In Vivo ^{31}P Nuclear Magnetic Resonance Spectroscopy in Patients with Old Myocardial Infarction

KENICHI MITSUNAMI, M.D., MAMORU OKADA, M.D., TOHRU INOUE, M.D.
MASATAKA HACHISUKA, M.D., MASAHIKO KINOSHITA, M.D.
AND TOSHIRO INUBUSHI, PH.D.*

To assess the usefulness of in vivo ^{31}P nuclear magnetic resonance (NMR) spectroscopy, we investigated spectra from the myocardium in 6 patients with old Q-wave infarction (QMI), 6 with old non-Q-wave infarction (NQMI) and 9 controls by ECG-gated depth-resolved surface-coil spectroscopy. External hexamethylphosphoric triamide (HMPT) was used to quantify the signal intensities. Left ventricular weight in the region of interest (LVW) was estimated from ^1H magnetic resonance images. The extent score by ^{201}Tl scintigraphy was determined in 3 QMI and 4 NQMI patients. No significant differences were found among the 3 groups in peak area ratios of ^{31}P NMR spectra to phosphocreatine (PCr) or adenosine triphosphate (ATP). Compared with controls, significant reductions were observed in values for the peak areas of PCr normalized by the standard HMPT (PCr/HMPT) or by both HMPT and LVW (PCr/HMPT/LVW) for QMI patients ($p < 0.05$), and in ATP/HMPT and ATP/HMPT/LVW for QMI and NQMI patients ($p < 0.01$). There was a significant negative correlation between ATP/HMPT and the ^{201}Tl scintigraphy extent score ($p < 0.05$). These findings suggest that in vivo ^{31}P NMR spectroscopy can detect high-energy phosphate reduction in the infarcted myocardium and may be useful in evaluating myocardial viability. (*Jpn Circ J* 1992; **56**: 614–619)

PHOSPHORUS (^{31}P) nuclear magnetic resonance (NMR) spectroscopy is unique as a noninvasive method for measurement of adenosine triphosphate (ATP) and phosphocreatine (PCr) metabolism, which is intimately involved with the viability of myocardial cells. Development of whole-body NMR spectrometers and NMR localization techniques that spatially resolve the distribution of metabolites have made human heart studies feasible, with applications in myocardial infarction¹ and cardio-

myopathy^{2–3}. Up to the present time, however, no applications that assess human myocardial viability have been reported.

We therefore applied ^{31}P NMR spectroscopy to patients with old myocardial infarction who, supposedly, have less volume of viable myocardium than normal, and studied the ability of ^{31}P NMR spectroscopy to detect high-energy phosphate reduction in the myocardium, and thus to assess myocardial viability.

METHODS

Twelve male patients between the ages of 40 and 78 (mean $\pm$ standard deviation [SD],

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*The First Department of Internal Medicine and *Molecular Neurobiology Research Center, Shiga University of Medical Science*

Mailing address: Kenichi Mitsunami, M.D., The First Department of Internal Medicine, Shiga University of Medical Science, Tsukinowa-cho, Seta, Otsu 520-21, Japan

Fig.1. Representative human cardiac ^{31}P NMR spectra of Q-wave infarction group (A), non-Q-wave infarction group (B), and the control group (C). The ordinate scales are identical for all 3 cases. The peaks identified are those of phosphomonoester (PME), inorganic phosphate (Pi), phosphodiester (PDE), phosphocreatine (PCr), and γ , α , and β -phosphates of adenosine triphosphate (γ -ATP, α -ATP, and β -ATP).

59 ± 10) with old myocardial infarction (more than 2 months after the onset of symptoms) and 9 healthy male volunteers between the ages of 27 to 42 (33 ± 5) without any clinical evidence of cardiovascular disease were included in the study. Informed consent was obtained from all subjects. All patients had a history of definite elevation of serum creatine kinase in the acute phase, and coronary angiographic evidence of significant stenosis in the chronic phase. They were divided into Q-wave and non-Q-wave myocardial infarction groups. The Q-wave myocardial infarction (QMI) group consisted of 6 patients (age, 62 ± 10) who had abnormal Q waves in 2 or more leads of the standard 12-lead electrocardiogram (ECG) at the time of the NMR study. The non-Q-wave myocardial infarction (NQMI) group consisted of the remaining 6 patients (age, 56 ± 10). Five patients in the QMI group demonstrated anterior infarction, the other had inferolateral infarction. Five patients in the NQMI group had anterior infarction, the remaining one was posterolateral.

