Importance of satellite cells in the strength recovery after eccentric contraction-induced muscle injury

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Satellite cells are involved in the replacement and/or repair of damaged fibers after other forms of traumatic injury (19), and Smith et al. (22) provided data suggesting that satellite cells may aid in the repair of fibers after injurious eccentric contractions. Thus it seems likely satellite cells contribute to the strength recovery after eccentric contraction-induced injury. However, a clear dissociation exists between the amount of histological damage incurred and the strength lost in eccentric contraction-injured muscles (27). As one example, McCully and Faulkner (16) reported 37% of the muscle fibers showed signs of degeneration due to eccentric contraction-induced injury when the reduction in maximal isometric tetanic force was proportionally larger (78%). This discrepancy between histopathology and strength loss may be partially explained by limitations associated with histological measurements of eccentric contraction-injured muscle (27) and/or the fact that excitation-contraction (E-C) coupling failure is responsible for a large part of the strength decrement (28). Because relatively few fibers degenerate when the strength loss is large (13, 15, 16), it is not unreasonable to suggest that elimination of satellite cell proliferation would have a minimal effect on the recovery of strength after eccentric contraction-induced injury.

Unlike the dissociation between histopathology and force production, the restoration of contractile protein and strength after eccentric contraction-induced injury is closely related in the later stages of recovery (10). In fact, the restoration of contractile protein content in muscles injured with eccentric contractions parallels strength recovery from 14 days until the muscle has functionally recovered, i.e., 28 days postinjury (10). Indications that satellite cells may play a role in the restoration of contractile protein in eccentric contraction-injured muscle are provided in models of skeletal muscle regeneration and hypertrophy, which require the restoration and/or addition of contractile protein (1, 7). The importance of satellite cell proliferation in these adaptations is evident when these models are used in conjunction with irradiation. Irradiation before the onset of a stimulus inducing regeneration or hypertrophy inhibits the normal adaptive response, attributing the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
utable to the inhibition of satellite cell proliferation (8, 21).

Because satellite cells fuse to form new myofibers and/or fuse with damaged or overloaded tissue to replace or add force-generating structures, i.e., contractile protein, it seems likely satellite cells would also contribute to the strength recovery that follows eccentric contraction-induced injury. However, to our knowledge, this has not been previously demonstrated. Therefore, the purpose of this study was to test the hypothesis that the elimination of satellite cell proliferation with irradiation would prevent normal strength recovery after eccentric contraction-induced injury.

METHODS

Animals. Female ICR mice (n = 40), 8–12 wk old, were purchased from Harlan Laboratories. They were housed 4–6/ cage at 20–23°C with a 12:12-h dark-light cycle. The animal care procedures and experimental protocol met the guidelines set by the American Physiological Society and were approved by the Texas A&M University Laboratory Animal Care and Use Committee.

Experimental design. To test if satellite cells are necessary for muscle strength recovery after eccentric contraction-induced injury, a stimulating nerve cuff was implanted on the common peroneal nerve in the left leg of mice, and the animals were subjected to one of the following four treatments: 1) irradiation and eccentric contraction-induced injury (Irr/Inj, n = 11), 2) eccentric contraction-induced injury only (Inj, n = 10), 3) irradiation only (Irr, n = 11), or 4) no intervention [control (Con), n = 8]. Strength of the anterior crural muscle group was assessed by measuring in vivo maximal isometric torque production at the ankle. The primary muscle in this group is the tibialis anterior (TA), which contributes 89% of the torque (26). Two rounds of experiments had to be completed to obtain sufficient numbers of replicates to test the hypothesis. In both rounds, torque was measured at 300 Hz seven times after the irradiation procedure: preinjury, immediately postinjury (0 days), and 7, 14, 21, 28, and 35 days postinjury. After the first round of measurements, the data suggested that E-C coupling restoration might be different between Inj and Irr/Inj groups. Therefore, to estimate E-C coupling recovery, in vivo isometric torque was measured at a low frequency (40 Hz) in animals in the two Inj groups of the second round in addition to the 300-Hz torque measurements. Muscle wet weights of the TA and extensor digitorum longus (EDL) muscles from some (n ≥ 8) animals of the four groups were determined 49–56 days after the injury induction.

