Quantification of Experimental Myocardial Infarction Using Nuclear Magnetic Resonance Imaging and Paramagnetic Ion Contrast Enhancement in Excised Canine Hearts

MARK R. GOLDMAN, M.D., THOMAS J. BRADY, M.D., IAN L. PYKETT, PH.D.,
C. TYLER BURT, PH.D., FERDINANDO S. BUONANNO, M.D., J. PHILIP KISTLER, M.D.,
JEFFREY H. NEWHOUSE, M.D., WALDO S. HINSHAW, PH.D., AND GERALD M. POHOST, M.D.

SUMMARY Determination of myocardial infarct size is important for clinical management of patients with ischemic heart disease and for research on limiting infarct size. Nuclear magnetic resonance (NMR) imaging permits tomographic depiction of the distribution of mobile tissue protons. NMR images have demonstrated high spatial resolution and contrast. To evaluate the potential of this technique in measuring myocardial infarct size, NMR imaging was performed in six canine hearts excised 24 hours after circumflex coronary artery ligation. Before sacrifice, the dogs received i.v. manganous chloride (0.05 mmol/kg). After NMR imaging, the hearts were sectioned and the myocardial slices were stained with triphenyl tetrazolium chloride. The pathologically determined infarct size was compared with the infarct size measured by NMR imaging. The correlation was good (regression line slope 1.06; \( r = 0.94 \)). We conclude that NMR imaging with paramagnetic contrast agents can be used to determine infarct size in excised hearts.

THE ACCURATE determination of myocardial infarct size has been a topic of major interest over the past decade. Previous work has demonstrated that prognosis depends upon infarct size. Careful measurement of infarct size is important in determining the efficacy of interventions designed to limit infarction. Methods used clinically to quantify infarct size rely on assessment of wall motion (radionuclide angiography or ultrasound), myocardial perfusion (thallium-201), radioisotopic labeling of necrotic myocardium (technetium-99m stannous pyrophosphate), or release of intracellular enzymes into the bloodstream (CK-MB). Nuclear magnetic resonance (NMR) imaging is a noninvasive, high-resolution technique that uses no ionizing radiation and allows tomographic depiction of the distribution of mobile tissue protons. Image contrast can be enhanced by using paramagnetic substances that are markers of myocardial blood flow and alter NMR properties of tissue in their distribution. We have used NMR imaging with paramagnetic ion contrast enhancement to detect acute myocardial infarction in excised canine hearts. To evaluate the potential utility of NMR imaging in quantifying myocardial infarction, we studied a canine model of myocardial infarction with paramagnetic ion contrast enhancement using manganous ion.

Methods

Infarct Production

Six adult mongrel dogs of either sex that weighed 15–20 kg were anesthetized with intravenous pentobarbital, 60 mg/kg (Veterinary Lab Inc.), intubated, and ventilated with 100% oxygen by positive pressure ventilator (Bird Corp.). The pericardium was opened through a left lateral thoracotomy, and the left circumflex coronary artery was isolated and ligated with 3-0 silk suture. The thoracotomy was closed and pleural air was evacuated. One gram of cephaloridine (Eli Lilly and Co.), and 1 g of procainamide (E.R. Squibb and Sons) were administered intramuscularly. The dogs were allowed to recover. Twenty-four hours after coronary ligation, the dogs were reanesthetized with i.v. pentobarbital, 60 mg/kg, and the thoracotomy was reopened. Through an i.v. line placed in a hindlimb, 20 mM of manganous chloride solution (Alfa Products) were infused over 5 minutes to a total dose of 0.05 mmol/kg. Ten minutes after the completion of the manganous chloride infusion, the hearts were excised and the ventricles were emptied, rinsed with physiologic saline, and left empty. A small rubber glove was placed in the left ventricle to preserve its shape.

