Duchenne muscular dystrophy: focus on pharmaceutical and nutritional interventions

Medicine in Focus

Duchenne Muscular Dystrophy: focus on pharmaceutical and nutritional interventions

1Radley HG, 2De Luca A, 3Lynch GS & 1Grounds MD.

1School of Anatomy and Human Biology, University of Western Australia, Crawley, Australia.
2Unit of Pharmacology, Department of Pharmacobiology, University of Bari, Bari, Italy.
3Basic and Clinical Myology Laboratory, Department of Physiology, University of Melbourne, Parkville, Australia.

Address all correspondence to Miranda Grounds: Telephone +61 8 6488 3486, Fax: +61 8 6488 3486, Email mgrounds@anhb.uwa.edu.au

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Abstract.

Duchenne muscular dystrophy is a lethal X-linked muscle disease resulting from a defect in the muscle membrane protein dystrophin. The absence of dystrophin leads to muscle membrane fragility, muscle death (necrosis) and eventual replacement of skeletal muscle by fat and fibrous connective tissue. Extensive muscle wasting and respiratory failure results in premature death often by the early 20s. This short review evaluates drug and nutritional interventions designed to reduce the severity of muscular dystrophy, while awaiting the outcome of research into therapies to correct the fundamental gene defect.
Combinations of dietary supplementation with amino-acids such as creatine, specific anti-inflammatory drugs and perhaps drugs that target ion channels might have immediate realistic clinical benefits although rigorous research is required to determine optimal combinations of such interventions.

**Duchenne muscular dystrophy and the mdx mouse model of DMD**

There are many forms of muscular dystrophy but only Duchenne muscular dystrophy (DMD) is discussed here. DMD is an X-linked lethal muscle wasting disorder, affecting approximately 1/3500 male births. The disease is caused by a mutation in the gene that encodes for the sub-sarcolemmal protein dystrophin (Biggar, 2006). Dystrophin links the muscle cytoskeleton through a membrane complex to the extracellular matrix. Dystrophic myofibres are susceptible to damage during mechanical contraction, damage leads to myofibre necrosis and ultimately the replacement of myofibres by fibrous and fatty connective tissue (due to failed regeneration). While the genetic defect was identified in 1987, the specific mechanism of myofibre damage is still unclear (Whitehead, Yeung, & Allen, 2006) and there is still no effective treatment for DMD. Therapeutic approaches for DMD fall into three main strategies (i) replacement of dystrophin by genetic, cell transplantation or molecular interventions; (ii) enhancement of muscle regeneration or reduction of fibrosis to combat failed regeneration and (iii) reduced muscle necrosis. This latter approach is the main focus of this review which outlines pharmacological interventions and nutritional supplementation as potential therapies to reduce myofibre necrosis in DMD. Most experimental studies use mdx mice and therefore data from this model,
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along with clinical studies, form the basis of this review. DMD primarily affects skeletal and cardiac muscle, and in addition other tissues (Biggar, 2006), but only the effects on skeletal muscle will be addressed.

The mdx mouse is the most widely used animal model for DMD. The absence of dystrophin results in a distinct disease progression with an acute onset of skeletal muscle necrosis around 3 weeks of age in young mdx mice (Figure 1), necrosis then decreases significantly after 4-6 weeks to a relatively low level in adult mice (McGeachie, Grounds, Partridge, & Morgan, 1993): the pathology is far more benign than in DMD. The acute onset of myofibre necrosis provides an excellent model to study therapeutic interventions to prevent or reduce necrosis. In contrast, reduced necrosis can be difficult to detect in adult mice where there is little active myofibre breakdown but high cumulative muscle pathology. For this reason, exercise is often used to induce muscle damage enabling potential therapeutic interventions to be evaluated in adult mdx mice (De Luca et al., 2003; Granchelli, Pollina, & Hudecki, 2000; Payne et al., 2006). The simplest form of exercise is voluntary wheel running, where muscle necrosis in the quadriceps is doubled (from ~6 to 12%) after 48 hours (Hodgetts, Radley, Davies, & Grounds, 2006; Radley & Grounds, 2006). Forced exercise greatly increases muscle damage with the most severe injury resulting from forced downhill running (eccentric exercise), although such severe muscle damage caused by eccentric exercise is a poor model for pre-clinical drug screening. The symptoms of dystrophopathy are cumulative, with fibrosis becoming increasingly pronounced in older (15 months+) mdx mice. Symptoms are most
severe in the mdx diaphragm that more closely resembles the severe pathology of DMD (Stedman et al., 1991).

