N-Acetylcysteine treatment of dystrophic mdx mice results in protein thiol modifications and inhibition of exercise induced myofibre necrosis

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Abstract

Oxidative stress is implicated as a factor that increases necrosis of skeletal muscles in Duchenne Muscular Dystrophy (DMD) and the dystrophic mdx mouse. Consequently, drugs that minimize oxidative stress are potential treatments for muscular dystrophy. This study examined the in vivo benefits to mdx mice of an antioxidant treatment with the cysteine precursor N-acetylcysteine (NAC), administered in drinking water. NAC was completely effective in preventing treadmill exercise-induced myofibre necrosis (assessed histologically) and the increased blood creatine kinase levels (a measure of sarcolemma leakiness) following exercise were significantly lower in the NAC treated mice. While NAC had no effect on malondialdehyde level or protein carbonylation (two indicators of irreversible oxidative damage), treatment with NAC for one week significantly decreased the oxidation of glutathione and protein thiols, and enhanced muscle protein thiol content. These data provide in vivo evidence for protective benefits of NAC treatment on dystropathology, potentially via protein thiol modifications.

Keywords: Duchenne Muscular Dystrophy; mdx Mouse; N-Acetylcysteine; Treadmill exercise; Oxidative stress; Protein thiol oxidation

1. Introduction

The muscular dystrophies are inherited muscle disorders characterised by progressive muscle wasting and weakness. The most common is Duchenne Muscular Dystrophy (DMD) a lethal, X-chromosome linked disease affecting about 1 in 3500–6000 boys worldwide (Reviewed in [1,2]). DMD and the milder Becker Muscular Dystrophy are caused by mutations in the dystrophin gene resulting in the absence or decreased functional dystrophin protein. Skeletal and cardiac myofibres lacking functional dystrophin have an increased susceptibility to sarcolemma damage after muscle contraction which ultimately leads to myofibre necrosis and regeneration [3,4]. Repeat cycles of widespread myofibre necrosis lead to the progressive loss of muscle mass and function in DMD, eventuating in premature death often due to respiratory or cardiac failure (Reviewed in [2,5]).

There is no definitive treatment for DMD and despite many negative side-effects, corticosteroids remain the standard pharmacological treatment to maintain muscle mass and strength, and help prolong life [2]. Numerous approaches to replace the defective dystrophin gene have been investigated, however they are not yet clinically established [6]. Until genetic or molecular therapies are available, pharmacological interventions may be useful as interim measures. Particularly attractive are pharmacological agents where the toxicological information is already documented for humans, as this should accelerate clinical adoption for DMD.
Myofibre necrosis is the primary pathological consequence in DMD, thus pharmacological interventions to prevent necrosis are appealing. The mechanism by which the absence of dystrophin leads to myofibre necrosis is not certain; but there is evidence that a lack of dystrophin causes myofibre membrane fragility, which leads to susceptibility to contraction induced injury and excess reactive oxygen species production, possibly mediated by increased calcium and inflammation [7,8]. Reactive oxygen species (ROS), such as hydroxyl radicals, can cause cellular damage by directly damaging macromolecules such as proteins, membrane lipids and DNA [9,10]. ROS, such as hydrogen peroxide, can also cause the reversible oxidation of protein thiols which can affect the function of many intracellular proteins including signal transduction proteins and transcription factors [10–12]. For example, ROS can mediate activation of the transcription factor nuclear factor kappa B (NFkB) [13] which itself regulates the expression of many genes, including those involved in the inflammatory and stress response [14] and protein thiol oxidation is elevated in dystrophic mdx muscles [12]. Whether it is by direct damage of macromolecules or via reversible modification of protein thiols, ROS have the potential to be major contributors to the pathology associated with muscular dystrophies [15,16]. Consequently, strategies to minimize the generation or actions of ROS have the potential to be useful treatment options for DMD.