Surgical procedures. A stimulating nerve cuff was implanted on the left common peroneal nerve of the mice in all four groups while the animals were under pentobarbital sodium anesthesia (100 mg/kg ip) as described previously (25). Briefly, the nerve cuff was constructed from two Teflon-coated, multistranded 90% Pt-10% Ir wires of 0.15 mm diameter (Medwire-Sigmund Cohn, 10/l9/49FT; Mt. Vernon, NY). An incision was made through the biceps femoris muscle, and loops formed from 2.5-mm deinsulated segments of the two wires were placed around the common peroneal nerve. The proximal end of the nerve cuff was externalized in the dorsal cervical region.

Irradiation. Between 19 and 36 days after implantation of the nerve cuff, mice were anesthetized with fentanyl citrate (0.33 mg/kg), droperidol (16.7 mg/kg), and diazepam (5 mg/kg), and the left legs of mice in the two Irr groups were exposed to γ-irradiation from a cobalt-60 source (model NPTT-Series, Neutron Products). A dose of 2,500 rad was applied over 13.1 min. This dose has previously been shown to be effective in inhibiting satellite cell proliferation (1, 17, 20). The effectiveness of the irradiation was verified in cross sections cut from TA muscles excised 49–56 days after performing eccentric contractions. Irradiation effectiveness was further verified using the traumatic injury model described previously (24). Briefly, a metal rod precooled in dry ice was held to the superficial aspect of the TA muscle for 5 s. Freeze-injured TA muscles were removed at 5 or 10 days postinjury and studied histologically.

Isometric torque measurements. In vivo maximal isometric torques produced by the anterior crural muscles at the ankle were measured in animals of all four groups as described previously (13, 23). The left foot of the anesthetized mouse (fentanyl citrate, 0.33 mg/kg; droperidol, 16.7 mg/kg; and diazepam, 5 mg/kg) was positioned in the foot plate controlled by a servomotor (Cambridge Technology 300B). The isometric contractions were performed with the plantar surface of the foot perpendicular to the tibia. Peak torque production was optimized by varying stimulation voltage in a series of five to eight isometric contractions; the stimulations were 45–60 s apart and consisted of 200-ms trains of 0.1-ms pulses at 300 Hz. As stated above, Irr/Inj and Inj mice in the second round of experiments also performed an isometric contraction at a low frequency (40 Hz) 90 s after the determination of peak torque. Low- to high-frequency torque ratios have previously been interpreted as an indicator for E-C coupling failure (5).

Eccentric contraction injury induction. Two minutes after optimization of peak torque, the left anterior crural muscles were injured as described previously (11, 13). The foot was fixed passively dorsiflexed 20° from the perpendicular position about the ankle. The muscles were then stimulated using 120-ms trains of 0.1-ms pulses at 300 Hz. An isometric contraction was performed for the first 100 ms of the stimulation, followed by 20 ms plantar flexion movement (eccentric contraction) over a 40° arc at 2000°/s. The foot was then passively returned to the perpendicular position. There were 12 s between contractions, so the duration of the protocol was ~30 min. Two minutes after the 150th contraction, a postprotocol isometric contraction at 300 Hz was performed. The animals in the second round also performed a postprotocol isometric contraction at 40 Hz after the 300 Hz contraction as described above.

Tissue collection and processing. At 49 or 56 days postinjury, animals were anesthetized with pentobarbital sodium, and TA and EDL muscles were dissected free of connective tissue, blotted dry, and quickly weighed using an analytical balance. After removal of the muscles, animals were euthanized with an overdose of pentobarbital sodium (200 mg/kg ip).