**INSERT FIGURE 1.**

Numerous parameters are measured to assess the in vivo effects of various interventions. In mdx mice, measurements on whole animals are combined with extensive tissue analysis. Physiological parameters such as Rotarod tests (to test motor co-ordination and fatigue resistance) and grip-strength tests (to measure the maximum amount of force an animal applies by grasping) assess changes in muscle endurance, muscle strength and overall functional capacity. Further physiological tests are conducted in vivo and on isolated muscles in situ or in vitro. Blood sampling and serum creatine kinase (CK) levels provide a qualitative indicator of muscle damage. Histological assessment of tissue sections quantifies cumulative muscle necrosis and regeneration, along with leaky myofibres, and immunohistochemical staining identifies changes in location and levels of specific proteins. Other measurements include alterations in channel (Ca$^{2+}$ and Cl$^-$) function that contribute (or sensitise) to disrupted calcium homeostasis and to muscle necrosis (De Luca et al., 2003; Whitehead et al., 2006). A positive result with mdx mice can eventually lead to clinical studies in DMD patients. In humans the main parameters measured are muscle strength, functional tests and CK levels. The Cooperative International Neuromuscular Research Group (CINRG) performs clinical trials on young DMD patients with various compounds, some of which showed positive results in an early screening program on dystrophic mice (Granchelli et al., 2000): while
some of these trials have been published, ongoing results are available on

**Steroids and anti-inflammatory drugs**

Until a cure for DMD is found, treatment will involve the administration of
corticosteroids combined with interventions to alleviate cardiac and respiratory
problems. Corticosteroids have a catabolic effect on muscle (non-exercised
muscle) and act to preserve existing muscle fibres and reduce inflammation,
although their exact mechanism of action in dystrophic skeletal muscle is
unknown. The two main corticosteroids used to treat DMD, Prednisone and
Deflazacort, both seem equally effective in delaying the progression of muscle
wasting. Unfortunately both are associated with adverse side-effects although
these, particularly weight gain, are less severe with Deflazacort. Side-effects of
Deflazacort include an increase in appetite requiring strict dietary control,
retarded growth resulting in short stature and asymptomatic cataracts. It is
recommended that daily calcium and vitamin D supplementation is taken in
conjunction with corticosteroids to maintain bone mineral density and reduce
bone fractures (Biggar, 2006). The administration of steroids is not a cure for
DMD but a therapy to improve quality of life and prolong lifespan.

DMD is characterised by aggressive inflammation and there is strong evidence
that this contributes to myofibre necrosis both in vitro and in vivo [reviewed in
(Tidball, 2005; Tidball & Wehling-Henricks, 2005)]. Other immunosuppressive
drugs have demonstrated benefits in mdx mice, leading to increasing
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recognition for the damaging role of inflammation in DMD. Early clinical trials with the immunosuppressive drug cyclosporine in DMD returned promising results as 8 weeks of treatment (5mg/kg/day) resulted in a significant increase in muscle force generation (Sharma, Mynhier, & Miller, 1993) and cyclosporine reduced the dystropathology in mdx mice (De Luca et al., 2005). However, cyclosporine exerts multiple dose-dependent effects and a correct dosage must be established especially when administered to young dystrophic patients during muscle development. These results are of clinical relevance, as immunosuppression may be required for enhancing efficiency of future gene/cell therapies.

Another potential anti-inflammatory drug that has attracted attention is pentoxifylline. Pentoxifylline has a wide range of anti-inflammatory and anti-coagulant effects; it reduces TNFα production in vitro (Vary et al., 1999), reduces fibrosis and may also play a role in normalising blood flow in dystrophic muscle. On the basis of increased muscle strength in exercised adult mdx mice (Granchelli et al., 2000) pentoxifylline is now the subject of two CINRG trials in DMD patients (one completed recently). However, a recent study in mdx mice involving pentoxifylline (16mg/kg/day) administration for 4 weeks failed to reduce fibrosis or improve the contractile force of the diaphragm muscle (Gosselin & Williams, 2006), this study does not support the use of pentoxifylline as an antifibrotic drug in DMD patients. However, pentoxifylline counteracts, both in vitro and in vivo, the abnormal activity of calcium channels responsible for high sarcolemmal calcium permeability of dystrophic myofibres,
suggesting a possible amelioration of dystrophic condition through alternative pathways (Rolland et al., 2006). Both cyclosporine and pentoxifylline (like corticosteroids) affect many cellular events and can have severe adverse side effects, careful animal studies will be helpful in this regard.

