Iranian Veterinary Surgery Association

Iranian Journal of Veterinary Surgery

Journal homepage: www.ivsajournals.com

Original Article

Effect of Botulinum Toxin A - Lidocaine on Experimental Abdominal Wall Defect Healing in Rats

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ARTICLE INFO

Article History:

Received 1 January 2022
Revised 6 March 2022
Accepted 16 March 2022
Online 16 March 2022

Keywords:

Botulinum Toxin A
Abdominal wall defect
Healing
Rat

ABSTRACT

The aim of this study was to investigate the effects of Botulinum Toxin A (Botox) and lidocaine on the repair of abdominal muscle defects. This study was conducted on 40 rats in four groups. Animals during experimental abdominal wall defect creation and before closing the defect in the Botox group, 5 units of Botox, in the lidocaine group 1 ml of 1% lidocaine, in the Botox-lidocaine group 10 units of Botox with lidocaine with a final volume of 1 cc and in the control group received normal saline in a volume of 1 ml. Each group was divided into two subgroups. Each subgroup animal was sacrificed on days 6 and 12, respectively, after surgery, and after taking a sample, it was examined macroscopically and microscopically. In macroscopic evaluation on both days 6 and 12 after surgery, the highest muscle tension (stretching of the muscles in the nipple area and a significant reduction in abdominal volume) belonged to the normal saline group and the lowest to the Botox and Botox-lidocaine groups. In microscopy on day 6, in normal saline and lidocaine groups, a wider area of granulation tissue and a large number of inflammatory cells were observed, and in the Botox and Botox-lidocaine groups, a less limited amount of granulation tissue was observed. On day 12, in the control and lidocaine groups, a large level of granulation tissue and fibroblasts was observed, while in the Botox and Botox-lidocaine groups, a very small amount of connective tissue was seen at the incision site. Animals receiving normal saline had significantly more inflammation than the groups receiving Botox and Botox lidocaine. According to the results, it seems that topical administration of Botox-lidocaine combination can be a good solution to reduce suture tension and prevent rupture of abdominal sutures.

Introduction

An abnormal protrusion of an organ, such as the intestine or part of an organ, from a defect or normal opening outside its normal location is called a hernia.[1] Abdominal hernias are mainly secondary to trauma (such as horns and amlidents or bites); however, they are more often congenital like umbilical hernias.[2] Most abdominal hernias (muscle defects) can be treated by suturing torn abdominal muscles. In rare cases,

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www.ivsajournals.com© Iranian Journal of Veterinary Surgery, 2022
https://doi.org/10.30500/IVSA.2022.322517.1291

artificial muscles are used to repair defects that do not allow the two sides of the muscle or peritoneum to meet.[2-4] The biggest and most common challenge of closing hernias is tearing or reopening the hernia area. This is due to the pressure exerted by the viscera and the sutures being stretched.[4] Stress and tension on the site also delay wound healing.[5,6] Thus far, several methods have been proposed, such as the use of different suture patterns, Botox, and the use of local anesthetics in the incision line to reduce the likelihood of hernia opening.[4] Previously, wound strength from the midline laparotomy in four different suture patterns in rats has been evaluated. Based on the results, no significant correlation was observed between single, simple-overall, simple and universal double-loop suture patterns.[6] In a study botulinum toxin type A was injected into the lateral wall of the abdomen one month before surgery, making the wound easier to close.[7] In another human study, the role of botulinum toxin A in the management of abdominal hernias was investigated. In this study, at least two weeks before surgery, Botox was injected into the muscles of the lateral abdominal wall, indicating good effects on reducing tension on the abdominal muscles.[8] In a study using botulinum toxin A, it was found that the use of this toxin, four weeks before abdominal hernia repair surgeries, reduced traction on the defective site due to paralysis of lateral abdominal muscles.[9] Botox injections two weeks before surgery in humans also indicated that the use of Botox two weeks before surgery was a safe process causing paralysis, relaxation and elongation of the muscle, which may be a useful aid in reconstructive surgery.

