Contrast-Enhanced Magnetic Resonance Imaging of Myocardium at Risk
Distinction Between Reversible and Irreversible Injury Throughout Infarct Healing

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OBJECTIVES We sought to determine the relationship of delayed hyperenhancement by contrast magnetic resonance imaging (MRI) to viable and nonviable myocardium within the region at risk throughout infarct healing.

BACKGROUND The relationship of delayed MRI contrast enhancement patterns to injured but viable myocardium within the ischemic bed at risk has not been established.

METHODS We compared in vivo and ex vivo MRI contrast enhancement to histopathologic tissue sections encompassing the entire left ventricle in dogs (n = 24) subjected to infarction with (n = 12) and without (n = 12) reperfusion at 4 h, 1 day, 3 days, 10 days, 4 weeks and 8 weeks. In vivo MR imaging was performed 30 min after contrast injection.

RESULTS The sizes and shapes of in vivo myocardial regions of elevated image intensity (828 ± 132% of remote) were the same as those observed ex vivo (241 slices, r = 0.99, bias = 0.05 ± 1.6% of left ventricle [LV]). Comparison of ex vivo MRI to triphenyltetrazolium chloride–stained sections demonstrated that the spatial extent of hyperenhancement was the same as the spatial extent of infarction at every stage of healing (510 slices, lowest r = 0.95, largest bias = 1.7 ± 2.9% of LV). Conversely, hyperenhanced regions were smaller than the ischemic bed at risk defined by fluorescent microparticles at every stage of healing (239 slices, 35 ± 24% of risk region, p < 0.001). Image intensities of viable myocardium within the risk region were the same as those of remote, normal myocardium (102 ± 9% of remote, p = NS).

CONCLUSIONS Delayed contrast enhancement by MRI distinguishes between viable and nonviable regions within the myocardium at risk throughout infarct healing.

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There is no actual table or diagram present in the text. The text is a scientific article discussing the use of contrast-enhanced MRI for distinguishing between reversible and irreversible myocardial injury throughout infarct healing. The article presents methods, results, and conclusions related to the use of MRI in assessing myocardial injury.

During an acute myocardial infarction, myocytes within the ischemic bed do not all die simultaneously, but rather, irreversible injury develops as a transmural wavefront occurring first in the subendocardium and then extending toward the subepicardium (1,2). Clinically, the distinction between reversible and irreversible injury within the risk region is important since selecting the appropriate course of action following an ischemic event depends strongly on whether or not infarction is already transmural or whether some or all of the risk region contains viable myocardium in jeopardy of a future event. This distinction between infarcted myocardium and injured but viable myocardium, however, is often difficult to make, in part because the severity of the contractile dysfunction in the infarct zone does not reflect the transmural extent of necrosis (3).

In a recent study by our group (4), we found a strong association between irreversible myocardial injury and delayed (>5 min) hyperenhancement in magnetic resonance images (MRI) following administration of Gd-DTPA. Hyperenhancement occurred in infarcted regions but was not observed in regions subjected to purely reversible ischemic injury (15 min coronary occlusion) despite the presence of myocardial stunning at the time of the MRI. Other recent reports, however, suggest a different perspective. Rogers et al. (5) studied Gd-DTPA enhancement in 17 patients and found that hyperenhanced regions observed one week after reperfused infarction partially recovered contractile function by seven weeks. The authors interpreted this finding as evidence that hyperenhancement can occur in viable myocardial regions. Pislaru et al. (6) studied an investigational “necrosis avid” MRI contrast agent, Gadophrin-2, in a canine model of acute infarction based in part on the assumption that FDA-approved contrast agents such as Gd-DTPA do not distinguish between reversible and irreversible ischemic injury.

While the physiologic interpretation of contrast-enhanced MRI (ceMRI) remains to be fully elucidated, the quality of the images continues to improve. Segmented inversion-recovery MRI pulse sequences now typically result in hyperenhanced image intensities that are 300% to 500% greater than nonhyperenhanced regions (4), greatly reducing observer subjectivity regarding the shape and transmural extent of hyperenhanced regions. In patients with ischemic heart disease, cine MRI is easily combined with contrast MRI to examine regional wall motion and contrast en-
enhancement with perfect registration in multiple short and long axis views. Considering the wide availability of MRI scanners and the routine use of Gd-DTPA and similar agents for noncardiac clinical indications (5,7,8), these recent improvements in image quality underscore the importance of establishing the relationship of delayed myocardial hyperenhancement to the underlying pathophysiology.

