Assessment of Myocardial Salvage After Ischemia and Reperfusion Using Magnetic Resonance Imaging and Spectroscopy

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To test the hypothesis that contrast-enhanced magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) can differentiate reversible from irreversible myocardial injury, these modalities were used to study ischemia and reperfusion in a rat model. The presence of ischemia and reperfusion were confirmed with radiolabeled microspheres (n=6). Groups of animals were subjected to either 16 (n=17), 30 (n=14), 60 (n=11), or 90 (n=14) minutes of left coronary artery (LCA) occlusion and 60 minutes reperfusion. After albumin–gadolinium (Gd)-DTPA injection, contrast-enhanced, T₁-weighted, spin-echo proton images were acquired at baseline and every 16 minutes during LCA occlusion and reperfusion. In separate experiments, 3¹P phosphorus (3¹P) spectra were acquired at similar time points during ischemia and reperfusion. After 16 minutes occlusion, normally perfused myocardium enhanced significantly compared with ischemic myocardium on MRI (104±7.9% vs. 61±11.0%, p<0.05, n=5, mean±SEM, % of baseline value). MRS showed reduced phosphocreatine (PCr) and adenosine triphosphate (ATP) (58.8±2.4%, p≤0.01; 81.4±2.4, p≤0.01, n=12). After 16 or 30 minutes ischemia, reflow resulted in uniform MRI signal intensity of the ischemic zone compared with normal myocardium (93.5±11.3 vs. 80.9±7.0, p=NS, n=11, % of baseline value at 30 minutes reperfusion) and PCr recovery on MRS (94.3±4.0%, p=NS, n=20, % baseline value at 30 minutes reflow). After 60 and 90 minutes ischemia, reflow resulted in marked enhancement of reperfused compared with normal myocardium on MRI (254.0±30.0 vs. 78.3±9.2, p≤0.01, n=10) and no recovery of PCr on MRS (64.1±3.0, p=NS, n=14). Triphenyltetrazolium chloride (TTC) staining revealed transmural myocardial infarction (MI) in all hearts subjected to 60 or 90 minutes ischemia and reflow, and small nontransmural MIs in only 2/11 hearts subjected to 16 or 30 minutes ischemia and reperfusion. Thus, 1) MRI with albumin–Gd-DTPA is useful for identifying myocardial ischemia by enhancing the contrast between normally perfused and ischemic myocardia; 2) MRI with albumin–Gd-DTPA is useful for identifying reperfusion after myocardial ischemia; and 3) after reperfusion, reversible can be distinguished from irreversible myocardial injury by characteristic findings on MRI and MRS. (Circulation 1989;80:969–982)

With current efforts to limit infarct size with intravenous thrombolytic agents, noninvasive techniques must be developed to detect coronary reperfusion and evaluate the impact of reperfusion on myocardial salvage. Although various clinical parameters and radionuclide imaging techniques have been described to assess coronary reperfusion, these generally lack sensitivity and may not provide information soon enough to allow alternative interventions.1,2

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Recently, magnetic resonance imaging (MRI) has been useful in identifying ischemic and reperfused myocardia. Both ex vivo\textsuperscript{3,4} and in vivo\textsuperscript{5} studies have shown increased T\textsubscript{2} values and increased signal intensity of reperfused infarcted myocardium compared with normal or ischemic myocardium. However, these changes were not always sufficient to permit reliable assessment of reperfusion. Other investigators have shown that gadolinium diethylentriamine pentaacetic acid (Gd-DTPA), a paramagnetic contrast agent, is useful for early identification of myocardial ischemia\textsuperscript{6-8} and reperfusion\textsuperscript{9-12} by increasing the contrast among normal, ischemic, and infarcted/reperfused myocardial tissue.

Although Gd-DTPA provides a significant advantage over magnetic imaging without contrast agents, in terms of detecting myocardial ischemia and reperfusion, the fact that this agent diffuses easily into the extravascular space and is rapidly cleared from normal myocardium limits its usefulness as a marker of myocardial blood flow. Furthermore, accumulation of Gd-DTPA is, at least partially, a function of the size of interstitial space and, hence, the amount of edema.\textsuperscript{13,14}

Previous studies from this laboratory demonstrate that albumin–Gd-DTPA, a macromolecular paramagnetic contrast agent largely confined to the intravascular space, enhances normal myocardium for prolonged periods of time after intravascular infusion.\textsuperscript{15} This marker allows for the identification of myocardial ischemia and may also allow for the assessment of reperfusion.\textsuperscript{16} The present study was designed to assess the ability of MRI with albumin–Gd-DTPA 1) to identify ischemic myocardium early after coronary occlusion in an animal model of ischemia and reperfusion, 2) to differentiate irreversibly damaged from salvaged myocardium after reperfusion, and 3) to correlate contrast-enhanced MRI changes with those observed in \textsuperscript{31}phosphorus (\textsuperscript{31}P) magnetic resonance spectroscopy (MRS).

Methods

Animal Model of Acute Myocardial Ischemia and Reperfusion

A rodent model of reversible myocardial ischemia with reperfusion was used as described previously.\textsuperscript{16,17} After anesthesia induction (pento-barbital, 40 mg/kg i.p.), an i.v. catheter was inserted into the internal jugular vein, and a tracheostomy was performed allowing ventilation on a Harvard rodent respirator. The thorax was opened by median sternotomy and a reversible coronary artery snare occluder was placed around the proximal left coronary artery (LCA). After visually testing the snare during a brief period of occlusion and reperfusion, the thoracotomy was closed and the animal placed in the bore of a superconducting magnet for MRI or MRS.