To estimate possible differences in fiber cross-sectional areas and numbers of centrally located nuclei in the injured muscles, several TA muscles from Irr/Inj (n = 3) and Inj (n = 3) mice taken 49–56 days after injury were coated in OCT embedding medium, immersed in isopentane chilled in liquid nitrogen, and stored at −80°C until use. Three cross sections (10 μm thick) ~250 μm apart were cut in a cryostat at −20°C and stained with hematoxylin and eosin. The perimeters of six separate groups of 50 fibers each (i.e., 300 fibers) were traced in each of the three muscle cross sections from each mouse, and the total fiber cross-sectional area for the 50 fibers was measured using an Olympus BX-60 microscope with image analysis software (BioQuant, version 3.0; R & M
Biometrics. Interstitial tissue area was excluded in this analysis using thresholding of staining intensity. Thus, from this analysis, the average fiber cross-sectional area for each mouse was determined from a total of 900 fibers. Also, the total number of fibers with centrally located nuclei was counted in each of the three muscle cross sections from each animal, and from these counts the average number of fibers with central nuclei per muscle was calculated.

In preliminary experiments to determine the effectiveness of the irradiation procedure, hematoxylin- and eosin-stained sections of freeze-injured TA muscles from irradiated and nonirradiated mice were inspected at 5 and 10 days after injury. One TA muscle was processed from each of the two groups at each time.

Statistical analysis. The effects of irradiation, injury, and time on in vivo maximal isometric torque production were evaluated using an irradiation (irradiated, nonirradiated) × injury (injured, noninjured) × time (preinjury, immediately postinjury, 7, 14, 21, 28, and 35 days) ANOVA with repeated measures over time. The effects of irradiation, injury, and time on body weight were evaluated using an irradiation × injury × time (preirradiation through 35 days postinjury) ANOVA with repeated measures over time. Analysis of low-frequency (40 Hz) to high-frequency (300 Hz) torque ratios were conducted using an irradiation × injury × time (preinjury, immediately postinjury, 7, 14, and 21 days) ANOVA with repeated measures over time. Differences among TA and EDL muscle wet weights, respectively, were analyzed with a one-way ANOVA. A general linear model was applied to account for unequal cell sizes. Fiber cross-sectional area was analyzed with a Student’s t-test. Student-Newman-Keuls post hoc tests were used where appropriate. An α-level of 0.05 was used for all analyses. Results are presented as means ± SE.

RESULTS

Effectiveness of irradiation. Forty-nine to 56 days after eccentric contraction-induced injury, nonirradiated TA muscle had many fibers with centrally located nuclei (91 ± 19 fibers per cross section, n = 3) indicative of regeneration and satellite cell proliferation. In contrast, muscles irradiated before the injurious bout of eccentric contractions had few fibers with centrally located nuclei (6 ± 2 fibers per cross section, n = 3), providing evidence the irradiation was effective in attenuating satellite cell proliferation (Fig. 1, B and D). Furthermore, freeze-injured TA muscles that were not irradiated showed signs of regeneration evidenced by small fibers with central nuclei in the area of freeze injury 5 days postinjury and relatively larger fibers with central nuclei 10 days postinjury, whereas freeze-injured TA muscles that were irradiated had few fibers with central nuclei at these time points (Fig. 1, A and C).

Torque measurements. Before the protocol of 150 eccentric contractions, the peak isometric tetanic torques produced by the anterior crural muscles were not different among the groups (3.09 ± 0.06 N·m × 10⁻²; n = 40) (Fig. 2). Immediately after the eccentric contractions, Irr/Inj and Inj peak isometric tetanic torques had decreased by 49 and 51% from preinjury, respectively, and there was no difference between the two groups. At 7 days postinjury the torques in the two Inj groups were not different and were 31–35% lower than preinjury levels. However, between 7 and 14 days there was a marked difference between groups in their recovery, with the Inj and Irr/Inj mice recovering 21 and 6% of their preinjury strength, respectively. Irr/Inj torque was 16–25% less than Inj between 14 and 35 days after the injury.