NMR Imaging

The prototype NMR imaging instrument (Technicare Corporation) consists of a horizontal-bore superconducting magnet operating at a field strength of 1.44 tesla, which corresponds to a proton NMR resonance frequency of 61.4 MHz; radiofrequency coils around the sample for stimulating and recording NMR signals; and a computer for signal processing and image reconstruction. The hearts were placed in a horizontal cylindrical carrier, which was inserted into the bore of the magnet. The long axis of the left ventricle was parallel to the bore of the magnet (i.e., parallel to the magnetic field). NMR images of the heart were obtained as tomograms oriented transversely to the long axis of the left ventricle using the steady-state free precession (SSFP) method of data collection described elsewhere. The radiofrequency pulse was 52°, and the

From the Cardiac Unit, Department of Medicine, and the Departments of Neurology and Radiology, Massachusetts General Hospital, Boston, Massachusetts.

Dr. Pohost is an Established Investigator of the American Heart Association.

Address for correspondence: Mark R. Goldman, M.D., NMR Imaging Project, Research 301, Massachusetts General Hospital, Boston, Massachusetts 02114.

Received January 7, 1981; revision accepted April 16, 1982.

pulse repetition interval was 7.3 msec. Tomograms were obtained from apex to base at 0.5-cm intervals by moving the carrier containing the heart stepwise through the sensitive plane between image acquisitions. Images were produced using a projection-reconstruction technique; data were acquired in a 256 × 256-pixel array and interpolated to 512 × 512-pixel display. Image data acquisition time was 2.3 minutes per tomogram.

Pathology Studies

After imaging, the hearts were sectioned transverse to the long axis of the left ventricle from apex to base at 1-cm intervals. The myocardial slices were stained with triphenyl tetrazolium chloride (TTC) (Sigma Chemical Co.) by immersion for 30 minutes in a solution consisting of 10 g of TTC dissolved in 500 ml of 5% dextrose in water at 37°C. This technique differentiates viable from necrotic myocardium in experimental myocardial infarction. The TTC-stained myocardial slices were then photographed with a video camera (COHU Instruments) through red, green, and blue filters sequentially; the images were digitized using a video frame buffer (DeAnza Instruments) and stored in a computer (VAX II/780, Digital Equipment Corp.). The super-imposed videographs were displayed on the video frame buffer system.

Data Analysis

The videographs of the pathologic sections were photographed from the video display and enlarged approximately fourfold by optical projection. The area of the entire left ventricular myocardium and the area of the myocardial infarct were determined by digital planimetry of each slice using a sonic x-y digitizer (Graf Pen, Science Accessories Corp.) interfaced to a computer (Sigma III, Xerox Corp.). The size of the infarct was expressed both as a fraction of the left ventricular area occupied by the infarct for a slice-by-slice comparison and as the percent volume of the entire left ventricle for an overall comparison for each dog. The fractional volumes used for the ventricle-by-ventricle comparisons were obtained by summing the fractional areas of the infarcts for each slice in a given ventricle and dividing by the number of myocardial slices in that ventricle. The corresponding NMR images were analyzed the same way; they were photographed, enlarged by optical projection and subjected to digital planimetry to determine the infarct size, and expressed as the fractional area or volume of the left ventricle occupied by infarct.

To test for reproducibility of planimetering infarct size, the planimetry of the infarcts from both the NMR images and the pathologic slices were performed by two independent observers and compared. The mean difference in infarct size between the two observers was 0.36 ± 0.38% (± sd). The correlation between infarct size determined by the two observers was y = 0.999x + 0.6% (r = 0.996).

Statistical Analysis

Linear regression was performed by the least-squares method. Significance between the regression line and the line of identity was determined by analysis of variance of the correlation coefficient. Significance was assumed if p < 0.05.

Results

All dogs tolerated the manganous chloride infusion without a change in heart rate or ECG. Circumflex coronary artery ligation produced posterior infarcts in all dogs. One apical section of one heart had no identifiable infarct; the remaining sections had easily delineated infarcts of 7–30% in fractional area. The infarcts constituted 2.8–21% of the total left ventricular myocardial volume.