MRI and MRS Protocol

Groups of animals were subjected to either 16, 30, 60, or 90 minutes of LCA occlusion followed by 60 minutes of reperfusion. During ischemia and reperfusion, MR images or spectra were acquired at baseline, after 2 minutes of ischemia, every 15 minutes during ischemia, after 2 minutes of reflow, and every 15 minutes thereafter for 1 hour of reperfusion. Imaging and spectroscopy experiments were performed on separate groups of animals.

MRI

MR images of the rat myocardium were acquired with a General Electric CSI 2.0 Tesla (85.6 MHz proton resonance frequency), chemical-shift imaging spectrometer (General Electric Medical Systems, Fremont, California). An EKG gated, two-dimensional (128×256 pixels) Fourier-transform spin-warp proton-imaging sequence (TE-15 msec) was used yielding single-slice (3-mm thick), four-phase cycled image acquisitions for each of 128 views. Transverse images were obtained at the level of the left ventricle over an 80×80-mm field of view. The image acquisitions were gated using an Accusync EKG monitor (Advance Medical Research, Milford, Connecticut), using the R wave to gate the CSI pulse-sequence trigger with a variable delay (average 15–20 msec) so that acquisitions were timed to early cardiac systole. With image acquisition gated to consecutive heart beats, the resulting range for TR times was 250–300 msec.

After acquisition of a baseline image without contrast agent, the LCA was occluded and albumin–Gd-DTPA (160 ml/kg) was injected i.v. through a tail vein. Animals (n=22) were subjected to 16 (n=5), 30 (n=6), 60 (n=6), or 90 minutes (n=5) of myocardial ischemia followed by 60 minutes of reperfusion. Repeat T\textsubscript{1}-weighted transverse or coronary images of the heart were acquired every 15 minutes during ischemia and reperfusion. A control group of animals (n=8) was subjected to 90 minutes of LCA occlusion and 60 minutes of reperfusion as described herein. However, the contrast agent was withheld until after 30 minutes of reflow. MR images were acquired at the same times as during the regular imaging protocol.

To control ventricular arrhythmias, lidocaine (10 mg/kg) was injected i.v. before induction of ischemia and, again, before reperfusion. All rats were killed by cardiectomy after imaging. The hearts were then sliced transversely from apex to base and incubated in a 1% solution of triphenyltetrazolium chloride (TTC) as outlined (see Histological Staining).

MR Image Analysis

MRI intensity values from the MR images were measured with GE CSI software, using the contour video algorithm, from regions of interest (ROI) (minimum of 20×20 pixels) defined in the ischemic
left ventricular free wall, left ventricular cavity directly adjacent to the ischemic region, the non-ischemic ventricular septum, and the oil phantom. The ROI was kept at constant volume in all images for each rat studied. To allow for possible drift in image characteristics, the intensities were normalized to the phantom for each image using the equation: relative intensity = intensity/sample intensity of phantom. The signal enhancement of normal and ischemic myocardia was computed according to the equation: signal enhancement (\%) = (SI post - SI pre/SI pre) × 100, in which SI pre and post represent the normalized signal intensity before and after administration of albumin–Gd-DTPA. All data analyses were done by two observers blinded to the duration of ischemia to which the animals were subjected.

**MRS**

Spectroscopy experiments were performed in animals that had a modified solenoid coil acutely implanted around the heart similar to the implant method described by Koretsky et al.\(^{18}\) \(^{31}\)P MR spectra were acquired at 97.3 MHz on a 5.6 Tesla superconducting magnet with a 7.6-cm bore (Nalorac Cryogenics Corp, Concord, California), interfaced with a home-built spectrometer using a Nicolet 1180/293B data system. Spectra were acquired over 4 minutes (184 scans) using a 10-\(\mu\)sec pulse width with a 1-sec interpulse delay. Data were collected in 4 K data points and Gaussian multiplied to give a line broadening of 20 Hz before Fourier transformation. After baseline correction using a convolution difference algorithm, spectral peak areas were quantified using the NTCCAP simulation routine (Nicolet, Fremont, California). All data analyses were done with the observer blinded to the duration of ischemia to which the animals were subjected.

Animals were subjected to 16 minutes (n=12), 30 minutes (n=8), 60 minutes (n=5), or 90 minutes (n=9) of ischemia followed by 60 minutes of reflow. Spectra were acquired prior to ischemia, immediately after ischemia, every 15 minutes during ischemia, immediately after reperfusion, and every 15 minutes during the 1-hour reperfusion period as described previously.

**Histochemical Staining**

At the end of each experiment, the hearts (n=24) were sliced transversely from apex to base in 2-mm thick slices. The tissue samples were then incubated in TTC for 5–10 minutes until viable myocardium was stained brick red.\(^{19}\) Infarcted myocardium fails to stain with TTC. It should be noted that the animals were killed approximately 1 hour after the MR experiment was completed, yielding a total reperfusion time of approximately 2 hours.