Other anti-inflammatory drugs such as oxatomide (Granchelli et al., 2000) and cromolyn (Granchelli, Avosso, Hudecki, & Pollina, 1996; Radley & Grounds, 2006) that block mast cell degranulation are used widely for clinical treatment of allergies such as asthma and show benefits in mdx mice, indicating that mast cell products (including TNFα) have detrimental effects in dystrophic muscle. A recent CINRG trial with oxatomide (based on the study by (Granchelli et al., 2000)) in DMD showed some minor benefits.

**Specific anti-cytokine drugs**

Another promising approach involves targeting specific aspects of the inflammatory response (rather than the broadly acting anti-inflammatory drugs) in order to reduce muscle necrosis. While systemic depletion of specific inflammatory cells may not be clinically viable, modulation of specific cytokines has been very successful clinically in several severe inflammatory disorders. Tumour Necrosis Factor–alpha (TNFα) is a key pro-inflammatory cytokine that stimulates the inflammatory response, and pharmacological blockade of TNFα activity with the neutralising antibody infliximab (Remicade) is highly effective clinically at reducing symptoms of inflammatory diseases such as Rheumatoid Arthritis and Crohn’s Disease (Feldman & Maini, 2003). Similar successful
clinical blockade of TNFα by the drug etanercept (Enbrel) results from the use of soluble receptors to TNFα. In mdx mice, infliximab delays and reduces the necrosis of dystrophic muscle in young mdx mice (Grounds & Torrisi, 2004). A protective effect of TNFα blockade is reinforced by two recent studies using etanercept, that clearly reduces muscle necrosis in young mdx mice (Hodgetts et al., 2006; Pierno et al., 2006) and in exercised adult mdx mice (Hodgetts et al., 2006) with additional physiological benefits on muscle strength, chloride channel function and reduced CK levels being demonstrated in chronically-treated exercised adult mdx mice (Pierno et al., 2006). Such emerging highly specific anti-inflammatory drugs designed for use in other clinical conditions, appear an attractive alternative (to steroids) for DMD, although their potential to reduce the severity of DMD remains to be determined. It may be possible to limit the use of these drugs to periods of intensive muscle growth in boys when muscle damage and deterioration can be especially pronounced. Patients undergoing long-term anti-cytokine treatment must be monitored carefully for serious infections, as is the case for any immunosuppressive drug.

**Other pharmaceutical interventions**

**Anti-oxidants.** It is widely recognised that high levels of reactive oxygen species can damage tissues, including skeletal muscle (Rando, 2002; Whitehead et al., 2006). Antioxidants that reduce oxidative damage in cells, such as Coenzyme Q10 (CoQ10) and green tea extract [(−)-epigallocatechin gallate] are the subject of recent research in mdx mice and DMD patients. Green tea extract supplemented diets fed to mdx mice (from birth), significantly
reduced muscle damage (necrosis and regeneration) in the EDL muscle of 4 week old mice and improved muscle function in 8 week old mice after 5 weeks of treatment (Buetler, Renard, Offord, Schneider, & Ruegg, 2002; Dorchies et al., 2006). CoQ10 is essential for several enzymatic steps in the production of energy and functions as an antioxidant. CoQ10 was the subject of a CINRG pilot study in DMD patients to assess the effectiveness and safety in combination with steroid treatment. CoQ10 increased strength in some muscle groups and a larger follow up study was recommended. This larger study, that is currently being conducted by CINRG, is a 13-month, prospective, randomized study comparing daily prednisone treatment (0.75mg/kg/day), CoQ10 (>2.5 ug/mL) and a combined treatment (prednisone and CoQ10) in older non-ambulatory patients.

**Anabolic effects of β₂-agonist drugs.** Anabolic agents result in a net increase in protein content and muscle size and this is usually (but not always) associated with increased strength. Although commonly recognised as asthma drugs, high doses of some β₂-agonists have anabolic effects on muscle and thus the potential to slow muscle degeneration. A 3 month pilot trial of the β₂-agonist albuterol given to patients with fascioscapulohumeral disease improved maximum voluntary strength. This was followed by a year long trial where patients were treated with up to 16 mg of albuterol twice daily resulting in improved muscle mass and grip strength. Albuterol administered for 28 weeks (Fowler, Graves, Wetzel, & Spencer, 2004) to boys with DMD produced a modest increase in strength with no reported side effects. It is noted that
studies with β2-agonists in mdx mice have returned inconsistent results (Dupont-Versteegden, Katz, & McCarter, 1995; Lynch, Hinkle, & Faulkner, 2000) and that β2-agonists are associated with numerous undesirable side effects including increased heart rate and tremors, which have limited their therapeutic potential. More recently synthesised β-agonists such as formoterol, have anabolic effects on skeletal muscle with minimal cardiac side-effects (Ryall, Sillence, & Lynch, 2006) when administered at micro-molar doses in mice and thus may offer a greater potential for DMD (Harcourt, Schertzer, Ryall, & Lynch, 2006).

