Assessment of Postreperfusion Myocardial Hemorrhage Using Proton NMR Imaging at 1.5 T

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Background. Intramyocardial hemorrhage occurs frequently after reperfusion of acute myocardial infarction. However, its significance has not yet been established, mainly because of the lack of methods for detecting such hemorrhage. The following ex vivo study was carried out to assess the potential of nuclear magnetic resonance (NMR) imaging to detect and quantitate postreperfusion intramyocardial hemorrhage.

Methods and Results. Sixteen adult mongrel dogs underwent 3 hours of coronary occlusion followed by 1 hour of reperfusion, and three dogs underwent 4 hours of occlusion without reperfusion. Radiolabeled microspheres and $^{51}$Cr-labeled red blood cells were used to assess flow and evaluate the extent of hemorrhage. These results were later compared with both NMR and histology. Spin-echo NMR imaging was performed on the excised hearts using a 1.5-T system. Macroscopic assessment of the sliced myocardium revealed the existence of hemorrhage in 14 of the 16 dogs that underwent reperfusion but in none of those with occlusion only. In all 16 dogs with reperfusion, zones of increased signal intensity (SI) ratio ($1.68 \pm 0.41$ compared with control, $p < 0.05$) were seen in regions relating to the distribution of the occluded coronary artery, whereas in 13 of the 16 dogs, areas of decreased SI within the zone of increased SI ratio ($0.81 \pm 0.16$ compared with control, $p < 0.05$) were also seen, corresponding to regions with macroscopic hemorrhage. In contrast, in the three dogs without reperfusion, no macroscopic hemorrhage was observed, and likewise, no NMR zones of reduced SI were detected. Hemorrhage size by NMR (decreased SI zones), correlated well with hemorrhage size calculated from tissue slices ($r=0.96$, SEE $= 0.92\%$, $p < 0.01$), or by $^{51}$Cr labeling ($r=0.78$, SEE $= 1.5$, $p = 0.1$). In the reperfusion group, $T_2$ relaxation times in the infarcted hemorrhagic zone ($58 \pm 9$ msec) were significantly lower than the infarcted zones without hemorrhage ($98 \pm 13$ msec, $p < 0.001$). In contrast, when compared with control ($964 \pm 72$ msec), $T_1$ relaxation times were significantly increased in both infarct zones, either with ($1,284 \pm 176$ msec) or without ($1,266 \pm 103$ msec) hemorrhage. The selective shortening of $T_2$ relaxation times in the hemorrhagic regions is consistent with the paramagnetic effects of deoxyhemoglobin.

Conclusions. NMR imaging may provide a noninvasive approach for the detection and quantitation of intramyocardial hemorrhage. This observation may provide a means to further characterize pathological processes associated with acute myocardial infarction and assess the role of myocardial hemorrhage after reperfusion therapy. (Circulation 1992;86:1018-1025)

Key words • nuclear magnetic imaging • therapy, reperfusion

Intramyocardial hemorrhage results from disruption of capillary integrity during myocardial ischemic insult and necrosis followed by leakage of red blood cells into the interstitium during the reflow period. Recent animal studies have shown that such hemorrhage occurs frequently in acutely reperfused myocardial infarction, and in most cases is localized within the necrotic zone. The clinical significance of this observation, however, is still unclear, largely due to the lack of methods for detecting hemorrhage in vivo. Thus, no data are available regarding the incidence of hemorrhage or its effects on myocardial remodeling and performance.

Nuclear magnetic resonance (NMR) imaging has a potential for tissue characterization based on changes in relaxation times $T_1$ and $T_2$, which are reflected as changes in image signal intensity. Several studies have demonstrated an increase in both $T_1$ and $T_2$ after an ischemic insult. Recent NMR studies from patients with acute intracerebral hemorrhage have demonstrated that hemorrhage can alter the relaxation parameters; however, the effect of hemorrhage on myocardial relaxation parameters has not previously been assessed.