^{31}P NMR experiments were performed with a 1.5-T, 1-m bore imaging-spectroscopy system (Signa; General Electric Medical Systems, Milwaukee). Subjects were ex-

amined in the supine position. A home-built, 15-cm diameter, circular, ^{31}P - ^1H (proton) double-tuned surface coil for both transmission and detection was placed precisely over the anterior region of the left ventricle guided by conventional ^1H NMR imaging. An ECG was used to monitor heart rhythm and to provide a trigger for synchronous acquisition of NMR data. Spectra were spatially localized to the myocardium with depth-resolved surface-coil spectroscopy (DRESS)⁴ with a 2.5-cm-thick section. Magnetic field homogeneity was optimized by shimming focused on the water signal in the region of interest (ROI). Typical line widths were 0.3-0.6 ppm. Each spectrum represented an average of 256 free induction decays which were acquired on every other heart beat at end-diastole and end-systole (350 msec after R wave of ECG). NMR pulse power was kept constant for all subjects at a level that nuclei approximately received a 90° flip angle at a depth of 6 cm on phantom studies. The total study time was 45-75 min per subject. Immediately after acquisition from every subject, reference vial containing 1 ml (5.72 mmole) of hexamethylphosphoric triamide (HMPT) in a phantom was placed at exactly the center

Fig.2. Cardiac phosphocreatine levels (in arbitrary units) calculated from systolic and diastolic ^{31}P NMR spectral areas corrected for saturation, with normalization by external ^{31}P reference (PCr/HMPT in the upper panels) and also by left ventricular weight in the region of interest (PCr/HMPT/LVW in the lower panels). NQMI: non-Q-wave infarction group, QMI: Q-wave infarction group.

of the previous ROI. A ^{31}P spectrum was then acquired at an NMR pulse repetition time (TR) of 2 sec, under the same surface coil position and shimming condition as for each particular subject, and served as a standard for normalization of signal intensities.

Integrated areas of the resonances of phosphomonoester (PME), inorganic phosphate (Pi), phosphodiester (PDE), PCr, γ , α , and β -phosphates of ATP (γ , α , and β -ATP), and HMPT were measured after applying 10-Hz line-broadening exponential filter and fitting the peaks to Gaussian or Lorentzian lines with a GEN-1280 data processing station (General Electric, Fremont, Calif.). Each metabolite area was subsequently divided by the corresponding HMPT area for normalization. As for PCr and β -ATP peaks, NMR saturation factors (SF) were calculated from the formulas: $\text{SF} = 1 - 0.769 e^{-0.307\text{TR}}$ for PCr and

$\text{SF} = 1 - 0.633 e^{-0.517\text{TR}}$ for β -ATP, which were derived from the cardiac spectra of normal volunteers acquired at TR's of 1, 2, 3, and 15 sec in our laboratory, and were used for the compensation for signal saturation. This correction assumes that metabolites in the normal and infarcted myocardium have the same spin-lattice relaxation times. Left ventricular weight (LVW) in the ROI was estimated from ^1H magnetic resonance images, assuming that the left ventricular mass in the ROI was enclosed by two spherical surfaces, namely, epicardial and endocardial and that the specific gravity of the myocardial tissue was 1.05. Levels of ATP were represented by the β -ATP, since it is the least contaminated ATP resonance.

Myocardial single photon emission computed tomography was performed in 3 QMI and 4 NQMI patients with a gamma camera (GCA-901A, Toshiba Medical, Tokyo)

Fig.3. Cardiac ATP levels (in arbitrary units) calculated from systolic and diastolic ^{31}P NMR spectral areas of β -ATP corrected for saturation, with normalization by external ^{31}P reference (ATP/HMPT in the upper panels) and also by left ventricular weight in the region of interest (ATP/HMPT/LVW in the lower panels). NQMI: non-Q-wave infarction group, QMI: Q-wave infarction group.

equipped with a low energy, general purpose collimator interfaced to a dedicated computer (GMS-550U, Toshiba Medical) after an intravenous injection of 74 to 111 MBq of ^{201}Tl within one month (before or after) the NMR study. All patients were in stable condition during these periods. The computerized 2-dimensional polar map display proposed by Garcia et al⁵ and Caldwell et al⁶ was used to quantify the size of the perfusion defect in either the rest or the delayed image. The extent score was calculated as follows: extent score = (number of points with a value less than 2 SD's below the normal mean activity) / (total number of points in the polar map).

Data are presented as mean $\pm$ SD. We used the SPSS/PC+ system (SPSS Inc., Chicago) on an IBM PS/2 computer for all statistical analyses. The Scheffé test was ap-

plied to detect significant differences defined by the analysis of variance among the three groups⁷. The significance of differences between systolic and diastolic spectra was tested by Student's paired t test. Correlation between NMR spectra and the ^{201}Tl scintigraphy extent score was assessed by linear regression analysis. A probability (p) value of less than 0.05 was considered to be significant.