**Pathology**

Myocardial samples from animals subjected to 30, 60, and 90 minutes of ischemia, followed by 2 hours of reperfusion, were fixed in formalin and stained with hematoxylin and eosin (H and E) for light microscopy. The samples were then reviewed by two observers blinded to their source for presence of myocardial hemorrhage and contraction bands. In addition, tissue samples from six animal preparations not subjected to myocardial ischemia were examined as controls.

**\(T_1\) Determinations**

\(T_1\)-Relaxation times were measured in vitro using a 0.25 Tesla NMR Spectrometer (Praxis-II, Praxis Corp, San Antonio, Texas) at 37°C in nons ischemic (as defined by TTC staining) (n=16), ischemic but not infarcted (n=16), and infarcted myocardial samples (n=10) from animals that had been injected with albumin–Gd-DTPA. Nonischemic samples were also obtained from animals that had not been injected with albumin–Gd-DTPA (n=6). The \(T_1\) values were obtained using a saturation recovery sequence from the resulting free-induction decay curves (32 measurements from a sequence of increasing interpulse delays). The data were fitted to a monoexponential curve using nonlinear least-squares regression analysis. Correlation values for the observed spectrometer data were ≥0.998 in all cases.

**Blood Flow by Radiolabeled Microspheres**

The induction of myocardial ischemia and reperfusion were confirmed by radiolabeled microspheres in six animals. Each animal was prepared with a nonengaged snare occluder around the LCA as described previously. Approximately 100,000 15-\(\mu\)m microspheres labeled with \(^{65}\)Zn, \(^{57}\)Co, \(^{113}\)Sn, \(^{153}\)Gd, \(^{85}\)Sr, or \(^{114}\)In (selected in random order) were injected into the left atrium and flushed with 0.2 cc of saline. Separate microsphere samples were injected before snare closure, after 2 minutes of snare closure, after 85 minutes of closure, after 2 minutes of snare opening and presumed reperfusion, and after 15 minutes of snare opening. Afterward, the animals were killed by cardiotomy, and the hearts were sliced transversely from apex to base and incubated in TTC (see Histochemical Staining). The central ischemic region, the border zone of myocardial ischemia (as defined by TTC staining), the nonischemic posterior left ventricular wall, and the right ventricular free wall were dissected, weighed, fixed in 10% formalin, and counted by an automated gamma counter. The relative myocardial flow of each tissue sample was expressed relative to that of nonischemic myocardium using the equation: relative myocardial blood flow (\%) = C/g sample/C/g nonischemic septum. Relative blood flows were expressed as mean percentages±SEM.

**Production and Characterization of Contrast Agent**

Human serum albumin was labeled with Gd-DTPA following methods described previously.\(^{15}\) An average of 20 Gd-DTPA molecules were cova-
lently conjugated to each albumin molecule by reaction with cyclic anhydride of DTPA and subsequent addition of Gd(NTA)₂. The dialyzed albumin–Gd-DTPA complex was characterized using standard methods. The T₁ and T₂ relaxivities (b₁ and b₂) measured in water at 0.25 T and 37°C were b₁ = 273.1 mM/sec; and b₂ = 387.7 mM/sec relative to carrier concentration. The T₁ and T₂ relaxivities per Gd³⁺ ion were 15 and 22 mM respectively. A dose of 160 mg/kg was given i.v. for all MRI experiments. Albumin–¹⁵³Gd-DTPA, a radiotracer derivative of the MR contrast agent used for distribution studies, was synthesized using the same protocol, yielding a specific activity of 0.093 μCi/mg albumin-bound Gd (see following section).

Myocardial Albumin–¹⁵³Gd-DTPA Distribution

The tissue distribution of albumin–¹⁵³Gd-DTPA was examined after 15 minutes of ischemia (n=9), and after 15 minutes of ischemia followed by 15 minutes of reperfusion (n=5), to confirm the myocardial distribution of the agent by an independent detection technique. With the exception of imaging, the animal preparation and the protocol were identical to those used during the 16 minute ischemia/reperfusion imaging protocol. In animals subjected to 16 minutes of ischemia, albumin–¹⁵³Gd-DTPA (2.4 μCi) mixed with cold-carrier albumin–Gd-DTPA (160 mg/kg) was injected into the tail vein of the anesthetized rat immediately after coronary occlusion. The ischemic (pale discolored) portion of the anterior wall was painted with India ink for identification during the subsequent dissection. After 16 minutes of ischemia, the animal was killed by cardiotomy. Animals subjected to 16 minutes of ischemia and reflow were prepared in the same way and killed by cardiotomy after 16 minutes reflow. The left ventricle was dissected from the great vessels, atria, and right ventricle, and sliced transversely from apex to base. Sections containing ischemic and nonischemic myocardia were dissected using the India ink marker and divided into portions representing the ischemic subendocardium, ischemic subepicardium, nonischemic subendocardium, and nonischemic subepicardium. These tissue samples were rinsed with normal saline, blotted, weighed, and counted by a gamma well counter. Myocardial distribution of albumin–¹⁵³Gd-DTPA was analyzed according to the following equation:

\[
\text{Distribution (\%)} = \frac{(C/g \ LV \ sample \ N) \times 100}{(C/g \ LV \ sample \ 1) + (C/g \ LV \ sample \ 2)}
\]

in which LV sample N denotes the counts per gram (C/g) of the individual tissue sample in question.