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The purpose of the present study in an ex vivo canine model of occlusion and reperfusion was 1) to assess the potential of NMR imaging to detect and quantitate intramyocardial hemorrhage and 2) to determine the mechanism responsible for the changes in NMR signal intensity and relaxation times after reperfusion and hemorrhage.

Methods

Animal Model

Twenty-four adult mongrel dogs (15–25 kg) were anesthetized with sodium pentobarbital (20 mg/kg) and ventilated with a Harvard pump using 0.25–0.5% halothane, 50% nitrous oxide and oxygen. Five dogs died before the protocol was completed, leaving 19 in the study group. After anesthesia, the chest was opened through a left lateral incision (four intercostal space), and the hearts were exposed. A left atrial catheter was introduced via the atrial appendage for injection of microspheres, and a second catheter introduced through the femoral artery into the distal aorta for pressure monitoring and microspheres reference blood withdrawal. Arterial blood gases were continuously monitored to assess the adequacy of ventilation.

Occlusion Protocol

Animals were assigned either to the reperfusion group (3 hours of occlusion and 1 hour of reperfusion, n=16) or the no reperfusion group (4 hours of occlusion, n=3). After injection of microspheres for baseline blood flow determination, a coronary artery was identified and a temporary occluder was positioned distal to the first marginal or the first septal perforator artery. Ventricular arrhythmias were controlled with repeated boluses of intravenous procanamide hydrochloride (100 mg) and lidocaine hydrochloride (40 mg). Bretyllium tosylate (5 mg/kg) was added if necessary. After stabilization, 100 ml of blood was withdrawn through the femoral artery catheter and used for 15Cr labeling of red blood cells. Blood volume was replaced with 0.9% saline.

Two hours and 45 minutes after occlusion, a second set of microspheres was injected through the left atrial line, and chromium-labeled blood was injected through the femoral vein to assess the degree of postreperfusion hemorrhage. Fifteen minutes later, the occluder was gradually released over a 2-minute interval to allow controlled reperfusion.

Forty-five minutes after reperfusion, a third set of microspheres was administered, and 15 minutes later, hearts were arrested by an intravenous injection of saturated KCl solution. Hearts were then excised, drained of blood, and rinsed with saline to remove remaining blood. The atria and surrounding excess fat tissue were trimmed, and the ventricles were filled with rubber gloves to prevent collapse during NMR imaging.

NMR Imaging

The excised hearts were imaged using a 1.5-T NMR imaging system (Philips Medical Systems North America, Shelton, Conn.). Hearts were positioned in the center of a 32-cm head coil, and multiple tomographic slices of the left ventricle were obtained in the short-axis view.

Two pulse sequences were used for imaging and relaxation time calculation: 1) spin echo sequence (TR=1,000 msec, TE=50 msec with three consecutive echoes), and 2) a combined interleaved spin-echo and inversion-recovery sequence as described by In den Keef et al.14 Acquisition parameters with this sequence were: TR_{IR}=2,000 msec, TR_{SE}=1,000 msec, IR=400 msec, TE=50 msec, where TR_{IR}=repetition time of inversion–recovery sequence, TR_{SE}=repetition time of the spin-echo sequence, IR=inversion delay time, and TE=echo time. All images were acquired using a slice thickness of 5 mm, matrix resolution of 128×256, two measurements, and a field of view of 10 cm.

For each heart, left ventricular (LV) borders were planimetered using the first echo in the spin-echo images. The LV mass was calculated using a simplified Simpson’s reconstruction approach according to the following equation:

\[ \text{LV mass (grams)} = \Sigma (\text{EPI-ENDO})/(\text{ST} + \text{SG})(1.06) \]

where EPI and ENDO are the areas enclosed by the epicardium and endocardium respectively, ST is slice thickness, SG is the interslice gap, and 1.06 is myocardial specific gravity (grams per milliliter).

Infarct size by NMR was analyzed by observers blinded to the experimental results. The entire myocardium was defined by planimetry of the first spin-echo image (TE=50 msec), whereas the myocardial region with increased signal intensity (SI) on the second spin echo image (TE=100 msec) was used to define infarcted regions. To assure analysis in a systematic fashion, the image contrast was windowed so that the signal from the normal myocardium (opposite the area with increased SI) would be null. The outer borders of the zones with increased SI surrounding zones with decreased SI were then planimetered using a track ball. Infarct mass was then calculated in a similar way to LV mass and expressed as a percentage of total LV mass. Using the same method, zones with decreased SI were planimetered to assess hemorrhage size.