A pharmacological agent that targets ROS is the orally available N-acetylcysteine (NAC); NAC has antioxidant properties [17] and it has been used clinically to treat various conditions, including acetaminophen overdose (Reviewed in [18]). NAC can exert its antioxidant properties directly, as it contains a thiol which can directly scavenge some types of ROS [17–19] and NAC is also a precursor of l-cysteine which is required in the synthesis of the major intracellular antioxidant glutathione [20,21]. NAC has shown promise as an antioxidant to decrease contraction induced injury in the mdx mouse model of DMD, as treatment of EDL muscles isolated from mdx mice with 20 mM NAC improved force production and significantly decreased the impact of eccentric contractions on myofibre membrane integrity [22]. Our objective was to test whether NAC could prevent contraction induced injury in vivo. We establish that NAC, when taken orally, decreased myofibre membrane damage and necrosis in exercised mdx mice. Consistent with the actions of NAC as an antioxidant, we document decreased oxidative stress, specifically related to protein thiol oxidation in dystrophic muscles of mdx mice treated with NAC.

2. Materials and methods

2.1. Animal procedures

All experiments were carried out on adult male dystrophic mdx (C57Bl/10ScSn-mdx/mdx) and non-dystrophic control C57Bl/10ScSn (C57) mice (the parental strain for mdx). All groups were sampled at 12 weeks of age. Treatments with NAC were begun at 6 weeks (for 6 weeks of treatment) or 11 weeks (for 1 week of treatment). All mice were obtained from the Animal Resource Centre, Murdoch, Western Australia. They were maintained at the University of Western Australia on a 12-h light/dark cycle, under standard conditions, with free access to food and drinking water. All animal experiments were conducted in strict accordance with the guidelines of the National Health and Medical Research Council Code of practice for the care and use of animals for scientific purposes (2004), and the Animal Welfare act of Western Australia (2002), and were approved by the Animal Ethics committee at the University of Western Australia. For consistency, all experiments (exercise and sampling) were started at 8 am and completed by 11 am each day.

2.2. NAC treatment

NAC (Sigma–Aldrich) was administered as either a 1% drinking water solution for 6 weeks as previously described [22], or as a short term high dosage regime of 4% drinking water solution for one week, where mice consumed about 2 g of NAC/kg per day [23].

2.3. Exercise protocol

In order to initiate contraction induced myofibre membrane damage and myofibre necrosis, mdx mice were exercised for one single 30 min exercise session on a horizontal rodent treadmill (Columbus Instruments USA), according to a protocol established by our laboratory group [24,25]. In brief, groups of 3 or 4 mdx mice were (1) settled for 2 min on the stationary treadmill belt, (2) acclimatized with gentle walking for 2 min at a speed of 4 m/min, followed immediately by (3) a warm–up of 8 min at 8 m/min and then (4) the main exercise session for 30 min at 12 m/min. Mice were sampled either immediately after exercise (0 min) or 24 h after treadmill exercise. Muscle necrosis was quantified after 24 h, since experiments in our laboratory demonstrate that exercise-induced necrosis of mdx muscles is equivalent at 24 and 48 h [25].

2.4. Tissue collection and image acquisition

All mice were sacrificed at 12 weeks of age by cervical dislocation while under terminal anesthesia (2% v/v Attane isoflurane Bomac Australia). Various muscles were collected (while mice were under terminal anesthesia) and immediately snap frozen in liquid nitrogen for biochemical analysis, or prepared for histology. For histology, quadriceps muscles were cut in half and mounted on cork board using tragacanth gum (Sigma–Aldrich). Muscles were quenched in isopentane cooled in liquid nitrogen and stored at −80 °C until used for sectioning. Transverse sections (5–8 μm) were cut through the mid-region of each muscle on a Leica CM3050S cryostat, as per [24].
were routinely stained with Haematoxylin and Eosin (H&E). For morphological analysis, non-overlapping tiled images of transverse muscle sections provided a picture of the entire muscle cross section. Images were acquired using a Leica DM RBE microscope, a personal computer, a Hitachi HVC2OM digital camera, Image Pro Plus 4.5.1 software and Vexta stage movement software. Tiled images were taken at 10× magnification.

2.5. Histological image analysis

Histological analysis of muscle necrosis was carried out on whole muscle cross sections of the quadriceps muscle. Muscle morphology was drawn manually by the researcher using Image Pro Plus 4.5.1 software. The area occupied by necrotic myofibres (i.e. myofibres with fragmented sarcoplasm and/or areas of inflammatory cells) was measured as a percentage (area) of the whole muscle section. All section analysis was done ‘blind’. Histological analysis was completed as per the TREAT-NMD recommended standard protocol “Histological measurements of dystrophic muscle – M.1.2.007” http://www.treat-nmd.eu/research/preclinical/SOPs/. Due to the level of variation in myofibre necrosis in 12 week old mdx mice [25] histological data for untreated mdx mice was pooled data from previous experiments (n = 60 for unexercised mice and n = 25 for exercised mice).