Statistics

All values are expressed as mean±SEM. Comparisons of MRI signal intensities at various time points and concentrations of high energy phosphate metabolates (PCr, ATP) were assessed for significance with a one-factor analysis of variance (ANOVA) with repeated measures using the Scheffe’s test. Differences in T₁ determinations and albumin–¹⁵³Gd-DTPA distribution were assessed using the unpaired t test. Statistical significance was defined as a p value of ≤0.05.

Results

Confirmation of Myocardial Ischemia and Reperfusion

Microsphere data confirming the induction of regional ischemia in this model are shown in Figure 1. After 2 minutes of coronary occlusion, normalized flow in the central ischemic zone and border zone was reduced to 3.9±1.08% and 18.2±4.8% (p≤0.01, n=6, mean±SEM) of baseline value, respectively. After 85 minutes of coronary occlusion, flow to the central zone and border zone were 15.8±5.1% (p≤0.01) and 27.1±5.1% (p≤0.05) of baseline values, respectively. Myocardial blood flow to the central ischemic zone and border zone returned to baseline values at 2 minutes (77.5±19.8%, 98.0±31%) and 15 minutes (99.5±16.1%, 116.2±41%) after release of the occluder (p=NS). Blood flow to the nonischemic right myocardium was not significantly changed during the period of ischemia and reperfusion.

MRI

Myocardial enhancement patterns produced by albumin–Gd-DTPA during ischemia and reperfusion are shown in Figures 2–4. During periods of coronary occlusion, signal intensity of the ischemic segment was significantly reduced compared with normally perfused segments (p<0.05) (Figures 2A–D and 3B). This differential in enhancement between normal and ischemic myocardia was observed for all periods of ischemia (16–90 minutes) and in all animals (n=22). There was consistently an area of marked increase in signal intensity in the left ventricular cavity adjacent to the ischemic myocardium during periods of coronary occlusion (Figures 2A–D and 3B).
Figure 2. MRI enhancement of ischemic and nonischemic myocardium on T$_1$-weighted images during various periods of ischemia and reperfusion. Panel A: Sixteen minutes of ischemia followed by 60 minutes of reperfusion. Albumin–Gd-DTPA given immediately after onset of ischemia. There was significant enhancement of normally perfused myocardium during ischemia. Ischemic myocardium was not significantly enhanced. There was marked enhancement within the LV cavity adjacent to ischemic myocardium during coronary occlusion. After reflow, ischemic and nonischemic zones had uniform enhancement. Panel B: Thirty minutes of ischemia followed by 60 minutes of reperfusion. Normal myocardium enhanced significantly during coronary occlusion, whereas ischemic myocardium did not. The LV cavity adjacent to ischemic myocardium showed marked enhancement during coronary occlusion. After reflow, enhancement of ischemic and nonischemic myocardium was uniform. Panel C: Sixty minutes of ischemia followed by 30 minutes of reperfusion. Nonischemic myocardium enhanced uniformly during coronary occlusion and reperfusion. Ischemic myocardium not significantly enhanced during coronary occlusion but enhanced markedly following reperfusion. Panel D: Ninety minutes of ischemia followed by 45 minutes of reperfusion. Ischemic myocardium did not enhance significantly during coronary occlusion but enhanced markedly during reperfusion. Panel E: Ninety minutes of ischemia followed by 60 minutes of reperfusion. Albumin–Gd-DTPA not given until after 30 minutes of reperfusion. No significant enhancement of ischemic or nonischemic myocardium noted during coronary occlusion or reperfusion, prior to albumin–Gd-DTPA administration. After albumin–Gd-DTPA injection, ischemic and reperfused myocardium became markedly enhanced.
and 3B). This was attributed to stasis of intracavitary blood adjacent to ischemic myocardium.

After coronary reperfusion, two patterns of myocardial enhancement emerged, depending on duration of ischemia, in animals injected with albumin-Gd-DTPA. In animals subjected to 16 or 30 minutes of myocardial ischemia, reperfusion resulted in a uniform and equal signal intensity
Figure 3. $T_1$-weighted images of heart subjected to 30 minutes of ischemia and reperfusion. Panel A: Baseline image before ischemia and before contrast administration. Panel B: Following left coronary occlusion and administration of albumin-Gd-DTPA. Note area of nonenhanced, ischemic myocardium (arrows) and superenhanced signal from left ventricular cavity adjacent to ischemic myocardium. Panel C: After 15 minutes of reperfusion. Note uniform enhancement of previously ischemic and nonischemic myocardium.

within the ischemic and nonischemic zones (Figures 2A, 2B, and 3C). In these animals, there were no significant differences between myocardial enhancements within the ischemic and nonischemic myocardia. Enhanced signal from within the left ventricular cavity adjacent to the previously

Figure 4. $T_1$-Weighted images of heart subjected to 90 minutes of ischemia and 15 minutes of reperfusion. Note superenhancement of previously ischemic and reperfused myocardium.
ischemic myocardium returned to normal low levels (Figures 2A, 2B, and 3C) after reperfusion. However, animals subjected to 60 or 90 minutes of myocardial ischemia showed increased contrast enhancement within the ischemic zone compared with nonischemic myocardium after reperfusion (Figures 2C, 2D, and 4). Thus, a zone of bright signal intensity within the reperfused myocardium could be distinguished from normal myocardium. The signal intensity of nonischemic myocardium remained unchanged throughout the course of coronary occlusion and reperfusion in animals subjected to short-term (16–30 minutes; Figures 2A and 2B) and long-term myocardial ischemia (60 and 90 minutes; Figures 2C and 2D).

