

## Original Article

# Effects of botulinum toxin type A on healing of injured skeletal muscles

Ramin A. Shokravi, Mohammad Moshref, Behnam Eslami, Farhad Aghmasheh

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Islamic Azad University, Tehran, Iran

**Address for correspondence:** Dr. Farhad Aghmasheh, No. 15, Resalat Street, North Naderi Ave., Qazvin, Iran.

E-mail: farhadaghamasheh@hotmail.com

### ABSTRACT

**Objectives:** (1) Evaluation of microscopic healing of skeletal muscle fibers after injuries, especially the arrangement of new muscle fibers and scar tissue diameter in the injury region. (2) Evaluation of alterations in microscopy of the healing procedure within skeletal muscles after injury following botulinum toxin type A (BTX -A) induced muscle immobilization. **Materials and Methods:** The study was done on 12 white lab rabbits of either sex in a 6-month period. **Results:** The immobilization of skeletal muscle fibers as a result of the use of BTX-A after injury caused a qualitative increase in fibrous tissue formation in the area of injury, and the BTX-A-induced immobilization for a period of 6 months led to muscle atrophy.

### KEY WORDS

Botulinum toxin, skeletal muscles, wound healing

Skeletal muscles, which form a large volume of body mass, play a great role in body movements. Furthermore, because of the support offered to the superficial structures, they are important to the quality and appearance of these structures, especially the face. In recent years, many studies have been done to improve healing mechanisms in this important part of the body.

Although the healing of human skeletal muscle that occurs after acute trauma has not been extensively studied, the available evidence shows that muscle healing, like healing of the other vascularised tissues, proceeds through inflammation, repair and remodelling. At the same time that the myofibres regenerate, fibroblasts produce the factors that are necessary to repair the intercellular matrix of the muscle. However, the intercellular matrix rebuilding can interfere with the orderly regeneration of the myofibres, and this may be the cause of the disorganized mass of scar and partially regenerated

myofibres seen microscopically. This type of tissue may restore the continuity of the muscle but may not restore contractile function.<sup>[1]</sup>

The present study aims at finding new methods to reduce formation of this unfavourable fibrous tissue within skeletal muscle after experimental injury. We temporarily immobilized the experimentally injured muscle using BTX-A, thereby eliminating tension, and histologically evaluated the effect of eliminating tension in the healing area during the healing process.

### MATERIALS AND METHODS

Twelve white rabbits of either sex, weighing between 1.8 and 2.5 kg, were caged individually and maintained on standard rabbit chow, cabbage supplement, bread and water as needed. They were divided into two groups (main and control), each consisting of six rabbits (three

male and three female). In both groups, the gastrocnemius muscle was selected for surgery (three muscles in the right leg and three in the left leg in each group). Seventy-six hours before the surgery, in the main group, 50 MU (Mouse Unit) of Dysport™ (botulinum toxin type A)<sup>[2]</sup> was injected into skeletal muscle using a 1-ml insulin syringe (15 MU in each head and 20 MU in the muscle belly); so on the surgery day, the selected muscle was immobilized by Dysport™ injection.

Three days later, surgical anaesthesia (20-30 min), good relaxation and a sleep time between 60 and 120 min were made available under ketamine (25 mg/kg IM) and xylazine (5 mg/kg IM) injections in the opposite leg. Then the region was washed with liquid soap and povidone-iodine 10% and shaved by a sharp blade in a symmetrical 4 cm<sup>2</sup> area. Then, full-thickness skin was incised through a vertical incision with a no. 15 blade and the skeletal muscle exposed. After that, the belly of the muscle was horizontally divided into two equal parts. These two parts were then sutured with four separate 4-0 resorbable chromic gut sutures. The overlying fascia was sutured separately with four interrupted resorbable sutures. Finally, the skin was sutured continuously with 4-0 silk.

