The role of reactive oxygen species in the hearts of dystrophin-deficient mdx mice

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Williams IA, Allen DG. The role of reactive oxygen species in the hearts of dystrophin-deficient mdx mice. Am J Physiol Heart Circ Physiol 293: H1969–H1977, 2007. First published June 15, 2007; doi:10.1152/ajpheart.00489.2007.—Duchenne muscular dystrophy (DMD) is caused by deficiency of the cytoskeletal protein dystrophin. Oxidative stress is thought to contribute to the skeletal muscle damage in DMD; however, little is known about the role of oxidative damage in the pathogenesis of the heart failure that occurs in DMD patients. The dystrophin-deficient (mdx) mouse is an animal model of DMD that also lacks dystrophin. The current study investigates the role of the antioxidant N-acetylcysteine (NAC) on mdx cardiomyocyte function, Ca\textsuperscript{2+} handling, and the cardiac inflammatory response. Treated mice received 1% NAC in their drinking water for 6 wk. NAC had no effect on wild-type (WT) mice. Immunohistochemistry experiments revealed that mdx mice had increased dihydroethidine (DHE) staining, an indicator of superoxide production; NAC-treatment reduced DHE staining in mdx hearts. NAC treatment attenuated abnormalities in mdx cardiomyocyte Ca\textsuperscript{2+} handling. Mdx cardiomyocytes had decreased fractional shortening and decreased Ca\textsuperscript{2+} sensitivity; NAC treatment returned mdx fractional shortening to WT values but did not affect the Ca\textsuperscript{2+} sensitivity. Immunohistochemistry experiments revealed that mdx hearts had increased levels of collagen type III and the macrophage-specific protein, CD68; NAC-treatment returned collagen type III and CD68 expression close to WT values. Finally, mdx hearts had increased NADPH oxidase activity, suggesting it could be a possible source of increased reactive oxygen species in mdx mice. This study is the first to demonstrate that oxidative damage may be involved in the pathogenesis of the heart failure that occurs in mdx mice. Therapies designed to reduce oxidative damage might be beneficial to DMD patients with heart failure.

calcium handling; dilated cardiomyopathy; Duchenne muscular dystrophy

DUCHEEN MUSCULAR DYSTROPHY (DMD) is a fatal X-linked disease that affects 1 in 3,500 male births (12). The underlying defect in DMD is the absence of the protein dystrophin. In addition to skeletal muscle damage and atrophy, DMD patients develop a dilated cardiomyopathy that is the cause of death in 30% of patients (15).

Increasing evidence suggests that reactive oxygen species (ROS) may play a critical role in the pathogenesis of dilated cardiomyopathies. In dilated cardiomyopathies, an imbalance exists between ROS production (23) and myocardial antioxidant stress defense mechanisms (2, 45), leading to increased ROS-induced myocardial injury with disease progression (29). Raised ROS levels lead to oxidation of various proteins, resulting in myocardial dysfunction. Myocardial proteins affected by ROS include sarcoplasmic, excitation-contraction (EC) coupling (64), and contractile (19) and stress-sensitive signaling proteins (1). Increased ROS can also lead to inflammation and fibrosis via ROS-induced activation of proinflammatory pathways (54). Inflammation and abnormalities in the function of the above proteins occur in dilated cardiomyopathies. Thus raised ROS levels may contribute to the dysfunction that occurs in dilated cardiomyopathies.

Various enzymatic sources within the myocardium are capable of producing excess ROS, including the mitochondria, nitric oxide synthase (NOS), xanthine oxidase (XO), and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidases are multimeric enzymes that contain a membrane-bound core, comprising a catalytic gp91phox subunit and a p22phox subunit, and four regulatory subunits: p67phox, rac1, p47phox, and p40phox (37). In dilated cardiomyopathies, NADPH oxidase activity and subunit protein expression are increased (34). Although it is not known whether NADPH oxidase activity is increased in the myocardium in DMD, it appears to be a good candidate for ROS production. This is because alterations in caveolae occur in DMD (43) and also are known to affect NADPH oxidase activation (28). Furthermore, it is possible that the increased elasticity (38) and susceptibility of dystrophin-deficient (mdx) myocytes to stretch (63) lead to increased NADPH oxidase activity (26).

