THE CONTRIBUTION OF SARCOLEMMLAL REPAIR TO RECOVERY AFTER AN ACUTE SKELETAL MUSCLE STRAIN

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Introduction: Muscle injuries resulting from forceful muscle contractions are routinely encountered in orthopedics. Typically, a single, lengthening ("eccentric") muscle contraction is the cause of such injuries. Despite the significant morbidity associated with muscle injuries, treatment strategies vary greatly, mainly due to the lack of a clear understanding of the mechanisms of injury and recovery. An increased understanding of these mechanisms may lead to the development of specific interventions that will decrease morbidity and accelerate the restoration of function. Studies using repeated lengthening contractions indicate that myogenesis via satellite cell (SC) proliferation is necessary for recovery of function.

We hypothesized that rescaling of the sarcolema (muscle plasma membrane and its associated proteins) could provide an alternative mechanism for recovery in the absence of myogenesis.

Methods: Our use of rats was approved by the University of Maryland Institutional Animal Care and Use Committee. We induced an injury in the tibialis anterior muscle (TA) of adult, male Sprague Dawley rats (397 ± 24 g) by forcibly rotating 90° of plantar flexion starting with the foot orthogonal to the tibia, at an angular velocity of 900°/s, while the anterior tibial muscles (dorsiflexors) were stimulated maximally to produce a fused tetanic contraction. Muscles were activated by directly stimulating the peroneal nerve, after it was dissected free through a small incision and clamped with a bipolar subminiature electrode. This injury model leads to a reproducible, transient disruption of function and structure of the TA, both of which completely recover within 21 days. We studied maximal tension (P0), our measure of function, by attaching the tendon of the TA to a load cell and activating it as described in the injury protocol. We exposed the TAs of one group of rats (n=12) to a single dose of ionizing radiation (25-Gy at 2.5 Gy/min) prior to injury, in order to inhibit proliferation of SCs and myogenesis after injury. We compared functional recovery (3, 7 and 21 days after injury) in this group to another group (n = 12), in which TAs were not irradiated before injury.

In a third in a group of animals (n = 12), we tested whether or not irradiation alone affected function. We used RT-PCR, which assessed the expression of MyoD and myogenin (markers of muscle regeneration), and western blots, which assessed the expression of M-cadherin (marker for SC) and proliferating cell nuclear antigen or PCNA (marker for cell proliferation) to evaluate the effects of irradiation on myogenesis. We studied sarcolemmal rescaling in a separate group of animals (n = 9), which were injected with Evan’s blue (EBD), a membrane-impermeable dye, 24 hrs before injury. We compared the continued presence of EBD-positive fibers 3 and 7 days after injury, and the decreasing percentage of myofibers with loss of dystrophin, indicated that, most of the fibers injured in our protocol do not undergo regeneration, but instead are likely to recover in part as a result of rapid rescaling of their membranes.

Results: Irradiation inhibited myogenesis, as indicated by the absence of muscle-specific regenerative markers. Nevertheless, contractile function in irradiated and injured TAs recovered to control values over a time course that was identical to recovery of non-irradiated, injured TAs (Table 1).

<table>
<thead>
<tr>
<th>Days after injury</th>
<th>Irradiated and Injured (N)</th>
<th>Injured Only (N)</th>
<th>Irradiated Only (N)</th>
<th>Non irradiated, Non Injured Control (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.82 ± 0.06*</td>
<td>2.83 ± 1.03*</td>
<td>7.43 ± 0.65</td>
<td>8.24 ± 1.30</td>
</tr>
<tr>
<td>7</td>
<td>6.70 ± 1.40</td>
<td>6.07 ± 1.23</td>
<td>7.61 ± 0.40</td>
<td>8.64 ± 0.97</td>
</tr>
<tr>
<td>21</td>
<td>8.64 ± 0.97</td>
<td>8.84 ± 0.54</td>
<td>8.69 ± 1.34</td>
<td>8.24 ± 1.30</td>
</tr>
</tbody>
</table>

Discussion: Our results suggest that, unlike recovery from injuries induced by repetitive lengthening contractions, recovery from a single, maximal lengthening contraction does not require myogenesis. They further suggest that membrane rescaling occurs shortly after injury and well before the recovery of function. Thus, recovery from a single, maximal lengthening contraction may not involve the formation of new myofibers, but may require rescaling of the membranes of injured fibers. These findings indicate that the mechanism of recovery following injury may differ based on the mode of injury.

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References