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Severe burn increased skeletal muscle loss in *mdx* mutant mice

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ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form
29 January 2016
Accepted 26 February 2016
Available online 4 March 2016

Keywords:
Thermal injury
Skeletal muscle
Cell death
Cell proliferation
Myogenesis
Preexisting muscle disease

ABSTRACT

Background: Severe burn causes muscle mass loss and atrophy. The balance between muscle cell death and growth maintains tissue homeostasis. We hypothesize that preexisting cellular structural defects will exacerbate skeletal muscle mass loss after burn. Using a Duchenne muscular dystrophy (*mdx*) mutant mouse, we investigated whether severe burn caused more damage in skeletal muscle with preexisting muscle disease.

Methods: The *mdx* mice and wild-type (WT) mice received 25% total body surface area scald burn. Gastrocnemius (GM), tibialis anterior, and gluteus muscles were obtained at days 1 and 3 after burn. GM muscle function was measured on day 3. Animals without burn served as controls.

Results: Wet tissue weight significantly decreased in tibialis anterior and gluteus in both *mdx* and WT mice after burn ($P < 0.05$). The ratio of muscle to body weight decreased in *mdx* mutant mice ($P < 0.05$) but not WT. Isometric force was significantly lower in *mdx* GM, and this difference persisted after burn ($P < 0.05$). Caspase-3 activity increased significantly after burn in both the groups, whereas HMGB1 expression was higher in burn *mdx* mice ($P < 0.05$). Proliferating cell nuclear antigen decreased significantly in *mdx* mice ($P < 0.05$). Myogenic markers pax7, myoD, and myogenin increased after burn in both the groups and were higher in *mdx* mice ($P < 0.05$).

Conclusions: More muscle loss occurred in response to severe burn in *mdx* mutant mice. Cell turnover in *mdx* mice after burn is differed from WT. Although markers of myogenic activation are elevated in *mdx* mutant mice, the underlying muscle pathophysiology is less tolerant of traumatic injury.

Published by Elsevier Inc.

Introduction

The hypermetabolic response to severe burn causes muscle loss to exceed muscle gain resulting in a negative net balance of muscle tissue.[1] The mechanisms of muscle loss after burn are not completely understood. Severe burn results in increased resting energy expenditure due to elevated stress hormones, production of acute phase proteins, and increased immunologic mediators.[2] Prior studies have demonstrated that increased whole body protein turnover, specifically from breakdown of skeletal muscle, provides amino acid substrates for wound healing after burn.[3] However, shunting of amino

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0022-4804/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jss.2016.02.037

acids to the burn wound cannot account for muscle loss entirely as net protein loss with elevated catabolism is known to persist 9 mo-1 y after burn, well after wound closure.[4,5]

At the molecular level, it has been observed that caspases and the activated ubiquitin–proteasome pathway leads to accelerated degradation of myofibrillar proteins in the acute phase after burn.[6] Specifically, the atrophy-related ubiquitin E3 ligases MuRF-1 and atrogin-1 have been noted to have an 8- and 3-fold increase, respectively 48 h after burn.[7] E3 ubiquitin ligases and caspases are regulated through the PI3K pathway, a target of IGF-I and insulin.[8]

Skeletal muscle damage is not just limited to depletion of proteins. It has been noted by Duan *et al*. that skeletal muscle cell apoptosis is induced on the first day after burn with a peak at 4 d after injury. Elevation of circulating apoptotic ligands and caspases and an increase in muscle tissue proapoptotic genes and proteins suggest a mechanism for this finding.[9]

To maintain muscle homeostasis, muscle cell regeneration must compensate for cell death. Myogenesis after burn is not compensatory to cell loss leading to atrophy.[10] Myogenesis is dependent on muscle progenitor satellite cells. Satellite cells are a small population of quiescent cells found between the sarcolemma and basal lamina. In response to external stimulation, activated satellite cells first proliferate and then differentiate to fuse into new fibers in the process of repair.[11]

The role of myogenesis after burn is not clear. Wu *et al*.[12] have observed satellite cell activation in muscles of severe burn rat models. However, Duan *et al*.[13] showed that proliferative activity of myoblasts decreased in the tibialis anterior (TA) on the first day after burn, suggesting an inhibition of muscle cell growth. After burn, several pathways at the hormonal, cellular, and molecular levels control protein turnover, apoptosis, and myogenesis, all contributing to the end result of muscle atrophy.

