EXPERIMENTAL STUDY

Acta Orthop Traumatol Turc 2015;49(4):447–452
doi: 10.3944/AOTT.2015.14.0296

Effect of intramuscular botulinum toxin-A in a rat rotator cuff repair model: an experimental study

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Objective: Rotator cuff repair is associated with multiple complications, significant morbidity, and re-intervention, which could be mitigated by postoperative chemodeneveration with botulinum toxin-A (BTX-A). This study evaluated the antinociceptive and paralytic effects of BTX-A on an experimental supraspinatus repair rat model and its effect on functional outcomes (running performance).

Methods: Thirty rats were grouped into the surgical repair group (group A), repair + intramuscular BTX-A group (group B), or control group (group C). At the end of the 3-month follow-up, running performance of the rats on a motorized treadmill was evaluated in four time periods (0–30 min, 30–60 min, 60–90 min, and 90–110 min), and penalty points (i.e., number of shock stimuli per lane) were recorded. Afterwards, the supraspinatus muscles were removed and evaluated histologically.

Results: Regarding running performance, group B received significantly fewer penalty points than did group A ($p<0.05$). The penalty points received were not significantly different between groups B and C in the first three time periods, but were significantly higher in group B at the 90–110-min interval than in group C. On necropsy, all repaired tendons were intact, with no sign of failure at the repair site. Histological evaluation revealed marked degeneration and necrosis of muscles in both repair groups, which was much less evident in group B. Groups A and B had less fatty infiltration than group C.

Conclusion: BTX-A injections resulted in a better function based on running performance, probably due to decreased tissue tension at the repair site and less pain. Further studies on humans are needed to demonstrate this effect clinically.

Keywords: Animal study, antinociceptive effect; botulinum toxin A (BTX-A); rotator cuff injury; shoulder rehabilitation.

Rotator cuff tear commonly affects the middle aged and elderly populations. It usually requires surgical treatment and causes significant morbidity.[1] Postoperative healing is influenced by many factors,[2] and the most common postoperative complications are re-tear and shoulder stiffness, which have been associated with significant morbidity and re-intervention. Postoperative rehabilitation is important for improving functional

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Submitted: August 21, 2014 **Accepted:** January 03, 2015

Available online at
www.aott.org.tr
doi: 10.3944/AOTT.2015.14.0296
QR (Quick Response) Code

outcomes. Controlled motion at the repair site is essential for optimal tendon healing without adhesion.[3] However, excessive force generated during rehabilitation or co-contractions secondary to pain may cause repair failure.[4] Furthermore, meta-analyses have revealed that early postoperative rehabilitation (versus delayed rehabilitation) does not have significantly positive, long-term functional outcomes;[5,6] this indicates that an optimal rehabilitation protocol is yet to be established.

Botulinum toxin type A (BTX-A) is a neurotoxin that blocks acetylcholine exocytosis at the neuromuscular junction, resulting in a chemical denervation (chemodenervation) of the muscle. BTX-A produces selective muscle weakness when injected intramuscularly. The paralytic effect starts 2–5 days after administration and lasts for 2–3 months.[7] This property is used for symptomatic relief related to spasticity.[8] Major areas of use of BTX-A are gait problems and joint contractures in cerebral palsy, cervical dystonia, hemifacial spasm, focal spasticity, hyperhidrosis, ophthalmological and otolaryngeal disorders, as well as some cosmetic conditions. BTX-A also inhibits substance P, which is a potent neurotransmitter involved in the activation of inflammation. It also inhibits the release of acetylcholine, substance P, calcitonin gene-related peptide, vasoactive intestinal peptide, and neuropeptide Y. Botulinum toxin decreases the release of these peptides and eventually causes pain relief.[9] Botulinum toxin injections may also reduce pain associated with excessive muscle contraction and related disorders, given its independent antinociceptive effect.[10]

Beneficial effects of BTX-A following tendon repair were well reported previously on rat and rabbit Achilles tendon repair models, without use of an immobilization tool[11,12]—for example, tendon unloading by chemodenervation resulted in a reduction of the muscle force responsible for repair failure and subsequently better rupture resistance at follow-up.[11]

In this study, we investigated the paralytic and antinociceptive effects of BTX-A after the repair of an iatrogenically lacerated supraspinatus tendon and its beneficial role, both functional and histological, on tendon healing using a supraspinatus repair rat model.