In the present study, we evaluated cMRI of postischemic myocardium following administration of Gd-DTPA at different times throughout infarct healing. Dogs were subjected to either transient 90-min or permanent coronary artery occlusion, imaged in vivo by MRI, and sacrificed at either 4 h, 1 day, 3 days, 10 days, 4 weeks or 8 weeks. All hearts were imaged ex vivo at 500 × 500 × 500-μm spatial resolution and then sectioned into registered contiguous 2-mm-thick tissue slices encompassing the entire left ventricle (LV). Regions of MRI hyperenhancement were compared to regions at risk of infarction defined by fluorescent microparticles, to infarcted regions defined by triphenyltetrazolium chloride (TTC) staining and to histology by light microscopy.

METHODS

Experimental preparation. Twenty-eight 25- to 30-kg mongrel dogs were studied. The care and treatment of all animals was in accordance with the Position of the American Heart Association on Research Animal Use, adopted November 15, 1984. Under sterile technique, a lateral thoracotomy was performed, the pericardium was opened, and the proximal left anterior descending or left circumflex artery was carefully dissected and occluded to produce an infarction. In one group of animals (n = 12), the artery was permanently occluded with a snare ligature; in another group (n = 16), the artery was occluded for 90 min with a removable soft occluder, and then reperfusion was restored. In animals to be studied at one day and beyond, the chest was then sutured closed, and the animals were allowed to recover.

MRI and experimental protocol. Animals were studied at 4 h, 1 day, 3 days, 10 days, 4 weeks and 8 weeks.

In vivo imaging. In vivo MRI images were acquired for all time points except 4 h (due to scanner scheduling limitations). Animals were anesthetized, intubated, transported to the MRI facility and imaged in the right lateral decubitus position (1.5T Siemens Symphony; Erlangen, Germany). Approximately 30 min after 0.3 mmol/kg Gd-DTPA i.v., contiguous 5-mm-thick short-axis T₁-weighted images were acquired from base to apex (ca. 15 per heart) using a segmented inversion-recovery pulse sequence described in detail elsewhere (9). Animals were then sacrificed and the hearts removed for further study. In the animals with reperfused infarcts, the coronary artery was reoccluded before sacrifice and 120 ml of 1.3% fluorescent microparticles (1 to 10 μm in diameter; Duke Scientific Corporation; Palo Alto, California) was injected into the left atrium to identify the myocardium at risk of infarction (10–12).

To test whether or not the observed enhancement patterns were specific to the use of Gd-DTPA, eight animals were imaged on consecutive days using Gd-HP-DO3A on the first day and Gd-DTPA on the next day. Gd-HP-DO3A (ProHance; Bracco Diagnostics, Inc.; Milan, Italy) is another clinically approved contrast agent that has a molecular weight (ca. 800 daltons), T₁ relaxivity (ca. 4 \text{ mmol}^{-1} \text{ s}^{-1}), and biologic distribution (extracellular) similar to Gd-DTPA. Two animals were studied at each of four time points (3 days, 10 days, 4 weeks and 8 weeks).

Ex vivo imaging. After excision, hearts were quickly immersed in cold (4°C) saline solution, rinsed, and ventricular cavities were blotted dry. Balloons containing 99.9% deuterated water (D₂O) were placed in the ventricular cavities and three fiduciary markers defining the short axis of the heart were glued to the epicardium near the base. The hearts were then suspended vertically in a standard knee coil and three-dimensional gradient-echo images (TR = 20 ms, TE = 3.2 ms) were acquired with a spatial resolution of 500 × 500 × 500 μm. T₁-weighting was achieved by the use of a large flip angle (70°).

Histochemical staining. Following ex vivo imaging, hearts were cooled and made partially stiff by short, repeated immersions in 95% ethanol precooled to −80°C and then sectioned into 2-mm-thick short axis slices from base to apex (ca. 25 per heart) using a commercial rotating meat slicer along the plane defined by the three epicardial markers. All slices were stained with 2% TTC and photographed under room light to determine infarct size and under ultraviolet light to determine the myocardium at risk.

Light microscopy. In 24 animals, one transmural block of tissue was obtained from the infarct territory and a second transmural block was obtained from a normal region opposite the infarct territory. Each tissue block was photographed under room light (for TTC) and ultraviolet light (for fluorescent microparticles) and then stained with hematoxylin and eosin (H&E) and Masson’s trichrome.