Chen *et al*. and Farooque *et al*. examined the effect of Botox on W-Plasty deep scars and showed that the use of botulinum toxin A in the cutting line reduced traction.[10,11] The effects of botulinum toxin A on wound healing and facial scarring and cleft palate repair have also been studied, demonstrating the effectiveness of this drug by relaxing muscles and reducing traction on the wound edges. In human, the effect of pre-surgery Botox injection (7-14 days) on improving the defect closure process and laparoscopic abdominal hernia surgery has also been evaluated. In this study, Botox was injected into a predetermined area of the abdominal muscle before surgery and was found to increase the muscle length.[12] Lien *et al*. examined the effect of Botox on the abdominal wall muscle contraction compared to normal saline in a shear hernia model in rats.[13] In this study, the hernias was seen in both groups, but it was significantly lower in the group receiving Botox.[13] By injecting Botox into the lateral muscle of the human abdomen, the effect of botulinum toxin A as a supplement on abdominal wall regeneration in hernia repair has been investigated. In this study, CT scan showed that Botox injection before surgery increased the muscle length and possibly made it easier to close the defect.[14] Botox was used to repair a hernia in a 19-year-old girl and was clinically effective in reducing the hernia recurrence.[15,16]

Researchers have studied the effect of diluted Botox on epinephrine-containing lidocaine on skin rejuvenation. Given that lidocaine causes an immediate muscle relaxation and epinephrine causes vascular contraction and long-term absorption of the drug from the site, this assumption is likely to cause a longer lasting effect and provide better performance of Botox.[17]

Gassner *et al*. showed that addition of a local anesthetic to botox enhanced the effects of botulinum toxin type A.[18] Botox causes muscle paralysis and muscle relaxation by blocking nerves. The lidocaine addition to botulinum toxin type A casued to rapid paralyze of the injected muscle, so that the muscle relaxation was faster and better compared to the Botox diluted in normal saline.[18] Considering the challenge of reopening the hernia area or rupture of the abdominal wall after surgery in humans and particularly heavy animals on the one hand and the need to provide practical solutions on the other hand, this study aimed to investigate the effect of botulinum toxin type A (Botox) alone or in combination with lidocaine on repairing the experimental defect of abdominal wall muscles in a rat model.

Materials and Methods

Animals

In this study, 40 adults male Wistar rats with an average weight of 250-300 g from the animal house of the Faculty of Veterinary Medicine of Shahid Chamran University of Ahvaz were used. The temperature of the animal housing was adjusted to 23 ± 3° C with standard humidity. The animals were exposed to 12 hours of light and 12 hours of darkness. This cycle of darkness and light was regulated by an automatic system. During this period, the rats were fed with a special food for rats in the form of compressed capsules prepared from the same animal house. The individual cages were thoroughly washed and disinfected with alcohol before surgery. An independent 120 ml drinking bowl was

provided for each cage. All surgeries were performed by one person at a specific time of day. The animals were divided into four equal groups as follows: Group 1: (S) Saline injection; Group 2 (B): Botox injection; Group 3 (L): Lidocaine injection; Group 4 (BL): Botox and lidocaine combination injection.

Surgery

On the day of the experiment, all animals were anesthetized after being transferred to the operating room by intraperitoneal administration of a combination of 10 mg/kg xylazine 2% (Alfasan, Woerden, The Netherlands) and 100 mg/kg ketamine 10% (Alfasan, Woerden, The Netherlands). After being transferred to the operating table, the abdominal area was clipped and prepared routinly for aseptic surgery. Then, a sharp incision was made on the skin in front of and behind the umbilicus scar. After dissecting the subcutaneous tissues and making an incision in the white line of the abdomen, a 3x2 cm full thickness strip of ventral abdominal muscles is removed So that it includes 1 cm on each side of the midline of the abdomen. Then, the experimental defect was sutured using a 3-0 sized absorbable suture with a Mayo-Mattress pattern. Then, each group received appropriate treatment. Finally, the skin was sutured using a 2-0 sized nylone suture with a simple interrupted pattern.

Injection Considerations

Group 1: Saline with a final volume of one ml and in equal amounts in marginal deficient muscles. Group 2: Botox (Dysport , Ipsen Biopharm Limited, UK) (10 units per patient) with a final volume of one ml and an equal amount created in the marginal muscle of the defect13 Group 3: 1% lidocaine with a final volume of one ml and in equal amounts in at the edge of the remaining muscles. Group 4: a combination of Botox (10 units per patient) and lidocaine 1% in equal proportions, with a final volume of one ml and in equal amounts in the marginal muscle of the defect.13 Ketoprofen (5 mg/kg, subcutaneously) and enrofloxacin (10 mg/kg, subcutaneously) were administered for three days.