Materials and methods

All animal procedures were performed at Ankara University Animal Laboratory under the approval and monitoring of the Institutional Animal Studies and Ethics Committee of Ankara University. Thirty Wistar rats (age, 12–14 weeks; mean weight, 232 g) were divided in three groups: the surgical repair group (group A), the repair + BTX-A injection group (group B), and the control group (group C; normal rats without any intervention that served as the baseline for functional and histological assessments).

After standard anesthesia and preparation, a long lateral incision was made on the left shoulder girdle. The subacromial space was exposed by retracting the fibers of the deltoid muscle. An iatrogenic, transverse, full-thickness laceration of the supraspinatus tendon was made at its insertion on the humerus using a no. 11 surgical blade. A 0.5-mm K-wire was used to make a hole on the lateral aspect of tuberculum majus. A transosseous fixation was performed by passing a 5.0 prolene suture from the humeral tunnel. The suture was passed from the supraspinatus tendon and tied. After repair, 9 U/kg of BTX-A[8] was injected intramuscularly into the repaired muscle belly of group B rats. Standard care was given to all subjects with no restriction of motion.

At the end of the 3-month follow-up, running performance was evaluated on a motorized treadmill (connected to a computer), enabling the coordination of speed, slope, and aversive stimuli by a computer (Manufacturer: Ankara University Mechanical Engineering) (Figure 1). There were four separated lanes, each of which had an electric grid at the beginning and a fan at the end. The fans were used for heat dissipation and to keep the rats running toward the end. All rats were familiarized with the treadmill for 5 min before each experiment. Four rats could run at the same time for each selected group (Figure 2). A low dose of electrical foot shock as an aversive stimulus was used to keep the rats running. This shock stimulus for every lane was recorded by the computer as a penalty point (1 point per shock). The evaluation program was executed as follows: 5 days

Fig. 1. Computerized and motorized treadmill with rats running. Asterisk (*) shows shock grid, to keep rats running and to record penalty points. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 1. Histopathological evaluation scale.

Histopathological changes in muscle	Score
No histopathological change	0
<10% of changes in ten consecutive high power fields	1
10–50% of changes in ten consecutive high power fields	2
>50% of changes in ten consecutive high power fields	3

Changes refers to the following types of changes: Degeneration and necrosis, capillarization, and regeneration.

of adaptation exercise, 3 days of rest, and 1 day of final running.[13] During the exercise adaptation period, rats showing good running performance were enrolled in real exercise protocols. Rats that received too many penalties, exerted resistance to walking and sniff behavior, and avoided running by remaining somewhere between the wall of the lanes and the electric grid to avoid the shock were excluded from the study (one from each group). On the final run, the penalty points earned by each rat were noted for the following time periods: 0–30 min, 31–60 min, 61–90 min, and 91–110 min.

The rats were euthanized, and the supraspinatus muscles, along with the corresponding attachments to the bone, were resected. After the standard preparation, the sections were stained with hematoxylin and eosin to examine the cell morphology, degeneration, necrosis, capillarization, and fatty infiltration. A histopathological scoring system was used, assigning a score from 0 to 3 based on the extent of the following types of changes: degeneration and necrosis, capillarization, and regeneration (Table 1).

Results regarding physiological tests were analyzed using SPSS 19.0 software. The normal distribution of values was examined by the Shapiro–Wilk test. Descriptive statistics of penalty points were represented as median (range). Comparisons between groups were made by the Kruskal–Wallis test, and comparisons between two groups were made by Mann–Whitney U-test. Histological scores were also analyzed by the Kruskal–Wallis test. A p value of <0.05 was accepted as statistically significant in all comparisons.

