The relationship between proton nuclear magnetic resonance relaxation parameters and myocardial perfusion with acute coronary arterial occlusion and reperfusion

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ABSTRACT Previous investigators have demonstrated that acute myocardial ischemia, whether followed by reperfusion or not, is associated with prolongation of the proton nuclear magnetic resonance (NMR) relaxation times T1 and T2. Therefore, the relationship between the severity of ischemia and alterations of these relaxation times has not been assessed. In our studies, changes in T1 and T2 were compared with myocardial perfusion in dogs both before and during 30 and 60 min of coronary arterial occlusion. In addition, studies were performed to assess the impact of reperfusion on relaxation times after 30 and 60 min of coronary occlusion. In the reperfusion studies the relationship between myocardial relaxation times and flow during the reocclusion, occlusion, and the 15 min reperfusion periods were studied. In the occlusion-only preparations, there was a significant inverse relationship between the relaxation times and flow during occlusion. In the reperfusion preparations there was a significant direct relationship between T1 and T2 and flow during the period of reperfusion. There was no significant relationship between the relaxation times and flow during occlusion in groups that subsequently underwent reperfusion, and no relationship between T1 and T2 and reocclusion flow in either the occlusion-only or reperfusion groups. These data suggest that the proton relaxation times provide an indication of the severity of an ischemic insult. Since NMR images can be generated that are sensitive to differences in these relaxation parameters, NMR imaging may provide a means to assess noninvasively the severity of the myocardial ischemic insult.


PROTON NUCLEAR MAGNETIC RESONANCE (NMR) imaging is a noninvasive approach capable of generating high-resolution tomographic or three-dimensional images of the heart without ionizing radiation or contrast agents. The intensity of structures depicted in proton NMR images is related to several biophysical properties of tissue. These properties include proton density and the relaxation times T1 and T2 and are known to be affected by the concentration and compartmentalization of water, the concentration and type of lipid, macromolecular environment, temperature, and other factors affecting the chemical milieu. Ischemic and reperfusion-associated insults can lead to alterations in such physicochemical properties of myocardium. Accordingly, NMR imaging techniques that emphasize alterations in the relaxation times might provide insight into the myocardial response to an ischemic insult.

Previous work suggests that T1 and T2 tend to lengthen with coronary arterial occlusion of 30 min or longer. In addition, T1-weighted images from our laboratory delineated myocardium in the distribution of an occluded coronary artery with prolonged T1. However, the relationship between the severity of acute ischemic insult and changes in T1 and T2 has not been defined. The purpose of this study was to examine the relationship between the relaxation times and the severity of the ischemic insult associated with coronary arterial occlusion of 30 and 60 min in duration with and without reperfusion.
Methods

Canine preparation. Adult mongrel dogs were anesthetized with intravenous pentobarbital. The dogs were then intubated with a No. 9 endotracheal tube and ventilated with 97.3% oxygen and 2.7% carbon dioxide by means of a Harvard ventilator. One gram of procainamide was administered intramuscularly and infusion of lidocaine (2 mg/ml) was maintained throughout the procedure. A left thoracotomy and pericardiotomy were performed and the left anterior descending coronary artery (LAD) was isolated. A loose 0 silk tie was then placed around the LAD. A 19-gauge catheter was inserted into the aortic arch via the internal mammary artery and aortic pressure was monitored. A 23-gauge catheter was placed in the left atrium. A 19-gauge catheter was placed in the aortic arch to allow microsphere reference samples to be obtained via a Holter pump for the 2 min after administration of microspheres. To measure baseline myocardial perfusion, 4 to 5 million (30 μCi) 15 μm 113Sn-labeled microspheres (New England Nuclear Corp.) were injected into the 23-gauge catheter and reference samples were acquired as described above. In animals undergoing reperfusion, the silk tie was removed and reperfusion was permitted for 15 min. Two minutes before the end of the reperfusion period, 4 to 5 million (30 μCi) 15 μm 46Sc-labeled microspheres (New England Nuclear Corp.) were injected and reference samples collected. The dogs were then killed with an intravenous injection of potassium chloride either after the occlusion period or after reperfusion and their hearts were excised. Transmural myocardial samples weighing approximately 2 g were taken from the following visually identified regions: a central ischemic region in the midst of the territory supplied by the LAD distal to its occlusion; a peripheral region immediately adjacent to the central region, and two nonischemic regions from the left ventricular wall. Each sample was divided into epicardial and endocardial halves and placed into closed vials. Samples were handled with techniques within the guidelines suggested by Beall11 to minimize alterations in relaxation times resulting from tissue handling and storage.

Measurement of proton relaxation times. The proton relaxation times, T1 and T2, were determined for each sample with an IBM PC-20 Multi-spec (IBM Instruments, Danbury, CT). NMR measurements were made at a radio frequency of approximately 20 MHz with a phase-sensitive detector at a probe temperature of 40°C. Ninety and 180 degree radio frequency pulse widths as well as phase were determined for each myocardial sample. T1 was determined with an inversion-recovery pulse sequence using 18 τu values ranging from 20 msec to 2.56 sec.12 T2 was determined with a Carr-Purcell-Meiboom-Gill pulse sequence with 10 spin echoes ranging from 4 to 40 msec after the 90 degree pulse.13 Values for T1 and T2 were calculated automatically and displayed by the PC-20.