Data analysis. IN VIVO HYPERENHANCEMENT. Image intensities were measured in hyperenhanced and remote, nonhyperenhanced regions of myocardium using a software package (NIH Image, National Institute of Health, Bethesda, Maryland). Hyperenhancement was defined as an image intensity >3 SDs above the mean of remote myocardium. The spatial extent of hyperenhancement was expressed as a percentage of the LV on a slice-by-slice basis. Consistent with previous analyses (4,13), the measurement
of the spatial extent of hyperenhancement included subendocardial regions of hypoenhancement within the core of the hyperenhanced region if these regions were present.

IN VIVO VERSUS EX VIVO HYPERENHANCEMENT. For each animal, the ex vivo images were registered to the in vivo images for blinded comparison of hyperenhancement patterns. First, the short-axis slice locations acquired in vivo were displayed on an in vivo long-axis image (Fig. 2). Next, each high resolution ex vivo data set was loaded into a multiplanar reconstruction tool (MPR; Siemens Medical Systems; Erlangen, Germany) and contiguous 5-mm-thick short-axis ex vivo images were extracted from the three dimensional data set according to the slice positions displayed in the in vivo long-axis image. A separate observer measured hyperenhanced regions in the same manner used for the in vivo images.

EX VIVO HYPERENHANCEMENT VERSUS INFARCT SIZE AND REGIONS AT RISK. Each three-dimensional ex vivo MRI dataset was also rotated to the short-axis orientation defined by the three epicardial markers and images were extracted at the same slice locations as the histologic slices. An observer blinded to the MRI results measured infarct size using the TTC-stained slices and another observer blinded to the TTC results analyzed the MRIs for the spatial extent of hyperenhancement. The risk regions were analyzed in a similar manner based on photographs taken under ultraviolet light. In all cases, regions were expressed as a percentage of the LV on a slice-by-slice basis. Regions-of-interest (ROI) were also drawn on the ex vivo MRIs to determine the image intensities corresponding to the following: 1) infarcted myocardium (TTC negative); 2) at risk but not infarcted myocardium (fluorescent negative and TTC positive); and 3) normal myocardium (fluorescent positive and TTC positive) remote from the infarction site. The ROIs were placed only on those MRIs in which the corresponding histologic slices clearly demonstrated the presence of all three regions within the same short-axis slice.

LIGHT MICROSCOPY. Pairs of H&E- and trichrome-stained tissue sections were presented to a pathologist (J.W.L.) blinded to the results of the MRI, TTC and fluorescent microparticle examinations. The pathologist examined each section for the presence or absence of irreversible injury and, if present, outlined regions without viable myocytes by hand on the glass slides. Irreversible injury was defined using standard histologic criteria such as coagulation and contraction band necrosis acutely and collagen replacement chronically. Based on these outlines, the percent surface area of each irreversibly injured region was calculated for later comparison to TTC-negative regions and to regions without fluorescent microparticles based on photographs of the same tissue blocks taken before H&E and trichrome staining (see “Experimental Protocol”).

Statistical analysis. All results were expressed as mean ± SD. The method of Bland and Altman (14) was used to compare in vivo versus ex vivo hyperenhancement, hyperenhancement with infarct size and hyperenhancement with the region at risk. Image intensities amongst regions were compared using repeated measures analysis of variance with Bonferroni correction. All tests were two tailed and p < 0.05 was considered significant.

RESULTS

In vivo and ex vivo contrast enhancement. Hyperenhancement was observed in vivo at 1 day, 3 days, 10 days, 4 weeks and 8 weeks after nonreperfused (top row) and reperfused (bottom row) myocardial infarction.

![Figure 1.](image-url) Hyperenhancement was observed at 1 day, 3 days, 10 days, 4 weeks and 8 weeks after nonreperfused (top row) and reperfused (bottom row) myocardial infarction.
(241 slices, lowest r = 0.99), the mean difference in area was nearly zero (largest bias = 0.70% of LV area) and the limits of agreement (LOA) were narrow (largest LOA = ±3.1% of LV area).