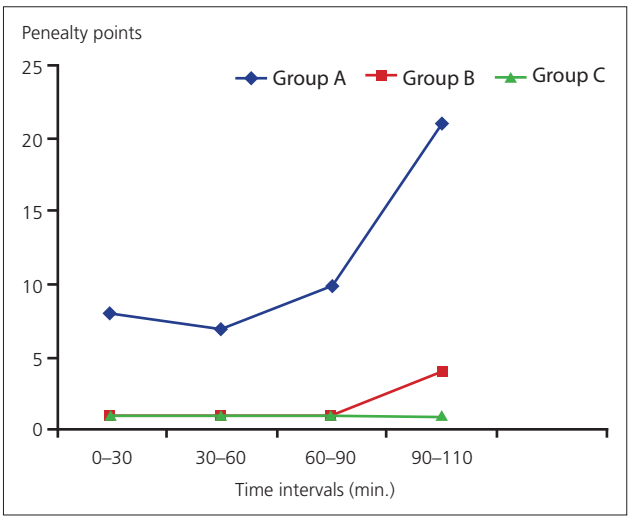

Fig. 2. Graph showing distribution of penalty points. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Results

The penalty points earned in each time period are summarized in Table 2. Rats in group A received significantly more penalty points than the rats in the other groups in all time periods. The median penalty points earned by the rats in groups B and C were similar in first three time periods, but were significantly higher in group B in the 90–110-min interval than in group C (Figure 3a).

A total of 30 specimens were obtained from the rats in all groups for histological analysis. In group A, we observed degeneration and cell necrosis more often than in group B and less capillarization compared to the other groups (Figure 3b). We also observed fatty infiltration in group A (Figure 3c). In group B, we observed primarily regenerative changes, and in some focal areas, degeneration and necrosis of muscle cells (Figure 3d). No marked histological change was observed in group C. Histopathological grading scores are summarized in Table 3. The difference between group A and B was significant; likewise, the difference between both treatment groups and control were also significant ($p<0.05$).

Table 2. Penalty points of groups in each time interval.

		Penalty points gained (median)			
		0–30 min	**31–60 min**	**61–90 min**	**90–110 min**
Group A	n=9	8 (7–10)	7 (7–8)	10 (10–11)	21 (21–22)
Group B	n=9	1 (0–1)	1 (0–1)	1 (0–2)	4 (4–7)
Group C	n=9	1 (0–1)	1 (0–1)	1 (0–2)	1 (1–2)
p-value		**<0.001***	**<0.001***	**<0.001***	**<0.001***

*Statistically significant.

