



NADP Stimulates DNA Repair after Thermodynamic Stress in Cardiomyocytes of Aging MDX Mice



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Abstract

Using thermodynamic stress we aim to analyze the possibility of regulating the DNA double strand (DSBs) formation in heart tissue of aging mdx mice by intraperitoneal injections of NADP⁺ which is shown to mediate key regulators of oxygen stress and cell death.

Keywords: Heart; Cardiomyocytes; Dynamic Stress; DNA Repair; MDX Mice

Introduction

Nowadays there is a significant increase in the survival value of patients due to the drug therapy which, in turn, causes a significant increase of the incidence rate of heart failure and cardiomyopathy developing due to cardiomyocytes death [1]. Thus, one of the main issues of cardiomyocyte biology is their survival under rough circumstances arising provided that there is pathology of the myocardium.

One of the models with genetically determined myocardial dystrophy are mdx mice, where the absence of dystrophin synthesis is accompanied by the development of oxidative stress and of cardiomyopathy signs in the heart of aging mdx mice [2,3].

One of the oxidative stress manifestations in the heart of mdx mice is the constant presence of DNA fragments 65kbp in the left ventricles myocardial cells [4]. Low molecular DNA fragments were observed after the so-called thermodynamic stress (TDS) [5]. Formation of low molecular weight DNA fragments coincides with appearance of DNA double-strand breaks (DSBs). DNA endonuclease activity, mitochondrial damage and amyloid deposits in the myocardium of mdx mice [6-8].

One hour after TDS of young mdx mice the fraction of cardiomyocyte nuclei with DNA DSBs reaches 46 % while the proportion of labeled non-muscle cells reaches 9%. In 24h the proportion of nuclei with DSB decreases to 9% and 2% respectively, indicating the involvement of DNA repair in the survival of mdx mice cardiomyocytes after TDS. There was also the loss of cardiomyocytes in the myocardium after the TDS [6].

There is increasing evidence that the pool of nicotinamide adenine dinucleotide (NAD⁺) and its derivatives is depleted under stress conditions and during aging [9,10]. We suggested that increased DNA damage and oxygen stress in cardiomyocytes of mdx mice after TDS also lead to the decrease of NAD⁺ pool in cells. Here we focused our efforts on the effects of exogenous NADP⁺ on the DSB in myocardial cells after DS in aging mdx mice.

Materials and Methods

Identification was performed on 12-month-old male and female mdx mice carrying mutation in DMD gene (n=12) (RRID: IMSR_HAR: 1217). All procedures were approved by the local Ethics Committee of the Institute of Experimental Medicine RAS (St. Petersburg, Russia). All reagents were purchased from SIGMA-Aldrich (St. Louis, MO, USA) if not indicated otherwise.

Animals were submitted to TDS -- swimming for 5 min in 12 °C water. There upon animals were given a single intraperitoneal dose of NADP⁺ solution in water (700mg/kg weight). In 24h animals were deeply anesthetized and sacrificed by cervical dislocation. Slides of left cardiac ventricles for histological examination were sectioned into 10-µm thickness using cryostat (Bright Instrument Co LTD, GB).

To reveal DNA DSB the phosphorylation product of histone H2AX was detected by anti-γH2AX antibodies [11]. Tissue sections were incubated overnight at 4 °C with polyclonal rabbit antibodies to γ-H2AX (Abcam, Cambridge, CA, USA, Abcam Cat# ab11174, RRID: AB_297813) at 1:200 dilution, then incubated for 20 min in biotinylated goat anti-rabbit IgG (Vector Lab, Burlingame,

CA), followed by incubation with horseradish peroxidase (HRP)-streptavidin for 20min. The antibody binding sites were visualized through reaction with DAB-H₂O₂ solution. Results obtained were presented as the means \pm the standard error of measurement (SEM) of at least three animals per group. The total number of cells per each myocardium was about 1000. When counting the mean values, the number of studied hearts was taken as the number of observations. One-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test were used to determine statistical significance between groups of young and aging mice. Differences were considered significant at $p < 0.05$.

Results

In the left ventricle myocardium of aging mdx mice, the fraction of nuclei with a positive staining for γ -H2AX in the absence of stress is $10.1 \pm 1\%$ (Table 1), which is higher than that of the DSBs accumulation in the myocardium of 3-month-old animals ($6.7 \pm 0.2\%$ ($p < 0.05$)). In young mdx mice TDS leads to the significant increase in the proportion of nuclei with a positive staining for γ -H2AX during first hour (42%), but then the number returns to the baseline within 24h, which indicates a sufficiently effective DNA reparation. In aging mdx mice TDS leads to the increase in the proportion of γ -H2AX-positive nuclei to $15.9 \pm 3\%$ after 1 h and $26 \pm 6\%$ after 24h. Such dynamics of DSB accumulation doesn't coincide with pattern of DSB in young mdx mice after TDS and C57Bl/6 mice after x-ray irradiation, where 20% of myocardial cell nuclei retain DSB in 24h.

Table 1: Proportion of γ -H2AX-positive nuclei in cardiomyocytes of mdx mice after thermodynamic stress (TDS).

Exposure	Time after TDS, h	
	1	24
No exposure (control)	$10.1 \pm 1\%$	
DS	$15.9 \pm 3\%*$	$26 \pm 6\%*$
DS, followed by NADP+ administration	$11.2 \pm 2\%*$	$10.9 \pm 1\%*$

* $p < 0.05$ against control

After NADP+ administration the fraction of γ -H2AX-positive nuclei in myocardium of aging mdx mice does not change from the original value and remains at the same level during first 24h after DS

(see table). Thus, myocardium of aging mdx mice is characterized by higher degree of DSB accumulation in comparison with young animals. We observed marked accumulation of DSB during first 24h after DS. The administration of NADP+ prevents this accumulation which may be explained by the intracellular NADP+ and NAD+ pool restoration leading to the decrease of oxygen stress level in cardiomyocytes. The results suggest that exogenous NADP+ may be used as potential antioxidant in DMD treatment for preventing myocardium damage.

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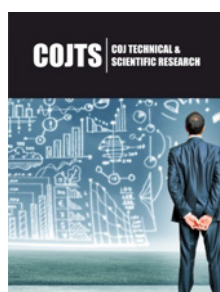
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