ROS have been implicated in the pathogenesis of the skeletal muscle damage that occurs in DMD (41). However, no studies have investigated the role of ROS in the dilated cardiomyopathy associated with DMD. The present study was performed on the mdx mouse, which is an animal model of DMD. The mdx mouse also lacks the protein dystrophin and develops a dilated cardiomyopathy similar to boys with DMD (40, 58). The aim of the current study was to determine whether the EC-coupling defects, cardiac inflammation, and fibrosis observed in mdx animals could be prevented by antioxidant therapy.

METHODS

Animals. Male wild-type (WT; C57BL/10ScSn) and mdx mice, which occur in this strain of mice, were obtained from the Animal Resource Centre (Perth, Australia). All experiments were approved by the Animal Ethics Committee of the University of Sydney (Sydney, Australia).

Treatment with the antioxidant N-acetylcysteine. All investigations were performed on 9-wk-old mice. Mice were divided into four groups: control mice (WT and mdx) and mice that received 1% N-acetylcysteine (NAC; WT-NAC and mdx-NAC) in their drinking water from 3 until 9 wk of age (i.e., for 6 wk). Antioxidant treatment was started when mice were weaned at 3 wk of age to prevent the...
activation of pathogenic processes that start from an early age in mdx mice (36).

**Measurement of superoxide.** Superoxide levels were measured using the superoxide-sensitive dye dihydroethidium (DHE) (56). DHE is cell permeable and, in the presence of superoxide, is converted to the fluorescent product ethidium bromide (EtBR), which is trapped by the fluorescent product ethidium bromide (EtBR), which is trapped by DHE (56). EtBR fluorescence was measured using fluorescence microscopy.

**Isolation of cardiac myocytes and measurement of intracellular Ca$^{2+}$ concentration.** Isolation of single ventricular myocytes and measurement and calibration of intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) was based on previous methods (59). Changes in [Ca$^{2+}$]i were measured using the fluorescent indicator fluo-4 (Molecular Probes).

**Measurement of cardiomyocyte Ca$^{2+}$ sensitivity and fractional shortening.** Measurements of cardiomyocyte Ca$^{2+}$ sensitivity and fractional shortening were based on previous methods (20, 49). Cell length was determined using the fluorescent signal provided by fluo-4 and measured using repeated confocal scans along the long axis of cardiomyocytes. Cell length was determined as the difference between the length at which fluorescence increased and decreased. Ca$^{2+}$ sensitivity was calculated by plotting [Ca$^{2+}$]i versus cell length during a contraction (Fig. 3A); the [Ca$^{2+}$]i/length trajectory during the relaxation phase was used to determine [Ca$^{2+}$] i at 50% cell shortening (Fig. 3B), which was used to estimate Ca$^{2+}$ sensitivity.

**Preparation of heart homogenate.** The relative levels of troponin I (TnI), caveolin-3, rac1, and gp91phox were determined by quantitative immunoblotting. Preparation of cardiac tissue homogenates was based on previous methods (59). Heart sample homogenates were separated using SDS-PAGE (Bio-Rad) and transferred to a nitrocellulose membrane in a Mini Trans-Blot Transfer Cell (Bio-Rad). Membranes were incubated with antibodies against TnI (1:1,000); 200 μg protein; Chemicon), caveolin 3 (1:1,000; 5 μg protein; BD Biosciences), rac1 (1:1,000; 80 μg protein; BD Biosciences), and gp91phox (1:500; 200 μg protein; BD Biosciences). Subsequently, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies and developed using an enhanced chemiluminescence detection system (Amersham BioSciences). Total protein loading was normalized using a Bradford assay (Bio-Rad). All bands were normalized to the protein GAPDH.

**Immunohistochemistry.** Immunohistochemistry was based on previous methods (59). Heart cross-sections (10 μm) were incubated with either collagen type III primary antibody (1:80; Chemicon) or CD68 primary antibody (1:200; AbD Serotec). Sections were then incubated with fluorescence-labeled secondary antibody. In situ fluorescence was assessed using fluorescence microscopy. CD68 data are presented as the number of pixels exceeding a threshold divided by the total number of pixels.

