Normal High Energy Phosphate Ratios in “Stunned” Human Myocardium

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Objectives. We sought to investigate whether alterations in cardiac high energy phosphates occur in postischemic “stunned” human myocardium.

Background. Transient postischemic myocardial dysfunction is a common phenomenon that occurs in a variety of clinical settings in the absence of necrosis, and its pathogenesis is still unclear. Cardiac high energy phosphates are reduced during ischemia, and persistently altered myocardial high energy phosphate metabolism has been suggested as a mechanism contributing to stunning.

Methods. We studied 29 patients with a first anterior myocardial infarction (MI) who underwent successful reperfusion within 6 h of the onset of chest pain. These patients underwent 31P magnetic resonance spectroscopy (MRS) a mean of 4 days after MI for measurement of left ventricular contractility and relative high energy phosphate metabolites. Twenty-one patients underwent a second 31P MRS study a mean of 39 days after MI. Eight volunteers served as control subjects.

Results. Global and infarct area wall motion scores improved significantly between the early and late studies. No difference was found between early cardiac phosphocreatine (PCr)/beta-adenosine triphosphate (beta-ATP) ratios in patients and control subjects ([mean ± SD] 1.51 ± 0.17 vs. 1.61 ± 0.18, respectively, p = 0.17) or between early and late study results in patients (1.51 ± 0.17 vs. 1.53 ± 0.17, respectively, p = 0.6). For alpha of 0.05, the study had a 90% power to detect a 9% difference.

Conclusions. The results of this study demonstrate normal myocardial PCr/ATP ratios in patients with myocardial stunning after reperfusion and suggest that relative cardiac high energy phosphates are not depleted in stunned human myocardium.

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on at least three anterior wall leads on the admission electrocardiogram and developed Q waves on the follow-up ECG. Creatine kinase-MB fraction levels peaked at 103 ± 56 U/liter (mean ± SD) (normal value <10 U/liter) an average of 12 ± 4 h after the onset of symptoms. Twenty-seven patients received intravenous thrombolytic agents, whereas the other 2 underwent primary percutaneous transluminal coronary angioplasty.

All 29 patients underwent MRS studies before hospital discharge an average of 4 ± 2 days after MI. Cardiac catheterization was performed in all patients to confirm coronary patency. The first MRS study and coronary angiography were performed <2 days apart. No patient had clinical or laboratory signs of coronary reocclusion between the studies. Twenty-one patients had a repeat MRS study during follow-up (mean 39 ± 37 days after MI). Three patients died during admission, and five refused to participate in the follow-up study. Eight normal volunteers <40 years old, with no history or findings of heart disease, served as control subjects for the MRI spectroscopic studies. Table 1 shows the clinical characteristics of the final 21 study patients.

Cardiac catheterization. Cardiac catheterization was performed through the right brachial artery, using standard techniques. Coronary angiography and left ventriculography were performed in all patients. Arterial patency, defined as Thrombolysis in Myocardial Infarction (TIMI) grade 3 flow or better in the infarct-related artery was assessed in blinded manner by two independent observers.

MRS studies. MRS studies of the heart were performed with a Gyroscan ACS imaging and spectroscopy system (Philips Medical Systems), with an effective magnetic field of 1.5 tesla. Subjects were placed in the bore of the magnet in the supine position. A surface radiofrequency coil tuned to the 31P resonance frequency (63.83 MHz) was placed over the precordium. Spectra were obtained from the apical and anterior walls of the left ventricle, using the image-selected in vivo spectroscopy (ISIS) technique (9) as a localization technique for selecting a volume of interest from which the signal was obtained (nominal volume ~24 cm3). Signal acquisition was gated by the ECG with a repetition time of 2,500 ms. Trigger delay was 200 ms; 512 measurements were obtained over ~21.5 min. After processing, the area under each peak was determined by manual fitting with computer assistance. Skeletal muscle was consistently avoided when positioning the spectroscopic region of interest with MRI guidance. We corrected for blood contamination of the spectra using the 2,3-diphosphoglycerate (2,3-DPG) doublet resonances from blood as reference. A blood ATP signal corresponding to 15% of the integrated signal from the two 2,3-DPG peaks was subtracted from the beta-ATP value before calculation of metabolite ratios (10), which were corrected for partial saturation effects (11). An exponential multiplication function with a line-broadening factor of 12 Hz was applied to the signal before Fourier transformation to improve the signal to noise ratio (mean of 13 for PCr and 9.5 for ATP). For baseline correction, we applied polynomial subtraction. The PCr/beta-ATP ratio was calculated for each patient. Inorganic phosphate could not be measured because of its low concentration and overlap with the 2,3-DPG peak from the blood. Reproducibility of MRS measures of the cardiac PCr/ATP ratio was evaluated in separate studies 1 week apart in a small number of normal volunteers, and consistent findings were observed (1.68 ± 0.04 vs. 1.69 ± 0.02).

