The effect of N-acetylcysteine (NAC) on contractile function and protein-thiol oxidation in skeletal muscles of mdx mice

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Duchenne Muscular Dystrophy (DMD) is a fatal X-linked recessive disease caused by an absence of the muscle membrane protein dystrophin. The disease is characterized by severe muscle weakness and necrotic death of muscle fibres. Although the mechanisms underlying this severe myopathy are not fully understood, excessive reactive oxygen species (ROS) generation has been implicated as a cause of cell death. Elevated ROS production could also significantly impair contractile function by increasing muscle protein thiol oxidation, thereby contributing to the muscle weakness seen in DMD patients. We hypothesize that ROS induced contractile dysfunction contributes to dystrophic muscle weakness in the mdx mouse model of DMD, and will be attenuated by treatment with the antioxidant N-acetylcysteine (NAC).

Six week old dystrophic, mdx mice (n=8) and non-dystrophic, C57 mice (n=8) were treated with 2% NAC in drinking water for six weeks and compared to untreated mdx (n=8) and C57 (n=8) control mice. Grip strength and body weight were measured weekly during the treatment period. After six weeks of treatment, the 12 week old mice were anaesthetized (sodium pentobarbitone; 40 mg/kg; i.p.) and the *extensor digitorum longus* (EDL) and the diaphragm muscles were excised and mounted in an *in vitro* muscle test system for analysis of contractile function. Contralateral hind limb muscles were surgically removed and snap-frozen in liquid nitrogen for analysis of protein thiol-oxidation.

In mdx mice, NAC treatment significantly increased the normalized grip strength by 36% and increased maximum specific force in isolated EDL compared to untreated mice (NAC = 13.1 ± 1.2 N/cm^2; Untreated = 9.8 ± 0.8 N/cm^2, P<0.05). However, there was no significant difference in maximum specific force from diaphragm muscles between NAC treated and untreated mdx mice. NAC treatment also significantly reduced myosin protein-thiol oxidation (NAC = 10.6 ± 0.8%; Untreated = 13.7 ± 0.8%, P<0.05), but not actin protein-thiol oxidation, in mdx mice.

In non-dystrophic C57 mice, NAC treatment significantly increased normalized grip strength by 26.5%, but had no significant effect on maximum specific force in either EDL or diaphragm muscles. Furthermore, there were no significant differences in either myosin or actin protein-thiol oxidation, between NAC treated and untreated C57 mice.

These data suggest that oxidative stress contributes to contractile dysfunction in dystrophic muscle *via* modification of thiol groups on the contractile protein myosin. Furthermore, in the EDL muscles of mdx mice, this muscle weakness may be ameliorated with the anti-oxidant, NAC.