**NADPH oxidase assay.** NADPH oxidase activity was measured in total left ventricular homogenate using a lucigenin-enhanced chemiluminescence assay (23). Left ventricular tissue was mechanically homogenized in ice-cold buffer A containing (in mM) 150 NaCl, 0.05 Tris·HCl, 0.025 EGTA, 0.025 EDTA, a protease inhibitor cocktail (1%; Sigma), a phosphatase inhibitor (0.5%; Sigma), and calpain inhibitor 1 (0.35%; Sigma). Experiments were performed at 37°C in lysis buffer A with 2 mg/ml total protein, NADPH (200 μM), and a low concentration of lucigenin (10 μM). Light emission from 600-μl aliquots was measured using a photomultiplier-based luminometer. Activity was also measured in the presence of the antioxidants 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron, 5 mM) or NAC (10 mM). The following agents were also used to assess potential sources of superoxide production: a flavoprotein inhibitor, diphenyleneiodonium (DPI, 10 μM); a NOS inhibitor, $N^\omega$-nitro-$l$-arginine methyl ester hydrochloride (l-NAME, 100 μM); a mitochondrial site I electron transport inhibitor, rotenone (20 μM); or a XO inhibitor, oxypurinol (100 μM).

**Statistical analysis.** Data are expressed as means ± SE; n, number of experiments. Differences between means were analyzed using one-way ANOVA or with the Student’s t-test.

## RESULTS

**ROS are increased in mdx hearts.** To ascertain whether ROS levels are increased in mdx hearts, we measured the levels of DHE fluorescence in mdx and WT heart sections. When compared with WT hearts, mdx hearts displayed an increase (19 ± 4%) in EtBr fluorescence, reflecting an increase in superoxide levels (Fig. 1). We hypothesized that, as superoxide levels are augmented in mdx mice, treatment with the antioxidant NAC (1% wt/vol) may prevent or reduce some of the abnormalities observed in these animals. The use of NAC and its dosage were based on findings from previous trials (13). In mdx hearts, NAC treatment returned EtBr fluorescence to WT levels (Fig. 1). NAC treatment had no effect on WT hearts.

**Effect of NAC on intracellular Ca$^{2+}$ handling.** We have previously shown that intracellular Ca$^{2+}$ handling is abnormal in mdx cardiomyocytes (59). To determine whether ROS are involved in the Ca$^{2+}$-handling defects in mdx cardiomyocytes, we recorded Ca$^{2+}$ transients from WT, WT-NAC, mdx, and mdx-NAC mice, NAC treatment had no effect on mdx or WT-resting [Ca$^{2+}$]i (Fig. 2A). NAC treatment corrected the time to peak and returned the amplitude of the Ca$^{2+}$ transient and the decay constant of mdx cardiomyocytes close to WT values (Fig. 2). NAC treatment had no effect on WT Ca$^{2+}$ transients.

**Effect of NAC on cardiomyocyte myofilament Ca$^{2+}$ sensitivity and fractional shortening.** Since ROS have been shown to influence cardiomyocyte myofilament Ca$^{2+}$ sensitivity (19) and whole heart fractional shortening (21), we investigated whether the raised ROS in mdx hearts affected these parameters. In mdx cardiomyocytes, Figure 3A shows a cell shortening versus [Ca$^{2+}$]i trajectory for one contraction. The arrow shows the relaxation phase during which [Ca$^{2+}$]i, and cell shortening are thought to be close to their steady-state equilibrium (49). Figure 3B shows the [Ca$^{2+}$]i/length trajectory during the relaxation phase during which [Ca$^{2+}$]i, and cell shortening values (Fig. 2). NAC-treatment had no effect on WT Ca$^{2+}$ transients.

![Fig. 1. Effect of N-acetylcysteine (NAC) treatment on superoxide levels in wild-type (WT) and dystrophin-deficient (mdx) hearts (n = 5 experiments). NS, not significant. *P < 0.05 and ***P < 0.001 between groups.](http://ajpheart.physiology.org/)
laxation phase in a WT, mdx, and mdx NAC-treated cardiomyocyte. The [Ca\textsuperscript{2+}] at which 50% shortening occurred is greater in mdx than in WT (Fig. 3C), indicating a decrease in Ca\textsuperscript{2+} sensitivity. 

**Effect of NAC on cardiac inflammation and fibrosis.** Mdx mouse hearts have increased inflammation and fibrosis (58). Increased ROS levels may promote the inflammation and fibrosis found in mdx hearts via ROS-induced increases in proinflammatory proteins (31, 54). To test this hypothesis, we investigated whether NAC treatment reduced signs of inflammation and fibrosis in mdx mouse heart sections. Immunohistochemistry experiments revealed that mdx hearts had increased levels of a marker of inflammatory responses (Fig. 5; the macrophage-specific protein CD68) and of collagen type III expression close to WT values.