Maximal isometric torque production by Inj mice that were not irradiated had recovered to control values by 28 days postinjury. The immediate strength loss and prolonged strength recovery by Inj animals were similar to previous reports of in vivo (13, 26), in situ (2),
and in vitro (10, 11, 13) measurements of force recovery after eccentric contraction-induced injury.

Low- to high-frequency torque ratio. The low-frequency (40 Hz) to high-frequency (300 Hz) torque ratios for the Irr/Inj and Inj animals were depressed to similar extents immediately (~5- to 6-fold) and 7 days (~2-fold) postinjury (Fig. 3). There were no differences in the low- to high torque frequency ratios between the two injured groups at any time.

Body weight, muscle wet weights, and fiber cross-sectional area. The body weights of all groups were unchanged throughout the experimental period, and there were no differences in body weights among the groups at any time point studied. The initial and final body weights of the mice are shown in Table 1. There were no differences 49–56 days postinjury in wet weights of TA or EDL muscles among Inj, Irr, Con, and contralateral control muscles (Table 1). However, Irr/Inj TA and EDL muscles weighed 16–21% less than Inj, Irr, Con, and contralateral control muscles. At 49–56 days postinjury, fiber cross-sectional area was 9% less in Irr/Inj muscles compared with Inj TA muscles, although this difference was not statistically significant. It is emphasized that these are estimates only because of the small number of animals included in the fiber size measurements.

Table 1. Body weights and muscle wet weights

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight, g</th>
<th>Initial body</th>
<th>Final body</th>
<th>Experimental TA, mg</th>
<th>Contrlateral TA, mg</th>
<th>Experimental EDL, mg</th>
<th>Contrlateral EDL, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irr/Inj</td>
<td>31.2 ± 0.6</td>
<td>31.5 ± 0.9 (n = 10)</td>
<td>31.8 ± 0.8 (n = 10)</td>
<td>15.2 ± 2.3 (n = 8)</td>
<td>62.6 ± 2.3 (n = 9)</td>
<td>11.5 ± 0.3 (n = 9)</td>
<td>11.1 ± 0.4 (n = 8)</td>
</tr>
<tr>
<td>Inj</td>
<td>31.7 ± 0.6</td>
<td>31.5 ± 0.9 (n = 11)</td>
<td>31.8 ± 0.8 (n = 10)</td>
<td>15.9 ± 2.3 (n = 9)</td>
<td>65.0 ± 2.2 (n = 8)</td>
<td>11.5 ± 0.3 (n = 9)</td>
<td>11.4 ± 0.3 (n = 8)</td>
</tr>
<tr>
<td>Irr</td>
<td>31.2 ± 0.6</td>
<td>31.5 ± 0.9 (n = 11)</td>
<td>31.8 ± 0.8 (n = 10)</td>
<td>15.2 ± 2.3 (n = 8)</td>
<td>62.6 ± 2.3 (n = 9)</td>
<td>11.5 ± 0.3 (n = 9)</td>
<td>11.1 ± 0.4 (n = 8)</td>
</tr>
<tr>
<td>Con</td>
<td>30.6 ± 0.9</td>
<td>31.5 ± 0.9 (n = 8)</td>
<td>31.8 ± 0.8 (n = 8)</td>
<td>15.2 ± 2.3 (n = 8)</td>
<td>62.6 ± 2.2 (n = 6)</td>
<td>11.5 ± 0.3 (n = 9)</td>
<td>11.1 ± 0.4 (n = 8)</td>
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Values are means ± SE. Groups: Irr/Inj, irradiation and eccentric contraction-induced injury; Inj, eccentric contraction-induced injury only; Irr, irradiation only; Con, no intervention (control). TA, tibialis anterior; EDL, extensor digitorum longus. * Different from control (P < 0.05).