Histopathologic Examination

Tissue sampling (blocked ressection of skin and muscles) was performed on the 6th and 12th days (five rats in each time) after surgery under euthanasia with over-dose of anesthetic. The samples were placed in a container containing 10% formalin buffer. Then, in the pathology laboratory, the collected samples were routinely prepared and stained with hematoxylin and eosin and Masson Trichromeand then were examined microscopically. In a microscopic examination, various repair factors, such as granulation tissue formation, new vascular formation, presence of inflammatory cells, and location of muscle fibers, were evaluated semi-quantitatively. Qualitative analysis was performed in such a way that to evaluate epithelization (formation of new epithelium, welding of the repair site) if completely conducted with +++ with part of it uncoated with ++, the epithelialization process was started, but the surface uncovered range was rated with +.

To evaluate the inflammatory process if it has a large level of inflammation (more than 75%) with +++, if it has a moderate level of inflammation (25% to 75%) with ++, if it has a small level (less than 25%) with + scored. Finally, after collecting data, the SPSS software version 26 (IBM Corporation, NY, USA) was used to review the data. One-way analysis of variance with a Tukey post-test was used to compare the data obtained from the study groups. Analysis of variance with reproducibility with an LSD post-test was also used for differences between the groups. The results were presented as mean ± standard deviation and values of $p < 0.05$ were considered significant.

Results

Macroscopic Examination

In the observations of the animals on the 6th day after surgery, the highest muscle tension (muscle stretch and significant reduction in abdominal volume) belonged to the normal saline group, and the lowest belonged to Botox and Botox lidocaine groups. This trend was also observed in the observation of animals on day 12 after surgery. On the 6th day after surgery, a short length of the skin suture was teared in one of the normal saline animals due to excessive traction.

Microscopic Examination:
Qualitative Evaluation

On microscopic examination, skin sections on day six exhibited the formation of granulation tissue at the incision site, around the muscles, and suture sections. This tissue was recognizable despite a large number of fibroblasts, and blood capillaries. The amount of granulation tissue was different between different groups. In the two groups of normal saline and lidocaine, a wider area of granulation tissue was observed. There were also a large number of pink

connective fibers seen in Mason's Trichrome staining in blue (Figures 1 and 2). In the two groups of Botox and Botox-lidocaine, a limited amount of granulation tissue was observed, and the amount of collagen fibers produced was lower (Figures 3 and 4). In the groups of normal saline and lidocaine, a large number of inflammatory cells were observed between the structures of granulation tissue and around muscle groups.

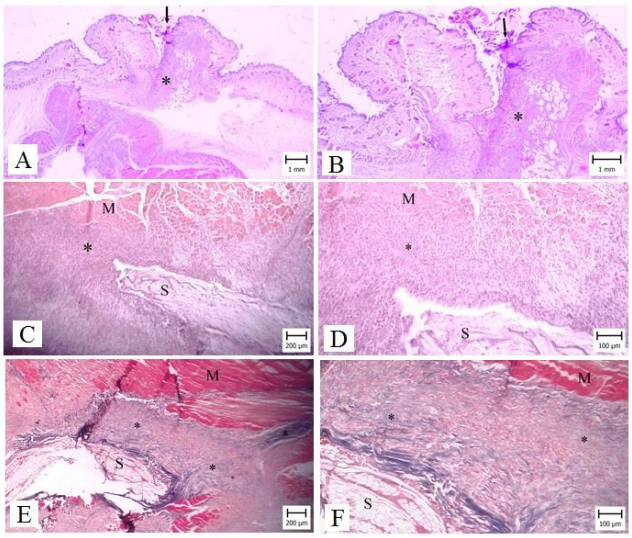

Figure 1. Normal saline group. Day 6. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the size of the granulation tissue area at the incision site and around the suture section (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

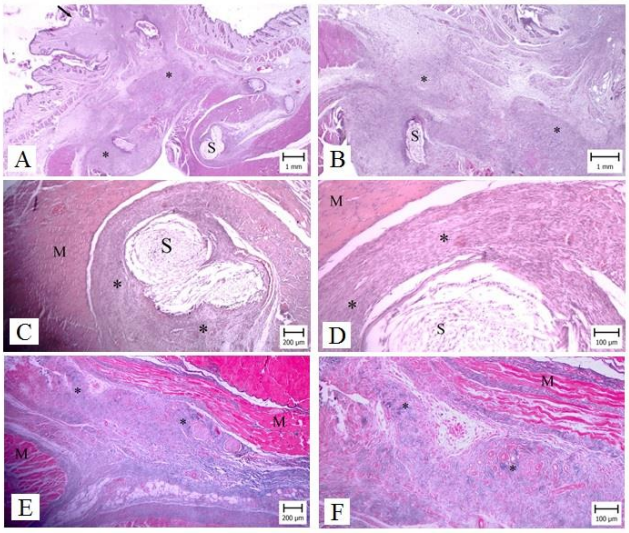

Figure 2. Lidocaine group. Day 6. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the size of the granulation tissue area at the incision site and around the suture section (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