During surgery, bleeding was controlled by fastening a tourniquet at the most upper part of the leg. After surgery, the area was washed with povidone-iodine 10% and a mixture of gentamicin and spectinomycin. The ear on the same side was punched for marking, and the area was sprayed with chloramphenicol spray. The leg wound was dressed with cotton balls and reinforced with gauze sponge and circumferential wraps of cotton bandage. All the above-mentioned stages except for the Dysport™ injection were similar in the main and control

groups. At each daily dressing change, wound area was irrigated with povidone-iodine 10%, and 0.3-0.5 ml of a mixture of spectinomycin and gentamicin was injected intramuscularly for 5 days. After 7 days, skin sutures were removed and rabbits maintained together in a large cage for 5 months. After 1 month of BTX-A effects disappearing - assessed by reappearance of movement of the gastrocnemius muscle - rabbits were sacrificed by injection of a minimum of 4 ml thiopentone 5% IV and, immediately, en bloc biopsy from surgical area was performed. The biopsy specimens were transferred to formalin 10% for fixation; and after 3 days, ten 5- $\mu$ m cross sections from each sample were prepared and stained with haematoxylin-eosin (H and E). Samples were evaluated by the pathologist with Nikon Fuji  $\times$  HC300zi and calibrated in Photoshop 6 software. The results are displayed in the result table.

## RESULTS

Inflammation was observed in the surgical area in 66.7% of specimen in both main and control groups even after 5 months. In 50% of samples, the inflammation was mild and chronic. In one out of six cases of each group, an acute focal inflammatory area was found far from the incision line [Figure 1]. Further studies showed that it existed because of teeth-biting irritation.

In all samples (12 cases), fibrous tissue formation was seen. The average diameter of fibrous tissue in the main group was  $99.17 \pm 3.87 \mu\text{m}$ ; and for the control group, the average diameter was  $69.5 \pm 1.78 \mu\text{m}$ . These results indicate that a 6-month immobilization period of a healing incision in skeletal muscle may lead to an increase in fibrous tissue formation in the incision line;

**Table 1: Result table**

001	M	2500 gr	20-25 $\mu\text{m}$	+	√	105 $\mu\text{m}$	Regular	+
002	F	1900 gr	20-35 $\mu\text{m}$	+++	√	100 $\mu\text{m}$	Regular	+
003	M	2100 gr	20-35 $\mu\text{m}$	+	√	102 $\mu\text{m}$	Irregular	+
004	F	2000 gr	35-40 $\mu\text{m}$	+	√	95 $\mu\text{m}$	Irregular	+
005	M	1800 gr	40-45 $\mu\text{m}$	-	√	96 $\mu\text{m}$	Regular	+
006	F	1950 gr	45-50 $\mu\text{m}$	+	√	97 $\mu\text{m}$	Irregular	-
007	M	1850 gr	45-50 $\mu\text{m}$	+	√	67 $\mu\text{m}$	Irregular	-
008	F	2000 gr	30-65 $\mu\text{m}$	++	√	72 $\mu\text{m}$	Irregular	-
009	F	2400 gr	20-30 $\mu\text{m}$	+	√	70 $\mu\text{m}$	Regular	-
010	M	2300 gr	20-35 $\mu\text{m}$	+++	√	69 $\mu\text{m}$	Irregular	-
011	F	2450 gr	45-50 $\mu\text{m}$	-	√	71 $\mu\text{m}$	Regular	-
012	M	1850 gr	20-45 $\mu\text{m}$	+	√	68 $\mu\text{m}$	Regular	-

Inflammation degrees: + - mild [up to 10 inflammatory cells in high power field view (400 $\times$ )]; ++ - moderate [up to 50 inflammatory cells in high power field view (400 $\times$ )]; +++ - severe [more than 50 inflammatory cells in high power field view (400 $\times$ )]; -- - less than 10 inflammatory cells in high power field view (400 $\times$ )

(001 to 006): Main group (with Dysport™ injection)

(007 to 0012): Control group

similar to findings of Jarvinen *et al.*<sup>[7]</sup> alluded to in the subsequent discussion.

The arrangement of collagen fibres in both groups was equally regular or irregular [Figures 2, 3].

In 83.3% (five of six cases) of main group, muscle atrophy and muscle fiber border degeneration after 6 months - i.e. after 1 month of remobilization - was seen, but none of the control group muscles showed atrophy [Figure 4].