To assess the influence of magnetic field dependence on NMR SI in the different infarcted zones, imaging was also performed using a 4.7-T system (Biospec, Bruker) (n=6), using a spin-echo sequence and the same parameters as the 1.5-T system.

Relaxation Time Calculation

For each dog, NMR slices that best demonstrated the infarct were used for relaxation time calculation. Mean signal intensities for normal tissue and for zones of infarct were derived from the images using a region of interest of 50–100 pixels (0.6–1.2 cm²). T1 values were derived from the changes in SI in the multiple echo times using a two-parameter fit:

\[ \text{SI} = A e^{-\text{TE}/T1} \]

where SI=signal intensity, A=spin density of water protons, and TE=echo time.

T1 values were determined from a combined spin-echo and inversion recovery sequence (available only on the 1.5-T system), using a method described by In den Keef et al.14 With this method, T1 can be calculated from a combined interleaved spin-echo and inversion-recovery sequence. Relaxation times were measured in
Morphological Evaluation

After imaging, hearts were cut into 1-cm-thick slices from base to apex. Heart slices were photographed before and after incubation for 20 minutes with 2% triphenyl tetrazolium chloride (TTC). Right ventricular tissue was removed, and the weight of LV and septum were obtained for each slice. Epicardial and endocardial borders as well as infarcted and hemorrhagic zones were traced using a sonic digitizer (Grafluid pen, Science Accessories Corp., Southport, Conn.) interfaced to a Hewlett-Packard 9825A computer programmed to calculate areas. Infarct size was evaluated on the TTC-stained slices and calculated as a percent of total LV mass. Hemorrhagic areas were digitized from the photographs of the freshly cut slices (before staining with TTC), and hemorrhage size was calculated as a percent of total LV mass and also as a percent of infarct size.

In nine dogs, the slice best demonstrating the infarct was selected, and a 3-mm-thick transmural slice including both lateral borders of the infarct was fixed in 10% phosphate-buffered formalin. The tissue was embedded in paraffin, and 6-μm-thick sections were stained with hematoxylin and eosin and Gomori aldehyde fuchsin trichrome.

Evaluation of Blood Flow

Three sets of approximately 2–5×10⁶ polystyrene nonbiodegradable, radiolabeled 15±1.0-μ tracer microspheres (New England Nuclear) were injected through the left atrial cannula over a 20–30-second interval. Microparticles were labeled with ¹⁶Ce, ⁸⁵Sr or ⁴⁶Sc and suspended in 10% dextran with 0.05% Tween 80 to prevent aggregation. Reference blood flow withdrawal from the descending aorta was started 10–15 seconds before microsphere injection and continued for 3 minutes using a Buchler peristaltic pump. Blood tubes were weighed and corrected for specific gravity (1.06) to determine withdrawal rate (8–10 ml/min). For blood flow analysis, each slice of the ventricle was divided into two transmural sections and further divided into epicardial, midwall, and endocardial samples weighing 0.5–1.5 g each. Care was taken to divide at junctions between necrotic core, hemorrhagic zones, and normal myocardium. The location of each sample was used to generate a map of each myocardial slice. Samples were placed in plastic tubes and counted in a LKB 1282 CompuGamma counter. Counts were corrected for spillover. Regional blood flow (milliliters per gram per minute) in each sample was determined from the corrected counts using the following equation: Q₉=(Cᵢ·Qᵢ)/Cᵣ, where Q₀ is tissue blood flow, Cᵢ is corrected tissue radioactivity, Qᵢ is withdrawal rate of the reference blood flow sample, and Cᵣ is radioactivity in the reference flow sample.