Furthermore, a large number of giant muscle cells were observed around the muscle groups on day 12. These cells were identified by a large number of nuclei in a red cytoplasm. On the surface of the incision site, the keratinocytes in two cases from the Botox-lidocaine group, two cases from the normal saline group, and one

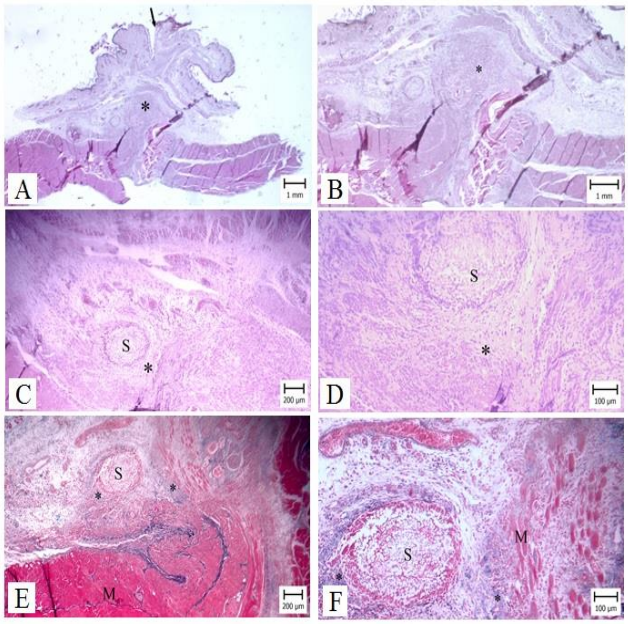

Figure 3. Botox group. Day 6. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the decreased size of the granulation tissue area at the incision site and around the suture section in comparison with Figures 1 and 2 (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

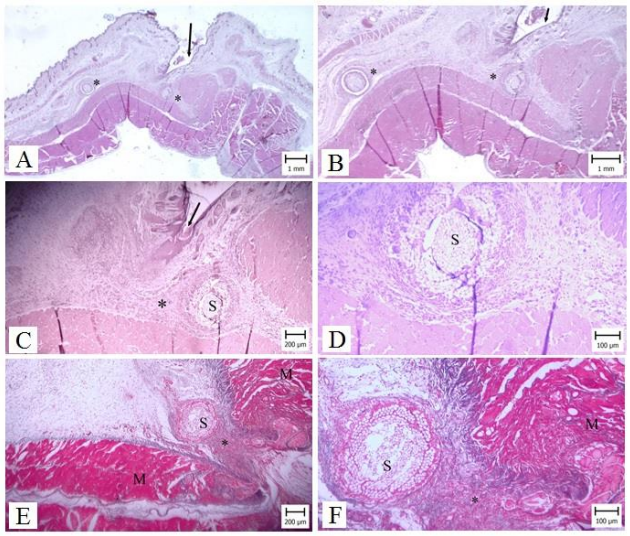

Figure 4. Botox and lidocaine group. Day 6. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the decreased size of the granulation tissue area at the incision site and around the suture section in comparison with Figures 1 and 2 (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

case from the lidocaine group were joined together and completely covered the incision surface. In other cases, part of the wound was still without epithelium. Granulomatous inflammation was observed around the suture sections, which was evident in the presence of giant cells and other mononuclear inflammatory cells. However, it should be noted that its thickness in the two groups of Botox and Botox-lidocaine was less than that of the two groups of normal saline and lidocaine. In the histopathological examination of the sections prepared on day 12, mature granulation tissue was observed in the incision line and around the muscle groups. This tissue was seen numerous fibroblasts and a small number of capillaries at the incision site. Collagen fibers were seen in Mason's Trichrome staining in blue, and the amount was different between different groups.

In control and lidocaine groups, a high level of these filaments and connective tissue was observed (Figures 5 and 6). While in the two groups of Botox and Botox-lidocaine, a very small amount of connective tissue was seen at the incision site. Evidence of muscle group regeneration was also observed. This was evident despite small diameter muscle sections (Figures 7 and 8).

Regarding epidermal repair, in five mice of Botox and lidocaine groups, one case from the Botox group, two cases from the lidocaine group and four mice from the normal saline group, the deficient level was completely covered by new keratinocytes. In other mice, a low level of defect remained that was not covered, and inflammatory scabs were still observed.

