Spectroscopy

^{31}P-Magnetic Resonance Spectroscopy Studies of Cardiac Transplant Patients at Rest

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ABSTRACT

Studies in animal models and patients have suggested that ^{31}P-magnetic resonance spectroscopy (MRS) may be useful in diagnosing transplant rejection, but such studies often are confounded by the late inclusion of patients after transplantation. The present study examined the utility of ^{31}P-MRS in the diagnosis of acute allograft rejection during the first posttransplant month. Thirteen recent heart transplant recipients underwent 57 resting ^{31}P-MRS studies within 24 hr of a biopsy. Subjects lay supine with a 10-cm surface coil placed over the heart. A 1-dimensionsal chemical shift imaging protocol was used to collect spectral information. Spectra from the heart were weighted for distance from the coil and summed before analysis. ANOVA and Duncan's multiple range test were used to analyze the data comparing phosphocreatine (PCr)/ATP ratios with biopsy scores. Transplant patients had significantly lower myocardial PCr/ATP ratios when compared with a normal control group (1.27 ± 0.27 versus 1.61 ± 0.22, $p < 0.001$). However, when the patient group was classified by biopsy score, the expected order of score, $0 > 1 > 2 > 3$, was not obtained. Rather, the order was $2 > 0 > 1 > 3$. Although the difference between scores 2 and 3 was significant (1.46 versus 1.14, alpha $= 0.05$ level), the lower three groups were statistically indistinguishable. In addition, the PCr/ATP ratios were not predictive of future biopsies. Although significantly lower than normal control subjects, resting myocardial PCr/ATP ratios of transplant subjects are not useful in assessing the

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level of rejection. It is suggested that the measurement may be more predictive in mildly exercised myocardium.

KEY WORDS: Heart; Magnetic resonance spectroscopy; Rejection; Transplantation.

INTRODUCTION

The current gold standard for assessing human cardiac allograft rejection is repeated endomyocardial biopsies. A noninvasive method for the clinical detection of rejection still remains a worthy goal. Preliminary studies in both animals (1–4) and humans (5–9) using ^{31}P-magnetic resonance spectroscopy (MRS) have suggested that alterations in bioenergetics of transplanted myocardium do occur during the rejection process. The mechanism of these alterations and whether they are of clinical importance remains unknown. We have performed over 175 MRS studies in heart transplant patients, and the present work summarizes the final phase I work involving 57 studies in 13 patients. Phase I is defined as the direct comparison between *resting* ^{31}P-MRS and endomyocardial biopsies. Other laboratories have conducted similar studies and have found no correlation between the phosphocreatine (PCr)/ATP ratio and the endomyocardial biopsy results; however, these studies might have suffered from the various times posttransplantation that the studies were first performed. This time range could vary from weeks to years and hence might suffer from artifacts generated by combining an analysis of both acute and chronic rejection. The present study sought to remove that parameter from the analysis equation by studying patients soon postoperatively, beginning from 2 to 3 weeks after the operation. In addition, the animal models indicated that the ^{31}P-MRS data might respond more rapidly than the more traditional methods of analysis. Therefore, the data were analyzed both directly with the corresponding biopsy obtained within 24 hr and two later periods ("lag period") that might better correlate with the MRS data obtained. Thus, resting ^{31}P-spectra were obtained and the PCr/ATP ratios were evaluated against the traditional biopsy result and subsequently analyzed to determine if a lag period of correlation between biopsy and PCr/ATP ratio might be occurring.

MATERIALS AND METHODS

Thirteen recent (<2 weeks) heart transplant recipients (12 men and 1 woman, aged 54 ± 10 yr, range, 33–67) gave informed consent to undergo a series of MRS exams (two to five) within 24 hr of a biopsy but before having any augmented immunosuppression therapy implemented. Fifty-seven MRS transplant studies were performed on a Philips ACS 1.5-T system. In addition, 16 normal volunteers (5 men and 11 women, aged 33 ± 8 yr, range, 22–53) were also studied. While in the supine position, electrocardiogram wires were placed on the subject's chest for gating. A 10-cm transmit/receive ^{31}P surface coil was then positioned over the apex of the heart and secured with a Velcro strap to reduce respiratory artifacts. Coil positioning was confirmed by scout imaging using a typical routine multislice spin-echo pulse sequence (TE 30 msec, TR 530 msec, slice thickness 9 mm, field of view 400 mm, acquisition matrix 204 × 256). A voxel containing the anterior left ventricular wall (approximately 200 ml) was selected such that the anterior boundary was parallel to the chest wall to minimize chest muscle contamination. The voxel's water signal was used for shimming with the body coil. A cardiac-gated 1-dimensionsal chemical shift imaging (1D-CSI) sequence with image-selected in vivo spectroscopy column selection and adiabatic pulses was performed in the anteroposterior direction with 32 1-cm slices, 1024 points, SW = 2000 Hz, TR = 3 sec minimum, NA = 48. The outer profiles were sampled less often, making the entire MRS study 75–90 min.