Using the pathology map drawn for each slice, the mean blood flow in the myocardium opposite to the occluded artery was calculated and defined as blood flow in the control myocardium. The area at risk was defined as the weight of tissue in the vascular bed supplied by the occluded artery with blood flow less than 40% of blood flow in the control myocardium.¹⁶

¹⁵Cr Labeling

Red blood cells were labeled with ¹⁵Cr using a modification of a previously reported technique.³⁷ Whole blood (100 ml) was mixed with anticoagulant citrate dextrose solution (ACD, Squibb Diagnostics) and 400 μCi of labeled sodium chromate (¹⁵Cr, Amersham). The mixture was then slowly rotated for 30 minutes. Unbound radioactive chromate was reduced at the end of this period by addition of 100 mg ascorbic acid. The labeled solution was then centrifuged at 700 rpm for 10 minutes, the supernatant was removed, and cells were resuspended with isotonic saline to approximately the same volume. This washing process was repeated three times. The final suspension was injected via the left femoral vein 15 minutes before reperfusion. Tissue pieces were counted in a multichannel gamma wall counter (LKB Compugamma) as described for regional blood flow analysis. Corrected counts per gram of wet tissue were calculated for analysis. Intramyocardial hemorrhage in the zones with occlusion flow less than 1 SD of mean flow in the nonischemic tissue was defined as regions with ¹⁵Cr counts greater than mean±2SD of counts in nonischemic regions.

Statistical Analysis

Signal intensities in the different infarcted regions were recorded and compared with the nonischemic wall opposite to the infarct. Changes in signal intensity were presented as the ratio of ischemic over nonischemic myocardium, which defines tissue contrast. All data are presented as mean±SD. Comparison of T₁ and T₂ relaxation times and regional blood flow in the different regions was performed using ANOVA with a Bonferroni correction. Results were considered significant at the 0.05 level.

Infarct and hemorrhage size determined from slice photographs were correlated with the same parameters obtained from the NMR images, using a least squares linear regression analysis. Because obvious difficulties in direct comparison between NMR images and the corresponding pathological slices do exist, the entire volume of hemorrhage in all the pathology slices was compared with the volume of decreased signal intensity in NMR imaging. Similarly, hemorrhage size determined from ¹⁵Cr-labeled red blood cells was correlated with the volume of hemorrhage observed both by pathological analysis and NMR imaging.

Results

Gross Pathology

In all dogs studied, a distinct region of nonstained myocardium could be identified on the TTC-stained tissue slices. In the reperfusion group, infarct size determined by TTC analysis correlated well but underestimated the weight of tissue with occlusion blood flow less than 40% of flow in the nonischemic regions (r=0.93, SEE=3.0, p<0.01). For the 12 dogs for which both TTC and microsphere data were available, area at risk (defined as the weight of tissue in the vascular bed supplied by the occluded artery with <40% of nonischemic tissue blood flow in the vascular bed supplied by the occluded artery) was 16.5±7.6%, whereas TTC infarct size 11.6±5.9%.
In 14 of the 16 reperfusion dogs, grossly visible myocardial hemorrhage was present. In contrast, no hemorrhage was observed in any of the three dogs with occlusion only. The extent of hemorrhage, ranging from 0.5% to 13.6% of total LV mass (mean, 3.4±4.1), correlated well with infarct size determined by TTC staining (r=0.81, SEE=2.32, y=−2.5+0.48x, p<0.001). Hemorrhage was located in the subendocardial central "core" of the infarct, extending toward the midwall in larger hemorrhages.

**Histological Evaluation**

One dog without reflow and eight dogs with reperfusion were examined histologically. In all dogs, subendocardial to transmural areas of contraction band necrosis were present. The subendocardium was generally totally necrotic in the ischemic zone, but midwall and subepimyocardial areas usually consisted of multifocal islands of necrosis within histologically normal appearing tissue. The region of necrosis often extended epicardially beyond the region of unstained tissue observed by the TTC technique, indicating a possible underestimation of infarct size by TTC.

Hemorrhage was not identified histologically in the nonreperfused dog or in the one reperfused dog without grossly identified hemorrhage. The other dogs had histologically identifiable hemorrhage that subjectively correlated well with the macroscopic assessment of hemorrhage size.