In a control group (n=8), animals were subjected to 90 minutes of ischemia followed by reperfusion, but contrast infusion was withheld until after 30 minutes of reperfusion. There were no significant differences in signal intensities among ischemic, nonischemic, and reperfused myocardia. However, after i.v. administration of albumin–Gd-DTPA, reperfused myocardium demonstrated significantly increased signal intensity (Figure 2E). All eight control animals had evidence of myocardial infarction (MI) by TTC staining.

**T₁-Relaxation Values**

Myocardial T₁ determinations in normal and reperfused myocardia are shown in Figure 5. The mean T₁ value of normal myocardium was 472±6.7 msec (n=6) prior to contrast enhancement. Administration of albumin–Gd-DTPA caused a significant reduction in the T₁ values of normal myocardium (303.0±6.1 msec, n=16, p<0.01). The T₁ values of noninfarcted myocardium subjected to short periods (16 or 30 minutes) of ischemia and reperfusion were not significantly different from enhanced normal myocardium (308.5±20.7 msec, n=10, p=NS). However, T₁ of reperfused infarcted myocardium was reduced to 189.3±9.8 msec (n=10, p<0.01).

**Figure 5. Myocardial T₁ values (msec) with and without ischemia.**

**Figure 6. Myocardial distribution of albumin–¹⁵³Gd-DTPA.** Panel A: After 16 minutes of LCA occlusion, Albumin–¹⁵³Gd-DTPA distribution reduced in ischemic (Isch) segments compared with nonischemic (NI) segments. There was no significant difference in distribution of this agent between ischemic subendocardial (endo) and ischemic (epi) subepicardial segments. Panel B: After 16 minutes of LCA occlusion and 15 minutes of reperfusion. There were no significant differences in albumin–¹⁵³Gd-DTPA distribution among ischemic and nonischemic segments.

**Myocardial Distribution of Albumin–¹⁵³Gadolinium-DTPA**

Myocardial distribution of albumin–¹⁵³Gd-DTPA is shown in Figure 6. After 16 minutes of ischemia without reperfusion (Figure 6A), the concentration of the radiolabeled contrast agent was significantly lower in both the ischemic subendocardium and ischemic subepicardium (18.5±1.55% and 20.13±1.14%) compared with normally perfused posterior wall (30.7±1.44%, p≤0.05). There was no significant difference in the concentration of this radiolabeled contrast agent between the ischemic subendocardial and subepicardial segments (Figure 6A). Thus, no gradient distribution of this agent was evident across ischemic myocardium. After 15 minutes of ischemia and reperfusion, the concentration of albumin–¹⁵³Gd-DTPA was not significantly different between the reperfused and normal segments (Figure 6B).

**Spectroscopy Data**

The effects of varying periods of myocardial ischemia and reperfusion on phosphocreatine (PCr) and intracellular adenosine triphosphate (ATP) are shown in Figures 7–9. Two minutes after the onset of myocardial ischemia, the level of cardiac PCr was decreased to 61.7±5.8 control values (n=34,
There is ischemic decline progressively during myocardial ischemia (Figures 7A–D). It is important to note that the PCr and ATP resonances during coronary occlusion represent contributions from both ischemic and nonischemic myocardium. Reperfusion resulted in significant recovery of PCr after 16 or 30 minutes of reperfusion.
Histopathologic and Histochemical Data

Histopathology and the histochemical staining data are summarized in Tables 1 and 2. Animals not subjected to ischemia and reperfusion (S1–S5) showed no histopathologic evidence of hemorrhage or contraction-band necrosis and no evidence of myocardial infarction on TTC staining. Animals subjected to 30 minutes of myocardial ischemia followed by reperfusion demonstrated small areas of necrosis in 2/5 animals. Both of these animals showed MRI enhancement of the infarcted segments after reperfusion. Four of six animals subjected to 30 minutes of ischemia followed by reperfusion showed no evidence of hemorrhage, contraction-band necrosis, infarction by TTC staining, or MRI enhancement on reperfusion. Of 11 animals subjected to 60 or 90 minutes of ischemia and reperfusion, histochemical (TTC) evidence of MI was noted in 8/8 cases, and histopathologic evidence of contraction-band necrosis or myocardial ischemia (Figures 7A and 7B). No PCr recovery was noted after 60 or 90 minutes of myocardial ischemia and reperfusion. There was no recovery of β-ATP following 16, 30, 60, or 90 minutes of myocardial ischemia (Figures 7A–D). During the first 30 minutes of reperfusion there was a trend toward β-ATP recovery in the animals subjected to 16 or 30 minutes of myocardial ischemia, but this failed to reach statistical significance.