Spectral data were transferred to a Sun Sparc10 for processing using Philips software (Sunspec and FIT-MASTERS) that included apodization (both Gaussian and exponential) and convolution difference before Fourier transformation. The two to four cardiac spectra that showed no chest wall contamination were weighted for distance from the coil, summed, and analyzed. The ratio of the resonance areas of PCr and ATP was obtained and corrected for both T1 differences (determined from fully relaxed cardiac spectra) and blood contamination (determined by the levels of 2,3-diphosphoglycerate and subtracting the appropriate amount of ATP as calculated from a previously run blood standard).

Statistical Analysis

At each time point, the data consisted of a bivariate pair made up of PCr/ATP and biopsy score. The main hypothesis to be tested was whether the PCr/ATP score 0, 7, or 14 days before the biopsy could predict the biopsy
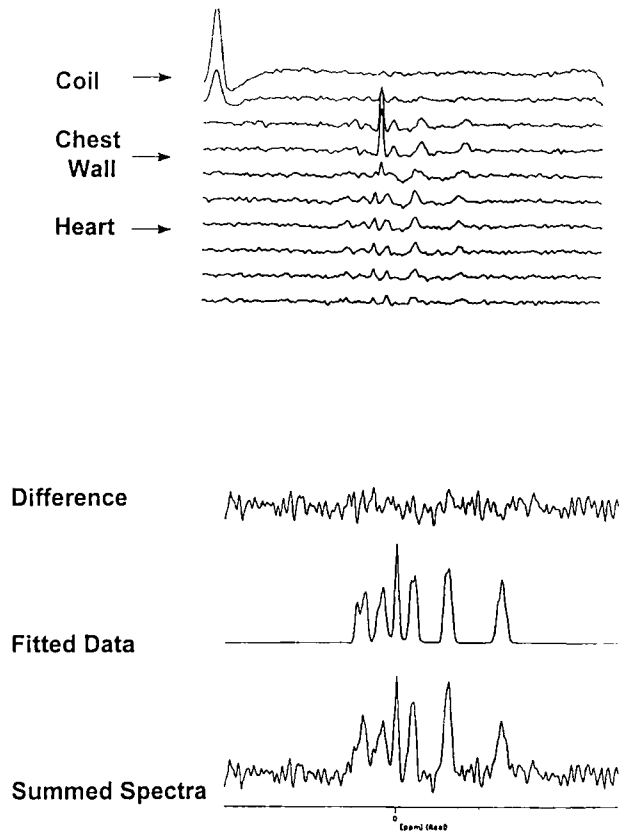
result. In seven cases, there were data missing at either 7 or 14 days before the observed biopsy, and in these cases, values were estimated from the available PCr/ATP data by linear interpolation. The biopsies were graded using the 1991 Billingham/International Society for Heart and Lung Transplantation rejection scoring system. For purposes of analysis, four biopsy classes were considered, namely 0, 1, 2, and 3. In any level, no distinction was made between the subclasses A and B.

Two approaches were used to investigate the question of interest. First, the data were grouped according to their biopsy reading, taking the particular "lag" into consideration. Thus, for example, in one case, the value of the biopsy 7 days after the PCr/ATP measurement was used to classify the measurement. In some cases, a single subject contributed more than one observation to a grouping, and in these cases those observations were averaged so that there was only one observation per subject in a cluster. Once the data were grouped, one-way ANOVA models were used to investigate the hypothesis. If the hypothesis was true, then some group differences should be seen. In cases where a significant overall ANOVA was found ($p < 0.05$), multiple comparisons among the means were made using Duncan's multiple range test.