Microsphere analysis. The myocardial and aortic reference arterial blood samples were weighed and activities were determined in a Packard Gamma Scintillation Counter.13 The perfusion to each myocardial specimen was then computed with a blood flow analysis program on a VAX 11/780 (Digital Equipment Corp., Maynard, MA).

Data analysis. The relationships between flow and proton NMR relaxation parameters were evaluated with multiple linear regression analysis and analysis of covariance using the statistical packages RS/1 (Bolt, Beraneck, and Newman Corp., Cambridge, MA) and BMDP (University of California, Los Angeles) implemented on the VAX 11/780 computer system. Regression lines were considered significant at p < .05.

Results

Coronary arterial occlusion only. In the group undergoing occlusion and no reperfusion (both 30 and 60 min subgroups) there were significant inverse correlations between the relaxation parameters and flow during occlusion. There was no correlation between relaxation parameters and preocclusion flow.

When all myocardial samples (epicardial and endocardial) were grouped together, there were significant correlations between flow during occlusion and T1 (r = -.57; p < .001) and T2 (r = -.35; p < .03) in the 30 min occlusion subgroup (figure 1) and between flow during occlusion and both T1 (r = -.56; p < .001) and T2 (r = -.50; p < .001) in the 60 min subgroup (figure 2, table 1).

In the endocardial samples of the 30 min occlusion subgroup there was significant correlation between flow during occlusion and both T1 (r = -.75; p < .001) and T2 (r = -.56; p < .01). In the epicardial samples from this group there was no significant correlation between any flow parameter and either relaxation time (figure 1). In the endocardial samples of the 60 min occlusion subgroup there were significant correlations again between flow during occlusion and both T1 (r = -.67; p < .002) and T2 (r = -.62; p < .005) but, in addition, in the epicardial samples there were significant correlations between flow during occlusion and both T1 (r = -.66; p < .002) and T2 (r = -.51; p < .025) (figure 2, tables 2 and 3).

Myocardial layer effect. To determine flow-independent differences of T1 and T2 vs myocardial layer, an analysis of covariance was applied after relaxation times were normalized for flow during occlusion. This analysis demonstrated that the epicardium and the endocardium represented significantly different populations at both 30 and 60 min of occlusion (p < .0001 for T1 and T2). Because of this, the correlations that ignore the distinction between epicardium and endocardium may not be as indicative of the underlying relationships between the relaxation times and occlusion flow than correlations assessing epicardial and endocardial samples separately.

Coronary arterial occlusion followed by reperfusion. In the reperfusion group there were significant correlations between relaxation times and flow during reperfusion but none between relaxation times and flow before or during occlusion.

When all myocardial samples (endocardial and epicardial) were considered together, significant correla-
FIGURE 1. Myocardial T1 (top) and T2 (bottom) vs flow during occlusion in dogs subjected to 30 min of LAD occlusion. The regression lines shown are for both epicardial (EPI) and endocardial (ENDO) samples combined. The regression equations are:

T1 = 683 - (34.1) Qo (r = -.57; p < .001); T2 = 49.9 - (2.74) Qo (r = -.35; p < .03). Mean values for normal myocardium are depicted by circles.

FIGURE 2. Myocardial T1 (top) and T2 (bottom) vs flow during occlusion in dogs subjected to 60 min of LAD occlusion. The regression lines shown are for epicardial (EPI) and endocardial (ENDO) samples combined. The regression equations are:

T1 = 696 - (51.1) Qo (r = -.56; p < .001); T2 = 52.1 - (5.59) Qo (r = -.50; p < .001). Mean values for normal myocardium are depicted by circles.
Occlusion only

T1
30 min  \( T_1 = 683 - (34.1)q_o \)  \(-.57\)  .001
60 min  \( T_1 = 696 - (51.1)q_o \)  \(-.56\)  .001
T2
30 min  \( T_2 = 49.9 - (2.74)q_o \)  \(-.35\)  .029
60 min  \( T_2 = 52.1 - (5.59)q_o \)  \(-.50\)  .001

Occlusion and 15 min reperfusion

T1
30 min  \( T_1 = 624 + (37.2)q_o \)  \(.77\)  .0001
60 min  \( T_1 = 639 + (23.8)q_o \)  \(.88\)  .0001
T2
30 min  \( T_2 = 47.3 + (2.20)q_o \)  \(.52\)  .001
60 min  \( T_2 = 46.1 + (1.47)q_o \)  \(.64\)  .0001

T1 and T2 are measured in milliseconds.

Myocardial layer effect. As in the occlusion-only groups, an analysis of covariance (where relaxation times were normalized for reperfusion flow) demonstrated that the epicardium and endocardium represented distinct populations when assessing T1 (p < .001). For T2, however, epicardial and endocardial populations were not significantly different.