Table 1. Data on Histopathology, Histochemical Staining, and MRI Enhancement in Individual Animals Subjected to LCA Occlusion and Reflow

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<td>0</td>
</tr>
<tr>
<td>CW28</td>
<td>90 I/R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CW31</td>
<td>90 I/R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. Summary of Histopathology, Histochemical Changes, and MRI Enhancement for Various Time Periods of Ischemia and Reperfusion

<table>
<thead>
<tr>
<th>Time period</th>
<th>Hemorrhage</th>
<th>Contraction bands</th>
<th>TTC infarct</th>
<th>MRI enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min Isch</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>—</td>
</tr>
<tr>
<td>16 min I/R</td>
<td>—</td>
<td>—</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>30 min I/R</td>
<td>2/6</td>
<td>1/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>60 min I/R</td>
<td>5/6</td>
<td>4/6</td>
<td>3/3</td>
<td>5/6</td>
</tr>
<tr>
<td>90 min I/R</td>
<td>5/5</td>
<td>4/5</td>
<td>4/4</td>
<td>5/5</td>
</tr>
</tbody>
</table>
dial hemorrhage was noted in 11/11 animals. Marked MRI enhancement of the infarcted tissue was noted in 10/10 animals that showed myocardial hemorrhage on histopathologic examination.

Relationship Between Histopathologic/Histochemical Changes and MRI Enhancement

The presence of marked myocardial enhancement on MRI after ischemia and reperfusion correlated strongly with the presence of hemorrhage and contraction-band necrosis on histopathologic staining. Of 13 hearts that showed evidence of either myocardial hemorrhage or contraction bands, 12 displayed persistent postreperfusion enhancement by MRI after administration of albumin–Gd-DTPA (92% sensitivity). The presence of supernormal persistent enhancement after reperfusion was highly sensitive and specific for the presence of MI with TTC staining. Persistent enhancement was seen in 9/10 hearts that displayed evidence of MI by TTC staining (90% sensitivity). The absence of enhancement by MRI was noted in 9/9 hearts that displayed no evidence of MI on TTC staining (100% specificity).

Relationship Between Myocardial Enhancement by MRI and MRS

There was good correlation between the absence of recovery of PCr by MRS and the presence of persistently marked myocardial enhancement with albumin–Gd-DTPA following reperfusion. In the 20 animals subjected to short periods (16 and 30 minutes) of myocardial ischemia followed by reperfusion, recovery of PCr (≥80% baseline values) was noted in all animals. Persistent myocardial enhancement on MRI was noted in only 2/11 animals subjected to 16 or 30 minutes of ischemia and reperfusion. In both cases, the areas of persistent enhancement were small, and the size of infarcted myocardium was qualitatively small on TTC staining. In contrast, animals subjected to 60 and 90 minutes of ischemia failed to demonstrate PCr recovery on reperfusion, and all showed persistent contrast enhancement by MRI after administration of albumin–Gd-DTPA.

Discussion

The results of this study demonstrate four major findings: 1) Albumin-Gd-DTPA-enhanced MRI is useful for identifying the presence of myocardial ischemia in this model by enhancing the contrast difference between normally perfused and ischemic myocardia; 2) cardiac MRI with albumin–Gd-DTPA identifies reperfusion after myocardial ischemia; 3) reversible myocardial injury results in a uniform and equal enhancement of ischemic and normally perfused myocardia on MRI and recovery of PCr on MRS after reperfusion (these MRI and MRS patterns correlate with the absence of, or minimal, infarction on TTC staining); 4) after reperfusion, irreversible myocardial injury is characterized by transmural enhancement on MRI using albumin–Gd-DTPA and lack of PCr recovery on MRS. These MR patterns correlate with the presence of infarcted myocardium by TTC staining, myocardial hemorrhage, and contraction-band necrosis on histopathologic examination.

Previous studies have demonstrated that MRI is capable of differentiating ischemic from reperfused myocardia in situations of prolonged (>3 hours) coronary occlusion and reflow.3-5 Johnston et al3 showed that reperfused myocardial infarctions in dogs exhibited significant increases in signal intensities on T1-weighted images after 3 hours of coronary occlusion and reperfusion. By comparison, dogs subjected to 3 hours of ischemia without reperfusion showed only a small increase in signal intensity on T2-weighted spin-echo MR images. Tsmolokoff and coworkers6 found similar results after 5 hours of ischemia and reperfusion. The increased signal intensities of these images were judged most likely to be secondary to prolongation of T2-relaxation times due to increases in tissue-water content. Soon after reperfusion, however, changes in T2 are relatively small. Image interpretation may be complicated by the fact that MI, in the absence of reperfusion, may also cause myocardial edema with increases in T2 times and signal intensities.11 Hence, it is unclear whether differences in signal intensity on T2-weighted images, alone, will be sufficient to differentiate reperfused from nonreperfused ischemic myocardia.

The assessment of myocardial ischemia and reperfusion by MRI has previously been addressed by several studies using Gd-DTPA, a small molecular paramagnetic contrast agent.6-12 This FDA-approved drug diffuses rapidly from blood into the extravascular interstitial space (50% first-pass clearance) and, thus, may be suboptimal as a marker of blood volume or perfusion. Accumulation of Gd-DTPA in any tissue depends, in part, on perfusion but also on the size of the interstitial space and edema. Peshock et al10 demonstrated that Gd-DTPA, administered i.v. to a canine model of myocardial ischemia and reperfusion, produced a significant increase in signal intensity of reperfused infarcted tissue compared with noninfarcted myocardium. Wesbey et al6 demonstrated that administration of Gd-DTPA improved the contrast between infarcted and normal myocardia on T1-weighted spin-echo images by MRI. The period of ischemia in this study was 24 hours. Another study by de Roos et al8 assessed the value of Gd-DTPA for detection of acute MI in humans. The authors found that infarct definition was substantially improved after Gd-DTPA administration in five patients examined 2–17 days after their MIs.