**Potentials sources of ROS in mdx cardiomyocytes.** To investigate potential sources of increased ROS production in mdx hearts, we measured NADPH oxidase activity using a lucigenin-enhanced chemiluminescence assay. NADPH oxidase activity was increased 1.4-fold in 
dxr hearts (Fig. 6, A and B). NAC treatment returned CD68 and collagen III expression close to WT values.

**Fig. 2. Effect of NAC treatment on Ca\textsuperscript{2+} transients in mdx and WT ventricular myocytes. A: sample traces of Ca\textsuperscript{2+} transients. B: amplitude of Ca\textsuperscript{2+} transients. C: time to peak of Ca\textsuperscript{2+} transients. D: decay constant of Ca\textsuperscript{2+} transients (n = 20 experiments). *P < 0.05, **P < 0.01, ***P < 0.001 between groups.**
increased in mdx hearts (Fig. 7, E and F). NAC treatment had no effect on the protein expression of any NADPH oxidase proteins or caveolin-3.

**DISCUSSION**

It is currently unknown why the absence of the protein dystrophin leads to heart failure in DMD. In skeletal muscle there is good evidence that abnormalities in ROS levels are involved in the pathology of DMD (41). However, no studies have explored the involvement of ROS in the dilated cardiomyopathy that occurs in DMD. The present study found that increased ROS levels in mdx cardiomyocytes were associated with abnormal intracellular Ca\(^{2+}\) handling, myofilament dysfunction, cardiac inflammation, and fibrosis. The fact that NAC

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**Fig. 3. Effect of NAC treatment on fractional shortening and Ca\(^{2+}\) sensitivity in mdx and WT ventricular myocytes.**

- **A**: trace showing [Ca\(^{2+}\)] against cell length during a contraction. The arrow shows the relaxation phase.
- **B**: intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)],/length trajectory during the relaxation phase of a Ca\(^{2+}\) transient. The [Ca\(^{2+}\)], at 50% cell shortening (Ca\(_{50}\)) was used as an estimate of Ca\(^{2+}\) sensitivity.
- **C**: group Ca\(^{2+}\) sensitivity data.
- **D**: grouped fractional shortening data. WT, n = 11; WT-NAC, n = 5; mdx, n = 20; mdx-NAC, n = 11 experiments. *P < 0.05 and **P < 0.001 between groups.

**Fig. 4. Troponin I (TnI) degradation in mdx and WT hearts.**

- **A**: sample blots showing native TnI and TnI degradation expression levels.
- **B**: group data showing native TnI expression in WT and mdx hearts.
- **C**: group data showing TnI degradation product expression in WT, WT-NAC, mdx, and mdx-NAC hearts (n ≥ 6 experiments). *P < 0.05 between groups.
NAC treatment corrected or improved all of the Ca\textsuperscript{2+} abnormalities in mdx cardiac muscle from dilated cardiomyopathy patients (45). The current study found that superoxide levels were increased in mdx heart sections compared with those in WT hearts; this finding correlates well with observations from mdx and DMD skeletal muscle (41) and cardiac muscle from dilated cardiomyopathy patients (45).

NAC treatment returned superoxide levels in mdx hearts back to WT values, suggesting that the beneficial effects of NAC treatment on myocardial function were a result of its antioxidant properties. The antioxidant capabilities of NAC treatment are the result of its ability to stimulate GSH synthesis, enhance glutathione-S-transferase activity, and act directly as a ROS scavenger (30). NAC treatment had no effect on any of the parameters measured in WT hearts, supporting previous findings that NAC only supports GSH synthesis under conditions of increased oxidative stress (30).

Why do ROS affect Ca\textsuperscript{2+} transients? It is well established that intracellular Ca\textsuperscript{2+} handling is defective in dilated cardiomyopathies (32). We have also shown that Ca\textsuperscript{2+} handling is abnormal in mdx hearts (59). The current study found that NAC treatment corrected or improved all of the Ca\textsuperscript{2+}-handling defects in mdx cardiomyocytes. ROS are known to modulate the function of many of the proteins involved in EC coupling (64). Thus it is probable that NAC treatment corrected the Ca\textsuperscript{2+}-handling defects in mdx hearts by preventing oxidation of EC-coupling proteins, such as the cardiac ryanodine receptor (RyR2), sarco(endo)plasmic reticulum Ca\textsuperscript{2+} ATPase 2 (SERCA2), and phospholamban (PLN).