2.6. Blood collection and plasma creatine kinase (CK) assay

While mice were under terminal anaesthesia, whole blood (about 0.5 ml) was collected via cardiac puncture using a 27.5 gauge tuberculin syringe (Sigma–Aldrich), into a 1.5 ml tube. Blood samples were immediately spun down in a refrigerated centrifuged for 5 min (12000g), plasma was removed and aliquoted. Blood plasma CK activity was determined in duplicate using the CK-NAC kit (Randox Laboratories) and analyses kinetically using a BioTek Powerwave XS Spectrophotometer using the KC4 (v 3.4) program. In order to confirm the sampling method and analytical method, levels of CK were also assayed in the control (non-dystrophic) C57 mice. These levels were very low (approximately 400 units/L) and comparable to previous reports [26].

2.7. Carbonylated protein

Oxidative damage to proteins in mdx gastrocnemius muscles was determined by measuring the carbonyl content with 2,4-dinitrophenylhydrazine (DNPH) as previously described [10,27,28]. Frozen muscles were crushed under liquid nitrogen, before protein was extracted with 20% TCA/acetone. The protein pellets were washed in acetone and ethanol, precipitated, dried, re-suspended in 10 mM DNPH in 2 M HCl and incubated for 30 min at room temperature in the dark. Proteins were washed with ethyl acetate/ethanol (1:1), dissolved in 6 M guanidine hydrochloride, and absorbance was measured at 370 nm. Protein concentration (mg/ml) was determined using the Bio-Rad Bradford protein assay. Carbonyl concentrations are expressed as nmol of carbonyl per mg protein.

2.8. Malondialdehyde levels

Malondialdehyde (MDA) was measured by High performance liquid chromatography (HPLC) with slight modifications to the method of Seljeskog (2006) [29]. Quadriceps muscle was ground under liquid nitrogen before the addition of 100 μl per mg of 5% perchloric acid. This was vortexed and incubated for 1 h at 4 °C. After centrifugation, 150 μl of supernatant was removed and mixed with 150 μl of 40 mM TBA. Samples and MDA standards were then incubated at 50 °C for 60 min, 250 μl butanol was added and separation of the upper butanol layer was achieved with a C18 column (5 μl, 4.6 × 150 mm, Dionex) using a Dionex Ultimate 3000 HPLC system. MDA concentrations are expressed as nmol of MDA per mg tissue.

2.9. Glutathione assay

The levels of reduced and oxidized glutathione were assayed using HPLC with dansyl derivatives as fluorescent markers, based on Jones (1998) with slight modifications [30]. Protein was extracted from ground quadriceps muscles by the addition (1 ml solution per 100 mg tissue) of 10% perchloric acid containing 0.2 M boric acid (Sigma–Aldrich) and 20 μM γ-glutamylglutamate (internal standard). Separation was achieved using a 3-Aminopropyl column (5 μm, 100 × 2 mm, Phenomenex) using a Dionex Ultimate 3000 HPLC system. Total glutathione was calculated by adding reduced glutathione to twice the oxidized glutathione, and was expressed as nmol per mg tissue. Percentage oxidized was calculated by dividing oxidized glutathione by the total glutathione and multiplying by 100.

2.10. Protein thiols

Reduced and oxidized protein thiols were measured as described by [31]. Frozen quadriceps muscle was crushed under liquid nitrogen, before protein was extracted with 20% TCA/acetone. Protein was solubilized in 0.5% sodium dodecyl sulphate, 0.5 M tris at pH 7.3 (SDS buffer) and protein thiols were labeled with the fluorescent dye BODIPY FL-N-(2-aminoethyl) maleimide (FLM, Invitrogen). Following removal of the unbound dye using ethanol, protein was re-solubilized in SDS buffer, pH 7 and oxidized thiols were reduced with tris(2-carboxyethyl)phosphine (TCEP, Sigma–Aldrich) before the subsequent unlabeled reduced thiols were labeled with a second fluorescent dye Texas Red C2-maleimide (Invitrogen). The sample was washed in ethanol and resuspended in SDS buffer. Samples were read using a fluorescent plate reader (Fluostar Optima) with wavelengths set at excitation 485, emission
520 for FLM and excitation 595, emission 610 for texas red. A standard curve for each dye was created using ovalbumin and results were expressed per mg of protein, quantified using Detergent Compatible protein assay (BioRad).