Magnetic resonance (MR) images of the heart were obtained with the same equipment and the 1H body coil. Cine MR imaging of the left ventricle was performed using a gradient-echo imaging sequence with a flip angle of 35°, an echo time of 6 ms and a repetition time equal to the RR interval. The acquisition matrix was 128 × 128, and the slice thickness was 10 mm oriented along the long axis of the left ventricle. From these images, we derived an imaging plane for subsequent left ventricular (LV) short-axis cine MR images.

For the purpose of evaluating regional contractility, the left ventricle was divided into segments using the model suggested by the American Society of Echocardiography (12). The short-axis cine MR images were transferred to a workstation, and a region of interest was selected for each segment. The volume of each segment was obtained using a computer-assisted semiautomated method (Amira, Tocnius Medical Systems, San Diego, Calif.). From these images, we derived an imaging plane for subsequent short-axis cine MR images.

<table>
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<tr>
<th>Pt No.</th>
<th>Age (yr)/Gender</th>
<th>Δt MRS (days)</th>
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F = female; M = male; MRS = magnetic resonance spectroscopy; PMI = postmyocardial infarction; Pt = patient; Δt = difference in time between early and follow-up studies.

**Table 1. Patient Characteristics**
The ventricle was divided into 16 segments, as proposed by the American Society of Echocardiography (12). Regional wall motion was assessed in a blinded manner by two observers using a six-point scoring system as follows: 0 = hyperkinesia; 1 = normal function; 2 = mild hypokinesia; 3 = hypokinesia; 4 = akinesia; 5 = dyskinesia (13). A global left ventricle function score was calculated as the average of regional scores. The observers had no knowledge of the time of the study (early or late) or the clinical characteristics of the patients. Each segment assessed for contractility represented at least 12 to 18 cm³ of tissue. Because 18 of 21 patients had three regions of dysfunction, and MRS studies were performed at the same examination without repositioning of the subject, the LV region interrogated metabolically with spectroscopy (nominal size 24 cm³) was well contained in the region of dysfunction (24 to 54 cm³).

Statistical analysis. Results are expressed as mean value ± SD. Comparisons between values obtained during the early and late studies were performed with the Wilcoxon test. Calculations were computed using the Statistical Analysis System (SAS Institute). During the design of the study, we calculated that a sample size of 16 patients would be necessary to demonstrate a 10% difference in PCr/ATP ratios between baseline and follow-up studies with a power of 90%. Therefore, a study group of 21 patients should have sufficient power to detect a 9% difference at the 0.05 level of confidence.

Results

Regional wall motion. Mean global LV function score was 1.60 ± 0.35 during the in-hospital studies and improved spontaneously during follow-up to 1.20 ± 0.30 (p < 0.0001). Some regional wall motion scores (Fig. 1) also showed spontaneous improvement, decreasing from 2.46 ± 0.68 to 1.54 ± 0.78, from 2.0 ± 0.89 to 1.40 ± 0.75 and from 2.37 ± 0.71 to 1.41 ± 0.59 in the septal, anteroseptal and anterior regions, respectively (p < 0.001 for all differences). Other LV wall segments did not show significant changes in score between the in-hospital and follow-up studies.

31P MRS. Figure 2 shows a representative example of a 31P MR spectrum acquired 4 days after anterior MI. The mean myocardial PCr/beta-ATP ratio was similar between the early and late studies in all patients (1.51 ± 0.17 and 1.53 ± 0.17, p = 0.5) (Fig. 3). These values were similar to the mean PCr/beta-ATP ratio observed in normal volunteers (1.61 ± 0.18, p = 0.17) and to those reported by others (4,14). Because LV function scores did not return to normal levels in all patients, we also evaluated myocardial PCr/beta-ATP ratios in subsets of patients with complete (n = 8) and partial improvement (n = 13) of LV function. Early and late ratios were
1.51 ± 0.16 versus 1.56 ± 0.18 (p = 0.21) and 1.51 ± 0.18 versus 1.52 ± 0.19 (p = 0.7), respectively, in patients with complete and partial improvement in LV function scores. There was no difference between early and late ratios in both groups (p = 0.54). In addition, no correlation was found between early and late regional wall motion scores and PCr/beta-ATP ratios (p = 0.61 and 0.50, respectively). Thus, anterior myocardial PCr/beta-ATP ratios were normal in these patients with transient ventricular dysfunction after severe ischemic insult.

Discussion

Transient postischemic dysfunction was first described in 1975 by Heyndrickx et al. (15) in a canine model, and numerous clinical and experimental studies have since investigated this phenomenon. In 1982, Braunwald and Kloner (2) coined the term “stunned myocardium” for the reversibly injured, nonnecrotic myocardial tissue whose functional recovery is delayed after reperfusion (2,16).

