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Medicine in focus

Duchenne muscular dystrophy: Focus on pharmaceutical and nutritional interventions

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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.

Keywords: Duchenne muscular dystrophy; Mdx mouse; Pharmaceuticals; Nutritional supplements; Therapy

1. 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; (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

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model, 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 (Fig. 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

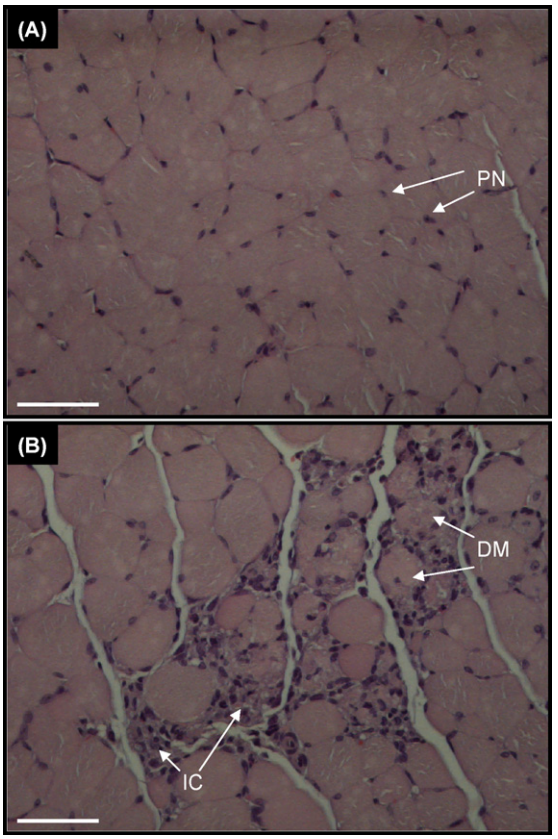

Fig. 1. Skeletal muscle necrosis in the mdx mouse. (A) Pre-necrotic (normal) skeletal muscle in the quadriceps muscle of a 20-day-old mdx mouse, characterized by healthy myofibres with peripheral nuclei (PN). (B) Necrotic skeletal muscle in the quadriceps muscle of a 23-day-old mdx mouse, characterized by infiltrating inflammatory cells (IC) and degenerating myofibres (DM). Transverse muscle sections stained with haematoxylin and eosin. Scale bar represents 50 μm.

enabling potential therapeutic interventions to be evaluated in adult mdx mice (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 h (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 dystropathology 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).

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). 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 http://www.cinrgresearch.org.

2. 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 prob-