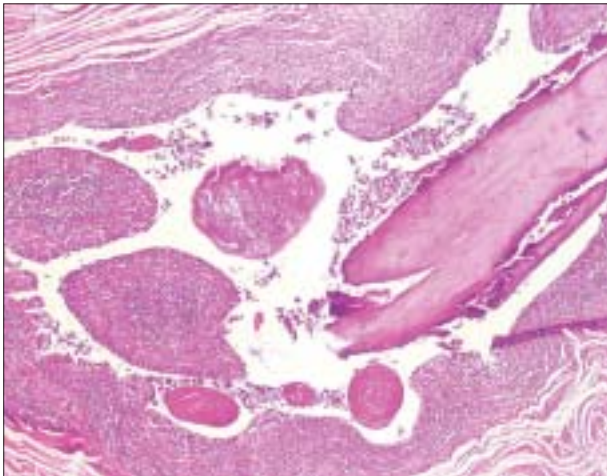
## DISCUSSION

There have been several reports in recent literature to suggest that immediate injection of botulinum toxin type A into the muscles underlying a wound can improve the cosmetic outcomes of cutaneous scars. After injection into the underlying muscle, BTX-A paralyzes the muscles and relieves tension in the wound area; it is therefore

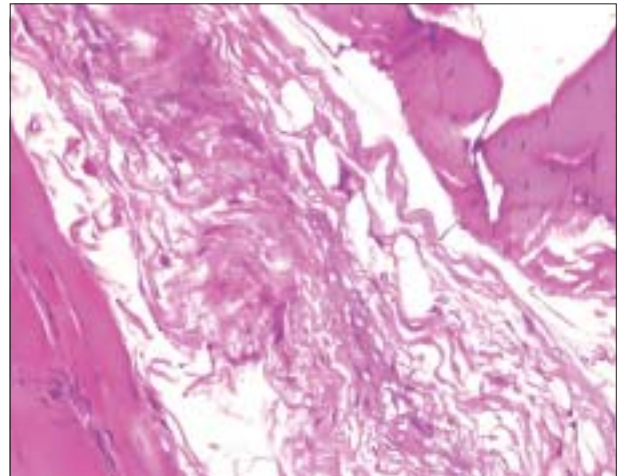
hypothesized that a more aesthetically appealing scar can be achieved.<sup>[3,4]</sup>

Using primate models, Gassner *et al.* have shown that surgical wounds that had been immobilized with botulinum toxin were rated as significantly better in appearance than the control wounds.<sup>[5]</sup> But can this BTX-induced reduction in tension be similarly effective in skeletal muscle wound healing?

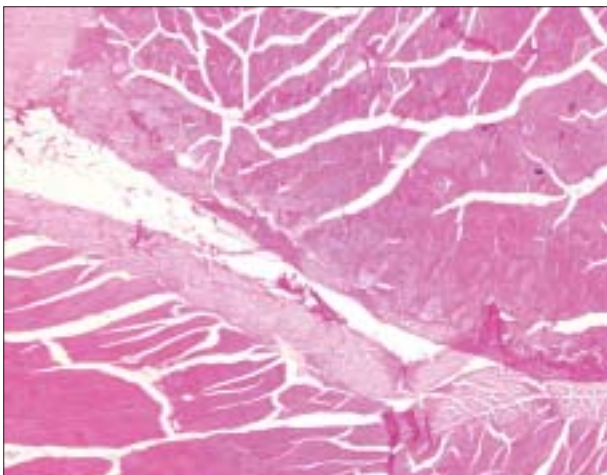
Jarvinen *et al.* have shown that immobilization following injury limits the size of the connective tissue area formed within the site of injury; immobilization for longer than 1 week is followed by marked atrophy of the injured muscle. Mobilization started immediately after injury is followed by a dense scar formation in the injury area, prohibiting muscle regeneration. When mobilization is started after a short period of immobilization, a better penetration of muscle fibre through the connective tissue is found.<sup>[6,7]</sup>