**Disturbed ion channels and drugs to inhibit proteases.** Damaged muscle membranes disturb the passage of calcium ions into the myofibre, and disrupted calcium homeostasis activates many enzymes, e.g. proteases, that cause additional damage and muscle necrosis. Ion channels that directly contribute to the pathological accumulation of calcium in dystrophic muscle are potential targets for drugs to treat DMD. There is evidence that some drugs, such as pentoxifylline, block exercise-sensitive calcium channels (Rolland et al., 2006) and antibiotics that block stretch activated channels reduce myofibre necrosis in mdx mice and CK levels in DMD boys (Whitehead et al., 2006). Calpains are calcium activated proteases that are increased in dystrophic muscle and may directly account for myofibre degeneration (Spencer, Croall, & Tidball, 1995). A new compound, BN 82270 (Ipsen) that has dual action as both a calpain inhibitor and an antioxidant (targeting both calpain and ROS induced muscle damage) increased muscle strength, decreased serum CK, and reduced
fibrosis of the mdx diaphragm suggesting a potential therapeutic effect with this new compound (Burdi et al., 2006). A promising new compound of Leupeptin / Carnitine (Myodur) has recently been proposed for clinical trials in DMD patients.

Beyond the diverse approaches mentioned already, there has been much research into substances to help reduce the dystropathology via maintenance or improvement of myofibre size, strength and function. However, strategies such as increasing IGF-1 or other growth factors, inhibition of myostatin, normalising nitric oxide production and the use of poloxamer (P188), do not readily translate into the clinical situation at present. Although these interventions (and many others) have shown promising effects in mdx mice, adverse or unknown long-term systemic effects currently limit their therapeutic potential.

**Chinese herbal medicine** is becoming increasingly popular as an alternative approach to reduce the severity of symptoms associated with many diseases. For example, ginseng has a diverse range of effects in vivo and a study that showed a reduction in exercise-induced damage in normal muscle after ginseng supplementation (Hsu, Ho, Lin, Su, & Hsu, 2005) suggests possible benefits for DMD. A review of traditional Chinese medicines, such as massage, acupuncture and capsules (that contained herbs and other ingredients) that claimed to alleviate symptoms in DMD patients, was conducted in Beijing in 2003 (Urtizberea, Fan, Vroom, Recan, & Kaplan, 2003). Due to the small number of cases no definitive conclusions could be drawn from this study,
although an overall mild frequency of contractures was noted and related possibly to the positive influence of acupuncture and massage. A follow-up study confirmed that high levels of glucocorticoids were present in the capsules and may account for the anecdotal improvement seen in patients (Courdier-Fruh, Barman, Wettstein, & Meier, 2003). Chinese herbal medicine was also found to improve locomotor activity in mdx mice (Chen, 2001). Traditional Chinese medicine is not formally regulated which potentially creates a large risk for incorrect dosing and dangerous content as medicines may unknowingly contain various heavy metals, herbicides, drugs, pesticides and microorganisms. Furthermore, adverse drug interactions with cumulative effects may result when administered to DMD boys already receiving steroid treatment.

**Nutritional interventions**

Deficiencies of many substances (Selenium, Vitamin E or vitamin D) can cause severe myopathies, suggesting the importance of nutritional supplementation to counteract these deficiencies: however it seems difficult to justify the application of such supplements to situations of adequate diet or DMD. Muscle wasting is associated with changes in the biochemistry of skeletal muscle that lead to reduced protein synthesis, increased protein breakdown (catabolism), and increased oxidative cell damage. The use of protein powders and specific amino acid supplements has been proposed for attenuating muscle protein loss and to provide a favourable environment for increasing protein synthesis and muscle mass, and has received much attention in sports medicine and ageing. Dietary supplementation is a form of protective therapy potentially available for
immediate use and broadly falls into three categories; anti-oxidants or Chinese herbs (both discussed above) and amino acid supplementation.