Hyperenhancement versus infarct size. Figure 3 compares ex vivo ceMRI to corresponding TTC-stained sections. The location and spatial extent of ceMRI hyperenhancement appeared to be the same as the infarct zone delineated by TTC. Linear regression analysis revealed a high correlation coefficient (510 slices, lowest r = 0.95). Bland-Altman analysis showed a high degree of agreement between MRI and TTC at every stage of infarct healing regardless of reperfusion status (largest bias = 1.7% of LV area; maximum LOA = bias ± 5.7% of LV area). Similarly, when total infarct size by TTC (mean, 8.0 ± 1.6% of LV; range, 0.5 to 29.2) was compared to the spatial extent of in vivo hyperenhancement (8.2 ± 1.5%; range, 1.0 to 30.5), a correlation coefficient of 0.96 was found and Bland-Altman analysis showed a high degree of agreement (bias: 0.24% of LV; limits of agreement of +8.0 and −7.6% of LV).

Hyperenhancement versus regions at risk. Areas of hyperenhancement were smaller than areas at risk (Fig. 4, 35 ± 24% of the risk region, largest bias = 17.9% of LV area), and the LOA were wide (maximum LOA = bias ± 16.7% of LV area).

ceMRI within the risk region. Figure 5 compares hyperenhanced, infarcted and risk regions of an animal with a reperfused acute infarction. The risk region (area without fluorescence) was significantly larger than the infarct (TTC negative). The MRI demonstrates that viable myocardium within the risk region (“at risk but not infarcted”) did not hyperenhance. Light microscopy confirmed that myocytes in the “at risk but not infarcted” region (region 2 of Fig. 5) were viable. Figure 6 compares ceMRI image intensities

Figure 2. Hyperenhancement observed in vivo was similar to that observed ex vivo.

Figure 3. The size and shape of hyperenhanced regions by MRI closely matched those of irreversible injury defined by TTC throughout infarct healing.
within the risk region to remote, normal myocardium. Within the risk region, image intensities were elevated in nonviable regions (243 ± 54% of remote, p < 0.001) but not in viable regions (102 ± 9% of remote, p = NS) for every stage of infarct healing.

**Light microscopy.** Of the 55 pairs of H&E- and trichrome-stained slides, the pathologist identified regions of irreversible injury in 33 of 33 paired slides from sections with TTC-negative regions and 0 of 22 paired slides from sections without TTC-negative regions. The percent surface areas of regions without viable myocytes identified by the pathologist by light microscopy were similar to the percent surface areas of TTC-negative regions (r = 0.99, bias = 1.19% of total tissue area) but were significantly smaller than the percent surface areas of regions without fluorescent microparticles (bias = 16.14%). Within irreversibly injured regions, the pathologist identified the full range of histologic features characteristic of infarcts at various stages of healing, including coagulation and contraction band necrosis (Fig. 5, arrows), neutrophils and macrophages in infarcts 4 h to three days old, granulation tissue in 10-day-old and four-week-old infarcts and mature collagenous scar at eight weeks.

**Gd-HP-DO3A versus Gd-DTPA.** For the eight animals in which in vivo ceMRI was performed using both contrast agents, no differences were detected in the spatial extent of hyperenhancement (92 slices, bias = 0.4 ± 3.2% of LV area, p = NS).

**DISCUSSION**

The main findings of this study were as follows: 1) the spatial extent of in vivo and ex vivo hyperenhancement was identical when the same slice thickness was used (5 mm); 2) the spatial extent of ex vivo hyperenhancement at high resolution (0.5 × 0.5 × 0.5 mm) was the same as infarct size defined by TTC; and 3) within the risk region, viable myocardium was distinguished from nonviable myocardium as nonhyperenhanced and hyperenhanced regions, respectively. These findings held throughout infarct healing and irrespective of reperfusion status.

**Comparison to previous studies.** Several previous studies by our group (13,15) and others (16–22) have compared contrast enhancement to infarct size using “thick” histologic sections cut by hand and typically not precisely oriented in the same plane as the images. In a recent report by our group (4), we developed a method by which high resolution ex vivo images could be accurately registered with “thin” (2 mm) histologic sections. The purpose of the current study was to apply this method to address three key issues raised but not addressed by our recent report (4) or by previous studies (16–22). First, we systematically compared in vivo to ex vivo enhancement to determine if the data comparing ex vivo images to histology can be used as a basis for the interpretation of in vivo images. Second, we used fluorescent microparticles to determine whether hyperenhancement occurs in viable regions at risk of infarction that surround infarcted regions. Third, we studied hyperenhancement throughout infarct healing because it remained unclear whether or not hyperenhancement would be observed at all stages of infarct healing and how hyperenhancement, if present, would relate to the profound cellular-
level changes that occur during the transition from acutely necrotic myocytes to collagenous scar. Study of these issues with a level of detail significantly beyond that of previous studies was motivated by observations of clearly defined, nontransmural regions of hyperenhancement in patients with coronary artery disease (4,9).

