In vivo cardiac ^{31}P-MRS in a mouse model of heart failure

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Introduction

Cardiomyopathies are often associated with an imbalance between energy demand and supply. Therefore, assessment of the myocardial energy status provides valuable insight in the underlying mechanisms of cardiac disease. Phosphorus-31 MR spectroscopy (^{31}P-MRS) offers a unique opportunity to non-invasively study the high energy phosphate metabolites that are of key importance for cardiac functioning: adenosine triphosphate (ATP) and phosphocreatine (PCr). Mouse models of cardiac disease are used extensively, but currently ^{31}P-MRS of the *in vivo* mouse heart is not widely available. Here, we demonstrate the use of 3D Image-Selected *In vivo* Spectroscopy (ISIS) [1] for non-invasive cardiac ^{31}P-MRS in the mouse [2], to investigate the myocardial energy status in a mouse model of heart failure.

Methods

Animal preparation - Male C57BL/6 mice underwent thoracic aortic constriction (TAC) surgery as described previously [3], inducing pressure-overload cardiomyopathy. Seven weeks after surgery, anesthetized mice (n = 5, 29.9 ± 2.3 g) were positioned prone in an animal support, with the heart above a custom-built, actively decoupled two-turn ^{31}P surface coil (Ø 15 mm) for signal reception. ECG and respiration were measured to monitor vital signs, and to enable cardiac triggering and respiratory gating. The setup was entered into a horizontal bore 9.4 T MR system (Bruker Biospin) equipped with a volume coil comprising a quadrature ^{1}H birdcage resonator and a linear ^{31}P birdcage resonator (Rapid Biomedical), which were used for ^{1}H MR imaging and shimming, and for RF transmission for ^{31}P-MRS, respectively. Healthy wild-type mice (n = 4, 24.2 ± 0.7 g) served as controls.

^{31}P-MRS - Cardiac cine ^{1}H MR images were made for reference purposes and to quantify left ventricular (LV) function. A ~6 mm cubic voxel was positioned to enclose the end-diastolic LV myocardium. Shimming of the voxel was done manually on the proton signal using an ECG triggered PRESS sequence. ECG triggered, respiratory gated 3D ISIS was performed on the same voxel (inversion pulses: 6.25 ms hyperbolic secant, bandwidth 37.5 ppm; excitation pulse: 1.2 ms sinc, bandwidth 32 ppm; both pulses on-resonance for γ-ATP; TR ≈ 2 seconds; 768 scans/96 cycles). Since steady-state of magnetization is essential for correct localization with the 3D ISIS addition/subtraction scheme, unlocalized dummy excitations were performed during respiratory gates to maintain a TR of ~2 seconds [4].

To determine the correction factor for partial saturation, metabolite T_1 values were measured with conventional unlocalized saturation-recovery experiments of the chest. *Ex vivo* spectra of fresh blood were acquired (37 °C; 1.2 ms sinc pulse; TR = 2000 ms; NA = 256) to establish to what extent ATP from blood contributes to the *in vivo* spectra.

Results

The MR protocol took ~3 hours to complete and was well tolerated by all mice. LV end-diastolic volume (LVEDV) and mass normalized to body weight (LVM/BW) were higher in TAC mice compared to controls (P < 0.01), whereas LV ejection fraction (EF) was reduced in TAC mice (P < 0.05, Table 1). Resonances from PCr (0.00 ppm) and ATP (γ, -2.48 ppm; α, -7.52 ppm; β, -16.26 ppm) could be distinguished in the cardiac ^{31}P-MR spectra (Figure 1A-B). Inorganic phosphate (P_i, 5.02 ppm) was obscured by diphosphoglycerate (2,3-DPG, ~5 ppm) arising from blood. Myocardial PCr/γ-ATP, corrected for partial saturation, was lower in TAC mice when compared to healthy controls (P < 0.05). *Ex vivo* ^{31}P-MRS of fresh blood revealed only a marginal contribution of ATP from the blood to the *in vivo* spectra (Figure 1C).

Discussion

Here, we demonstrate a non-invasive method for localized ^{31}P-MRS of the *in vivo* mouse heart. By maintaining a constant TR, signal contamination from tissue surrounding the heart was minimized. Importantly, using the same setup, anatomical and functional data could be obtained during the same experimental session. Combined, this is a powerful approach to investigate cardiac disease progression and the effects of therapeutic strategies in longitudinal study designs. With this method, we showed that decreased EF in TAC mice is accompanied by decreased PCr/γ-ATP, indicating a disturbed energy homeostasis in this mouse model of heart failure [5].

References

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	Control (n = 4)			TAC (n = 5)		
LVEDV [μL] **	61.8	±	6.0	91.7	±	19.0
EF [%] *	64.4	±	5.2	45.4	±	20.0
LVM/BW [mg/g] **	3.1	±	0.2	4.4	±	0.6
PCr/γ-ATP [-] *	1.1	±	0.2	0.8	±	0.2
T_1 PCr [s]	2.6	±	0.2	2.7	±	0.1
T_1 γ-ATP [s]	1.2	±	0.2	1.4	±	0.3

Table 1 Left ventricular functional and morphological parameters, and results from ^{31}P-MRS of the *in vivo* mouse heart. *, P < 0.05; **, P < 0.01.

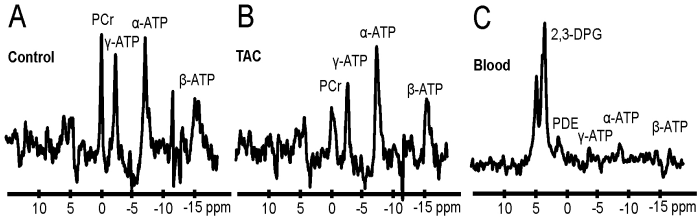

Figure 1 *In vivo* cardiac ^{31}P-MR spectra of (A) a healthy control mouse, and (B) a TAC mouse. C: *Ex vivo* ^{31}P-MR spectrum from fresh blood at 37°C. PDE; phosphodiesters.