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Complete removal of load is detrimental to rotator cuff healing

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Hypothesis: This study evaluated the effect of the mechanical environment on the healing rotator cuff by paralyzing the supraspinatus muscle in the operative shoulder of a rat model of rotator cuff injury and repair.

Methods: Unilateral shoulders of rats underwent a supraspinatus injury and repair. Botulinum toxin A was used to paralyze the muscle after repair. Postoperatively, 1 group was immobilized and 1 group was allowed free range of motion. Saline-injected, casted rats were used as the control group. Repairs were evaluated histologically, geometrically, and biomechanically.

Results: Specimens from the saline-injected rats had greater scar volume and cross-sectional area of the repair compared with the paralyzed groups. Structural properties were increased in the saline group compared with the paralyzed groups. Free range of motion (ie, uncasted group) resulted in modest improvements in biomechanical properties but did not obviate the effect of paralysis.

Conclusions: Complete removal of load was detrimental to rotator cuff healing, especially when combined with immobilization.

Level of evidence: Basic science study.

Keywords: Shoulder; rotator cuff; mechanobiology; tendon biomechanics; force

Rotator cuff repair, which typically requires tendon-to-bone healing, is one of the most commonly performed orthopedic operations. Rotator cuff tears are seen in approximately 30% of the population aged older than 65 years.[22] Given that rotator cuff disease is age-related, the number of tears are anticipated to continue to increase as the population ages. Recent research has demonstrated that a surprising number of rotator cuff repairs do not heal.[2,6,8,13–15] Repair site healing is characterized by poor-quality reparative scar, which remains of poor quality even at long postoperative time points.[7] The healing process is influenced by a number of environmental, biologic, and mechanical factors. In particular, the role of the mechanical environment, as influenced by postoperative rehabilitation, significantly affects rotator cuff healing.[1]

Increased force is beneficial to healing in a number of musculoskeletal tissues; for example, increased compression improves bone fracture healing,[20] and early mobilization improves anterior cruciate ligament healing.[21] Similarly, early range of motion improves flexor tendon

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healing.[9] Static stress has also been shown to be beneficial to medial collateral ligament healing.[12] In contrast to these results, exercise after supraspinatus injury and repair in a rodent model resulted in increased quantity but poorer-quality tissue compared with cast immobilization.[24] It was unclear, however, whether cast immobilization was beneficial due to a decrease in force or to a decrease in motion. In addition, control of the postoperative environment using cast or sling immobilization is difficult to apply clinically and relies on patient compliance for effectiveness. Optimizing the mechanical environment after rotator cuff repair would have important clinical implications and could potentially improve outcomes after rotator cuff repair.

Given these prior results, we undertook this study to evaluate the effect of muscle force and shoulder motion on healing at the rotator cuff insertion site. Specifically we addressed the research questions:

1. Can the benefits of cast immobilization be reproduced by paralyzing the repaired rotator cuff muscle and leaving the shoulder uncasted?
2. Does complete removal of muscle force and motion after injury and repair (through cast immobilization and muscle paralysis) delay tendon-to-bone healing?

We hypothesized that muscle paralysis without casting would result in healing comparable with casting alone, while complete removal of muscle force and motion through cast immobilization and muscle paralysis would delay healing, as demonstrated histologically and biomechanically.

Materials and methods

Animal injury model

All animal procedures were approved by the Institutional Animal Studies Committee. The supraspinatus tendons in unilateral shoulders of 108 adult male Sprague-Dawley rats weighing 400 to 450 grams were transected and repaired to the humeral head, as described previously.[7,23] Briefly, animals were anesthetized using isoflurane with an oxygen carrier by nose cone. After sterile preparation and draping, a 2-cm vertical incision was made over the scapulohumeral joint to expose the shoulder.