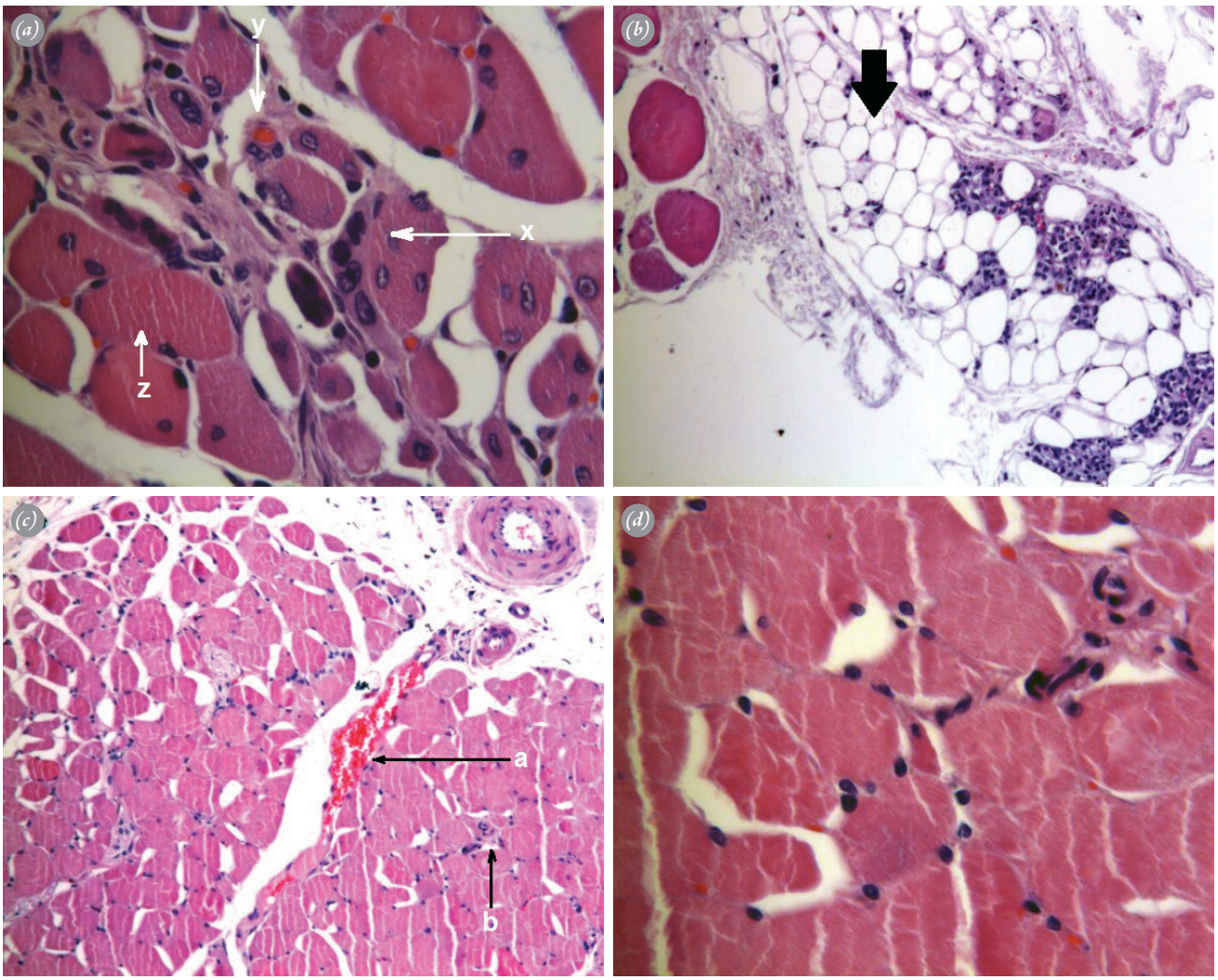

Fig. 3. **(a)** Group A; degeneration and necrosis (x) with minimal vascularization (y). Normal tissue (z). **(b)** Group A; arrow shows diffuse fatty tissue between necrotic muscle. **(c)** Group B; regenerative changes as early angiogenesis (a), with focal areas of necrosis and degeneration (b). **(d)** Normal muscle tissue in group C. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Discussion

Shoulder rehabilitation following rotator cuff tear surgery is undertaken in 4 phases. Phase I corresponds to the first 4–8 weeks (depending on the quality of the repair and comorbidities) and requires protection of the repair site with gentle mobilization of the joint during the inflammatory and repair periods. Phase II starts after 4–8 weeks with active assisted range of motion exercises during the remodeling phase. Phase III exercises start after 8–12 weeks following repair and focus on the initial strengthening of the repaired muscle. Finally, phase IV exercises begin at 12–16 weeks following repair for intense and advanced strengthening of tissue to promote return to competitive activity.[14] BTX-A injection acts mainly on rehabilitation phases I and II and partially on phase III. The predicted effects can be either beneficial or not.

During phase I, the effect of BTX-A injection following rotator cuff repair is two-fold. It decreases the muscle tone, thereby reducing the resistance to the repair method,[15] and it decreases pain and inflammation by direct inhibition of substance P.[16,17] Both these effects have been demonstrated in human studies.[15,17]

During the remodeling phase (corresponding to phases II–IV), active muscle contraction, without failure of the repair, is the main objective. In this phase, collagen reorientation requires stress on the repair site, and total muscle paralysis can have some potential drawbacks; however, controversial results have been reported. It was shown, both microscopically and biomechanically, that complete unloading after supraspinatus repair by chemodenervation with BTX and immobilization by cast, had a detrimental effect on tendon healing. Conversely,

Table 3. Results of histopathological evaluation scores.

