS, Parker MA, Klocke FJ, Kim RJ, Judd RM.** Infarct resorption, compensatory hypertrophy, and differing patterns of ventricular remodeling following myocardial infarctions of varying size. *J Am Coll Cardiol* 43: 2124–2131, 2004.
10. **Fieno DS, Kim RJ, Chen EL, Lomasney JW, Klocke FJ, Judd RM.** Contrast-enhanced magnetic resonance imaging of myocardium at risk: distinction between reversible and irreversible injury throughout infarct healing. *J Am Coll Cardiol* 36: 1985–1991, 2000.
11. **Flacke S, Allen JS, Chia JM, Wible JH, Periasamy MP, Adams MD, Adzamli IK, Lorenz CH.** Characterization of viable and nonviable myocardium at MR imaging: comparison of gadolinium-based extracellular and blood pool contrast materials versus manganese-based contrast materials in a rat myocardial infarction model. *Radiology* 226: 731–738, 2003.
12. **Fliss H, Gattinger D.** Apoptosis in ischemic and reperfused rat myocardium. *Circ Res* 79: 949–956, 1996.
13. **Franco F, Thomas GD, Giroir B, Bryant D, Bullock MC, Chwialkowski MC, Victor RG, Peshock RM.** Magnetic resonance imaging and invasive evaluation of development of heart failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. *Circulation* 99: 448–454, 1999.
14. **Jordan JE, Zhao ZQ, Vinten-Johansen J.** The role of neutrophils in myocardial ischemia-reperfusion injury. *Cardiovasc Res* 43: 860–878, 1999.
15. **Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P.** Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest* 74: 86–107, 1996.
16. **Kim RJ, Chen EL, Lima JA, Judd RM.** Myocardial Gd-DTPA kinetics determine MRI contrast enhancement and reflect the extent and severity of myocardial injury after acute reperfused infarction. *Circulation* 94: 3318–3326, 1996.
17. **Kuhn DE, Roy S, Radtke J, Khanna S, Sen CK.** Laser microdissection and capture of pure cardiomyocytes and fibroblasts from infarcted heart regions: perceived hyperoxia induces p21 in peri-infarct myocytes. *Am J Physiol Heart Circ Physiol* 292: H1245–H1253, 2007.
18. **Kurrelmeyer KM, Michael LH, Baumgarten G, Taffet GE, Peschon JJ, Sivasubramanian N, Entman ML, Mann DL.** Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. *Proc Natl Acad Sci USA* 97: 5456–5461, 2000.
19. **Naumova AV, Weiss RG, Chacko VP.** Regulation of murine myocardial energy metabolism during adrenergic stress studied by in vivo ^{31}P NMR spectroscopy. *Am J Physiol Heart Circ Physiol* 285: H1976–H1979, 2003.
20. **Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert SR, Cigola E, Anversa P.** Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. *J Mol Cell Cardiol* 28: 2005–2016, 1996.
21. **Oshinski JN, Yang Z, Jones JR, Mata JF, French BA.** Imaging time after Gd-DTPA injection is critical in using delayed enhancement to determine infarct size accurately with magnetic resonance imaging. *Circulation* 104: 2838–2842, 2001.
22. **Rochitte CE, Lima JA, Bluemke DA, Reeder SB, McVeigh ER, Furuta T, Becker LC, Melin JA.** Magnitude and time course of microvascular obstruction and tissue injury after acute myocardial infarction. *Circulation* 98: 1006–1014, 1998.
23. **Rogers WJ Jr, Kramer CM, Geskin G, Hu YL, Theobald TM, Vido DA, Petruolo S, Reichek N.** Early contrast-enhanced MRI predicts late functional recovery after reperfused myocardial infarction. *Circulation* 99: 744–750, 1999.
24. **Ross AJ, Yang Z, Berr SS, Gilson WD, Petersen WC, Oshinski JN, French BA.** Serial MRI evaluation of cardiac structure and function in mice after reperfused myocardial infarction. *Magn Reson Med* 47: 1158–1168, 2002.
25. **Roy S, Khanna S, Kuhn DE, Rink C, Williams WT, Zweier JL, Sen CK.** Transcriptome analysis of the ischemia-reperfused remodeling myocardium: temporal changes in inflammation and extracellular matrix. *Physiol Genomics* 25: 364–374, 2006.
26. **Roy S, Khanna S, Sen CK.** Perceived hyperoxia: oxygen-regulated signal transduction pathways in the heart. *Methods Enzymol* 381: 133–139, 2004.
27. **Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, Zweier JL, Sen CK.** Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. *J Biol Chem* 278: 47129–47135, 2003.
28. **Ruff J, Wiesmann F, Hiller KH, Voll S, von Kienlin M, Bauer WR, Rommel E, Neubauer S, Haase A.** Magnetic resonance microimaging for noninvasive quantification of myocardial function and mass in the mouse. *Magn Reson Med* 40: 43–48, 1998.
29. **Sen CK, Khanna S, Roy S.** Perceived hyperoxia: oxygen-induced remodeling of the reoxygenated heart. *Cardiovasc Res* 71: 280–288, 2006.
30. **Simonetti OP, Kim RJ, Fieno DS, Hillenbrand HB, Wu E, Bundy JM, Finn JP, Judd RM.** An improved MR imaging technique for the visualization of myocardial infarction. *Radiology* 218: 215–223, 2001.
31. **Suzuki H, Wildhirt SM, Dudek RR, Narayan KS, Bailey AH, Bing RJ.** Induction of apoptosis in myocardial infarction and its possible relationship to nitric oxide synthase in macrophages. *Tissue Cell* 28: 89–97, 1996.
32. **Taatjes DJ, Wadsworth MP, Zaman AK, Schneider DJ, Sobel BE.** A novel dual staining method for identification of apoptotic cells reveals a modest apoptotic response in infarcted mouse myocardium. *Histochem Cell Biol* 128: 275–283, 2007.
33. **Weiss RG, Chatham JC, Georgakopolous D, Charron MJ, Wallimann T, Kay L, Walzel B, Wang Y, Kass DA, Gerstenblith G, Chacko VP.** An increase in the myocardial PCr/ATP ratio in GLUT4 null mice. *FASEB J* 16: 613–615, 2002.
34. **Yang Z, Berr SS, Gilson WD, Toufektsian MC, French BA.** 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. *Circulation* 109: 1161–1167, 2004.
35. **Yang Z, Bove CM, French BA, Epstein FH, Berr SS, DiMaria JM, Gibson JJ, Carey RM, Kramer CM.** Angiotensin II type 2 receptor overexpression preserves left ventricular function after myocardial infarction. *Circulation* 106: 106–111, 2002.

36. **Yang Z, Cerniway RJ, Byford AM, Berr SS, French BA, Matherne GP.** Cardiac overexpression of A_1-adenosine receptor protects intact mice against myocardial infarction. *Am J Physiol Heart Circ Physiol* 282: H949–H955, 2002.
37. **Yang Z, French BA, Gilson WD, Ross AJ, Oshinski JN, Berr SS.** Cine magnetic resonance imaging of myocardial ischemia and reperfusion in mice. *Circulation* 103: E84, 2001.
38. **Young AA, French BA, Yang Z, Cowan BR, Gilson WD, Berr SS, Kramer CM, Epstein FH.** Reperfused myocardial infarction in mice: 3D mapping of late gadolinium enhancement and strain. *J Cardiovasc Magn Reson* 8: 685–692, 2006.
39. **Zweier JL, Flaherty JT, Weisfeldt ML.** Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci USA* 84: 1404–1407, 1987.