Although these studies support the effectiveness of MRI with Gd-DTPA for detection of acute MI and reperfusion, this technique may be limited by the fact that Gd-DTPA (MW=600 Daltons) diffuses out of the intravascular volume quite rapidly.15 Albumin–Gd-DTPA (MW=92,000 Daltons) has been shown to remain at near-uniform concentrations within the intravascular space for 1–3 hours after
i.v. administration, resulting in prolonged enhancement of organs that are normally well perfused. Schmiedl et al. showed the effectiveness of albumin–
Gd-DTPA in the early detection of acute (8 hours) MI and early reperfusion in the rat model. In contrast to previous work, the present study shows the effectiveness of albumin-Gd-DTPA-enhanced MR imaging in assessing myocardial salvage after varying periods of myocardial ischemia. In addition, this is the first study that correlates enhancement patterns observed on MRI with \(^{31}P\) MR spectroscopic changes in an animal model of ischemia and reperfusion.

Characteristic MRI-enhancement patterns using albumin–Gd-DTPA may serve to differentiate ischemic myocardium, reperfused myocardium without MI, and reperfused myocardium with irreversible injury. Acutely ischemic myocardium was characterized by decreased signal intensity compared with normally perfused myocardium. Adjacent to the zone of decreased signal intensity was an area of very high signal intensity (Figures 3B and 4B). Reperfused viable myocardium was characterized by uniform signal intensity compared with normal myocardium (Figure 3C). Reperfused infarcted myocardium was characterized by marked contrast enhancement of the reperfused zone compared with normal myocardium (Figure 4C).

To define whether the area of high signal intensity seen during coronary occlusion represented subendocardial enhancement or increased cavitary signal adjacent to ischemic myocardium, the myocardial distribution of albumin–
Gd-DTPA was measured after 16 minutes of ischemia without reperfusion. The fact that albumin–
Gd-DTPA activity was reduced in both ischemic subendocardial and subepicardial segments suggests that the increased signal intensity did not originate from subendocardial tissue. The most likely explanation is that this increased signal represents stagnant blood adjacent to the hypokinetic ischemic wall. Similar flow artifacts adjacent to ischemic myocardium have been noted by other investigators.

The patterns of contrast enhancement seen during myocardial ischemia or reperfusion are probably secondary to the effect of albumin–Gd-DTPA on tissue \(T_1\) values. After injection of the contrast agent, there was increased signal intensity in normally perfused myocardium and a corresponding decrease in the \(T_1\) of this tissue (Figure 5). There was significantly less enhancement of ischemic myocardium compared with normally perfused myocardium. Myocardial distribution of albumin–
Gd-DTPA indicates a reduced concentration of this paramagnetic agent within the ischemic region (Figure 6A). After reperfusion, there was a marked increase in the intensity of irreversibly damaged myocardium with a significant reduction of myocardial \(T_1\) in this region compared with normally perfused myocardium (Figure 5). Thus, the reduced tissue \(T_1\) and increased contrast enhancement in this region is probably attributable to accumulation of the paramagnetic agent within irreversibly damaged myocardium. It should be noted that in the absence of paramagnetic contrast agents, the \(T_1\) value of infarcted myocardium is increased, not decreased, over normal tissue \(T_1\).

There are several possible reasons for the increased signal intensity seen in irreversibly damaged myocardium after ischemia and reperfusion. One possible explanation is that this enhancement is due to a hyperemic response with significant coronary vasodilation in the previously ischemic myocardium. However, Klener et al. have demonstrated that, whereas reperfusion after relatively short periods (40 minutes) of ischemia results in a hyperemic response, prolonged (90 minutes) ischemia followed by reperfusion results in attenuation of this hyperemic response in a canine model. Furthermore, Wood and coworkers showed that the degree of hyperemia after reperfusion is inversely related to the extent of infarction. This is consistent with our observations. No significant hyperemia was noted by examination of the radiolabeled microsphere results in hearts with significant myocardial necrosis after 90 minutes of ischemia and reperfusion (Figure 1). Thus, it is unlikely that the marked increase in signal intensity after long-term ischemia and reflow can be explained by coronary vasodilation and hyperemia in this region.

An alternative explanation is that the increased signal intensity of the irreversibly injured and reperfused myocardium may result from albumin–Gd-DTPA leakage into damaged tissue due to loss of vascular integrity with respect to macromolecules in this region. This theory is supported by the observation that myocardial hemorrhage was noted in all hearts demonstrating increased enhancement following reperfusion (Tables 1 and 2). Furthermore, the contrast enhancement of the infarcted, reperfused tissue increased progressively over time (Figures 2C and 2D), suggesting that the macromolecular contrast agent accumulated progressively in this region. Other investigators have described changes in capillary permeability to erythrocytes after long periods of ischemia and reperfusion in the canine model.