RESULTS

Spectra from a patient who underwent cardiac transplantation is shown in Fig. 1 (top). The 1D-CSI approach enables one to clearly delineate where the coil is located due to the external reference vial, the chest wall, due to the inherently higher PCr signal in skeletal muscle, followed by several spectra derived wholly from cardiac muscle. The fitting of the summed spectra is shown in Fig. 1 (bottom), which displays both the raw spectrum, the fitted data, and then the difference between them, indicating the robustness of the fit. Using the 1D-CSI method described above, our control value for PCr/ATP was 1.61 ± 0.23 (Table 1). Compared with normal subjects, all transplants, regardless of biopsy score, had a significantly lower PCr/ATP ratio. However, there was no significant difference in the PCr/ATP ratios with biopsy scores ($p > 0.05$), but there was a trend to decreased PCr/ATP at the highest biopsy score. Over the course of 6-8 weeks, there was no correlation between biopsy scores and differences in the PCr/ATP within the same subject.

The means and sample sizes for each group and each lag are given in Table 1. For a lag of zero, the test for overall model adequacy was significant ($p < 0.017$). Ex-

Figure 1. A representative ^{31}P-MRS, cardiac-gated, 1D-CSI sequence with an image-selected in vivo spectroscopy column from 32 1-cm slices displaying selected spectra. Top: Sample data set illustrating the localization. Bottom: Fitting of the summed spectra.

amination of the group means revealed the following order: group 2 > group 0 > group 1 > group 3. The Duncan's test (adjusted for unequal sample sizes, alpha = 0.05) indicates that the mean for group 2 is greater than that for group 3 but no different from that for group 0. At the same time, group 0 does not differ from group 1 and group 1 does not differ from group 3. Under the study hypothesis, we would have expected the ordering 0 > 1 > 2 > 3. Thus, although there appear to be some differences, they are not supportive of the hypothesis. For 7- and 14-day lags, the overall ANOVA was not significant ($p = 0.57$ and $p = 0.69$, respectively) and no differences could be detected among the group means. In each case, the observed ordering of the means also failed to support the hypothesis of a relationship between the value of the PCr/ATP measurement and the biopsy score.

In the second approach to addressing the hypothesis,

Table 1

Comparison of Resting Myocardial PCr/ATP Ratios in Subjects with Heart Tranplants and Biopsy Scores

	Biopsy Score			
	0	1	2	3
All transplants time lag	1.37 ± .30 (19)*	1.20 ± .23 (21)†	1.30 ± .38 (4)‡	1.20 ± .27 (13)†
0 days	1.39 (9)	1.16 (9)	1.46 (3)	1.14 (9)
7 days	1.24 (7)	1.18 (7)	1.41 (3)	1.27 (7)
14 days	1.25 (5)	1.26 (7)	1.47 (3)	1.24 (6)

Results are presented as the means ± SD, with population size in parentheses.
Normal control values were 1.61 ± 0.22 (16).
*$p < 0.01$, † $p < 0.001$, ‡ $p < 0.05$, when compared with normal control values.

individual correlation coefficients were calculated for each subject with lags of 0, 7, and 14. This could only be done for a subset of subjects because some lacked sufficient sequential data to make this possible. For lag 0, there were eight subjects who qualified, and for lags of 7 and 14 days, there were seven. If the hypothesis that low PCr/ATP implied high biopsy score was true, we would expect these correlations to be negative. For the case of zero lag, six of eight subjects had negative correlations, but only one of these was statistically different from zero. For a lag of 7 days, only three of the seven were negative and none were statistically significant. Finally, for a lag of 14 days, four were negative with only one statistically significantly different from zero. Thus, these results do not support the hypothesis either. Both methods of analysis led to the same conclusion: There is no consistent predictive value for determining rejection in the PCr/ATP measurements of resting myocardium.

DISCUSSION

This study was designed to determine whether PCr/ATP ratios are useful in assessing human myocardial allograft rejection. Although there was a significant decrease in the PCr/ATP ratio in all transplants, it was not predictive of the level of rejection as measured by endomyocardial biopsy.

Of significant importance is the work of Herfkens et al. (10), who were the first group to study cardiac transplant patients using ^{31}P-MRS. They reported, albeit with few patients ($n = 6$), a tendency demonstrating that ^{31}P-nuclear magnetic resonance spectroscopic information

was encouraging for evaluating transplant rejection non-invasively.

The decrease observed in the present study is similar to that seen in patients with mildly rejection transplants by Bottomley et al. (8) and van Dobbenburgh et al. (9). Bottomley et al. reported anomalies in cardiac high-energy phosphate metabolism in rejecting and normal heart transplant patients as assessed by ^{31}P-MRS. Even though some of the NMR data appeared to correlate with moderately rejecting patients, others did not. Although a statistically significant difference was observed between rejecting myocardium and normal volunteers, the ability to obtain useful information regarding the discrimination between mild and moderate rejection was not indicated. In addition, this study examined a mixture of patients and did not discriminate between early acute rejection, late acute rejection, and chronic rejection.