The deltoid was removed from the cranial and lateral aspects of the acromion using electrocautery. The supraspinatus was exposed by supination of the forearm. A No. 11 blade was used to transect the supraspinatus tendon insertion from the bone. A 0.5-mm drill hole was created transversely in a cranial−caudal orientation through the proximal humerus. Fibrocartilage was removed from the insertion site on the bone using a 0.0625-inch burr. The tendon was grasped using a double-armed 5-0 Prolene suture (Ethicon, Somerville, NJ) with the use of a technique similar to a Mason-Allen method. The suture was passed through the drill hole and tied reapproximating the distal supraspinatus tendon to its anatomic position on the humeral head.

The rats were divided into 3 groups. The first group (Botox/casted) received a botulinum toxin A (Botox, Allergan, Irvine, CA) injection into the supraspinatus muscle belly after repair. A single intramuscular injection (9 U/kg) was applied to the ipsilateral supraspinatus muscle in 36 rats. This dose and method of delivery was chosen based on our pilot studies and reports in the literature.[3] These rats were immobilized in a cast postoperatively to preclude use of that upper extremity.

In a second group of 36 rats, an equal volume of saline was injected into the operative supraspinatus muscle (saline/casted group). This group was also immobilized in a cast and served as the control group, because our previous study showed that immobilization resulted in the best healing response compared with cage activity and exercise.[24]

In a third group of 36 rats, the same dose of botulinum toxin A was injected into the operative supraspinatus muscle, but no cast was applied (Botox group).

The contralateral shoulders were left untreated in all 3 groups. The rats were assigned to the three groups by means of systematic allocation. The sequence was frequently changed such that the surgeon (L. M. G.) was blinded to the group at the time of surgery.

Before skin closure, bupivacaine (2 mL) was injected into the surrounding soft tissues (eg, the subcutaneous skin, the deltoid and trapezius, the clavicular area) to provide a postoperative shoulder block.

Postoperative animal care was administered by an animal care technician. Rats were monitored for discomfort, distress, and pain. Animals that exhibited signs of distress, such as dyspnea or a decrease in body weight, were administered buprenorphine (0.01 to 0.05 mg/kg) subcutaneously every 12 hours.

Sixty rats were euthanized at 21 and 56 days after repair for biomechanical testing (10 per group). The rest were euthanized at 7, 14, 21, and 56 days for histologic analysis (4 per group). The time points were chosen to examine the proliferative (7 and 14 days) and the remodeling (21 and 56 days) phases of healing, as described in our previous reports.[7,23,24] There were no repair failures in any of the groups.

Histology-based assays

Four specimens were obtained from each group for scar volume and histologic analysis. Scar volume was measured using scans of the insertion site obtained with a cone beam microcomputed tomography scanner (Scanco Micro CT 40, Scanco Medical AG, Bassersdorf, Switzerland). Specimens were suspended in air within a plastic vial that provided a fully noncontact procedure for measurement of volume. Scar volume was determined from the start of the insertion to 3 mm proximal to the insertion. After measurement of scar volume, specimens were processed for histology.

The muscle tendon unit with its attachment to bone was processed using standard techniques. Specimens were fixed overnight in 4% paraformaldehyde and decalcified in 14% ethylenediaminetetraacetic acid. Specimens were embedded in paraffin, sectioned at 5 μm, and dried for 1 hour at 60°C. Sections were stained with toluidine blue to examine fibrocartilage formation, hematoxylin and eosin to examine cell morphology, Masson trichrome to examine fibrous tissue, and Picrosirius red to examine collagen organization. A pathologist (N. H.), blinded to group, evaluated the tissue sections for inflammation, fibrocartilage formation, vascular proliferation, fibrosis, collagen organization, and fibroblast proliferation.