**Blood Flow Analysis**

Myocardial blood flow in the nonischemic tissue during the occlusion period was 1.05±0.34 ml/g/min in the epimyocardium and tended to be slightly increased to 1.23±0.51 in the endomyocardium (Figure 1). Blood flow in the ischemic regions during occlusion (0.42±0.17 ml/g/min) was significantly reduced compared with the nonischemic regions but returned to control values 45 minutes after initiation of reflow. Blood flow was similarly reduced in the ischemic areas during occlusion in the dogs that did not undergo reperfusion.

**FIGURE 1.** Bar graph of regional blood flow in the different myocardial zones during occlusion and reperfusion. During occlusion, a significant transmural decrease in flow at the ischemic tissue was observed. During reperfusion, blood flow in the ischemic regions returned to levels slightly above the nonischemic regions. EPI, epimyocardium; MID, midwall; ENDO, endomyocardium. Results presented as mean±SEE.

**FIGURE 2.** Plot shows distribution of 51Cr counts in endocardial, midwall, and epimyocardial tissue of a dog with hemorrhage in 3.4% of left ventricle plus septum. Hemorrhage occurred mainly in subendocardial tissue with the most severe reduction of blood flow. The upper left part (surrounded by dashed lines) represents the low-flow regions used to determine hemorrhage size by 51Cr counts (as defined in "Methods").

**51Cr-Labeled Erythrocyte Evaluation of Hemorrhage**

Successful studies with 51Cr-labeled erythrocytes were completed in nine dogs. In one dog without reflow and three dogs with hemorrhagic mass, <1% of LV and septum, there were no samples with increased 51Cr label. In the other five dogs with hemorrhage size ranging from 1.1% to 8.0% of LV mass, increased 51Cr labeling was observed in samples with low occlusion flow (Figure 2). In these dogs, the weight of tissue samples with hemorrhage defined by 51Cr counts (area indicated in Figure 2) correlated well with the hemorrhage size calculated from the tissue slices (r=0.78, y=−0.76+1.04x, SEE=1.53, p=0.1).

**NMR Imaging**

*Ex vivo imaging: 1.5-T system.* NMR assessment of LV mass correlated well with LV mass determined by weighing and summing the slices of the LV for each heart (r=0.97, SEE=6.6, y=−2.3+1.02x, p<0.001). In all dogs in the reperfusion group, a zone of increased SI (1.68±0.41 compared with control, p<0.05) was seen in the second echo image (TE=100 msec), correlating with the distribution of the occluded coronary artery. However, in 13 of these 16 dogs, zones of decreased SI (mean, 0.81±0.16 compared with control, p<0.05) were also seen within the zone of increased SI. In contrast, in the nonreflow group, only zones of increased SI were observed.

NMR-determined infarct size correlated well but consistently overestimated infarct size by TTC (r=0.94, SEE=3.5, p<0.001). Similarly, a good correlation was found between infarct size determined by NMR and microsphere-determined myocardium at risk (r=0.88, SEE=3.6, p<0.01).

In the 13 reperfused hearts that exhibited decreased SI within the infarct zone, gross inspection of sliced myocardial slices revealed a close relation between the distribution of hemorrhage and the NMR zones of decreased SI (Figure 3). Zones of decreased SI were not observed in three of the hearts in the reperfused group;
A Pathology Slice  
B NMR Image  
C Microsphere Blood Flow Map  
D Cr-51 Distribution Map

FIGURE 3. Photomicrographs and schematic diagrams depict correlation between macroscopic findings (panel A) nuclear magnetic resonance (NMR) imaging (panel B), microsphere blood flow map (panel C) and $^{51}$Cr distribution map (panel D) in one of the reperfusion dogs. A good correlation is observed between the hemorrhagic regions observed visually (arrows) and the endomyocardial NMR zones of reduced signal intensity (arrows). Hemorrhage (detected visually and by the $^{51}$Cr) occurred into regions with the most severe flow reduction during occlusion.

two hearts did not have a visible macroscopic hemorrhage, and in the third, only a small hemorrhage was observed. The size of the NMR zones with decreased SI correlated closely but consistently underestimated hemorrhage size determined from tissue slices ($r=0.96$, SEE=0.92%, $p<0.001$) (Figure 4). Also, a significant correlation was found when hemorrhage size was expressed as a percent of the infarcted tissue ($r=0.84$, SEE=6.8%, $y=-2.65+0.68x$, $p<0.01$) (Figure 5).

