Myocardial Magnetic Resonance Imaging Contrast Agent Concentrations After Reversible and Irreversible Ischemic Injury

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Background—Discrepant reports have been published recently regarding the relationship of contrast-enhanced magnetic resonance image intensities to reversible and irreversible ischemic injury. Unlike image intensities, contrast agent concentrations provide data independent of the MRI technique. We used electron probe x-ray microanalysis (EPXMA) to simultaneously examine concentrations of Gd, Na, P, S, Cl, K, and Ca over a range of myocardial injuries.

Methods and Results—Reversible and irreversible injury were studied in 38 rabbits divided into 4 groups defined by occlusion and reperfusion time, as well as time the animals were euthanized. Gd-DTPA was administered, and the hearts were excised and rapidly frozen, cryosectioned, freeze-dried, and examined by EPXMA in up to 3 regions: remote, infarcted, and at risk but not infarcted. Infarcted regions were defined by anti-myoglobin antibody or triphenyltetrazolium chloride staining. Regions at risk were defined by fluorescent microparticles administered during occlusion.

Compared with remote regions, in acutely infarcted regions, Gd was increased (235 ± 24%, P < 0.005) in the same 50×100-μm areas in which Na was increased (154 ± 5%, P < 0.001) and K was decreased (52 ± 8%, P < 0.001). Similarly, in chronically infarcted regions, Gd was increased (472 ± 78%, P < 0.001) in areas in which Na was increased (332 ± 28%, P < 0.001) and K was decreased (47 ± 5%, P < 0.001). Also compared with remote regions, however, concentrations of Gd, Na, and K were not elevated after reperfusion in regions that were at risk but not infarcted (P = NS).

Conclusions—Regional elevations in myocardial MRI contrast agent concentrations are exclusively associated with irreversible ischemic injury defined histologically and by regional electrolyte concentrations. (Circulation. 2002;105:224-229.)

Key Words: magnetic resonance imaging ■ contrast media ■ infarction ■ ischemia

Contrast-enhanced magnetic resonance (MR) images of the heart are playing an increasing role in clinical cardiac imaging.1,2 Although the quality of these images has improved,3 discrepant reports continue to appear in the literature concerning their physiological interpretation.4–10 Data from our laboratory5,6 and others10 suggest that hyperenhanced myocardial regions observed after administration of extracellular MRI contrast agents such as Gd-DTPA are exclusively associated with irreversible ischemic injury, whereas results from other laboratories suggest a less specific8 or even contrary4,7,9 interpretation. These issues must be resolved before the full potential of contrast MRI can be recognized.

Discrepancies in the literature may be due in part to differences in technique. Most studies require registration either of images acquired during MRI scans on different days7,9 or of in vivo images to ex vivo histological tissue sections.4–6 In both cases, the results will be strongly affected by registration errors. In addition, older MRI techniques often resulted in poorer image quality1 that complicated interpretation.

One approach to addressing these issues is to directly study regional contrast agent concentrations rather than image intensities. This approach would eliminate issues such as image registration and limitations of in vivo image quality and would directly address the underlying physiology portrayed by contrast-enhanced MRI.

Contrast agent concentrations can be determined with a variety of techniques such as radiolabeling11 and inductively coupled plasma atomic emission spectroscopy (ICP-AES).12 Although useful, these approaches are limited by the need to acquire at least a few milligrams of tissue, and the results are directly affected by the locations from which the samples are taken. Ideally, one would like to measure myocardial tissue concentrations on the scale of tens of microns and relate these
Animal Groups

Regions Examined By EPXMA

Figure 1. A, Animal groups 1 to 4; reperfusion time is given by difference between occlusion time and time animals were killed, except for group 4, which was permanently occluded. B, Regions of short-axis slice examined by EPXMA.

to myocardial injury with comparable spatial resolution. In previous studies by our group\textsuperscript{13,14} and others,\textsuperscript{15,16} electron probe x-ray microanalysis (EPXMA) has been used to examine myocardial concentrations of endogenous elements such as sodium and potassium. The EPXMA technique is also commonly used to detect metals and other foreign materials in tissue.\textsuperscript{17} and in pilot experiments, we verified that EPXMA allows examination of the element present in most MRI contrast agents, gadolinium. This affords the opportunity to study regional Gd concentrations simultaneously with those of all endogenous nuclei sodium, phosphorus, sulfur, chlorine, potassium, and calcium over a range of reversible and irreversible myocardial injuries.

