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

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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.[1]

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

Original Article

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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) 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 cm² 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 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 main group was 99.17 ± 3.87 µm; and for the control group, the average diameter was 69.5 ± 1.78 µm. 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;

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 ± 3.87 µm; and for the control group, the average diameter was 69.5 ± 1.78 µm. 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

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Inflammation degrees: + - mild [up to 10 inflammatory cells in high power field view (400×)]; ++ - moderate [up to 50 inflammatory cells in high power field view (400×)]; +++ - severe [more than 50 inflammatory cells in high power field view (400×)]; -- less than 10 inflammatory cells in high power field view (400×)
similar to findings of Jarvinen et al.[7] 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 paralyses the muscles and relieves tension in the wound area; it is therefore hypothesized that a more aesthetically appealing scar can be achieved.[3,4]

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.[5] 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.[6,7]
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. 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.[8]

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.

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