NOX2 Inhibition Restores Contractility, Intracellular Calcium Handling and Reduces Arrhythmogenicity in Dystrophic Cardiomyopathy

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Dystrophic cardiomyopathy is the cardiac manifestation of Duchenne muscular dystrophy (DMD). Intracellular calcium (Ca²⁺) handling is abnormal in the cardiomyocytes of the mdx mouse, a model of DMD (1). We tested the hypothesis that NADPH oxidase (NOX2)-derived oxidative stress may cause disturbances in Ca²⁺ handling and contractility in mdx mice heart cells.

Mdx mice (19 months old, n= 35) and background controls mice (C57BL/10SnJ, n=45) were used for the study. To assess superoxide production, isolated cardiac myocytes from wild type and mdx myocytes were loaded with 2',7'-dichlorofluorescein diacetate (DCF), and treated with vehicle (DMSO) or the NOX inhibitors apocynin (100 µmol/L) or VAS2870 (20 µmol/L) (60 cells each group, from 4 hearts) and visualized using confocal microscopy. Contractility, assessed as sarcomere shortening and calcium was evaluated using fura-2 (2). NOX2 expression was evaluated by Western Blotting.

Fig 1. Effect of NOX inhibition superoxide production in mdx myocytes. ** p<0.001 vs. wt control, ***p<0.0001 vs. wt control, ††, p<0.001 vs. mdx control, †††, p<0.0001 vs. mdx control (ANOVA).

NOX2 expression was increased fivefold in the mdx hearts compared to wild type (n= 6 each group, p<0.005, t test) and NOX2 inhibition with apocynin and VAS2870 decreased superoxide production in mdx myocytes (Fig. 1). Next, we studied the impact of NOX2 inhibition on contractility and Ca²⁺ handling in mdx cardiomyocytes. Contractility was decreased in mdx myocytes (n=25) compared to wild type (n= 31, p<0.05, ANOVA). Pre-treatment with apocynin restored this response towards normal. In addition, the amplitude of evoked Ca²⁺ transients that was diminished in mdx myocytes (n= 21) compared to wild type (n=35), was also restored upon NOX2 inhibition (p<0.05, ANOVA). Total sarcoplasmic reticulum (SR) Ca²⁺ content was reduced in mdx myocytes (n=12 wild type and mdx, p<0.05, ANOVA). This content was normalized by apocynin treatment. At the same time, NOX2 inhibition decreased dramatically the production of spontaneous diastolic Ca²⁺ release events (p<0.05, ANOVA) and decreased the SR Ca²⁺ leak in mdx myocytes (p<0.05, ANOVA).

These results indicate that in mdx hearts, NOX2 inhibition increases contractility by improving the SR calcium handling. NOX2 inhibition reduced SR Ca²⁺ leak, probably reducing the sensitivity of the ryanodine receptor. Targeting NOX2 in dystrophic cardiomyopathies may help to restore heart function.
(2) Gonzalez DR et al (2010 *J Biol Chem*; **285**:28938-28945