The NMR images clearly showed regions of diminished signal intensity that corresponded to the areas of infarction (fig. 1). The fractional size of the infarct obtained from the NMR images was plotted against the pathologic infarct size obtained for the slice-by-slice comparison (fig. 2) and for the overall ventricle-by-ventricle comparison (fig. 3). There was good correlation between the two infarct sizing methods: the regression line slopes were 0.96 (r = 0.93) for the slice-by-slice comparison and 1.06 (r = 0.94) for the overall ventricle-by-ventricle comparison. The regression lines were not significantly different from the line of identity (p > 0.1).

Although the hearts were sliced at 1-cm intervals

Figure 1. (top) Myocardial slices, 1 cm thick, stained with triphenyl tetrazolium chloride from base (left) to apex (right) of an excised dog heart with a 24-hour-infarct. (bottom) The nuclear magnetic resonance images at corresponding levels of the unsliced heart. Note the crisp delineation of the infarct and high resolution of the internal cardiac anatomy.
corresponding to the NMR imaging planes, the plane of the pathologic section may not precisely match the plane of the NMR tomogram. Furthermore, the pathologic data are obtained from a surface (i.e., zero plane thickness), while the NMR images represent planes approximately 3 mm thick. Partial volume effects may therefore affect the correlation with pathology and increase the scatter of points.

Discussion

Accurate determination of infarct size is important in treating patients with coronary artery disease and is essential in evaluating techniques to limit infarction. Infarct size provides prognostic information and its measurement may assist in making therapeutic decisions.1-4 Noninvasive techniques for quantifying infarct size include electrocardiography, creatine phosphokinase release, echocardiography and radionuclide techniques.

NMR imaging is an attractive modality for clinical applications; it uses nonionizing magnetic and radiofrequency fields to generate high-resolution images. It is noninvasive, tomographic, and can acquire three-dimensional data. The present study demonstrates the potential value of this technique for the determination of infarct size. The data demonstrate that using the paramagnetic ion manganese for contrast enhancement, NMR imaging can be used to measure infarct size accurately in excised canine hearts 24 hours after coronary artery ligation. The left ventricle was empty in these experiments. The motion of blood through the beating heart markedly reduces its NMR signal intensity,5 but does not affect the appearance of the blood in x-ray tomography, for x-ray attenuation is independent of motion. Nevertheless, the lack of blood in the ventricular cavity provides a greater degree of contrast between the cavity and the myocardium than might be observed in vivo. Although blood motion should provide adequate contrast in imaging the beating heart, motion sensitivity may limit the accuracy of delineating myocardium from blood in the ventricular cavity. Using manganese as a paramagnetic contrast-enhancing agent may solve these problems, for manganese is actively taken up by viable myocardial cells and has a short half life in the blood pool, resulting in a high myocardium-to-blood ratio.

The ability to use proton NMR imaging to produce high-resolution tomograms of organs and live specimens relies on the ability of the mobile protons, which constitute much of biologic tissue, to absorb and re-emit radiofrequency radiation in the presence of a magnetic field. Appropriate application of magnetic field gradients allows spatial information to be encoded to the NMR signal, and image reconstruction is then possible.10 The capability of NMR imaging to exploit spatial variations in NMR properties such as relaxation times (the rate at which the excited protons relax back to equilibrium) has resulted in images that show tissue discrimination. Recently, NMR images of the brains of live human subjects have demonstrated the ability of NMR imaging to discriminate between gray and white matter.11,12 In the present study, excised hearts were used because the prototype NMR imaging system had a 3-inch bore and would only permit imaging of specimens the size of a dog heart. In addition, the imaging method was SSFP. The SSFP technique is useful because of its reported high efficiency and sensitivity, but...
motion such as that during normal cardiac contraction reduces the NMR signal to an unusable level. Other techniques of NMR image production, however, are not as sensitive to motion as SSFP, and images of the intact beating heart have been published.\textsuperscript{13}

Manganese was used as a contrast-enhancing agent in this study. The mechanism by which manganese delineates the infarct depends on the distribution of the ion and its effect on the NMR properties of the protons within that distribution. Radioactive tracer studies with gamma-emitting manganese-54 and positron-emitting manganese-52m have shown that intravenously administered manganese is a blood flow marker with a myocardium-to-blood ratio of greater than 40:15 minutes after injection.\textsuperscript{14} Regional studies comparing the activity of manganese-54 with labeled 15-\textmu microspheres demonstrate a good correlation.\textsuperscript{15}

The lateral border of a 24-hour-old canine infarct is sharply defined: the microcirculation is patent in the noninfarcted tissue and occluded in the infarct.\textsuperscript{16} There is a small zone, averaging less than 30\mu wide, in which the patency of the microcirculation is ambiguous, but it is of little consequence with regard to measuring infarct size. The distribution of manganese is then probably concordant with the microcirculation and manganese is taken up in the viable myocardium right up to the edge of the infarct.