Discussion

This study was designed to determine whether relationships exist between myocardial proton NMR relaxation properties and the severity of insult resulting from: (1) coronary arterial occlusion of 30 and 60 min and (2) coronary reperfusion of 15 min after 30 and 60 min of occlusion. Since these myocardial proton relaxation parameters (T1 and T2) can contribute substantially to image intensity and are potentially derivable by NMR imaging techniques, this investigation demonstrates the potential of NMR imaging for assessing the severity of myocardial ischemic and reperfusion insults through quantitation of regional myocardial relaxation times.

Coronary arterial occlusion: relaxation times vs flow.

Transmural assessment showed that both relaxation parameters (T1 and T2) correlated inversely with microsphere-determined myocardial perfusion in both the 30 and 60 min occlusion preparations. The linear regressions indicate the trends between relaxation times and flow, but the exact relationships between T1, T2, and flow are not known. Endocardial samples alone also demonstrated significant inverse correlations between both T1 and T2, and microsphere-determined myocardial blood flow after both 30 and 60 min of...
coronary arterial occlusion. Epicardial samples, however, demonstrated a significant inverse correlation in only the 60 min occlusion preparation. From previous studies of ischemic damage associated with coronary arterial occlusion, epicardial samples would be anticipated to develop less ischemic damage than endocardial samples. The differences in T1 and T2 alterations between epicardium and endocardium provide further evidence that such changes are related to the severity of ischemic insult.

The mechanisms responsible for prolonging the relaxation times in occlusion-only ischemic insults are not known, although factors affecting tissue water content and distribution are most likely involved. Previous investigators have shown that elevations of proton relaxation times are associated with increases in tissue water concentration in neoplastic tissues. Williams et al. demonstrated that significant T1 prolongation and elevated myocardial water concentrations were extant in myocardium subjected to at least 30 min of coronary arterial occlusion. Swelling of the myocytes and lifting of the sarcolemma have been demonstrated with electron microscopy in acutely ischemic myocardium. In addition, mitochondrial swelling is also known to take place, documenting changes in the intercompartmental distribution of tissue water. Although it is unclear what the mechanisms of changes in T1 and T2 are, there is little doubt that intercompartmental shifts in tissue water concomitant with alterations in the chemical environment (including changes in electrolyte and macromolecular concentrations) play a major role in the ischemia-induced alterations of the relaxation times.

**Coronary arterial occlusion with reperfusion: relaxation times vs flow.** In contrast to the inverse correlation between flow during occlusion and relaxation times in the occlusion-only preparations, T1 and T2 were directly related to the extent of reperfusion in the groups that underwent reperfusion. The return of blood flow to regions subjected to greater than 20 min of ischemia has been associated with massive cell swelling, cellular vacuolization, mitochondrial enlargement, and the deposition of calcium in the mitochondria. Changes in the compartmentalization and concentration of water resulting from postocclusion reperfusion likely affects T1 and T2. The influx of calcium from reperfusing blood may also affect tissue water balance and the chemistry of macromolecules in solution, thus affecting the relaxation times. Further investigation is required to allow a more precise understanding of the mechanisms of ischemia-related changes in relaxation times.
Imaging in vivo vs assessment of T1 and T2 in vitro. Although T1 and T2 can be evaluated in most organs with conventional proton NMR imaging techniques, cardiac motion interferes with precise measurement of relaxation parameters in current NMR imaging systems. This is because NMR signal intensity is very sensitive to motion in addition to proton density, T1, and T2. Synchronization of image acquisition with the cardiac cycle, i.e., “gating,” considerably reduces the impact of cardiac motion. Nevertheless, even the most up-to-date gated cardiac NMR imaging techniques are not yet capable of reliable quantitation of absolute relaxation times. Furthermore, even in gated imaging studies the disordered wall motion associated with a coronary occlusion make reliable derivation of myocardial T1 and T2 difficult. For example, a hypokinetic or akinetic wall would be expected to show greater signal intensity than normally contracting myocardium unrelated to differences in T1 and T2. Nevertheless, improvements in imaging technology will likely provide a means of differentiating the effects of myocardial motion from changes in relaxation times and will thus allow more reliable quantitation of T1 and T2 from cardiac NMR images in vivo. Methods used in this study can provide an assessment of the relative regional distribution of myocardial relaxation properties.

Clinical implications. We have demonstrated that alterations in T1 and T2 are related to the severity of the ischemic insult during occlusion and to the degree of reperfusion. Thus proton NMR imaging, in addition to its ability to provide high-resolution tomographic images of the heart, may provide a means of characterizing the severity and the location of the ischemic and reperfusion insult.

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References
2. Ling GN, Tucker M: Nuclear magnetic resonance relaxation and water contents in normal mouse and rat tissues and in cancer cells. JNCI 64: 1199, 1980
3. Block RE, Maxwell GP, Prudhomme DL, Hudson JL: High resolution proton magnetic resonance spectral characteristics of water, lipid and protein signals from three mouse cell populations. JNCI 58: 151, 1977
5. Beall PT, Asch BB, Chung DC, Medina D, Hazelwood CF: Distinction of normal, preneoplastic and neoplastic mouse mammary distal cell cultures by water nuclear magnetic resonance relaxation times. JNCI 64: 335, 1980
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