Relaxation Time: 1.5-T System

In the reperfusion group, $T_2$'s in the nonhemorrhagic infarct zones (98±13 msec; range, 76–112 msec) were significantly higher than in the infarcted zones with hemorrhage (58±9 msec; range, 48–73 msec) or control zones (56±4 msec; range, 50–64 msec) ($p<0.001$). In contrast, $T_1$ values were significantly increased in both nonhemorrhagic infarct (1,284±176 msec) and in zones with hemorrhage (1,266±103

FIGURE 4. Plot shows correlation of hemorrhage size presented as percent of left ventricular (LV) mass, as determined from tissue slices and nuclear magnetic resonance (NMR) zones of reduced signal intensity. There is underestimation of hemorrhage as determined by NMR. Dashed line represents the line of identity.

FIGURE 5. Plot shows correlation of hemorrhage size (presented as percent of infarct size) as determined from tissue slices and nuclear magnetic resonance (NMR) zones of reduced signal intensity. There is underestimation of hemorrhage as determined by NMR. Dashed line represents the line of identity.
msec) compared with control values (964±72 msec) (Figure 6).

Using these changes in relaxation times, the expected changes in SI were calculated. This was done using the modified equation for SI in spin-echo sequence

$$SI = e^{-TE/T2} (1 - e^{-TR/T1})$$

where TR=1,000 msec and TE=100. Good correlation was found between the expected and the observed changes in SI for both the infarcted ($r=0.75, y=0.3+0.79x, p<0.05$) and the hemorrhagic zones ($r=0.73, y=-0.19+1.18x, p=0.1$).

**Relaxation Time: 4.7-T System**

As expected, $T_1$ relaxation times with the 4.7-T system were lower than at 1.5-T system (control, 40±2 msec; infarct without hemorrhage, 61±10 msec; infarct with hemorrhage, 42±2 msec). Similar to the 1.5-T system, $T_8$ in the zone with reduced signal intensity were not significantly different from control.

**Discussion**

The present study demonstrates that intramyocardial hemorrhage occurs frequently in acutely reperfused myocardium and that NMR imaging can depict such hemorrhage as a zone of reduced SI. Macroscopic hemorrhage occurred in 14 of the 16 dogs undergoing reperfusion but in none of the three dogs with occlusion only. Hemorrhage occurred usually into the most ischemic tissue, and the extent of hemorrhage correlated with infarct size.

NMR imaging was able to detect hemorrhage as zones of decreased SI in 13 of the 14 dogs (93%) with macroscopic hemorrhage. Likewise, no zones of decreased SI were observed in nonhemorrhagic regions. In one dog with macroscopic hemorrhage that NMR failed to detect reduced SI zones, only a small hemorrhage was observed. Furthermore, despite the potential difficulties in assessing the volume of hemorrhage from cut surfaces of tissue slices, the size of the hemorrhage measured by NMR correlated well with but underestimated hemorrhage size assessed from tissue slices ($r=0.92$). Similarly, good correlation was found between NMR-determined hemorrhage size and the size of hemorrhage measured from $^{51}$Cr red blood cell labeling.

This study is consistent with previous studies demonstrating the usefulness of spin-echo NMR imaging for the detection and sizing of infarcted myocardium. However, it should be emphasized that NMR-determined infarct size correlated well but consistently overestimated infarct size determined by TTC staining.

**Mechanism for SI Changes**

NMR tissue contrast is an exponential function involving $T_1$ and $T_2$ relaxation times as well as proton density and velocity. Previous investigators have shown that the increase in SI in the infarcted tissue can be related to both $T_1$ and $T_2$ changes. Indeed, in this study, relaxation time measurements in the zone with increased SI and no hemorrhage revealed a significant increase in both $T_1$ and $T_2$ compared with control.