		Histopathological scores (mean±standard deviation)		
		Degeneration and necrosis	**Capillarization**	**Regeneration**
Group A	n=10	1.60±0.96	1.00±0.94	0.70±0.94
Group B	n=10	0.50±0.70	0.80±0.78	0.70±1.05
Group C	n=10	0.00	0.00	0.00
p-value		**<0.001***	**0.005***	**0.047***

*Statistically significant.

that same study also concluded that a certain level of controlled paralysis of the supraspinatus together with early motion may decrease repair failure, allowing sufficient tensile stimulus to promote healing.[18] Another experimental study reported that increased tension following repair was related to a decrease in failure properties and viscoelastic peak stress and an increase in tissue area and stiffness.[19] It was concluded that this effect may be due to increased tissue damage and aggravation of the scar tissue caused by excessive tension, thereby leading to the suggestion that repair tension should be minimized.[19] In another study, BTX-A led to improved collagen organization at the repair site at week 4, but also led to diffuse atrophy of the muscle at week 8, with no difference at week 24.[20] The studies described above have some common features: all of them were performed on rat models and all reported histological and biomechanical results. Although these studies reported confusing results, they all concluded that a decreased repair tension is necessary to minimize repair failure.

In our study, we evaluated the effect of BTX-A on function following cuff repair based on the running performance of rats at the end of tendon healing and remodeling periods by a standardized objective tool. We consider this method as the strength of this study. Our study showed that a better outcome was accomplished both histologically and functionally by the BTX-A injection. The fatty infiltration observed in group A (surgical repair without BTX-A injection) was not observed in group B (surgical repair with BTX-A injection). Also, group B showed a better running performance than group A and similar to the running performance observed in the control group (group C) (Figure 2). This may be attributed to either better tissue healing with tendon unloading or the antinociceptive effect on the muscle, which caused a diminished spasm of the repaired muscle and decreased functional capacity. The injection dosage and technique were similar as in previously reported studies. Since we did not use any method of immobilization postoperatively, the shoulder range of motion was not restricted. This active shoulder motion may have also contributed to the favorable outcome that we have obtained by preventing stiffness.

Although widely used, significant concerns are present regarding BTX-A use. One concern is the structural changes that can take place in immature muscle tissue (juvenile rats), such as structural changes in the myosin heavy chain (MHC) content and structure, together with decrease in the titin content (a structural protein of the sarcomere).[21] We used mature rats because this method is clinically intended for adults and elderly patients, so we consider that this effect was minor. Another concern is the lack of strengthening during phases III and IV of rehabilitation, which may overlap the paralysis period. This effect was not encountered in our study, but it should be investigated in detail. In the clinical setting, most of cuff tears are degenerative or delayed traumatic, and the clinical outcome of delayed strengthening by BTX-A is unknown. Theoretically, this can be a serious issue if the traumatic cuff tear affects a performance athlete for instance. Therefore, further investigations are necessary to clarify this point.

Our study methodology may be affected by some confounders. The first is the time of onset of the BTX-A paralysis, which begins at approximately 1–2 weeks following injection and lasts for about 3–6 months in rats.[22] Thus, during the inflammatory phase of tendon healing, tendon unloading was not evident in our study. Previous studies claim that early loading during the inflammatory phase may improve tendon healing,[23] but we did not encounter any case of repair failure. Therefore, we did not accept this effect as a concern. Another limitation is the model that we used. Athough the rat supraspinatus repair model is a commonly used model for cuff tear surgery experiments, its main drawback is the lack of demonstration of a chronic long standing tear with tendon degeneration and retraction, which is the usual scenario in the clinical setting. Our experimental model is of a fresh and iatrogenic rotator cuff laceration, which does not represent ordinary, chronic, retracted rotator cuff tear. Additional long-term experimental studies after complete resolution of muscle paralysis,

with physiological evaluation of the repaired muscle, are necessary.

Despite these concerns, our study reveals promising functional outcomes. Clinical studies with long-term follow-up and careful patient selection are necessary to determine the suitability of this approach for routine clinical use.

The antinociceptive and paralytic effects of BTX-A allowed the achievement of better functional outcomes by means of improved running capacity in a rat rotator cuff repair model. Further experimental studies with chronic tear models and long-term follow-up are needed before this rotator cuff tear treatment can be researched in humans.

Conflics of Interest: No conflicts declared.

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