ROS have been shown to increase RyR2 activity and expression levels (8, 46), which would be predicted to decrease the time to peak and increase the amplitude of Ca\textsuperscript{2+} transients, as we have found previously (59); thus this may provide an explanation as to why NAC treatment prevented the changes in the amplitude and time to peak of Ca\textsuperscript{2+} transients in mdx cardiomyocytes. ROS are also known to inhibit SERCA2 function, through both a direct action on SERCA2 (27, 61) and through its ability to attenuate PLN phosphorylation that preserves the inhibitory action of PLN on SERCA2 (51). It is therefore likely that NAC treatment increased the rate of recovery of Ca\textsuperscript{2+} transients through its ability to prevent oxidation of SERCA2 and PLN. This hypothesis is supported by the finding that NAC has been shown to increase PLN phosphorylation (5).

**Fractional shortening and Ca\textsuperscript{2+} sensitivity in mdx cardiomyocytes.** Mdx cardiomyocyte fractional shortening was found to be decreased. In support of previous findings from pressure-overloaded whole hearts (21), NAC treatment corrected fractional shortening in mdx cardiomyocytes, implicating a ROS-related pathology. The current study did not explore the mechanism by which oxidative stress reduced fractional shortening; however, possible mechanisms include posttranslational modification of the contractile proteins, either directly by ROS (10) or via ROS-induced phosphorylation (22).

Myofilament Ca\textsuperscript{2+} sensitivity was decreased in mdx mice, as shown in models of stunned (17) and infarcted myocardium (33). However, observations from dilated cardiomyopathy patients have shown Ca\textsuperscript{2+} sensitivity to be unchanged (7) or increased (47, 60). This disparity in the Ca\textsuperscript{2+} sensitivity in the dilated cardiomyopathy that occurs in DMD and other forms of dilated cardiomyopathy probably relates to differences in the primary aetiology of the diseases.

Although ROS have previously been shown to be capable of reducing Ca\textsuperscript{2+} sensitivity in ventricular muscle (19), NAC treatment did not significantly increase Ca\textsuperscript{2+} sensitivity in mdx cardiomyocytes or prevent TnI degradation. The inability of NAC to correct Ca\textsuperscript{2+}-sensitivity defects suggests that increased ROS are not involved in the changes in Ca\textsuperscript{2+} sensitivity. Thus an alternative mechanism may underlie the observed changes in Ca\textsuperscript{2+} sensitivity, such as altered myofilament-handling defects in mdx hearts.

**Fig. 5.** Effect of NAC treatment on mdx and WT cardiac macrophage levels. Group data show levels of the macrophage-specific protein CD68 (n ≥ 6 experiments). *P < 0.05 between groups.

**Fig. 6.** Effect of NAC treatment on mdx and WT cardiac collagen type III levels. A: representative images showing the distribution of collagen type III in heart tissue. B: quantified collagen type III levels in the tissue (n ≥ 6 experiments). *P < 0.05 and **P < 0.01 between groups. Bar = 100 \(\mu\)m.
Gene expression; posttranslational modification, such as phosphorylation (55); or proteolysis of the myofilament proteins (35). In support of the proteolysis hypothesis, the current study demonstrates that the changes in Ca\textsuperscript{2+}/H\textsubscript{11001} sensitivity in \textit{mdx} mice were also associated with an increase in TnI degradation. Although the current study did not demonstrate a causative link between Ca\textsuperscript{2+}/H\textsubscript{11001} sensitivity and TnI degradation, previous studies have supported this link (17, 18). The reduction in fractional shortening found in \textit{mdx} cardiomyocytes is probably not related to TnI degradation since NAC-treatment corrected the fractional shortening defect but did not prevent TnI degradation.

**Inflammation, fibrosis, and the role of ROS.** Antioxidant treatment prevented the increased myocardial inflammation and fibrosis found in \textit{mdx} mice, suggesting a role for ROS in this pathology. We hypothesized that increased ROS may lead to an increase in inflammatory cell aggregation, which could then lead to an increase in fibrosis. In cardiac muscle ROS can promote inflammatory cell aggregation by activating proinflammatory proteins, such as NF-κB (11, 54). ROS also increase endothelial cell adhesion molecules, such as VCAM-1 (31) and P-selectin (39), as well as increasing macrophage migration inhibitory factor, a cytokine that inhibits macrophage migration and concentrates them at the site of inflammation (53). Cardiac inflammation could then lead to cardiac fibrosis via inflammatory cell secretion of profibrotic proteins, such as transforming growth factor-β (57). It is important to note that inflammation is probably not an initiating factor in the \textit{mdx} pathology, since in skeletal muscle membrane, damage is observed before the appearance of macrophages (6). However, inflammation and cardiac fibrosis are potentially damaging, since increased collagen can lead to conduction defects and decreased systolic ejection due to myocardial heterogeneity (52).

