Creatine Kinase-Overexpression Improves Adriamycin-induced Dysfunction and in vivo ATP kinetics in Murine Hearts

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SYNOPSIS: Adriamycin (ADR) is a commonly used life-saving antineoplastic agent that also causes dose-dependent cardiotoxicity. Impaired energy metabolism may contribute to contractile dysfunction in human heart failure and may play a role in ADR-induced cardiotoxicity. We overexpressed the myofibrillar isoform of creatine kinase (CK-M), the major cardiac energy reserve reaction, to test the hypothesis that increasing CK-M expression would improve energy metabolism and, in turn, improve contractile function in dysfunctional ADR hearts. ^{1}H MRI and ^{31}P MRS results reveal that CK-M overexpression improves depressed CK energetics and cardiac dysfunction in ADR hearts.

INTRODUCTION: Adriamycin (ADR) is an antineoplastic agent often used for advanced solid tumors and several hematopoietic malignancies. However, ADR's therapeutic application is limited in part by adverse effects on cardiac function[1-2].There is some evidence that ADR adversely affects mitochondrial function[3-6] but there is no *in vivo* information on its impact on creatine kinase (CK), the prime energy reserve of the heart. Improving CK metabolism in ADR-induced cardiotoxicity by increasing CK expression is a logical means to test this hypothesis because reduced CK metabolism has been linked to human and experimental HF[7-8]. We created mice conditionally and cardiac-specifically overexpressing the myofibillar isoform of CK (CK-M-OE), the most abundant isoform, and administered ADR to them and non-transgenic littermates in a regimen previously shown to induce cardiotoxicity and contractile dysfunction[9]. We quantified the *in vivo* metabolic and contractile consequences of CK-M-OE in ADR hearts with ^{1}H MRI/^{31}P MRS.

MATERIALS AND METHODS: Experiments were carried out on a Bruker Biospec MRI/MRS spectrometer equipped with a 4.7T/40cm Oxford magnet, as previously described[9]. Intra-peritoneal injection of ADR (5mg/kg) was administered once a week for five weeks as described previously[9]. *In vivo* ^{1}H MRI was performed at 6 and 8 weeks, and ^{31}P MRS was performed at 7 weeks after ADR or placebo administration, on placebo-treated control (n=6), ADR-treated control (n=10 at 6wk); (n=8 at 8wk), and CK-M-OE placebo-treated (n=6) and CK-M-OE ADR-treated (n=7) mice. Multi-slice cine MR images were acquired of the entire left ventricle (LV) to assess LV mass, ventricular volumes and ejection fraction (EF)[9]. The *TRiST* method[10] was used to measure CK flux from [PCr]x(k_f), where k_f(CK pseudo-first order rate constant). [PCr] and [ATP] were evaluated *in vivo* as described previously[11]. Results are presented as mean ± SD. Comparisons of MRI- and MRS-derived measures of LV anatomy, function and metabolism among multiple groups were analyzed by one way ANOVA and pair wise comparisons were performed with the Tukey test.

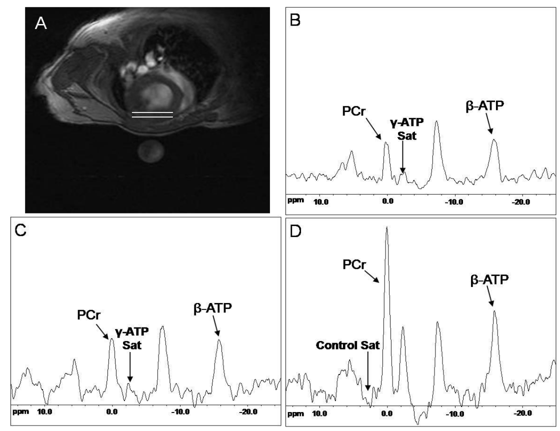

Fig.1: (A) Typical transverse ^{1}H MR image of a mouse thorax with ^{31}P MR cardiac voxel denoted by white lines (B) ^{31}P MR spectrum with γ-phosphate of ATP saturation with TR=1.5s, NEX=96, (C) γ-phosphate ATP saturation with TR=6s, NEX=32, and (D) control saturation spectrum with TR=10s and NEX=16. PCr; phosphocreatine, β-ATP; β-phosphate of adenosine triphosphate

RESULTS: A representative ^{1}H image and spatially-localized *TRiST* ^{31}P spectra are shown in Fig.1. CK-M overexpression did not alter baseline contractile function. However at 7 wk of ADR the mean PCr/ATP ratio, [PCr], k_f and CK flux were significantly reduced in ADR-treated hearts and this was associated with contractile dysfunction with significant reductions in EF and SV (Table 1).

In contrast, PCr/ATP, k_f and CK flux were significantly higher in CK-M-OE hearts receiving ADR than in control (Table 2). Importantly, after 8 weeks the EF and SV were significantly higher in CK-M-OE ADR mice than in control ADR mice (Table 1). Thus CK-M-OE improves depressed energetics in ADR hearts and this is associated with significant improvements in contractile function.

DISCUSSION: First, we observe that not only is cardiac PCr/ATP reduced after ADR, as previously reported[3], but that for the first time [PCr], k_f and CK flux are significantly reduced during ADR administration. Second, CK-M overexpression increases the rate of ATP synthesis through CK (CK flux) in placebo hearts but has no effect on PCr/ATP, [PCr] and [ATP]) (Table 2) or on contractile function (Table 1). Third, critically, CK-M overexpression improves cardiac energetics in ADR hearts and improves ADR-induced contractile dysfunction (Tables 1 & 2). Metabolic strategies, in particular those targeted at improving CK energy metabolism, promise a new avenue for treating or preventing cardiac dysfunction associated with ADR and thereby may allow continued or higher dose administration of this life-saving drug for some patients with malignancy.

Table 1	SV, µl	EF, %
Control	43.4±4	66.5±2
Control ADR (6wk)	43.4±5	60.1±4
Control ADR (8wk)	36.2±3$^{¢}$	51.7±7$^{\$,*,¢,\chi,\xi}$
CK-M Overexp	46.0±4	66.7±3
CK-M ADR (6wk)	40.8±4	61.8±5
CK-M ADR (8wk)	38.9±7	61.8±3

$^{\$}$, p<0.05 with compared to control, *, p<0.05 with compared to ADR treated control (6wk), $^{¢}$, p< 0.05, with compared to CK-M overexp, $^{\chi}$, p<0.01 with compared to ADR treated CK-M (6wk), $^{\xi}$, p<0.01 with compared to ADR treated CK-M (8wk)

Table 2	PCr/ATP	[PCr] µmol/g	[ATP] µmol/g	K_f s^{-1}	Ck_{flux} µmol/g/s
Control	1.92±0.15	9.94±1.5	4.81±1.0	0.32±0.03	3.16±0.47
Control ADR (7wk)	1.53±0.13$^{*,\$,¢}$	6.94±0.5*	3.74±0.2	0.27±0.01$^{\$,¢}$	1.90±0.11$^{*,\$,¢}$
CK-M overexp	1.96±0.02	8.47±2.0	4.21±0.9	0.54±0.09*	4.49±1.20*
CK-M ADR (7wk)	1.88±0.14	8.50±0.5	3.61±0.2	0.46±0.04$^{*,\$}$	3.88±0.44

*, p<0.05 with compared to control, $^{\$}$, p<0.05 with compared to CK-M overexp $^{¢}$, p<0.05 with compared to CK-M ADR (7wk)

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