**Amino acids.** Amino acids such as creatine, taurine, glutamine and L-arginine have all been trialed in the mdx mouse with some benefits on muscle strength or dystropathology. Creatine is directly involved in the energy supply of muscle cells and supplementation into the diet (10% w/w in chow) of both exercised adult mdx mice and pregnant mdx mothers increased strength and improved dystropathology (De Luca et al., 2003; Passaquin et al., 2002). Similar benefits were seen after intraperitoneal injection of 10mg/kg in exercised adult mice (Granchelli et al., 2000). A double-blinded randomized creatine monohydrate (0.10g/kg/day) trial in boys with DMD for 4 months showed increased hand grip strength and fat-free mass, independent of steroid usage (Tarnopolsky et al., 2004). A more recent clinical trial conducted by CINRG tested two amino-acids (creatine 5g/day and glutamine 0.6g/kg/day) in DMD boys aged 4-10 years (grouped as 4-7 years and 7-10 years). Although it did not significantly improve muscle strength, creatine was well tolerated by all patients and there was a trend towards less deterioration in other outcomes (Escolar et al., 2005). Creatine monohydrate supplementation in DMD is the subject of a recent review (Pearlman & Fielding, 2006) that concludes that it should be considered as a therapeutic agent for DMD, due to the potential for increased fat-free mass and increased muscle strength. However, additional long-term studies are required to elucidate the role of creatine in skeletal muscle growth and to accurately assess the degree to which creatine exerts protective musculoskeletal effects, without unwanted side effects such as weight gain and kidney problems.
Taurine is a free amino acid abundant in skeletal muscle that exerts a wide spectrum of actions, ranging from osmolyte control, antioxidant action and anti-inflammatory effects. In skeletal muscle, taurine modulates ion channel function and calcium homeostasis (Conte Camerino et al., 2004). Supplementation of taurine (10% w/w in chow) is relatively safe and counteracts exercise-induced weakness after chronic exercise and ameliorates gCl (macroscopic chloride conductance, an index of degeneration-regeneration) in EDL muscles of mdx mice (De Luca et al., 2003). Clinically, taurine has been used with varying degrees of success in a wide variety of conditions (Birdsall, 1998) and is present in commercial food and beverages claimed to work as energizers.

Screening of numerous amino acids for potential efficacy in the mdx mouse (Granchelli et al., 2000) found that intraperitoneal injections of glutamine (10mg/kg) and a glutamine and alanine combination (10mg/kg each) significantly improved whole body strength after 6 weeks of treadmill exercise. Clinical studies with glutamine show that oral supplementation (0.5g/kg/day) over 10 days also inhibits whole-body protein degradation in DMD patients (Mok et al., 2006). Combinations of multiple dietary supplements (including creatine), both with and without prednisolone have recently shown improved muscle strength and reduced fatigue in exercised mdx mice (Payne et al., 2006). Such studies build a good case for promising benefits from combined interventions.
An additional possible treatment for DMD would be to compensate for the loss of dystrophin and nitric oxide with pharmacological agents. Administration of L-arginine (the substrate for nitric oxide synthase) increases nitric oxide production and up regulates utrophin expression in mdx mice. Six weeks of L-arginine treatment (200 mg/kg – intraperitoneal injection) improved muscle dystrophopathology and decreased serum CK in mdx mice (Voisin, 2005) and when given in combination with Deflazacort (Archer, Vargas, & Anderson, 2006) L-arginine (0.375% in drinking water) spared limb muscles from exercise induced damage and increased the distance (km) run voluntarily by an individual mouse.

While amino acids have been proposed for many clinical conditions, possible benefits to DMD of supplementation with taurine, glutamine, alanine and arginine (alone or in combinations) remain to be formally evaluated.

**Conclusion.**

The protective interventions briefly outlined in this review are those with some immediate possibility for clinical application in the near future; either drugs already in clinical use for other purposes or nutritional supplements as summarised in Table 1. When developing therapies for DMD, the goal is to maintain or promote skeletal muscle mass and function but at the same time reduce any deleterious side effects, such as unwanted cardiovascular complications seen with powerful muscle anabolic agents. Early treatments
administered before the pathology manifests are expected to be the most efficacious. Aggressive inflammation is a secondary characteristic of the disease and specific anti-inflammatory drugs seem to be a logical progression in the search for an alternative to steroids. Dietary supplementation in conjunction with some anti-inflammatory drug seems particularly promising and requires further rigorous investigation.

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