**Methods**

**Experimental Preparation**

Thirty-eight 2.5-kg New Zealand White rabbits were studied. Four animal groups (1 to 4) were defined by occlusion and reperfusion times as shown in Figure 1. In each animal, EPXMA was performed in up to 3 regions: infarcted, remote, and at risk but not infarcted (RNI; see Figure 1). All animals were treated according to the standards given in the “Position of the American Heart Association on Research Animal Use” adopted November 15, 1984.

**Experimental Protocol**

After anesthesia and mechanical respiration, the chest was opened and the anterior branch of the left coronary artery was occluded. Groups 1, 2, and 3 were reperfused, whereas group 4 was not (Figure 1). In all groups, 50 mL of Gd-DTPA (Magnevist, Berlex) was infused into the jugular vein at a constant rate over a 20-minute period. The agent was allowed to circulate for 10 minutes. Sodium pentobarbital was then administered, and the hearts were quickly excised. In groups 1 and 2, the risk region was defined by fluorescent microparticles injected into the left atrium 1 minute before the animals were killed and immediately after the same coronary artery was reoccluded. The microparticles (zinc cadmium sulfide yellow fluorescent, 1 to 10 μm, or polymer microparticles, 10 μm, both from Duke Scientific Corp) were prepared in advance by dissolving 2 g in 10 mL of saline and emulsifier (0.1 mL of Tween 20, Sigma).

**Histology**

Immediately after heart removal, the heart was washed with saline, embedded in OCT (Sakura Finetek), and rapidly frozen by immersion in liquid nitrogen. In a cryomicrotome (−20°C), short-axis sections were cut (5 μm for histology and 15 μm for EPXMA). Consecutive 5-μm slices were stained with hematoxylin and eosin, trichrome, and mouse anti-human anti-myoglobin monoclonal antibody\textsuperscript{18} (ARP, Inc). The next consecutive 15-μm slice was mounted on a scanning electron microscope stub with double-sided adhesive carbon tape and freeze-dried for EPXMA. The next consecutive 1-mm-thick slab was cut by hand, thawed, and stained with triphenyltetrazolium chloride (TTC). One slice distal to the occlusion site was analyzed per animal.

Three regions were defined: infarcted, RNI, and remote. Infarcted regions were defined by the absence of cardiac myoglobin\textsuperscript{18} or a TTC-negative reaction. In scar tissue (group 4), infarcted regions were also defined on the basis of blue trichrome stain. RNI was defined as any region that was visible as indicated by the presence of cardiac myoglobin or a TTC-positive reaction despite an absence of flow by microparticles during occlusion.

**EPXMA: Spectroscopy**

The EPXMA techniques used were similar to those described in detail and validated by other groups\textsuperscript{15,16,19} and those reported previously by our group.\textsuperscript{13,14} In brief, we used an electron microscope (SEM-4500, Hitachi) and an EPXMA detector (Voyager X-ray detector, Noran Systems). In pilot experiments designed to determine whether Gd could be detected in the tissue, analysis of the x-ray peaks from the tissue was performed with standard software on our EPXMA system. Within each x-ray spectrum, the software identified between 3 and 5 x-ray peaks known to be uniquely associated with each region per animal were averaged. To ensure an adequate signal-to-noise ratio, the slice thickness was 15 μm, acquisition time was 600 seconds, and multiple spectra per region per animal were averaged. To ensure excitation of Gd, we used a higher acceleration voltage (20 kV) and beam current (50 μA) than are typically used for tissue samples. For quantification, the following peaks were used: K ($\lambda_{\alpha}$ 4.048 keV), Na ($\lambda_{\alpha}$ 3.307 keV), Mg ($\lambda_{\alpha}$ 1.253 keV), Ca ($\lambda_{\alpha}$ 1.504 keV), Cl ($\lambda_{\alpha}$ 0.351 keV), K ($\lambda_{\alpha}$ 0.842 keV), and Na ($\lambda_{\alpha}$ 0.747 keV).