The precise mechanism for this increases in $T_1$ and $T_2$ is not entirely clear. It seems, however, that an increase in myocardial water content may be an important factor in this mechanism. Thus, because myocardial edema at the periphery of the infarct can increase SI, overestimation of the true size of the infarct can result. However, because regions of necrosis by histology sometimes extended epicardially beyond the region unstained by TTC, underestimation of the true infarct size by TTC could also contribute to the difference.

In the hemorrhagic zones, we have also demonstrated that the decrease in SI was related to both $T_1$ and $T_2$ changes. As mentioned above, SI in a spin-echo pulse sequence is exponentially related to $T_1$ and $T_2$ relaxation times. In the current protocol, an increase in $T_2$ would increase SI, whereas an increase in $T_1$ would decrease it. Thus, contrast between adjacent tissues will depend on both the magnitude and the balance between $T_1$ and $T_2$ changes. In the infarcted hemorrhagic zones, $T_8$ were significantly decreased compared with the infarcted zones without hemorrhage (Figure 6). In contrast, $T_8$ were increased in both the necrotic and the hemorrhagic zones. This selective shortening of $T_2$ induced by the hemorrhage should lead to a relative decrease in SI compared with the surrounding infarct. However, as $T_8$ in the hemorrhagic zones were not significantly different from control values, the additional decrease in SI in the hemorrhagic zones relative to control was most likely due to the prolonged $T_1$ in the hemorrhagic regions. Indeed, with the imaging protocol used in the present study (i.e., TR=1,000 msec, TE=100 msec), the expected changes in SI calculated from the changes in $T_1$ and $T_2$ were in concordance with the observed SI measurements.

**Mechanism of Selective $T_2$ Changes**

The selective shortening of $T_2$ relaxation time in the hemorrhagic tissue is most likely related to the paramagnetic characteristics of deoxyhemoglobin. It is well known that oxyhemoglobin in red blood cells has only paired electrons and therefore lacks the ability to affect local magnetic fields. However, with interstitial hemorrhage and stagnation of blood in the capillaries, oxyhemoglobin is reduced to deoxyhemoglobin. The iron (Fe$^{2+}$) in the deoxyhemoglobin has four unpaired electrons, resulting in a strong paramagnetic effect. Thus, protons in water molecules that diffuse across the local magnetic field gradients created by the paramag-
netic substance will dephase (loose coherence) more rapidly, thus resulting in a selective shortening of \( T_2 \).

Because hemorrhage occurs only in the severely ischemic myocardium (Figure 1), \( T_2 \) values in these regions will be determined by the relative contribution of two opposing mechanisms: the first is an increase in \( T_2 \) caused by tissue edema; the second is a decrease in \( T_2 \) induced by the paramagnetic effect of deoxyhemoglobin. These opposing effects of necrosis and hemorrhage on normal tissue \( T_2 \) may lead to a gradation of \( T_2 \)'s in these areas resulting in a "patchy" appearance that has some similarities to the histological appearance of mixed necrosis, hemorrhage, and normal tissue in the periphery of myocardial infarction, especially after reperfusion.

In some regions, the opposing effects of infarct and hemorrhage on \( T_2 \) can result in "averaged" \( T_2 \) values that are not significantly different from control. Thus, with the increase in \( T_2 \)'s in the infarct, the amount of hemorrhage required to reduce \( T_2 \) and visible reduce signal intensity would be larger than if the hemorrhage occurred into noninfarcted myocardium with normal \( T_2 \). This is consistent with the underestimation of hemorrhage size using NMR-observed reduction in SI compared with direct observation of hemorrhage. Also, the routine use of 10-mm tomographic slices by NMR would tend to reduce sensitivity to detect hemorrhage due to partial volume effects, whereas using thinner tomographic slices (i.e., 5 mm) would improve NMR ability to detect hemorrhage.