The myocardial distribution of albumin–
Gd-DTPA after short periods of ischemia and reflow suggest that this macromolecular contrast agent may serve as a perfusion agent. As noted in Figure 6A, there was significant reduction in the myocardial distribution of radiolabeled albumin in the ischemic region compared with normally perfused myocardium after 16 minutes of coronary occlusion. However, on reperfusion, the distribution of albumin–
Gd-DTPA returned to normal in the previously ischemic region (Figure 6B). These findings correlate with the MRI data using albumin–Gd-DTPA, in which decreased contrast enhancement was observed within ischemic myocardium after 16 minutes of coronary occlusion. After reper-
fus, the signal intensity of the ischemic region normalized (Figures 2A, 2B, and 3C). Further comparisons, however, between this agent and radionuclide perfusion markers are necessary to fully assess the potential of albumin–Gd-DTPA as a marker of perfusion.

The changes seen on MRI were consistent with those detected by $^{31}$P MRS. Animals subjected to either 16 or 30 minutes of myocardial ischemia showed significant PCR recovery on MRS and uniform MRI-signal intensity between the ischemic and nonischemic segments after reflow. These findings generally agreed with the absence of myocardial infarction on TTC staining and an absence of histologic changes. However, in animals subjected to 60 or 90 minutes of ischemia, reperfusion resulted in no PCR recovery by MRI and a marked increase in the MRI-signal enhancement of ischemic myocardium compared with normal myocardium. These MR patterns correlated with evidence of irreversible damage on TTC staining and histologic evidence of contraction-band necrosis and myocardial hemorrhage. Thus, MRI with albumin–Gd-DTPA may be used to document the presence of coronary reperfusion, as well as myocardial salvage, and it also correlates with the presence or absence of recovery of PCR by MRS.

Comparison of the results of the MRS data and the histologic data suggests that the irreversible myocardial injury occurs after 30–60 minutes of myocardial ischemia in this model. After 30 minutes of myocardial ischemia, there was substantial salvage of myocardium as assessed by recovery of high energy phosphates and TTC staining. Two of six hearts showed evidence of MI on TTC staining. However, these two cases were limited to very small areas in the subendocardial region of the anterior wall. In contrast, in the vast majority of cases, 60 and 90 minutes of ischemia followed by reperfusion was associated with evidence of large transmural MIs as shown by spectroscopic, histochemical, and histopathologic data.

Potential limitations of this study include the performance of MRI and MRS in separate but parallel experiments on different animals. This was necessitated so that MRS could be performed at higher field strength (5.6 T) than available on the GE CSI spectrometer (2.0 T), therefore, permitting high spectral and temporal resolution. Thus, there is potential uncertainty about an absolute correlation between spectroscopic changes seen during ischemia and reperfusion and MRI enhancement after injection of albumin–Gd-DTPA. In characterizing the spectroscopic and imaging changes during various time periods of ischemia and reperfusion, however, it is quite clear that reversible myocardial ischemia results in recovery of PCR and normalization of the contrast-enhancement pattern seen during ischemia. Similarly, long periods (60 and 90 minutes) of myocardial ischemia followed by reperfusion result in the absence of PCR recovery by MRS and a marked increase in contrast enhancement of the irreversibly injured myocardium on MRI after reflow. A second potential limitation of this study is that the time course of this experiment was somewhat shorter than others using TTC staining as a standard for definition of MI. However, Vivaldi et al.\(^27\) have demonstrated that TTC may detect ischemic injury as early as 30 minutes after coronary occlusion in the rat. It should be noted that, in these previous studies, TTC staining was done after fixed coronary occlusions and not followed by reperfusion.\(^26,27\) The presence of reperfusion probably accelerates the histopathologic changes following ischemic injury by accelerating calcium-mediated injury and washing out substrates of oxidative phosphorylation from damaged mitochondria. Furthermore, the close association between the presence of infarction on TTC staining and the lack of recovery of high energy phosphates on MRS suggests that TTC provided a valid assessment of irreversible myocardial injury within the time period of this experimental protocol.

This study suggests that cardiac MRI with albumin–Gd-DTPA, as well as $^{31}$P MRS, is useful for assessing myocardial salvage after ischemia and reperfusion. This method could be used for assessing the potential protective effects of various pharmacologic agents in delaying the onset of irreversible myocardial injury or in decreasing the size of MI in this in vivo experimental model of ischemia and reperfusion.

This experimental study also suggests that MRI with a macromolecular contrast agent may have clinical use as well. This technique could be used to accurately diagnose the presence of myocardial ischemia in a patient with a suspected coronary occlusion and assess reperfusion after thrombolytic therapy. Furthermore, this technique may provide evidence for the presence of myocardial salvage after reperfusion. Currently, coronary angiography is the only technique that can reliably demonstrate the presence of coronary reperfusion after thrombolytic therapy in patients with acute MI. Because angiography is associated with well-defined complications (such as bleeding), there is a need for less invasive techniques providing this information.

This study demonstrates that with an in vivo model of myocardial ischemia and reperfusion: 1) MRI with albumin–Gd-DTPA identifies ischemic myocardium by enhancing the intensity difference between normally perfused and ischemic myocardia; 2) MRI with albumin–Gd-DTPA identifies reperfusion after myocardial ischemia; and 3) After reperfusion, myocardial salvage can be evaluated by characteristic findings on enhanced MRI and $^{31}$P MRS.

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