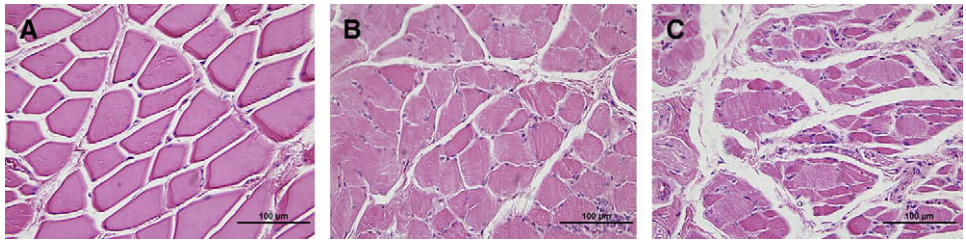

Figure 1 The supraspinatus muscle of the **(A)** saline/casted group shows only minimal atrophic changes, whereas that of the **(B)** Botox group shows a diffuse decrease of myofiber size and enlarged, pale nuclei, evidencing marked atrophic changes. **(C)** The myofibers of the Botox/casted group show more severe changes, such as a varying degree of advanced atrophy and degeneration (eg, fibril disruption, phagocytosis, etc). The perimysial connective tissue and the gap between the fascicles were increased.

Geometry and biomechanics

Ten specimens from each group were used for biomechanical testing, as previously described.[7,24] The tendon and humerus of each specimen were dissected free from overlying soft tissues. The deltoid muscle and acromion were removed. The supraspinatus was dissected subperiosteally from the supraspinatus fossa. The distal humerus was excised. Insertion site thickness was measured using a laser displacement sensor (Keyence LK-081, Woodcliff Lake, NJ). Width was measured using optical methods. The cross-sectional area was determined by assuming an elliptical cross-section. In this calculation, the width was used as the major axis of the ellipse and the thickness was used as the minor axis of the ellipse (ie, area $= \pi \times$ thickness $\times$ width).

The humerus was embedded in the testing apparatus with the use of polymethylmethacrylate. Testing was performed with the shoulder at 90° of abduction in a materials testing machine (Model 8841; Instron, Norwood, MA). The supraspinatus muscle was removed, and the tendon was clamped at its proximal end between 2 pieces of sandpaper. The sandpaper-tendon was clamped vertically in a soft-tissue clamp.

To ensure that the all samples began at the same load state (ie, at a consistently defined ''zero load'') we first performed a preload to 0.2 N. The preload was followed with 5 cycles of preconditioning at 5% grip-to-grip strain at a rate of 0.1%/s to define a consistent load history for each sample. A stress relaxation experiment was then performed consisting of a ramp to 5% strain at a fast rate of 100%/s and then relaxation for 5 minutes. A constant strain rate experiment consisting of a constant strain rate (0.1%/s to ensure quasistatic testing conditions) test to failure was then performed. Stress was calculated as tensile force divided by the initial cross-sectional area. Strain was determined optically by tracking stain lines. From these experiments we determined ultimate stress, tangent modulus (slope of the linear portion of the stress-strain curve), ultimate force, and stiffness (slope of the linear portion of the load-deformation curve).

Quasilinear viscoelastic properties were calculated from the stress relaxation testing, as described previously.[5,17,24] The nonlinear elastic behavior is described by parameters A and B. The time-varying relaxation characteristics are documented by the viscous parameters C, describing the total relaxation; τ_1, describing the early relaxation time constant, and τ_2, the late relaxation time constant. This model provides a detailed analysis of the viscoelastic tissue behavior.

Statistical analysis

Groups were compared using a 2-factor analysis of variance for time and treatment, followed by a Fisher least-squares differences post hoc test. The level of significance was set at $P < .05$. Histology-based results were qualitative in nature and were not statistically compared.

Results

Histology

Gross observations of the botulinum toxin injected shoulders demonstrated atrophy of the rotator cuff musculature. This was supported by histologic findings of atrophy at 14, 21, and 56 days (Figure 1). Muscle atrophy persisted in all botulinum toxin A injected shoulders through 56 days. Overall, specimens from the Botox group showed delayed healing compared with the Botox/casted and the saline/casted specimens. At 7 and 14 days, all specimens exhibited a disorganized tendon-to-bone interface (Figure 2). At 21 days, specimens from the Botox/casted and saline/casted groups started to become more organized, whereas the Botox group maintained a disorganized appearance (Figure 2). By 56 days, there were no major differences between any of the groups (Figure 2).