2.11. Statistics

Significant differences between groups were determined using one way ANOVA, and all data are presented as mean ± standard error of the mean. Significance was set at $p < 0.05$.

3. Results

3.1. Muscle damage (exercised mice)

We tested whether oral dosing with 1% NAC for 6 weeks could protect dystrophic muscle from exercise induced myofibre membrane damage and necrosis in vivo. In untreated mdx mice, a single 30 min treadmill exercise session caused a significant increase in necrosis by 24 h in the quadriceps muscle of mdx mice (Fig. 1A). NAC completely prevented this increase in exercise induced myofibre necrosis (Fig. 1A) with the level of necrosis being similar to that in unexercised NAC treated and untreated mdx mice (Fig. 1A).

We hypothesized that NAC would prevent myofibre necrosis by stabilizing dystrophic sarcolemma membranes (as measured by plasma creatine kinase levels). NAC did not affect CK levels in unexercised mice (Fig. 1B). Following exercise, there was a rapid elevation in plasma CK in untreated mdx mice (measured immediately after the exercise), indicative of membrane damage (Fig. 1B) and, while there was a trend for decreased CK levels, treatment with 1% NAC did not significantly attenuate the increase in CK released after exercise (Fig. 1B).

We tested whether an increase in NAC dosage to 4% would be more successful in preventing the exercise induced increase in plasma CK. After 1 week of treatment with 4% NAC, the level of CK was not affected in unexercised mdx mice. However, in exercised mdx mice treated with 4% NAC there was a significantly (about 2.5 fold) smaller increase in CK compared to untreated exercised mdx mice (Fig. 1B). The 4% NAC treatment also completely prevented the exercise-induced increase in myofibre necrosis, similar to the result for 1% NAC treatment (Fig. 1A).

Direct comparison between data for the 1% and 4% NAC groups after the exercise bout revealed no statistically significant difference between the 2 doses of NAC for CK levels or extent of myonecrosis.

3.2. Oxidative damage

Since NAC has antioxidant properties, we examined if the protective effects of NAC could be mediated by a decrease in oxidative stress. The protein carbonyl assay is commonly used as an indicator of irreversible oxidative damage to proteins [32]. Treatment with either 1% or 4% NAC did not change the levels of protein carbonylation in unexercised mdx gastrocnemius muscles (Fig. 2A). There was no significant change in protein carbonylation immediately after exercise (6.5 ± 0.5 nmol/mg protein, $n = 5$) nor at 24 h post-exercise (6.5 ± 0.8 nmol/mg protein, $n = 7$) from pre-exercise levels (4 ± 0.4 nmol/mg protein, $n = 6$).

MDA is widely used as a measure of irreversible oxidative damage to lipids [32]. Treatment with either 1% or 4% NAC did not cause a significant change in the levels of MDA in unexercised mdx quadriceps (Fig. 2B). MDA levels were not significantly changed immediately after exercise (0.37 ± 0.06 nmol/mg tissue, $n = 8$), nor at 24 h post-
exercise (0.28 ± 0.04 nmol/mg tissue, n = 8) compared with pre-exercise levels (0.38 ± 0.06 nmol/mg tissue, n = 9).

Taken together, these data indicate that the ability of NAC to protect mdx muscle from damage or necrosis was not mediated via the prevention of irreversible oxidative damage to proteins or lipids.

3.3. Thiol oxidation

NAC can scavenge reactive oxygen species indirectly by enhancing glutathione content [20]. However, the protective effects of NAC were not likely to be mediated by an increase in glutathione content as total glutathione content was not significantly altered in the quadriceps muscle by 1% or 4% NAC treatment (Fig. 3A). The percentage of oxidized glutathione can also be used as an indicator of oxidative stress [33]. Treating mdx mice with 1% NAC did not significantly decrease the percentage of glutathione in the oxidized form (Fig. 3B). However, 4% NAC significantly decreased the percentage of oxidized glutathione (Fig. 3B). These data are consistent with NAC acting as a thiol antioxidant in dystrophic muscle.