**Long-term Effects**

The paramagnetic effect of deoxyhemoglobin is thought to be the cause of decreased SI in patients with acute (less than 1 week) intracerebral hemorrhage.\(^1\)\(^2\)\(^3\)\(^4\) However, it has been shown that with time, deoxyhemoglobin is reduced to paramagnetic methemoglobin, which also leads to a reduction in \( T_1 \).\(^5\)\(^6\) Thus, later in the course of hemorrhage, SI can normalize or even increase, depending on the relative concentration of these constituents.\(^5\)\(^6\)\(^7\)\(^8\)

This observation may be important when assessing the long-term effects of reperfusion on relaxation times and myocardial SI. Wisenberg et al.\(^9\) reported "normalization" of \( T_2 \) values with a decrease in SI in the ischemic regions 5 days after reperfusion. This observation may in part be attributable to the paramagnetic effects of deoxyhemoglobin.

**Effects of Magnetic Field Strength on Ability to Detect Hemorrhage**

Since a paramagnetic effect increases as the square of the external magnetic field,\(^9\) the effect of deoxyhemoglobin is expected to become more significant at higher magnetic field strength. Thus, the ability to detect hemorrhage in myocardial infarction improves at higher field strength, as suggested by the present study. In addition, hemorrhage in myocardial infarction should be more difficult to detect at lower field strength, and this may account for the lack of visualization of reduced SI zones associated with hemorrhage in earlier ex vivo studies.

**Clinical Significance**

Intramyocardial hemorrhage occurs rarely during the natural course of myocardial infarction. However, with the introduction of thrombolysis and thus early reperfusion, the prevalence of myocardial hemorrhage has increased substantially.\(^10\)\(^11\) The clinical significance of this hemorrhage has not yet been established. Several studies have suggested that intramyocardial hemorrhage is involved in the so-called "reperfusion injury"\(^12\)\(^13\) and can lead to infarct expansion\(^14\) or impair the healing process,\(^15\) whereas others have shown that hemorrhage is confined only to severely ischemic myocardium and therefore does not contribute to infarct expansion.\(^16\)\(^17\)\(^18\) Several studies have demonstrated improved survival after later (>6 hours) reperfusion despite the lack of apparent limitation in infarct size.\(^19\)\(^20\) Accordingly, it has been postulated that reperfusion and myocardial hemorrhage may improve cardiac hemodynamics by "splinting" (increasing myocardial stiffness) the infarcted tissue and thus preventing infarct expansion and aneurysm formation.\(^21\)\(^22\)

The present study suggests that hemorrhage associated with myocardial infarction can be readily detected in the in vitro setting using appropriate NMR methods. First, the spin-echo approach with long echo delay times can be used to enhance contrast between the nonhemorrhagic and the hemorrhagic portions of the infarct due to the significant differences in \( T_2 \) between these two zones. Second, hemorrhage is better visualized at higher magnetic field strength. Finally, use of thinner tomographic slices reduces the effect of SI averaging and increases the likelihood of hemorrhage detection and the precision of quantitative assessment. However, in the clinical setting, detection of hemorrhage may be less accurate due to image degradation related to motion. Thus, clinical studies will be needed to assess the potential for in vivo detection and quantification of postreperfusion myocardial hemorrhage.

**Conclusions**

The present study demonstrates the potential of proton NMR imaging to depict postreperfusion myocardial hemorrhage as regions with reduced signal intensity. This decrease in signal intensity is related to a selective reduction in \( T_2 \) relaxation time, which is consistent with the paramagnetic effect of deoxyhemoglobin.

Further in vivo studies will be needed to assess the potential of NMR imaging to detect and quantitate myocardial hemorrhage in the clinical setting. This should assist to further characterize its clinical significance after reperfusion therapy.

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**References**

2. Klener RA, Ellis SG, Lange R, Braunwald E: Studies of experimental coronary artery reperfusion: Effects on infarct size, myo-
Gomori 12.


38. Braunwald E: Myocardial reperfusion, limitation of infarct size, reduction of left ventricular dysfunction, and improved survival: Should the paradigm be expanded? Circulation 1989;79:441–444
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