Osteoblastic and osteoclastic activity was also noted. At the early time points, all specimens exhibited increased activity of both osteoblasts and osteoclasts. The only difference between groups was seen at 56 days: The casted groups showed no osteoblast and osteoclast activity, whereas activity persisted at low levels in the Botox group. None of the groups at any time point exhibited polymorphonuclear leukocytes. High levels of mononuclear proliferation (histiocytes, lymphocytes, and plasma cells) and vascular angiogenesis were seen at early time points and decreased over time. No differences were seen between groups. Similarly, fibroblast proliferation and matrix deposition was highest at 7 and 14 days and gradually decreased to lower levels at 56 days. Fibroblast proliferation and matrix deposition was slightly higher in the Botox

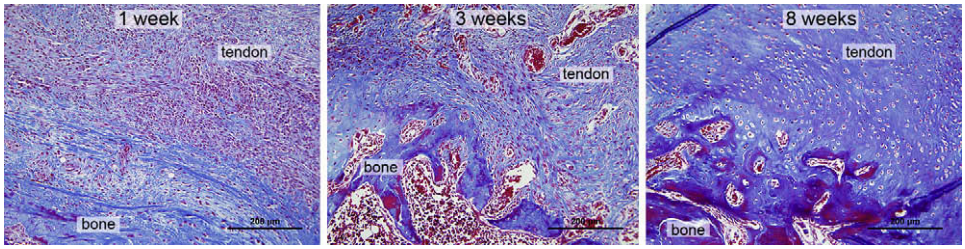

Figure 2 Fibrous and fibrocartilaginous organization at the interface between the healing tendon and bone improved over time. Inflammation and fibroblast proliferation was highest at the early time points and decreased over time. There were no differences between groups. The 1-week and 3-week photomicrographs were taken from the Botox group specimens and the 8-week micrograph was taken from the Botox/casted group (original magnification ×20; Masson Trichrome).

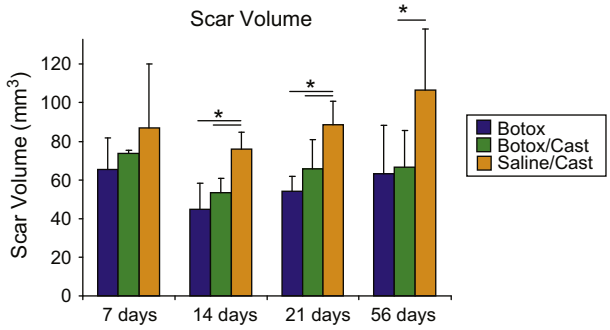

Figure 3 The saline/casted group had significantly greater scar volume at 14, 21, and 56 days compared with the Botox and Botox/casted groups (*$P < .05$). Data are presented with the standard deviation (*error bars*).

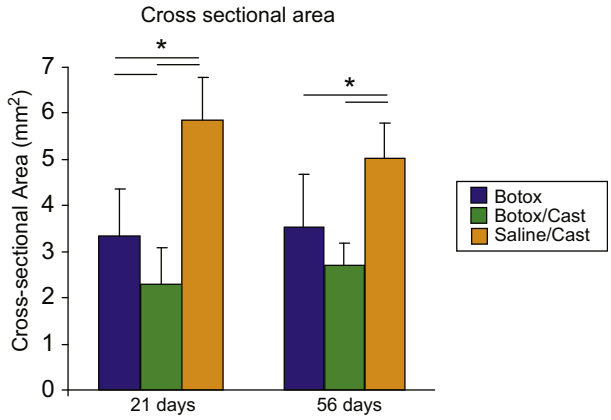

Figure 4 The cross-sectional area was greater in the saline/casted group at 21 and 56 days compared with the Botox and Botox/casted groups (*$P < .05$). Data are presented with the standard deviation (*error bars*).

group at 56 days, again demonstrating a slight delay in healing relative to the casted groups.