**Data Analysis**

The sizes of each peak in the EPXMA spectra were expressed as a peak-to-background (P/B) ratio as described by Hagler et al.\textsuperscript{19} P/B is proportional to the relative concentration of the element.\textsuperscript{19} The P/B ratios from spectra in the same animal and region (nominally 5) were averaged. To account for animal-to-animal variations in tissue
thickness, instrumentation drift, etc, P/B ratios from irreversibly (infarcted) or reversibly (RNI) injured regions were divided by the P/B ratios from the remote region of the same animal and expressed as percent of the remote region.

**EPXMA: Imaging**

To examine concentrations across the entire heart (eg, Figure 5), EPXMA spectra were acquired in a rectangular grid comprising 0.5×0.5-mm squares. Acquisition time for EPXMA images was ~8 hours. Composite “images” were constructed for which the grayscale intensity was determined by the elemental P/B at that location.

**Statistics**

Elemental concentrations in infarcted, RNI, and remote regions were compared with the paired t test on P/B ratios. Differences were considered significant at the P<0.05 level. The 95% CIs were calculated based on P/B ratios normalized to remote regions and corrected for the number of animals. The minimum difference in regional Gd concentrations that could be detected was calculated as 2 times the mean divided by the SEM of the differences in P/B of RNI and remote regions. The variation in Gd concentrations within infarcts of individual animals was assessed by calculation of the SEE of P/B in infarcted regions.

**Results**

Gd was detected in all animals and in all myocardial regions (infarcted, RNI, and remote; P<0.001 for each region). The minimum difference in Gd concentrations that could be detected was ±17% of the remote region.

Figure 2 is a typical example of acute irreversible injury in an animal from group 2. The short-axis slice stained for cardiac myoglobin (Figure 2A) shows a large lateral infarct. From this stain, the cartoon of Figure 2C was drawn. Figure 2D shows a photograph of the tissue mounted on a scanning electron microscope stub. Two spectra were obtained from within the squares shown in Figure 2D. Compared with the remote region (Figure 2E), concentrations of Gd, Na, and Cl were elevated in the infarcted region, whereas those of P and K were decreased (Figure 2F).

Figure 3 shows an example from an animal subjected to reversible injury (group 1). Regions perfused during the occlusion are shown by fluorescent microparticles in Figure 3C. As opposed to the case of irreversible injury (Figure 2), spectra from the region of reversible injury (RNI) look very similar to those of the remote region, both shown in Figure 3, with respect to all elements.

Figure 4 shows results for an animal with chronic irreversible injury (group 4). The trichrome stain (Figure 4A) contains scar on the left (blue) and viable myocardium on the right (red). Compared with the spectrum from the viable region (Figure 4C), concentrations of Gd, Na, and Cl were elevated in the scar (Figure 4D), whereas those of P and K were decreased. Figure 5 is an example of EPXMA imaging in a chronically infarcted heart of group 4. In the infarcted...
territory (blue region in trichrome stain), concentrations of Gd, Na, and Cl were elevated (high image intensity), whereas those of P and K were decreased (low image intensity).

Figure 6 summarizes the results from all animals and all elements. Remote concentration by definition is 100% (dashed line). Five floating bars are plotted per element, where the 2 left bars represent reversible injury and the 3 right bars represent irreversible injury. In reversible injury, no statistically significant differences of electrolyte or contrast agent concentrations were observed compared with remote concentrations (P=NS for all elements). Conversely, in acutely infarcted regions (groups 2 and 3), Gd was increased (235±24%, P<0.005), Na was increased (154±5%, P<0.001), and K was decreased (52±8%, P<0.001). In chronically infarcted regions (group 4), Gd was increased (472±78%, P<0.001), Na was increased (332±28%, P<0.001), and K was decreased (47±5%, P<0.001). Each of these differences was significant at the P<0.05 level after adjustment for multiple comparisons. In all groups, Gd concentrations followed those of Na and were inversely related to those of K. The variation in Gd concentrations within infarcts of individual animals was ±27%.

Discussion

We detected Gd in all myocardial regions (remote, infarcted, and RNI). Compared with remote normal myocardial regions in the same heart, the concentration of Gd was higher in regions of acute and chronic irreversible injury defined histologically. These elevations in Gd concentrations were observed in the same 50×100-μm regions that exhibited elevations in Na concentrations and reductions in K concentrations, again compared with remote normal regions of the same heart. Conversely, Gd concentrations in RNI regions were not different from Gd concentrations in remote normal regions of the same heart. These data strongly suggest that regional elevations in Gd concentrations are exclusively associated with irreversible ischemic injury at the postcontrast time point studied.

