Neuromuscular Disorders 19 (2009) 131–139

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Treatment with inhibitors of the NF-κB pathway improves whole body tension development in the mdx mouse

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ARTICLE INFO

Article history:
Received 9 June 2008
Received in revised form
11 September 2008
Accepted 13 October 2008

Keywords:
Muscular dystrophy
Mdx mouse
NF-κB
Ursodeoxycholic acid
Pyrollidine dithiocarbamate
Muscle function
Whole body tension

ABSTRACT

The whole body tension (WBT) method was used to evaluate the hypothesis that long term treatment with NF-κB inhibitors improves the total forward pulling tension exerted by the limb musculature of the mdx mouse. Mdx mice exhibited significantly reduced WBT values and more profound weakening during the course of generating multiple forward pulling movements than age-matched nondystrophic mice. Long term treatment with the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) did not significantly reduce nuclear p65 activation in the costal diaphragm, but increased WBT by 12% in mature (12 month) mice. Daily treatment (30 days) of 1 month old mdx mice with the inhibitor ursodeoxycholic acid (UDCA) reduced costal diaphragm nuclear p65 activation by 40% and increased WBT by 21%. These results indicate that treatment with NF-κB inhibitors improves WBT in the mdx mouse and further establishes the utility of the WBT procedure in assessing therapeutic efficacy.

1. Introduction

The mdx mouse [1] is a useful model for Duchenne and related muscular dystrophies because it fails to express the cytoskeletal protein dystrophin [2] and exhibits many of the phenotypic characteristics of human muscular dystrophy including skeletal muscle weakness [3–7], fiber necrosis, fibrosis, and macrophage infiltration [8,9], enhanced regeneration with associated centronucleation [10,11], and elevated plasma creatine kinase levels [1]. Gene screening studies on both the mdx mouse and on Duchenne muscular dystrophy indicate enhanced expression of several pro-inflammatory molecules [12,13] that are consistent with observed increases in the activation of the NF-κB pathway in both preparations [14,15].

The effect of passive stretch in increasing the activation of the NF-κB pathway [14], together with evidence indicating that a chronic history of passive stretch is particularly damaging to dystrophic muscle [16], led us to examine the effects of inhibitors of the NF-κB pathway on the phenotype of the chronically stretched mdx triangularis sterni (TS) muscle. These studies showed that daily treatment with pyrrolidine dithiocarbamate (PDTC) restored the resting membrane potential and improved the survival of striated TS muscle fibers in the mature (age 15 to 22 months at euthanasia) adult mdx mouse [17]. An independent series of investigations in a younger population (5 week old) of mdx mice showed that long term treatment with the same dose of PDTC (3 times weekly for 5 weeks) reduced necrosis in limb musculature, increased grip strength, and reduced grip strength fatigue [18]. More recently, Pan et al. [19] have reported beneficial effects of the NF-κB inhibitor, curcumin, on the structure and function of mdx limb musculature, and Acharyya et al. [20] have shown that mdx mice expressing reduced levels of p65 exhibited reduced pathology of the gastrocnemius muscle. Together these studies indicate that inhibition of the NF-κB pathway has an overall beneficial effect in treating Duchenne and related muscular dystrophies, and provide a rationale for developing and testing NF-κB inhibitors for potential clinical use.

Several years ago, this laboratory developed a noninvasive procedure to assess the overall body strength of mdx mice and provided the first evidence of skeletal muscle weakness in the mdx mouse [3]. The WBT (or "escape" test, [21]) measures the total forward pulling tension (FPT) exerted by individual mice as they repeatedly attempt to escape into a darkened tube, and has been used by several investigators to noninvasively measure muscle weakness in mdx mice and determine the therapeutic efficacy of

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doi:10.1016/j.nmd.2008.10.006

a variety of potential treatments for Duchenne muscular dystrophy [5,21–23] (Table 1). In contrast to measurements of grip strength [18] which are limited to determining the strength of distal forelimb musculature that are relatively spared in most myopathies, the WBT method measures the total force generated by the combined action of the proximal and distal musculature of all four limbs that are used to accelerate a mouse during a forward running movement. In the present study, WBT determinations were obtained from a larger sample in order to more fully determine the effect of aging on WBT over the approximate 2 year lifespan of both nondystrophic (C57 Bl10SnJ) and mdx mice.

The results indicate that mdx mice are significantly weaker than nondystrophic mice as early as 30 days of age and that this relative weakness continues until 1.5 to 2 years when the nondystrophic mice exhibit an age-dependent decline to approach the WBT levels seen in mdx mice. In addition, mdx mice exhibited enhanced weakening following a forward pulling maneuver. To examine the hypothesis that long term treatment with inhibitors of the NF-κB pathway improves limb muscle strength in the mdx mouse, young adult and mature mdx mice were treated with two inhibitors of the pathway that act by different mechanisms; PDTC and ursodeoxycholic acid (UDCA).