Geometry and biomechanics

The scar volume of the repaired tendon insertion was significantly higher in the saline/casted group compared with the Botox/casted and the Botox groups at 14, 21, and 56 days (Figure 3). The cross-sectional area of the healing insertion at 21 and 56 days was significantly higher in the saline/casted group compared with the other 2 groups. At 21 days, the Botox alone group had a higher cross-sectional area compared with the Botox/casted group (Figure 4).

Biomechanical testing demonstrated that the structural properties of the repaired tendons were significantly higher in the saline/casted group than in the Botox/casted group (Figure 5). Ultimate load was significantly higher in the saline/casted group compared with both Botox groups at 21 and 56 days. Ultimate load was significantly higher in the Botox group than in the Botox/casted group at 56 days. Stiffness was significantly higher in the saline/casted group compared with the Botox/casted group at 56 days.

Material properties, specifically ultimate stress and tangent modulus, showed no statistically significant differences when the Botox, Botox/casted, and saline/casted groups were compared (Figure 6). Quasilinear viscoelastic parameters A and B (representing nonlinear elastic behavior) were improved in the Botox/casted group compared with the saline/casted group at 21 days, but this difference was not seen at 56 days (Figure 7). Viscous parameter C and the early relaxation constant τ_1 were significantly higher in the Botox group than in the Botox/casted group at 56 days. The early relaxation constant τ_1 decreased over time in the Botox/casted and saline/casted groups. There were no differences between groups for the late relaxation constant τ_2 (Figure 8).

Discussion

Completely removing mechanical load from a healing rotator cuff insertion site is detrimental to healing. In this study we paralyzed the supraspinatus muscle with botulinum toxin A after supraspinatus injury and repair. Some of the shoulders were immobilized after repair to limit shoulder motion, whereas other rats were allowed free cage activity. Allowing free range of motion after paralysis did not obviate the effect of paralysis, but it did improve healing compared with paralysis and immobilization.

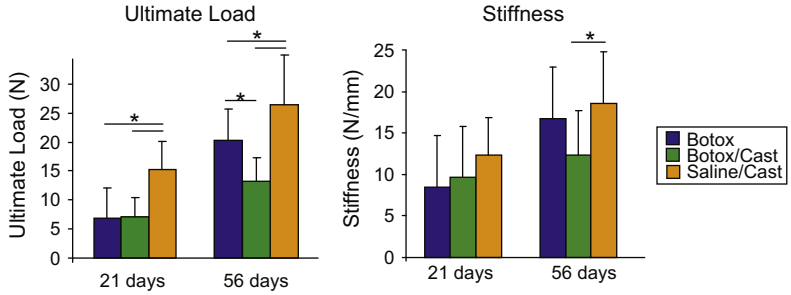

Figure 5 The ultimate load was higher in the saline/casted group at 21 and 56 days compared with the Botox and Botox/casted groups. Load in the Botox alone group was significantly higher than the Botox/casted group at 56 days. The saline/casted group was stiffer at 56 days compared with the Botox/casted group (*$P < .05$). Data are presented with the standard deviation (*error bars*).

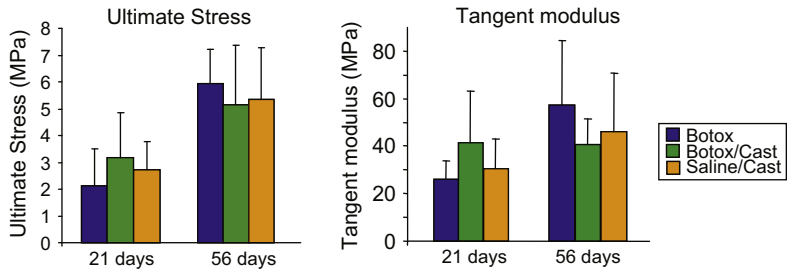

Figure 6 There were no differences in material properties between groups. Data are presented with the standard deviation (*error bars*). *QLV,* Quasilinear viscoelastic.

