Is ribose and creatine supplementation beneficial in chronic heart failure? An *in vivo* ^{1}H-MRS / ^{31}P-MRS / cine-MRI study in murine hearts

Kiterie M.E. Faller[1], Craig A. Lygate[1], Mahon L. Maguire[1], Debra J. Medway[1], Victoria L. Thornton[1], Stefan Neubauer[1], and Jurgen E. Schneider[1]
[1]*Cardiovascular medicine, University of Oxford, Oxford, Oxfordshire, United Kingdom*

Introduction

The failing heart is characterized by changes in myocardial energetics. Independantly of the aetiology of heart failure (HF) there is a decrease in creatine (Cr) and phosphocreatine (PCr) levels followed by total adenine nucleotides (TAN), with a drop in ATP only in advanced disease. Increasing Cr and/or total adenine nucleotide pool have been suggested as a therapeutic strategy[1]. It has previously been shown that ribose supplementation ameliorates total adenine nucleotide (TAN) pool depletion in acute models of HF[2]. However, data on chronic models are lacking. Thus, the aim of this study was to assess the effect of increased myocardial creatine and ribose levels on cardiac function and high-energy phosphate metabolism in a murine permanent coronary artery ligation (CAL) model by using advanced *in vivo* MR techniques including cine-MRI, ^{1}H-MRS and ^{31}P-MRS.

Material and methods

Protocol:

C57BL/6J mice (WT) and transgenic mice with elevated myocardial creatine levels (CrT-OE) were separated in four study groups:

- Group S: WT, sham surgery, no ribose
- Group I: WT, myocardial infarction (MI) surgery, no ribose
- Group R: WT, MI, receiving ribose in drinking water
- Group C: CrT-OE mice (preselected by ^{1}H-MRS[3] to provide a myocardial Cr range of 88-140 nmol/mg protein), MI, receiving ribose.

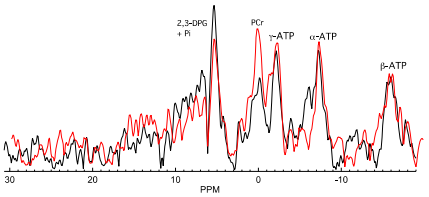

Figure 1: Representative cardiac ^{31}P-MRS spectra from mice from groups S (red) and I (black)

All mice then underwent CAL or sham surgery. Ribose supplementation was initiated the following day, and 8 weeks later mice underwent cardiac ^{31}P-MRS, cine-MRI[4] and LV cannulation. Hearts were then harvested and subjected to biochemical analysis (HPLC).

MR experiments:

All experiments were conducted on a 9.4T/210mm bore Magnex magnet with a Varian Direct Drive console.

^{31}P-MRS was acquired using an actively decoupled variable tune/match 14 mm diameter surface coil with a double tuned ^{1}H/^{31}P volume resonator (Rapid Biomedical, Germany) as previously described[5]. In brief, 2D CSI data were acquired *in vivo* in short axis orientation using acquisition weighting (Hanning) with an in plane voxel size of 2.3 x 2.3 mm (nominal resolution 3.8 x 3.8 mm, 13 x 13 PE steps) before zero filling in a 30 x 30 mm field of view, slice thickness of 5 mm with 8196 scans in total. Acquisitions were cardiac gated and a TR of ~250 ms (two cardiac cycles) was used with a 30° flip angle. Spectra were fitted using AMARES, corrected for saturation and blood contamination for myocardial spectra.

Results

We confirmed that ribose administration in drinking water increased myocardial ribose levels by 2 fold compared to the control group (p=0.004, n=5) without having any effect on the cardiac function of healthy C57BL/6J mice.

Infarcted groups were matched for infarct size using cine-MRI as shown in Table 1. Increasing myocardial creatine and ribose levels had no protective effect against the cardiac dysfunction observed in the infarcted hearts (Table 1).

Despite a decrease in Cr levels by 10 % following myocardial infarction in CrT-OE mice, [Cr] remained elevated compared to the sham group and the two other infarcted groups (Table 1). As expected, Cr levels were lower by 18 and 16 % in infarcted groups I and R compared to the sham group (Table1). Ribose feeding did not prevent TAN pool decrease secondary to MI (Table 1). Finally, no difference in PCr/ATP ratio was found between groups with the small number of data sets available to date (Figure 1 & Table 2).

Discussion

This work is the first robust test of ribose and creatine as potential therapeutic agents in chronic HF, since previous studies (using ribose only) have not controlled for obvious confounding factors such as matching for infarct size. Work is in progress to increase the group sizes to provide sufficient statistical power. However, our data so far suggest that the combination of creatine and ribose are not beneficial in chronic heart failure. New approaches are therefore needed to preserve TAN pool following MI. The non-invasive nature of the various techniques applied in this study including total Cr quantification (^{1}H-MRS), infarct size and LV mass measurement (cine-MRI) were crucial and allowed for a comprehensive and multiparametric characterization of this model. Finally, ^{31}P-MRS should prove to be an essential technique in evaluating PCr, a metabolite very difficult to assess *ex vivo* due to its extreme lability.

	Group S (n=16)	Group I (n=13)	Group R (n=4)	Group C (n=6)
Morphology				
Body weight (g)	25±3	24±2	24±2	23±1
LV weight (mg) (cine-MRI)	79±9	101±9***	117±3***	98±10***
Lung/BW (x 10^3)	5.7±0.9	6.6±1.6	7.9±1.7	7.5±2.2
Infarct size (%)	/	39±8	42±8	39±10
Functional parameters				
Ejection fraction (%)	69±8	26±6***	22±8***	28±8***
LVSP (mm Hg)	101±10	86±6***	83±3**	87±8**
LVEDP (mm Hg)	8±5	13±4*	15±3*	15±5*
dP/dt_{max} (mmHg/s)	8631±2457	4614±1380***	4420±588**	4903±1099**
Biochemistry (HPLC)				
[Cr] (nmol/mg prot)	80±6	68±6**	66±4*	113±16***
TAN (nmol/mg prot)	45±7	40±4	42±3	39±1

← *Table 1: Biochemical and cardiac function parameters. Data are expressed as Mean ± SD. * p<0.05, ** p<0.01, *** p<0.001 vs group S. LVESP: Left ventricular systolic pressure. LVEDP: left ventricular diastolic pressure.*

	Group S (n=8)	Group I (n=4)	Group R (n=2)	Group C (n=3)
Heart	1.4 ± 0.2	1.2 ± 0.3	1.4 ± 0.4	1.6 ± 0.4
Skeletal muscle (chest)	2.5 ± 0.3	2.3 ± 0.2	3.2 ± 0.3	2.5 ± 0.5

Table 2: PCr/β-ATP ratio assessed by ^{31}P-MRS. Data are expressed as Mean ± SE.

References: [1] Ingwall et al., *Eur J Heart Fail.* 2010;12:1268. [2] Zimmer, *Basic Res Cardiol.* 1992;87:303. [3] Schneider et al., *Magn Reson Med.* 2004;52:1029. [4] Schneider, *J Cardiovasc Magn Reson.* 2006;8:693. [5] Maguire et al., *Proc Intl Soc Mag Reson Med.* 2009 : 1785.