In vivo Murine Cardiac PCr and ATP Concentrations Measured by Magnetic Resonance Imaging and Spectroscopy

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SYNOPSIS: An in vivo 31P MRI and 31P MR spectroscopic method is proposed and validated for the measurement of in vivo high energy phosphate metabolite (PCr and ATP) concentrations in normal (n=7) and thoracic aorta constriction (TAC) (n=10) mouse hearts. The in vivo MR results for [ATP] are in good agreement with those obtained using an in vitro luminescent assay on perchloric acid extracts of the same hearts.

INTRODUCTION: Normal energy phosphate metabolism is critical for cardiac function and viability1,2. The combination of 31P magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) is uniquely able to assess the critical relationship between metabolism and function in the in vivo beating heart. 31P MRS/MRI studies in heart failure (HF) patients have documented reductions in the ratio of PCr/ATP, the concentrations of [ATP] and [PCr], and in ATP flux through the creatine kinase reaction1,4. Mouse models of HF offer a novel, appealing means to investigate the mechanistic links between altered energy metabolism and function because they allow gene-targeted metabolic interventions and mimic energetic aspects of human HF5. Although PCr/ATP measures have been validated for the in vivo mouse heart6,7; there has been no methods validated for measuring [ATP] or [PCr]. Thus there were two aims for this study. First, we sought to adapt human 31P MRS methods for quantifying [PCr] and [ATP] to the in vivo mouse heart and validate them by comparison with conventional invasive biochemical measures from a luminescent assay (LA). Second, we used the new 31P MRS method to test the hypothesis that in vivo myocardial [ATP] is reduced in the TAC model of murine HF.

MATERIALS AND METHODS: Experiments were carried out on a Bruker Biospec MRI/MRS spectrometer equipped with a 4.7T/40cm Oxford magnet and a 12cm (inner diameter) actively shielded gradient set, as previously described3,8. Studies were first performed on mice then, on the same day, on an external phantom using comparable protocols. More specifically, in vivo MRI/MRS studies were carried out on 7 control and 10 mice four weeks after TAC (weight 28-33g). Anesthesia was induced by -1% isoflurane, as previously described3,8. The probe included 22-mm 31P MRI and 13-mm 31P MRS coils. MRI were obtained with 31P MRI FLASH sequence (TE=1.5ms, TR=12ms, NEX=12) and spatially-localized 31P MRS with a one-dimensional chemical shift imaging (1D SCI) sequence (16 mm F.O.V., 16 phase encoding steps, NEX=64, TR =2000ms) using modified BIR4 90° adiabatic pulses. After completing the MRI/MRS study, the mice were sacrificed and the hearts immediately frozen in liquid nitrogen and later extracted with perchloric acid for metabolite measures. The 31P MRS/MRI studies in heart failure (HF) patients have documented reductions in the ratio of PCr/ATP, the concentrations of [ATP] and [PCr], and in ATP flux through the creatine kinase reaction1,4. Mouse models of HF offer a novel, appealing means to investigate the mechanistic links between altered energy metabolism and function because they allow gene-targeted metabolic interventions and mimic energetic aspects of human HF5. Although PCr/ATP measures have been validated for the in vivo mouse heart6,7; there has been no methods validated for measuring [ATP] or [PCr]. Thus there were two aims for this study. First, we sought to adapt human 31P MRS methods for quantifying [PCr] and [ATP] to the in vivo mouse heart and validate them by comparison with conventional invasive biochemical measures from a luminescent assay (LA). Second, we used the new 31P MRS method to test the hypothesis that in vivo myocardial [ATP] is reduced in the TAC model of murine HF.

RESULTS: Representative images and spatially-localized 31P spectra are shown for the mouse (Fig.1) and the phantom (Fig.2). The results of the 31P MRS and LA methods are summarized in Table 1. The mean in vivo [PCr] and [ATP] of roughly 10 and 5 µmol/g, respectively, agree with prior in vitro measures4. Critically, there is no significant difference in [ATP] determined by MRS and LA (p=ns). [PCr] is significantly higher by MRS (p<0.006) but this is likely due to the well recognized rapid degradation of PCr during sacrifice. [PCr] is significantly decreased in TAC mice as compared to controls by both MRS (p=0.001) as well as LA (p<0.0002). [ATP] is significantly decreased in TAC mice by MRS (p<0.03) as well as LA (p<0.03).

DISCUSSION: We present a novel, convenient, and accurate method of measuring cardiac 31P metabolite concentrations in the in vivo mouse heart from 1D CSI 31P MRS spectra. Our [ATP] results are consistent with the literature9 and with conventional invasive measures. This new MRS method does not add significantly to the time of an anesthesia mouse study. Although these are tissue rather than intracellular concentrations, tissue measures are obtained in human heart and are used, in part, to calculate ATP flux through the creatine kinase reaction. Cardiac creatine kinase [ATP] is reduced after TAC in the in vivo mouse heart.

REFERENCES:

Fig. 1: Axial 31P MRI of mouse (top) at the left ventricle (LV) and corresponding cardiac 31P spectrum (bottom).

Fig. 2: 31P MRI of phantom containing inorganic phosphate (top) and corresponding 31P spectrum (bottom).

Table 1. PCr and ATP concentrations obtained (µmol/g wet wt.) by MRS and LA method (Mean ± S.D.)

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>MRS method</th>
<th>Enzyme luminescent assay</th>
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<tbody>
<tr>
<td>[PCr]</td>
<td>Control</td>
<td>TAC</td>
</tr>
<tr>
<td>10.4 ± 1.4</td>
<td>6.7 ± 2.0</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>[ATP]</td>
<td>4.99 ± 0.9</td>
<td>4.0 ± 0.8</td>
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