As NAC is a thiol containing antioxidant we assessed whether NAC protected protein thiol groups from oxidation. Treating mdx mice with 1% NAC did not change the total protein thiol content of quadriceps muscle, nor the percentage of protein thiols oxidized (Table 1). However, increasing NAC to 4% significantly decreased the extent of protein thiol oxidation (19% untreated vs. 12% NAC treated) (Table 1). The decreased protein thiol oxidation was not a consequence of decreased oxidized protein thiol content; instead, it was a consequence of over a two fold increase in reduced protein thiol content, from 40 to 86 nmol/mg protein.

An increase in oxidized glutathione or protein thiol oxidation following exercise would implicate thiol oxidation
as a potential cause of membrane destabilization or necrosis in dystrophic muscle. For glutathione, the percentage of oxidized glutathione was not increased immediately after exercise (12.33 ± 1.77%, n = 7) nor at 24 h post-exercise (8.07 ± 1.59%, n = 9) from pre-exercise levels (10.05 ± 1.4%, n = 9). However, for protein thiols, there was a 30% increase in the percentage of protein thiol oxidation immediately after exercise (Table 1). We then examined the effect of 4% NAC treatment on protein thiol state following exercise in mdx quadriceps muscle (Table 1). Consistent with the observation in unexercised mice, the total amount of protein thiols content was increased in exercised mdx mice treated with 4% NAC. There was no significant difference in reduced protein thiol content or the percentage of oxidized protein thiols when muscle from 4% NAC treated mice after exercise was compared with untreated mice after exercise (Table 1). However, after exercise there was a significant increase in oxidised protein thiol content in muscles from 4% NAC treated, compared with untreated, mdx mice. Treatment with 1% NAC had no significant effect on protein thiol groups after treadmill exercise (Table 1). Together these data indicate that the protective properties could be a consequence of 4% NAC acting as a thiol antioxidant.

4. Discussion

The specific effect of NAC in prevention of myofibre necrosis in dystrophic muscle has not been previously reported in vivo. In this study we show that NAC prevented exercise induced myofibre necrosis, using two different dosing regimens of 1% NAC for 6 weeks or 4% NAC for 1 week. Prevention of exercised induced myofibre necrosis is an important outcome, because repeated bouts of myofibre necrosis eventually lead to the replacement of myofibres with fatty and fibrous connective tissue and thus a loss of function of dystrophic muscle [5,34].

Previous in vitro work indicated that NAC may protect dystrophic muscle from exercise induced myofibre necrosis by stabilizing fragile dystrophic sarcolemma membranes [22] since Evans Blue Dye uptake into isolated myofibres following stretched induced contractions was decreased with exposure to NAC. For the current in vivo experiments we used CK release into the blood to assess global myofibre membrane damage, since mdx mice have elevated blood CK levels [35] which are greatly exacerbated after treadmill exercise [26]. Exercise can be expected to affect some muscles more than others, so blood CK levels provide a convenient systemic measurement of leakiness for all muscles of the body. While there was a trend for attenuation in CK level following exercise after 1% NAC treatment, this was not statistically significant. This may be due to the high variation in CK levels following exercise in mdx mice [36], resulting in poor sensitivity to the protective effects of NAC (Type II error). However, when the dose of NAC was increased to 4%, there was a significant attenuation of the increased blood CK after treadmill exercise. These findings are important as they indicate that NAC can rapidly (within one week) protect dystrophic muscle from damage in vivo.

These data also imply that doses of NAC higher than 1% are more protective. This will need to be further tested with consistent treatment protocols to determine the optimal dose for in vivo treatment to reduce dystrophopathy (in conjunction with measurements of NAC tissue levels). Overall, our data and evidence from a previous study [22] are consistent with the concept that NAC stabilizes fragile dystrophic membranes, although how this occurs is not yet clear.

The beneficial actions of NAC may primarily reflect its antioxidant properties since oxidative stress has been identified as a possible cause of myofibre necrosis and wasting of dystrophic muscle [12,37]. ROS can cause irreversible damage to macromolecules such as membrane proteins and lipids, with extensive damage potentially leading to membrane permeability. Such irreversible oxidation of proteins can be assessed by measuring the protein carbonyl content of muscles and increased protein carbonyl content has been reported for both DMD biopsies and mdx muscles [10,38,39]. Oxidative damage to proteins can be caused by hydroxyl radicals, which can be prevented by NAC [18,19]. However, in the current study NAC treatment did not decrease protein carbonyl content in mdx muscle. Oxidative damage to lipids (lipid peroxidation) has also