The mechanism by which manganese increases the NMR signal in viable myocardium is complex, but is essentially due to the paramagnetic ion's effect on the relaxation times $T_1$ and $T_2$. The intensity of the NMR signal, $S$, in the SSFP mode for a given picture element\textsuperscript{6} is given by

$$S \propto \frac{\rho \sin \alpha}{\left( \frac{T_1}{T_2} + 1 \right) - \left( \frac{T_1}{T_2} - 1 \right) \cos \alpha}$$

where $S =$ signal intensity, $\rho =$ proton density, $\alpha =$ pulse angle (a constant for a given imaging experiment), $T_1 =$ spin-lattice relaxation time (a measure of the rate that the excited nuclei return to equilibrium relative to the static magnetic field) and $T_2 =$ spin-spin relaxation time (a measure of the rate of decay of the nuclear signal). If $T_1 > T_2$ (as is the case in many semisolids such as tissue), then

$$S \propto \frac{T_2}{T_1} \rho \sin \alpha.$$

Manganese dramatically shortens $T_1$ in myocardial tissue.\textsuperscript{19} Manganese probably differentially shortens $T_1$, relative to $T_2$, which results in increased signal intensity and image brightness. The infarct, which does not receive manganese, remains at its "normal" signal intensity and appears dark relative to the viable myocardium. This effect of manganese on myocardium contrasts with its effect in aqueous solution; in aqueous solution, the ion decreases $T_2$ proportionately much more than $T_1$, resulting in a darker image than pure water.

We previously demonstrated that when the SSFP method is used, manganese administration is necessary to delineate the infarct.\textsuperscript{5} Alternative pulse sequences might produce enough contrast between normal and infarcted tissue to discriminate with NMR imaging without the need for paramagnetic agents.

The extension of the results of the present study to human cardiac NMR imaging requires the consideration of two points. First, cardiac NMR imaging with alternative pulse sequences can be used to image the beating heart and can be gated to the cardiac cycle. High-resolution, gated cardiac tomographic images in man\textsuperscript{17} and high-speed (40-msec) cardiac images in rabbits\textsuperscript{18} have been produced, and the techniques are being evaluated and refined. Second, the potential toxicity of manganese or other paramagnetic agents must be considered. Information on the toxic effects of parenterally administered manganese is scanty. The dogs in our study did not suffer apparent hemodynamic changes during or shortly after manganese administration. Furthermore, similar imaging effects could probably be observed after much smaller doses of manganese. Other paramagnetic agents might produce similar results with no toxicity.

In conclusion, our data indicate that NMR imaging can produce tomograms of the distribution of mobile protons in tissue and can detect and quantify 24-hour-old canine myocardial infarcts when used in association with paramagnetic ion contrast enhancement. NMR images depict the infarct with high resolution in the excised heart. Anticipated future developments will include NMR imaging methods that can define infarct regions by inherent relaxation times. Clinical application of this approach appears likely in view of the initial success with gating.

Acknowledgment

The authors gratefully acknowledge John Newell and David Murphy for their expertise and help with our computer work and Luis Guerrero for help with surgical preparations. We also thank Beverly Volpe for help in preparation of the manuscript.

References

size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. Am Heart J 101: 593, 1981


Quantification of experimental myocardial infarction using nuclear magnetic resonance imaging and paramagnetic ion contrast enhancement in excised canine hearts.

Circulation. 1982;66:1012-1016
doi: 10.1161/01.CIR.66.5.1012

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/66/5/1012

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/