In 1993, van Dobbenburgh et al. also used a 1D-CSI approach for serial studies of cardiac transplant patients soon after surgery. Thirteen patients were examined 71 times within 12 hr of obtaining the endomyocardial biopsy. They reported that both nonrejecting and rejecting hearts by biopsy had similarly low PCr/ATP ratios, 1.03 and 1.06, respectively. Considering the PCr/ATP for normal control subjects to be approximately 1.80, these results in themselves are most interesting. But of more interest is a single "healthy" patient who displayed a PCr/ATP ration of 0.62, not only 21 days after transplantation, but had strikingly low ratios nine additional times, up to 156 days posttransplant, with ratios ranging from 0.73 to 1.19 and biopsy scores from 0 to 3A. They reported that regression analysis of all data points showed no correlation between biopsy and MRS results, yet there were temporary low PCr/ATP early after transplantation both in

nonrejecting and rejecting hearts. They concluded that the latter finding may obscure possible decreased PCr/ATP in rejecting hearts and attributed this lowering of PCr/ATP soon after surgery to the ischemic condition during hypothermic preservation and possibly reperfusion injury.

Recently, a non-MRS method assessed myocardial high-energy phosphate depletion by traditional wet chemical analysis in allograft rejection after orthotopic human heart transplantation. Benvenuti et al. (11) reported values for ATP concentrations that were significantly lower in both the moderate and severe rejection as indicated by biopsy. It is of special note that the ATP concentration returned to normal in seven cases when sequential analysis was performed after administration of augmented immunosuppression therapy. This study demonstrated that alterations of high-energy phosphate compounds during the rejection process do occur and thus adds further support in favor of the authenticity of the observed decreases in the PCr/ATP ratio as assessed by ³¹P-MRS. It should be noted that measurements of absolute concentrations of ATP and PCr by MRS, which are achievable, might be of greater value than PCr/ATP ratios alone. As with all previous studies in which ATP has been shown to decrease, this observation implicates ischemic involvement although metabolic alteration such as substrate utilization cannot be ruled out.

In principle, the use of ¹H-MRS in the heart should provide greater sensitivity, and some preliminary studies have been reported (12). The advantages of using ¹H-spectroscopy are primarily increased sensitivity. The disadvantages include creatine being the only visible major metabolite, the necessity of water suppression, and questions about the visibility of the creatine pool (13,14).

A comparison between biopsy and bioenergetics may not be expected to correspond directly because the comparison being made is between the host's immune system and the bioenergetics of the cell. One possible explanation for the observed change in the PCr/ATP ratio is the early stages of vascular rejection. This aspect of rejection has become an increasing factor in the transplant patient (15). It involves diffuse capillary endothelial cell swelling with increased capillary permeability, which may lead to a decrease in oxygen availability. These symptoms are not measured by the biopsy. The decrease in O_2 is reflected in the ³¹P-MRS spectrum through the creatine kinase reaction as a decrease in PCr/ATP ratio. An alternate explanation is that the equilibrium constant of the myocardial creatine kinase has changed due to the transplantation. To test this new hypothesis, one could stress the heart with some mild form of exercise. We have be-

gun studies using the "hand-grip" stress test pioneered by Weiss et al. (16). This method is convenient and less stressful overall than pharmacological methods, although the patients do need to be "coached" through the stress period. If oxygen were limiting, one would expect a further decrease in the PCr/ATP ratio. Alternatively, if the O_2 were not limiting, the PCr/ATP ratio would not change.

In summary, from three independent studies, one can conclude that the PCr/ATP ratio is lower in certain cardiac transplant patients. This decrease does not, unfortunately, correlate with the gold standard, the endomyocardial biopsy, thus making this approach unacceptable for detecting rejection clinically at the present time. However, the presence and persistence of lower PCr/ATP ratio in the transplanted heart beckons further investigation. Low PCr/ATP ratios in human myocardium are not normal, and additional studies to elucidate the mechanism for this lowering might ultimately have an impact on the cardiac transplant patient population. In any event, a noninvasive technique for detecting rejection, especially in the ever-increasing pediatric population, is still vitally needed.

ACKNOWLEDGMENT

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