RESULTS

QMI, NQMI, and control groups were found to be similar with respect to mean weight, height, body surface area, TR's at systolic and diastolic NMR acquisitions, and LVW ($29.2 \pm 6.1\text{g}$ for QMI, 34.1 ± 7.7 for NQMI, and 32.3 ± 3.5 for the control). Subjects in the control group were younger than

Fig.4. Cardiac ATP level (ATP/HMPT in arbitrary units) calculated from systolic ^{31}P NMR spectral areas of β -ATP corrected for saturation with normalization by external ^{31}P reference was compared with the extent score by ^{201}Tl scintigraphy in either the rest or the delayed image.

those in QMI ($p < 0.00001$) and NQMI ($p < 0.0005$) groups. There was no significant difference between TR's at systolic and diastolic acquisitions in each group.

Representative ^{31}P NMR spectra are shown in Fig. 1. The ordinate scales are identical for all 3 cases. There were no consistent, significant differences among the 3 groups in the relative ratios of PME, Pi, PDE, γ -ATP, and α -ATP to PCr or β -ATP. Systolic and diastolic PCr/ β -ATP ratios for QMI, NQMI, and the control groups were 1.86 ± 1.29 and 1.77 ± 1.67 , 1.58 ± 0.38 and 1.57 ± 0.34 , and 1.08 ± 0.25 and 1.16 ± 0.36 , respectively, showing no significant differences among the 3 groups or between systolic and diastolic values in each group.

The areas of PCr normalized by HMPT (PCr/HMPT in arbitrary units) for systole and diastole were 0.22 ± 0.07 and 0.17 ± 0.08 for QMI, 0.37 ± 0.21 and 0.30 ± 0.15 for NQMI, and 0.50 ± 0.14 and 0.53 ± 0.22 for the control. The areas of PCr normalized by both HMPT and LVW (PCr/HMPT/LVW in arbitrary units) for systole and diastole were 0.79 ± 0.37 and 0.64 ± 0.43 for QMI, 1.05 ± 0.45 and 0.87 ± 0.41 for NQMI, and 1.59 ± 0.53 and 1.68 ± 0.84 for the control. Significant decreases of PCr/HMPT and

PCr/HMPT/LVW were discerned in the QMI group compared with the control (Fig. 2).

The areas of β -ATP normalized by HMPT (ATP/HMPT in arbitrary units) for systole and diastole were 0.16 ± 0.08 and 0.14 ± 0.07 for QMI, 0.24 ± 0.13 and 0.21 ± 0.18 for NQMI, and 0.46 ± 0.06 and 0.46 ± 0.12 for the control. The areas of β -ATP normalized by both HMPT and LVW (ATP/HMPT/LVW in arbitrary units) for systole and diastole were 0.54 ± 0.24 and 0.46 ± 0.19 for QMI, 0.68 ± 0.30 and 0.61 ± 0.43 for NQMI, and 1.44 ± 0.24 and 1.43 ± 0.44 for the control. There were significant decreases of ATP/HMPT and ATP/HMPT/LVW in the QMI and NQMI groups compared with the control (Fig. 3). Linear regression analysis revealed significant negative correlation between ^{201}Tl scintigraphy extent score and ATP/HMPT in systole in 3 QMI and 4 NQMI patients (Fig. 4).

DISCUSSION

This study, using an external reference to quantify the signal intensities, suggests that in vivo ^{31}P NMR spectroscopy by DRESS technique can detect high-energy phosphate reduction in the human infarcted myocardium.

In general, NMR spectroscopy can estimate absolute metabolic contents in principle as long as corrections are made for NMR relaxation times, nonuniformities in the NMR excitation and detection fields, coil Q factors, and the actual volumes of samples contributing to each spectrum. Recently Bottomley and his associates quantified PCr and ATP concentrations in the normal myocardium by ^{31}P NMR spectroscopic imaging using a coaxial coplanar multiple NMR surface detection coil set and a ^{31}P concentration reference on the chest⁸ We were unable to acquire NMR spectra of a reference simultaneously with those of a subject, as Bottomley did, because the signal intensity of the ^{31}P concentration reference placed beside the chest wall was found to be unstable in our case. This was most probably due to radiofrequency field (B_1) inhomogeneity and/or eddy current. This prevented us from measuring the absolute metabolic concentra-

tions, because the Q factor of the surface coil is susceptible to loading conditions to the coil, which change from subject to reference.

Nevertheless we detected a relative reduction of high-energy phosphates in the infarcted myocardium making adequate correction for the nonuniformities in NMR excitation and detection fields using an external HMPT reference in the phantom. Thus, in vivo ^{31}P NMR spectroscopy seems to be useful in evaluating myocardial cell viability, although great efforts must be made before achieving one's goal. These include improving the sensitivity of ^{31}P NMR spectroscopy and further investigations into the linkage of ATP levels and myocardial cell viability in the face of the possibility of compartmentation of ATP.

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