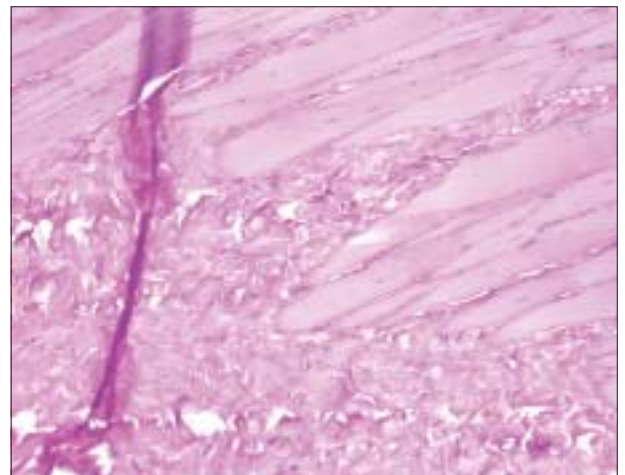
**Figure 1:** Note local calcifications included with mixed inflammatory tissue



**Figure 3:** Collagen fibers irregularities



**Figure 2:** Note regular fibrous band in incision line



**Figure 4:** Fibrous tissue penetration between muscle fibers and fiber atrophy

The study by Ansved *et al.* showed that the use of BTX-A in cervical dystonia treatment led to muscle atrophy in type 2B fibres in the muscles of legs.<sup>[8]</sup> They report that this atrophy is temporary and reversible. Also, Wyndaele and Van Dromme report muscular weakness as a side effect of BTX-A injection, but they emphasize on reversibility of this kind of muscle atrophy.<sup>[9]</sup>

We have demonstrated that an approximately 6-month-long period of immobilization of injured skeletal muscle fibres can lead to qualitative increase in scar tissue formation in healed skeletal muscle wounds, which may be functionally unfavourable. Further quantitative studies are needed to confirm the statistical significance of these findings.

Furthermore, our research indicates that muscle fibre atrophy after botulinum toxin type A injection is not rare, which seems to be a definite finding. Therefore, we do not, at present, recommend injection of this type of botulinum toxin preparation to improve healing of injured muscle, as induction of prolonged immobilization may lead to the production of an unfavourable scar in the healing area, associated with atrophic changes in the immobilized muscle.

## ACKNOWLEDGMENTS

The authors acknowledge gratefully the technical and scientific

assistance of Dr. Saeed Aghmasheh, Doctor of Veterinary Medicine, of the team.

## REFERENCES

1. Rockwood C, Charles A, Green DP. Fractures in adults. Lippincott-Reven: Philadelphia, USA; 1996. p. 293-304.
2. Brin MF. Botulinum toxin: Chemistry, pharmacology, toxicity and immunology. *Muscle Nerve Suppl* 1997;6:S146-68.
3. Wieder JM, Mory RL. Understanding botulinum toxin, surgical anatomy of the frown, forehead and periorcular Region. *Dermatol Surg* 1998;24:1172-4.
4. Sherris DA, Gassner HG. Botulinum toxin to minimize facial scarring. *Facial Plast Surg* 2002;18:35-9.
5. Gassner HG, Sherris DA, Otley CC. Treatment of facial wounds with botulinum toxin A improves cosmetic outcomes in primates. *Plast Reconstr Surg* 2000;105:1948-55.
6. Jarvinen MJ, Lehto MU. The effect of early mobilization and immobilization on the healing process following Muscle injuries. *Sports Med* 1993;15:78-89.
7. Jarvinen M, Sorvani T. Healing of a crush injury in rat striated muscle. 1. Description and testing of a new method of inducing a standard injury to the calf muscles. *Acta Pathol Microbiol Scand A* 1975;83:259-65.
8. Ansved T, Odergren T, Borg K. Muscle fiber atrophy in leg muscles after botulinum toxin type A treatment of cervical dystonia. *Neurology* 1997;48:1440-2.
9. Wyndaele JJ, Van Dromme SA. Muscular weakness as a side effect of Botulinum toxin injection for neurogenic detrusor over activity. *Spinal Cord* 2002;40:599-600.

**Source of Support:** Nil, **Conflict of Interest:** None declared.