**Timing of in vivo imaging.** All images were acquired approximately 30 min postcontrast for two reasons. First, in earlier studies (8,13,15), we found that “delayed” hyperenhancement is established by approximately 5 min postcontrast. Second, we have empirically observed that the endocardial border is often more easily detected at later times because the image intensity of the blood pool decreases at a faster rate than that of hyperenhanced myocardial regions. The data of the current study do not address the time course of first-pass contrast enhancement or the time course of contrast penetration in regions of microvascular damage (13,15).

**Contrast enhancement of the risk region.** We found that the myocardial region at risk is characterized by two different enhancement patterns: hyperenhanced and nonhyperenhanced. Hyperenhancement appeared in a subregion within the risk region and was exclusively associated with nonviable myocardium. Regions without hyperenhancement but within the risk region were found to contain viable myocytes both by TTC staining and light microscopy. The association of hyperenhancement to nonviable myocardium was observed throughout infarct healing and was unaffected by reperfusion status.

To our knowledge, our data are the first in which contrast enhancement within the risk region has been studied beyond 24 h postinfarct. One of the few studies to address this issue within the first 24 h is that of Schaefer et al. (16) who, unlike our current findings, reported that hyperenhancement overestimated infarct size. It should be noted, however, that the animals in the study by Schaefer et al. (16) received the MRI contrast agent during reactive hyperemia, suggesting that hyperenhancement may have occurred in hyperemic regions without infarction as well as in infarcted regions. In a smaller group of animals reported in the same article, Schaefer et al. (16) found that when the contrast agent was administered 90 min after reperfusion, the hyperemic response was no longer present and the spatial extent of hyperenhancement was much smaller (8.6% vs. 25.9% of LV) despite similar regions at risk and infarct sizes.

**Contrast enhancement of the infarct zone.** In agreement with previous findings, our data show hyperenhancement of acute infarcts 4 h to 3 days old both with (15–18,21,22) and without (19,20,23,24) reperfusion. For older infarcts, the data of some previous studies indicate that hyperenhancement is observed (8,22,25) whereas others do not (26,27). In the present study, we found that hyperenhancement persists at 10 days, 4 weeks and 8 weeks. Discrepant findings regarding whether or not older infarcts hyperenhance may relate to both the process of infarct healing and the MRI technique employed. Myocardial infarcts may shrink by as much as 400% during infarct healing (28) making older infarcts inherently more difficult to detect. In addition, pulse sequences with a more limited ability to distinguish differences in postcontrast longitudinal relaxation times (T1) may reduce an observer’s ability to detect infarcted regions (9).

In a previous study of two-day-old reperfused infarcts (13), we found that the spatial extent of in vivo hyperenhancement was 12% larger than infarcted regions. The method of our current study was developed specifically to improve our ability to examine this issue. In vivo spatial resolution was approximately eightfold higher (1.2 × 3.3 × 10 mm/1 × 1 × 5 mm × 8) and the number of histologic slices per animal was 25 compared to 5 in our previous study (13). Perhaps more importantly, the intermediate stage of ex vivo imaging greatly improved our ability to register indi-
vidual slices. When examined at this level of detail, the data indicate that the spatial extent of hyperenhancement is identical to infarct size. It is important to note that the spatial extent of hyperenhancement may appear slightly larger than infarct size when spatial resolution is not adequate to portray the three-dimensional shape of the infarct (4) (partial volume effect).

Subendocardial regions of hypoenhancement within larger regions of hyperenhancement were detected in 12 of the 24 animals. Impeded contrast penetration may relate to microvascular occlusion (13,18,29) in reperfused and nonreperfused infarcts and/or a closed infarct-related artery in the nonreperfused group.

In summary, when spatial resolution is adequate, regions “at risk but not infarcted” do not hyperenhance and hyperenhancement is identical to infarct size throughout infarct healing. We conclude that delayed contrast enhancement by MRI distinguishes between viable and nonviable regions within the myocardium at risk.

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