UDCA had been shown previously to reduce NF-κB transactivation in several mammalian cell lines by a mechanism that does not modify cytosolic NF-κB signaling components but involves interaction with glucocorticoid receptors (GR), nuclear translocation of the GR-UDCA complex, and inhibition of p65 transactivation [24]. In contrast to glucocorticoid activation of the GR, the nuclear UDCA-GR complex does not activate GR–response elements [24] and therefore would not be expected to produce the deleterious side-effects characteristic of long term exposure to glucocorticoids [25]. PDTC stabilizes cytosolic IκB-α [17,26] by a mechanism that involves inhibition of IκB-α ubiquitination [27] and has been shown to reduce nuclear NF-κB activation in mdx quadriceps following long term treatment [18]. The results indicate that long term treatment with either inhibitor improved WBT, and that the magnitude of improvement was positively related to the efficacy of the drug in reducing nuclear p65 activation in the mdx costal diaphragm.

2. Methods

2.1. PDTC and UDCA Treatment

Mdx (C57 Bl10-mdx) and nondystrophic (C57Bl10-SnJ) mice were obtained from Jackson laboratories and bred under conditions approved by the Institutional Animal Care and Use Committee (IACUC). All studies were performed in accordance with the guidelines provided by the IACUC. Male and female mdx mice received daily intraperitoneal (ip) injections of 50 mg/kg pyrollidine dithiocarbamate (PDTC; Sigma P8765) dissolved in sterile-filtered (0.2 μm) HEPES-buffered Ringers solution (147.5 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 11 mM glucose, 5 mM Hepes, pH 7.35). Age- and gender-matched groups of vehicle-treated mice received corresponding injections of HEPES Ringers [17]. The PDTC experiments were conducted on two series of mature adult mdx mice (9.5–16 month; 19–20 months) and on a series of young adult (1 month) mice. In each case, the mice in the experimental and vehicle-treated groups were matched according to age and gender with an equal number of males and females in each group. WBT tension measurements were obtained prior to initiating treatment and on multiple occasions following at least 3 weeks (20 days) of treatment.

Another group of 30 day old mdx mice were exposed to daily ip injections (30 day) of 40 mg/kg UDCA (Sigma #5127; 2 mg/ml; $N = 12$ experimental) in an isotonic saline vehicle (1.02% NaCl, pH 8.4,) or a synthetic taurine derivative of UDCA (tauroursodeoxycholic acid, TUDCA, SpA Laboratorio Farmacologico, Cenate Sotto, Italy; 6 mg/ml; $N = 4$ experimental) administered in a saline vehicle (NaCl 6 g/l, KCl 0.3 g/l, $CaCl_2$ 0.2 g/l, Na acetate 4 g/l, 300 mosM/l, pH 6.1). No differences in the effectiveness of the two closely related compounds was observed and the results were therefore combined and compared to a group of age- and gender-matched mdx mice treated with the appropriate vehicle solution.

2.2. Noninvasive measure for assessing whole body skeletal muscle function in mice

WBT measurements [3] were obtained by placing an approximately 0.5 inch square piece of adhesive tape around the tail about 1 inch from the tip. A small (2 cm) alligator clip was then attached to the adhesive tape around the tail and a second piece of adhesive was secured around the alligator clip to prevent slippage. The alligator clip was pre-fastened to a 3 inch long thread of 30 gauge flexible steel wire. The opposite end of the wire was attached to a metal hook that fitted directly into a Grass Force displacement transducer (FT103C) and the mouse was positioned at the mouth of a polyvinyl chloride tube (approximately 1 ft. long) that had been lined interiorly with a conventional aluminum screen (14 Mesh, .020 in.). The diameter of the tube was 1.5 inches for mature mice and 0.5 inches for the young adult mice (30–60 days of age). The procedure of attaching the clip to the mouse generally took less than 1 min.

After attaching the thread to the tail of the mouse, the mouse was gently led into the dark interior of the tube. The thread was attached to the transducer and the position of the transducer adjusted until the thread was taut. The tail was gently stroked with serrated forceps to cause the mouse to try to escape and the resultant increase in tension produced by each forward pull (“Forward Pulling Tension”, FPT) was recorded using a Grass Model 7 polygraph. Approximately 15–20 FPTs were recorded during each session. FPTs were expressed in grams and were divided by body weight to obtain WBT. A typical recording session was completed in approximately 5 min. The measurements were obtained during both the morning and afternoon hours from mice maintained on a 12:12 light dark cycle. In most cases, measurements were obtained from all relevant experimental groups (e.g. nondystrophic and mdx) during identical sessions.

Four parameters were routinely obtained from each WBT recording session. The mean force of the five maximal FPTs divided by the body weight is the WBT5, while the corresponding value for the top 10 FPTs is the WBT10 [3]. In addition, the ratio of the WBT10 to the WBT5 value is a measure of the consistency of FPT generation during a recording session and is termed the “Functional Reserve” (FR; [3]). A second measure of FPT consistency is

Table 1
Evaluation of the WBT5 indicates the overall reproducibility and consistency of the whole body tension method for assessing the total body strength of adult nondystrophic mice. The results obtained from four different laboratories indicate that nondystrophic mice exert WBT5 values of approximately 13–16 times body weight over a wide range of ages. *N* values represent the number of determinations and the number of mice used in each study.

	Age	N	WBT 5
Carlson and Makiejus [3]	1–2—5 months	56.42	13.8–14.1
	2.5—5 months	24.17	
	5—11 months	17.7	
Makiejus et al. [22]	>5 months	7.17	13.2
Hudecki et al. [5]	4—5 months	15	14.6
	5—6 months	15	15.6
Deconinck et al. [21]	3—4 months	9	14.5