Many mechanisms have been proposed to explain the pathogenesis of myocardial stunning. Insufficient production of energy by the mitochondria, abnormalities in the use of energy by the myofibrils, impaired response to sympathetic stimulation, extracellular matrix damage, calcium overload, excitation–contraction uncoupling and free radical production have all been suggested, but none have been conclusively proved (17–19), and most are difficult or impossible to investigate in humans under physiologic conditions.

Metabolic changes related to myocardial stunning. Severe abnormalities in metabolism and metabolite depletion are consistently observed after prolonged, irreversible ischemic injury, whereas metabolic abnormalities in reversibly stunned myocardium are more subtle (20,21). Canine models of myocardial stunning are characterized not only by modest nucleotide depletion that may persist for days but also by PCr levels that normalize early after reperfusion (22,23). This rapid resynthesis of PCr and essentially normal PCr/ATP levels have been taken as evidence that a defect in mitochondrial synthesis of ATP is not present in experimentally stunned myocardium (22,23).

Our objective in the present study was to evaluate whether alterations in myocardial PCr/ATP ratios were present and could contribute to postischemic dysfunction in humans. We observed contractile impairment that spontaneously improved during follow-up in patients who underwent rapid reperfusion for acute anterior MI. Myocardial high energy phosphates were measured using spatially localized 31P MRS, and no significant differences in cardiac PCr/beta-ATP ratios were found between our patients early after ischemia and normal volunteers. Similarly, this ratio did not change significantly in our patients during follow-up, despite a significant improvement in regional wall motion.

These findings are consistent with many previous observations of normal PCr/ATP recovery in experimentally stunned, noninfarcted myocardium. Several studies (24–26) in isolated hearts have demonstrated a rapid return of high energy phosphates to their baseline levels shortly after reperfusion, long before full contractile recovery. Similar observations of normal or high PCr/ATP ratios have been made in intact animals after coronary occlusion (27). Likewise, several investigations (28,29) show a dissociation between myocardial ATP levels and contractile function in stunned myocardium and an improvement in contractility of stunned myocardium during inotropic stimulation. Others (20) showed that the PCr/ATP ratio achieved supranormal levels 3 h after experimental reperfusion. An overshoot of the PCr/beta-ATP ratio was not observed here most likely because of the delay of several days between reperfusion and MRS evaluation. Thus, to our knowledge, these are the first observations of high energy phosphates in stunned human myocardium, with normal cardiac PCr/beta-ATP ratios consistent with those of previous experimental studies in postischemic, transiently dysfunctional noninfarcted myocardium.

Limitations of the study. A potential limitation of this study is that the region of interest included both subendocardial and subepicardial tissue; thus, differences in high energy phosphate ratios between regions of the myocardium could not be resolved. We cannot exclude the possibility that some degree of partial volume averaging from skeletal muscle, chamber blood and unstunned myocardium may have occurred, but we believe this to be relatively small and, if present, most likely similar between early and late studies. Another potential limitation is the use of PCr/beta-ATP ratios to index high energy phosphate metabolism rather than measures of absolute PCr or ATP concentrations or rates of high energy phosphate synthesis. PCr synthesis rates through the creatine kinase reaction have been measured in the human heart with 31P MRS (30), but such measures have not been made in patients with cardiac disease because the measures require high field (4 T) systems generally not available in hospital settings. Recently, Yabe et al. (31) used 31P MRS to measure the ATP and PCr content of human myocardium with reversible ischemia or scar diagnosed by exercise thallium scintigraphy, and a reduction in PCr was observed. However, measures of metabolite contents are not routinely performed and have not been rigorously validated with other techniques in the current setting. Therefore, the current data demonstrating normal cardiac PCr/beta-ATP ratios in stunned human myocardium cannot exclude the possibility of a parallel reduction in both PCr and ATP concentrations. However, such a similar reduction in both PCr and ATP after ischemia seems unlikely because PCr declines more dramatically than ATP during brief human cardiac ischemia (4,5), and PCr in animals recovers fully in viable tissues but not in nonviable myocardium (21,22). In our patients, high energy phosphate ratios would probably have reached new steady state levels by 4 days after MI, when the initial study was conducted. It is clear that the cardiac PCr/ATP ratio can be used to detect abnormalities in high energy phosphate metabolism in humans because this ratio is acutely reduced during exercise stress in patients with ischemic heart disease (4,5) and chronically reduced in subjects with
severe heart failure (7,8). We therefore suggest that the normal and unchanged cardiac PCr/beta-ATP ratios at rest in postischemic stunned human myocardium in the current study indicate that high energy phosphate depletion is not present and does not contribute to human myocardial stunning in this setting.

Conclusions. The primary pathophysiologic consequence of myocardial ischemia is altered metabolism, which contributes to impaired contractile function. Although a persistent abnormality in metabolism after ischemia could contribute to dysfunction during reperfusion, the observations of the present study demonstrate that myocardial PCr/beta-ATP ratios are not reduced in postischemic transiently dysfunctional human myocardium. These findings suggest that relative myocardial high energy phosphate depletion is not present in patients with myocardial stunning after reperfusion.

References