Human Duchenne's muscular dystrophy is a recessive X-chromosome–linked muscular dystrophy caused by a mutation in the dystrophin gene. Patients present with progressive proximal muscle weakness and loss of muscle mass eventually leading to paralysis. Dystrophin is a cytoplasmic protein that links myofilaments and structural proteins to the sarcolemma. Dmd^{mdx} mutant mice (*mdx*) lack dystrophin expression and have been extensively used as an animal model for the human muscular disease.[14,15] This rodent model is considered a less severe phenotype of the human disease, possibly due to the presence of a robust regenerative response or increased expression of utrophin and $\alpha 7\beta 1$-integrin, proteins that fulfill the same structural role as dystrophin.[16] To our knowledge, there is no information about the response to severe burn in the dystrophin defective *mdx* mouse. We hypothesize that preexisting cellular structural defects will exacerbate skeletal muscle mass loss after burn resulting in more severe atrophy. The aim of the present study was to investigate whether severe burn causes more damage in skeletal muscle with preexisting muscle disease.

Materials and methods

Adult male C57BL/10ScSn-Dmd^{mdx}/J mutant (*mdx*) mice (6-8 wk old) and age-matched wild-type (WT) control mice were purchased from Jackson Laboratory (Bar Harbor, ME) and allowed for 1-wk acclimation before the experiment. Mice were housed in a temperature-controlled room with a 12-h light/dark cycle and *ad libitum* laboratory chow and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center following the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Burned mice

Mice received 25% total body surface area (TBSA) scald burn under general anesthesia with 1.2% avertin (250 mg/kg) injected intraperitoneally (ip). The burn procedure was described in previous study.[17] Briefly, shaved mice received 1 mL of 0.9% saline injection subcutaneously along the spinal column. Mice were placed in a mold with an opening to expose a 12.5% TBSA. Mice were then immersed in 97°C water for 10 s on the dorsal and 2 s on the ventral side to receive a total 25% TBSA full-thickness scald burn. Mice received 1 mL of lactated Ringers solution (ip) for resuscitation and 0.05 mg/kg of Buprenorphine SR subcutaneously for analgesia. Sham animals underwent anesthesia and shaving with immersion in room temperature water (25°C). Hind limb muscles including gastrocnemius (GM), TA, and gluteus were obtained when mice were euthanized on days 1 and 3 after burn. Muscle tissue was weighed, halved, and either stored in 10% neutral buffered formalin for histologic process, or snap frozen in liquid nitrogen for further molecular biological analysis.

Muscle function test

Isometric contractile properties of the GM muscles were measured on day 3 after burn using the muscle level system with dynamic muscle control and analysis software (1305A Whole Animal System; Aurora Scientific, Inc). Under anesthesia, the GM muscle was gently dissected free of skin, fascia, and surrounding musculature. The Achilles tendon was sutured and attached to the lever arm of a Dual-Mode Servo Muscle Lever System (model 305c; Aurora Scientific, Inc). The hind limb was secured to the 806D *in-situ* rodent platform. Electrodes were implanted into the distal end of severed sciatic nerve.

The muscle stimulation protocol was followed in the previous study[18] with minor modifications. The GM was stimulated by the 701C Electrical Stimulator (Aurora Scientific, Inc) with a single twitch (0.2-ms impulse duration, 200 Hz frequency, 10 mA), and the muscle was stretched 0.2 mm every 25 s to reach the optimal length (Lo), where there was less than 2% change between twitches. The muscle isometric functions, twitch (Pt) and tetanic force (Po), were measured afterward. Muscle Po was obtained with a total 1 s of electric stimulation at 150 Hz with impulse duration of 0.2 ms and 75 pulses per train. Po measurement was done in triplicate with a 2 min of tension interval.

Muscle tissue histology

Fixed muscle tissues were paraffin embedded and sectioned longitudinally through the middle transverse plane. Tissue