Damage To The Plasma Membrane Of Rotator Cuff Muscle Fibers Following Surgical Repair

Max E. Davis, Patrick L. Stafford, BS, Matthew J. Jergensen, Asheesh Bedi, MD, Christopher L. Mendias, PhD.
University of Michigan Medical School, Department of Orthopaedic Surgery, Ann Arbor, MI, USA.

Disclosures:

Introduction: Large or massive tears of the rotator cuff are a common source of shoulder pain and disability, and can limit the mobility and negatively impact the quality of life of patients. Common pathophysiological changes that occur in patients with chronic rotator cuff tears include muscle fiber atrophy and shortening, fat accumulation and a decrease in the force generating capacity of muscle fibers. Even after surgical repair of these tears, an estimated 50% of patients still have substantial symptoms 6 months after the surgery and 40% have pathology 1 year post-surgery (1). Previous work in a sheep model of rotator cuff tears demonstrated that a slow, progressive lengthening of chronically torn rotator cuff muscles followed by surgical repair of the tendon reversed fatty degeneration and improved the gross morphological appearance of the cuff muscles (2). As patients with chronic rotator cuff tears often experience a shortening of the muscle fibers, it is possible that repairing the torn tendon to its original anatomical footprint induces a massive stretch-induced injury to the muscle that could contribute to poor outcomes after repair. Damage to the muscle fiber sarcolemma (the plasma membrane of muscle fibers) results in a persistent influx of calcium molecules, which induce muscle spasm and activate calcium-dependent proteases that degrade the muscle fibers. Evans Blue Dye (EBD) is a water soluble, membrane impermeable dye, and is often used to identify muscle fibers that have suffered an injury to their sarcolemma. We hypothesized that, compared with acutely torn and repaired rotator cuff muscles, the substantial acute stretch that is required to repair a chronically torn and shortened rotator cuff would result in significant muscle fiber damage as measured by an increase in EBD positive (EBD+) muscle fibers. To test this hypothesis, we injected EBD into rats that had undergone either acute or chronic rotator cuff tears and measured the amount of EBD+ fibers in each condition.

Methods: This study was approved by our IACUC. Male Sprague-Dawley retired breeder rats (n=25) were placed into 5 groups: control, sham surgery, acute tear no repair, acute tear and repair and chronic tear and repair with each modality performed bilaterally (Figure 1C). The sham group had the skin and deltoid over the rotator cuff opened but the supraspinatus tendon was not touched and was used to control for any damage caused by the surgical incisions. An intraperitoneal injection of 1 mg EBD/0.1 mL of PBS/10g of body mass was administered 24 hours before the harvest surgery. At harvest, the supraspinatus muscles of each rat were removed and the length of each muscle was measured with digital calipers. To determine EBD+ fibers the supraspinatus muscles were snap frozen in OCT and the mid-distal and mid-proximal areas of the muscle were cryosectioned and incubated with WGA-Lectin-AF488 to mark the extracellular matrix (ECM), DAPI to identify nuclei and the EBD, which is inherently fluorescent, was used to identify the fibers with a damaged sarcolemma. ImageJ software was used to quantify the number of EBD+ fibers and total fibers. A one-way ANOVA (p<0.05) and Tukey’s post-hoc sorting was used to evaluate the differences between groups.

Figure 1. (A) Diagram of the areas of histology; (B) Representative histology demonstrating undamaged and EBD+ muscle fibers; (C) Overview of the study design.
A

B

C

- SSP
- Mid-Proximal
- Mid-Distal
- INF

Control

Sham

Acute Tear No Repair

Acute Tear And Repair

Chronic Tear And Repair

Surgical Tear

Sham Surgery

Surgical Repair

EBD Injection

Harvest
Results: For supraspinatus muscle lengths, the muscles from the chronic tear and repair group was approximately 25% shorter than all other groups (Figure 2A). The number of EBD+ muscle fibers was generally greater at the mid-distal region of the muscle than the mid-proximal region. At the mid-distal end, while only a few fibers were observed in the control group, 27% of fibers were EBD+ in the sham group, and almost 90% of fibers were EBD+ in the remaining groups (Figure 2B). For the mid-proximal portion of the muscle, nearly 70% of fibers were EBD+ in the chronic tear and repair group while no significant differences were observed for any other groups (Figure 2C).

Figure 2 (A) Supraspinatus length after muscle harvest; Percent of EBD positive fibers in (B) mid-distal and (C) mid-proximal supraspinatus muscles for each of the 5 groups. Post-hoc sorting differences (p<0.05) indicated in graph, N=9 muscles per group.
Discussion: To our knowledge this is the first study that explored damage to the muscle fiber sarcolemma in acutely and chronically torn and repaired rotator cuff muscles. Across all surgical groups there was a clear gradient of injury, with the mid-distal portion experiencing much greater damage than the mid-proximal portion. While the findings supported our hypothesis that the chronic tear and repair group would experience the greatest amount of injury, nearly all fibers in this group demonstrated damage in the distal portion of the muscle. Furthermore, acute tear groups demonstrated similar levels of injury as the chronic group at the mid-distal portion of the muscle. The sham and all repair groups demonstrated damage at the mid-distal portion in the absence of physical handling or manipulation, suggesting that the inflammation that occurs at the shoulder joint as a result of the operative procedure may induce a secondary, pro-inflammatory cytokine mediated injury to the muscle fibers in this area. In all groups but the chronic tear and repair group, the mid-proximal portion appeared largely protected from injury. This could be due to physical distance from the shoulder joint or because the proximal portion is supported by lateral connections to the supraspinatus fossa, and these connections likely allow for improved lateral force transmission and subsequent protection of the sarcolemma from damage.

Significance: This study demonstrates that the repair of a chronically injured rotator cuff causes damage along the entire length of the muscle, with the distal end demonstrating greater damage. These results suggest that open or mini-open shoulder surgeries can induce damage to rotator cuff muscle fibers, independent of whether the cuff is manipulated during the surgery.

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References:

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