**Sources of raised ROS.** The finding that ROS play an important role in the heart failure that occurs in DMD raises the question, What is the source of increased ROS? Furthermore, what mechanism links absence of dystrophin to increased ROS?
production? In dilated cardiomyopathies, various sources of ROS production have been proposed, including NADPH oxidase, XO, NOS, and the mitochondria (37). We decided to study NADPH oxidase activity since this enzyme has been shown to have increased activity in dilated cardiomyopathy patients (23, 34). Furthermore, NADPH oxidase activity is altered by stretch and requires caveolae, both of which are altered in DMD (see below).

NADPH oxidase activity was increased 1.4-fold in mdx compared with WT hearts. NADPH oxidase, rather than dysfunctional NOS, XO, or mitochondrial production, was the source of NADPH-dependent ROS production, since ROS production was blocked by DPI but not by oxypurinol (XO inhibitor), rotenone (mitochondrial inhibitor), or 1-NAME (NOS inhibitor). The current study does not rule out other sources of ROS production; however, it does demonstrate that NADPH oxidase could be one source of increased ROS in mdx hearts.

NAC treatment did not reduce mdx NADPH oxidase activity, suggesting that the beneficial effects of NAC on mdx myocardial function and inflammation are not due to its ability to reduce ROS production through NADPH oxidase. Instead, it appears that NAC acts by enhancing endogenous ROS scavengers or directly scavenging excess ROS produced by NADPH oxidase. If raised activity of NADPH oxidase is not secondary to increased ROS, what is the primary mechanism augmenting its activity? Two possibilities, that may occur concurrently, are raised in the current study. The first mechanism relates to the finding that caveolae are increased in DMD (43). Caveolae are flask-shape plasma membrane invaginations that are formed by a family of proteins called caveolins, of which caveolin-3 is muscle specific (42). Dystrophin associates with caveolin-3 (48), and in DMD, where dystrophin is absent, caveolin-3 levels are augmented, leading to an increase in the number of caveolae (16). NADPH oxidase subunits have been shown to colocalize with caveolin-1 in vascular smooth muscle (24). Furthermore, caveolae have been shown to be important in the spatial regulation and agonist stimulation of NADPH oxidase (28, 62). Our finding that caveolin-3 expression is greater in mdx hearts may allow increased localization of gp91phox to caveolae, resulting in increased NADPH oxidase-dependent ROS production.

A second mechanism to account for increased NADPH oxidase activity relates to the possibility that the increased elasticity (38) and susceptibility of mdx myocytes to stretch (63) lead to increased activation of the enzyme. Stretch leads to both the activation of NADPH oxidase (26) and the regulatory subunit rac1 (28), in addition to increasing the expression of gp91phox (25).

Conclusion. Our data support the hypothesis that oxidative stress may mediate many of the pathological features of the dilated cardiomyopathy that occurs in mdx mice. This hypothesis is further supported by the finding that in vivo antioxidant treatments provide beneficial effects in mdx skeletal muscle (3, 4, 9). Unfortunately, clinical trials of antioxidants in DMD patients have shown no clear benefits (14, 50). However, these trials have been limited in size and duration and have failed to provide evidence that the ROS status of cells was altered. Furthermore, the studies were performed on patients who had already undergone severe muscle degeneration, which may limit the effectiveness of antioxidant treatment. In support of this latter point, we found that mdx mice treated with NAC from 6 to 9 wk of age (i.e., for only 3 wk) showed only marginal improvements in Ca2+ handling (I. A. Williams, unpublished data), suggesting that the starting time and/or duration of treatment is of importance. These findings suggest that antioxidants may be capable of preventing the development of the mdx pathology but may not be capable of reversing existing pathology.

In summary, increased oxidative stress may account for the changes in Ca2+ handling, myofilament dysfunction, and inflammation that occurs in mdx hearts. NADPH oxidase activity and expression are also increased in mdx hearts and may account in part for the increased ROS production found in DMD. Therapies designed to reduce oxidative damage might be beneficial to DMD patients with heart failure.

GRANTS

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REFERENCES


Oxidative Stress in Mdx Hearts