Cross-sectional area and scar volume was decreased in the paralyzed specimens. In addition, the structural properties of the tissue were decreased due to paralysis. The Botox group, however, had a significantly higher ultimate load compared with the Botox/casted group at the later time point. Material properties were not different when comparing the 3 groups, indicating that regardless of the mechanical environment, poor-quality scar tissue was generated. The highest structural properties were seen in the saline/casted group, but this was due to a greater quantity of tissue rather than to better quality tissue.

There was some indication that viscous (ie, time varying) properties were improved in the paralyzed uncasted repairs compared with the paralyzed casted repairs. As indicated by a higher time constant and a higher value for parameter C, paralyzed uncasted repairs were able to hold stress for longer durations than paralyzed casted repairs. This implies a slower stress relaxation rate and a better ability to sustain and transfer loads. Differences between groups, however, were not seen for the long-term relaxation constant.

Recent studies demonstrate that the failure of anatomic rotator cuff repair is a challenging clinical problem.[2,6,8,13–15] Factors affecting healing are partly environmental and biologic, but the mechanical environment also has significant influence. In most instances, the failure mechanism after rotator cuff repair is tendon pull out through the suture. Paralysis of the rotator cuff to minimize tendon pull out, therefore, is a compelling clinical option. This study, however, shows that a low level of controlled force is necessary for better healing.

Musculoskeletal tissues respond to stress, both in normal homeostatic conditions and in injured or healing conditions. Gomez et al[12] showed that increased static stress was beneficial to the properties of the healing medial collateral ligament. Increased cyclic stress, on the other hand, was detrimental to medial collateral ligament healing.[16] Therefore, although some force is beneficial to healing, excessive motion at the repair site may be harmful. Increased passive motion applied to flexor tendon injuries was beneficial to healing in a canine model.[9] The benefits in that model, however, are likely due to a decrease in adhesion formation and are not necessarily due to improvements in the properties of the healing tissue.

Some studies have looked specifically at the biomechanics of rotator cuff healing. One study that compared cast immobilization to cage activity and exercise found immobilization resulted in the strongest repairs.[24] In this study, no repair was completely unloaded because there was likely contraction of the supraspinatus providing some force across the repair site during the healing in the casted animals. The lower properties in specimens from the cage activity and exercise groups compared with the immobilization group may have been due to increased motion at the insertion or increased load across the repair site. Our study examined the role of these 2 factors by combining cast immobilization with muscle paralysis.

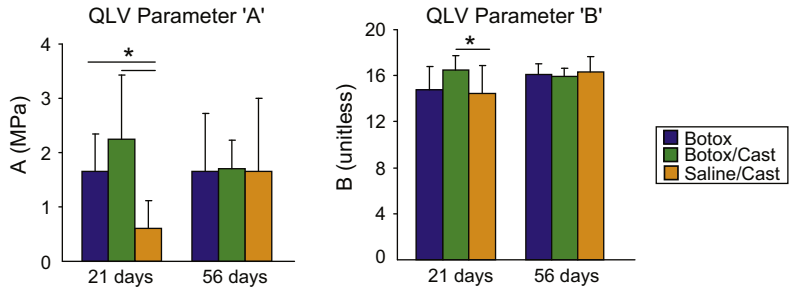

Figure 7 Elastic behavior was improved in the Botox/casted group at 21 days compared with the Botox and saline/casted groups (*$P <$ .05). Data are presented with the standard deviation (*error bars*). *QLV,* Quasilinear viscoelastic.