DISCUSSION

The purpose of this study was to determine if prevention of satellite cell proliferation by γ-irradiation would attenuate recovery of strength in muscles injured by eccentric contractions. The data show this to be the case. Loss of strength from injury was similar in irradiated and nonirradiated muscles through 7 days, but thereafter recovery of strength was markedly less in the irradiated muscles and still was not restored by 35 days after injury. The critical importance of satellite cells in the recovery process is apparent when one considers the numerous times the muscles are injured over a lifetime as indicated by delayed onset soreness and/or a loss in strength (18). Accumulation of strength losses every time a muscle was injured by overexertion would have disastrous consequences. Thus the results indicate that one essential role of satellite cells is to restore muscle strength after this common form of injury accruing from normal physiological activity.

The similarities in strength recovery between the injured groups through 7 days postinjury suggest that the recovery of muscle strength during the first week after injury does not require satellite cell proliferation. Similarly, Mitchell and Pavlath (17) recently reported that recovery of muscle mass in mice during the first week after hindlimb suspension occurs in the absence of satellite cell proliferation. We have shown that the recovery of strength during the first week postinjury occurs independently of contractile protein accretion in the injured EDL muscle (10). In fact, contractile protein content in the injured muscle is decreasing over this time period even as strength is recovering (10). This apparently contradictory observation can be explained by our observations that E-C coupling failure explains most of the strength loss in the first few days after injury and that the E-C coupling failure is resolved by 2 wk postinjury (11, 28). The data from the present study would indicate that satellite cell proliferation is not required for resolution of the E-C coupling failure. In addition, the low- to high-frequency ratios were similarly affected in the Irr/Inj and Inj groups, suggesting that the induction and resolution of the E-C coupling failure was unaffected by irradiation.
Loss of contractile protein in the EDL muscle after injury is maximal (~20%) at 2 wk postinjury (10). Thereafter, the rate of protein synthesis exceeds that of degradation, and both contractile protein content and force increase in parallel and are restored by 28 days after injury (10). Therefore, the recovery of contractile protein appeared to be the limiting factor in the later stages of force recovery (14–28 days) after eccentric contraction-induced injury in the EDL muscle. In the present study, the Inj muscle torque also recovered by 28 days after the injury but was still depressed 21% in the Irr/Inj mice. In light of the similarities in torque decrements and recoveries between this study and those reported by Ingalls et al. (10), it is tempting to speculate that the 21% lower torque that was observed 28 days postinjury in the Irr/Inj muscles was due to the failure to restore the previously reported 20% loss of contractile protein. Indirect support for this is provided by the observation that the mean weight of the irradiated injured muscles was ~20% less than that of the nonirradiated injured muscles (Table 1). Whether the decreased wet weight, and presumably contractile protein loss, in irradiated injured muscles is due to fiber loss and/or the loss of protein from surviving fibers is not known. McCully and Faulkner (16) reported both a decrease in fiber number and cross-sectional area after eccentric contraction-induced injury. Conversely, Ingalls et al. (10) reported no change in EDL fiber number but an ~20% loss of contractile protein employing the model of eccentric contraction-induced injury used in this study. The fact that there was only a 9% decrease (P > 0.05) in TA muscle fiber cross-sectional area in irradiated injured muscles (that lost ~20% of their mass and strength) in this study suggests there may have been a failure to replace lost fibers. Regardless of the mechanisms of muscle repair, inability of satellite cells to proliferate apparently deprives the injured muscles of the genetic apparatus required for restoration of normal functional capacity. It seems reasonable to assume that the failure of the Irr/Inj animals to return to normal strength results from attenuated resynthesis of proteins essential to the development and transmission of contractile force.

In conclusion, inhibition of satellite cell proliferation with γ-irradiation prevents approximately half of the normal torque recovery after eccentric contraction-induced injury. The satellite cell-dependent portion of the strength recovery occurs 1–2 wk postinjury and most likely results from a restoration of contractile protein that requires satellite cells to proliferate and presumably to mature and fuse with injured myofibers.

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DISCLOSURES

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REFERENCES