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Table 1

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Reduced*</th>
<th>Oxidised*</th>
<th>Total#</th>
<th>Percentage oxidised#</th>
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</thead>
<tbody>
<tr>
<td>Unexercised (9)</td>
<td>33 ± 2</td>
<td>8 ± 1</td>
<td>40 ± 3</td>
<td>19 ± 2</td>
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<td>Exercised (6)</td>
<td>39 ± 4</td>
<td>13 ± 2</td>
<td>51 ± 5</td>
<td>25 ± 2</td>
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<tr>
<td>1% NAC Unexercised (8)</td>
<td>60 ± 15</td>
<td>9 ± 1</td>
<td>69 ± 16</td>
<td>16 ± 2</td>
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<tr>
<td>Exercised (8)</td>
<td>43 ± 4</td>
<td>15 ± 1</td>
<td>58 ± 4</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>4% NAC Unexercised (4)</td>
<td>76 ± 10*</td>
<td>10 ± 1</td>
<td>86 ± 11*</td>
<td>12 ± 1*</td>
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<tr>
<td>Exercised (7)</td>
<td>58 ± 9**</td>
<td>20 ± 2*</td>
<td>78 ± 7**</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM nmols/mg protein.
# For each muscle, total protein thiol content and percentage of oxidized protein thiols were calculated as described in the methods.

** Significantly different from unexercised, untreated.
*** Significantly different from exercised, untreated.

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been reported in young mdx muscle [16,39] and irreversible damage to membrane lipids can be assessed by measuring malondialdehyde (MDA), generated as a consequence of lipid peroxidation. However, we did not observe a decrease in MDA level in mdx mice after either NAC treatment. Instead, there was a slight trend for an increase in both measures of oxidative damage (carbonylation and MDA), perhaps attributable to generation of damaging ROS (e.g. hydroxyl radicals) by metal catalysed reactions as observed for cysteine [40]. However, this was not significant in our experiments and was clearly outweighed by the demonstrated benefits of NAC in dystrophic mdx muscle for prevention of necrosis and creatine kinase release.

Another mechanism proposed for the antioxidant properties of NAC is up-regulation of the cellular antioxidant glutathione [20], since NAC is a precursor, via cysteine, for the synthesis of glutathione [17,41]. Glutathione, a non-protein containing thiol, is a naturally occurring antioxidant present at high concentrations (mM) in tissues, that can react directly with ROS. Of particular significance, glutathione is also an essential co-factor involved in the removal of hydrogen peroxide by glutathione peroxidase [9,42,43]. In our study, both 1% and 4% NAC treatment did not increase total glutathione in dystrophic myofibres. These observations imply that decreased myofibre necrosis caused by NAC was not a consequence of increased glutathione synthesis.

An effect of NAC treatment which has not been previously described was the increase in reduced protein thiols in mdx muscle. We propose that the protective properties of NAC are a consequence of this increase in reduced protein thiol content. As our data show, reduced protein thiols can become preferentially oxidized as a result of oxidative stress and so can be considered to be acting as antioxidants to decrease or buffer the levels of oxidative stress, somewhat analogous to glutathione [41]. In NAC treated mdx mice, 20 nmol/mg protein thiols were oxidized after exercise whereas only 10 nmol/mg protein thiols were oxidized in untreated mdx mice. A potential protective mechanism could involve preventing the oxidation of proteins critical to cell survival, or preventing oxidation of other non-protein molecular constituents of the dystrophic muscle that would otherwise lead to necrosis of the myofibres [41].

In summary, our novel in vivo data show that NAC protects dystrophic mdx muscle from exercise induced myofibre necrosis, the primary cause of severe muscle pathology seen in DMD. The exact mechanism responsible for these benefits of NAC is unclear at this stage, mainly because there is still uncertainty regarding the precise pathways leading to necrosis of dystrophic myofibres, although membrane permeability and oxidative stress have been strongly implicated. Our in vivo data provide evidence that the protective effect of NAC could be multifactorial, as NAC caused a decrease in membrane permeability and enhanced protein thiol content. NAC is highly attractive as a readily available, inexpensive and clinically proven drug with few adverse side effects. Our data strongly support further preclinical investigation into the potential dose of NAC to mitigate aspects of dystrophic pathology.

5. Author disclosure statement

The authors declare they have no conflicts of interest.

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