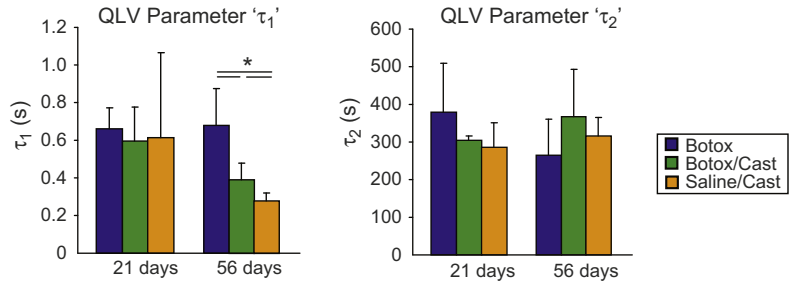

Figure 8 The early relaxation constant τ_1 decreased over time in the casted groups compared with the Botox alone group (*$P <$ .05). There were no changes in the late relaxation constant τ_2. Data are presented with the standard deviation (*error bars*). *QLV,* Quasilinear viscoelastic.

Other studies also looked at the effects of repair tension on healing.[10,11] Increased tension on repair was linearly related to changes in geometric and biomechanical properties of the repair. The cross-sectional area of the repair and stiffness of the healing tissue increased as the tension increased. Failure load and stress were negatively correlated with tension on the repair. Taken together, these studies suggest that tension force on the repair can stimulate tissue formation, but ultimately, may be detrimental to the healing properties of the repair. That study differed from ours in that it evaluated chronic tears; however, they similarly found that some force is beneficial.

Interestingly, after paralysis of the supraspinatus in our study, the uncasted group showed some improvement in mechanical properties compared with the paralyzed/casted group. The ultimate load was significantly higher in the Botox group compared with the Botox/casted group at 56 days. This indicates that paralysis coupled with free shoulder motion provided some stimulus for healing. Although motion did not completely obviate the effect of paralysis, it did provide some benefit. Contrary to the biomechanical results, the Botox alone group showed delayed healing histologically compared with the other 2 groups. It is possible that the Botox alone group would demonstrate even better healing at later time points compared with the casted groups because the healing insertion site continued to remodel. Decreasing the dose of botulinum toxin A to decrease but not eliminate muscle contraction is a future consideration for further optimizing the response.

Histologic observation in our study showed that the supraspinatus muscles injected with botulinum toxin had marked atrophy compared with those of the saline/casted group, which is consistent with other studies[3,4,18] (Figure 1). Only slight atrophic changes were noted in the supraspinatus muscle of the saline/casted group. The muscles in the Botox group showed diffuse atrophy of myofibers and enlarged, pale nuclei in hematoxylin and eosin stain. The muscles in the Botox/casted group showed more severe changes, which included varying degrees of atrophy and degeneration of myofibers, increased endomysial and perimysial connective tissue, and increased gap between the fascicles. This finding is more consistent with a degenerative muscle process rather than simple neurogenic atrophy. These findings suggest that chemical denervation combined with external immobilization may cause harmful effects on the muscle. Future work will attempt to optimize the botulinum toxin dosage and paralysis duration to achieve the benefits of repair site unloading without the disadvantage of muscle atrophy.

A strength of our study is the use of an established rodent model for rotator cuff injury and repair, which makes our findings particularly relevant to rotator cuff repair. The use of cast immobilization to optimize the healing environment has also been demonstrated previously.[24]

A limitation of our study is that this is a model of acute injury and repair. Most tears treated in humans involve chronic degeneration, followed by tendon tearing.[19] Thus, the biologic environment in our model is different from that most commonly seen clinically. However, an acute tear is more likely to heal than a chronic tear[7]; thus, the effects of paralysis may be underestimated in our model.

In summary, paralysis of the supraspinatus after injury and repair is detrimental to healing when combined with cast immobilization. Our findings suggest that a low level of controlled force is beneficial for healing. These findings have clinical relevance in terms of a postoperative rehabilitation program. Some controlled paralysis of the supraspinatus in combination with early range of motion may decrease the risk of tendon pull off while still allowing stimulus for healing.

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