Acute Infarcts

The finding that Gd concentrations were elevated in regions of acute infarction is consistent with other reports and with previous observations that MR image intensities are elevated in acute infarcts both with6,11,20–22 and without6,23–25 reperfusion. Because the data for Gd were acquired from the same regions as Na, P, S, Cl, K, and Ca, these new data for Gd can be compared with previous studies of the endogenous elements.

Chronic Infarcts

The present study is the first to report Gd concentrations in myocardial scar. The finding that Gd concentrations were
elevated in regions of myocardial scar (Figure 6) is consistent with several recent studies that found that MR image intensities were elevated in these regions, although some studies have not found elevated image intensities. The latter studies used older MR imaging techniques. There are relatively few previous studies that directly address electrolytic concentrations in myocardial scar. Using MR chemical shift imaging, Von Kienlin et al examined high-energy phosphates in chronic infarcts of rat hearts and found significant reductions. Horn et al showed that Na concentrations by nuclear MR spectroscopy and by ion chromatography were elevated in scar but not in reversibly injured myocardial regions. Similarly, in the present study, we found reduced P and elevated Na concentrations in scar by EPXMA (Figure 6). The finding that both Gd and Na concentrations are elevated in scar and in acute infarcts suggests a relationship between the mechanisms that govern Gd and those that govern Na.

Severe but Reversible Ischemic Injury
Our finding that Gd concentrations were not different in reversibly injured regions compared with remote normal regions in the same heart is consistent with previous observations that MR image intensities are isointense in injured but viable myocardium compared with remote myocardium in both dogs and humans. In view of the data showing that Gd concentrations were not different from normal regions after reversible ischemic injury, it appears that an event specific to myocyte death may be responsible for the elevated concentrations of Gd and consequently for the elevated image intensities observed after irreversible injury.

Electrolyte concentrations in regions of severe but reversible myocardial injury have been studied previously by a number of investigators. The data demonstrate that although electrolyte concentrations change during ischemia, they return to nearly normal levels after reperfusion in reversibly injured regions. For example, Jennings et al studied Na and K concentrations by atomic absorption spectrophotometry after a brief occlusion period (15 minutes) and found them to be similar to those of remote tissue after reperfusion. Similarly, in the present study, we found that Na and K concentrations were unchanged compared with remote myocardium in regions subjected to a 10-minute occlusion period, as well as in reversibly injured regions surrounding reperfused infarction (Figure 6). Myocardial edema after occlusion/reperfusion has been shown to increase extravascular volume in the absence of significant myocyte necrosis. This edema is primarily related to swelling of the myocyte and, in the presence of an intact sarcolemmal membrane, would not be expected to increase the distribution volume of Gd-DTPA.

Study Limitations
Sensitivity analysis revealed that the minimum difference in Gd concentrations that could be detected was 17%. Our data do not exclude the possibility that Gd concentrations in RNI regions differ from Gd concentrations in remote regions by <17%. By comparison, Gd concentrations in infarcted regions were >130% higher than in remote regions.

EPXMA required a higher dose than that used clinically. Because Gd-DTPA is biologically inert and is not actively transported, concentrations would be expected to be linearly dependent on dose. In this case, the regional differences (eg, ratio of infarcted over remote regions) reported in the present study are likely to be similar to those observed at other doses. The possibility that Gd distributions differ at higher doses cannot be excluded, however.

Absolute Gd concentrations vary with time after contrast agent administration. Although regional differences in Gd concentrations may vary to a lesser degree, we examined Gd concentrations at one specific time (20 minutes) after contrast administration that we considered sufficiently close to our clinical protocol of delayed hyperenhancement. The timing of contrast administration (infusion) differed from that used clinically (bolus), however, and conclusions about Gd concentrations at earlier or later time points after contrast administration cannot be drawn.

Acknowledgments
This work was supported by National Institutes of Health (National Heart, Lung and Blood Institute) grants R29-HL53411 and R01-HL63268 (Dr Judd). The authors thank Michelle Parker, MS, for the statistical analysis, Vinayak P. Dravid, PhD, for guidance regarding the EPXMA technique, and Francis J. Klocke, MD, for helpful discussions.

References


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Circulation. 2002;105:224-229
doi: 10.1161/hc0202.102016

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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