Semi-Quantitative Assessement

Inflammation: The inflammation feature at the surgical site was assessed by scoring the extent and severity of inflammation. On the 6th day after surgery, the animals in the normal saline group had significantly more inflammation than groups receiving Botox (p = 0.012) and Botox lidocaine (p = 0.005) (Table 1). The lowest rate of inflammation was observed on the 6th day after surgery (score 1) in animals receiving Botox and Botox lidocaine. In assessing the status of inflammation on the 12th day after surgery, animals receiving normal saline had significantly more inflammation than groups receiving Botox (p = 0.038) and Botox lidocaine (p = 0.008).) (Table 1). In terms of score, there was the least amount of inflammation (score 1) in all three groups of Botox, lidocaine and Botox lidocaine.

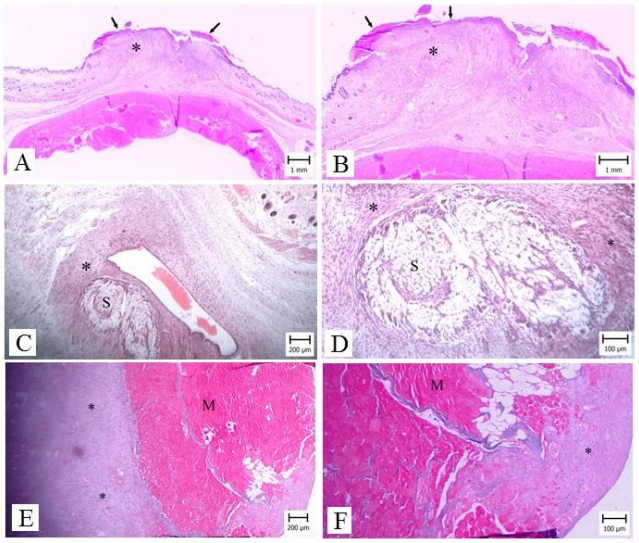

Figure 5. Normal saline group. Day 12. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the size of the granulation tissue area at the incision site and around the suture section (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

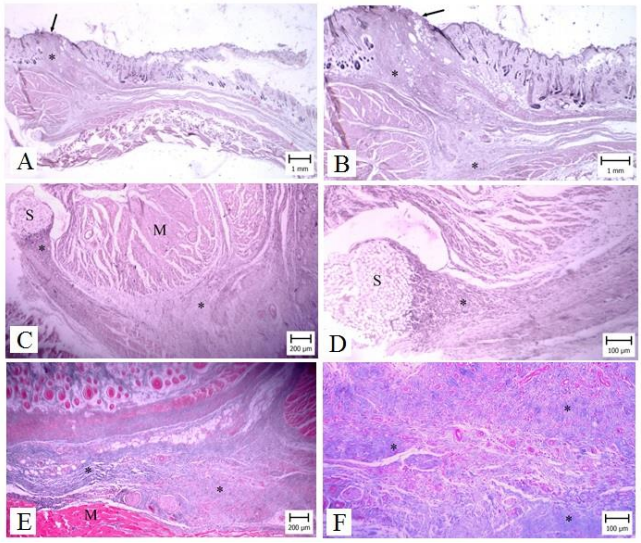

Figure 6. Lidocaine group. Day 12. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the size of the granulation tissue area at the incision site and around the suture section (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

Garanulation Tissue: The amount of granulation tissue at the surgical site was also evaluated, and the extent and severity of inflammation were measured. The lowest rate of granulation tissue formation was observed on the 6th day after surgery in the Botox lidocaine group, being significant only with the normal saline group (p = 0.010) and lidocaine (p = 0.001) (Table 1). The highest rate of granulation tissue formation was observed on the 6th day after surgery in the normal saline group, which had a significant

difference with the Botox lidocaine group (p = 0.010) (Table 1). On day 12 after surgery, the highest score of granulation tissue formation was assigned to the normal saline group, which was significant compared to Botox (p = 0.002) and Botox lidocaine (p = 0.020) groups (Table 1). The lowest mean score belonged to the Botox group, which was significant compared to normal saline (p = 0.002) and lidocaine (p = 0.020) groups (Table 1).

Epithelialization: Examination of new epithelium formation on the 6th day after surgery showed no statistically significant differences (p = 0.020). However, on the 12th day after surgery, all samples of the Botox lidocaine group received the maximum epithelialization score. At this time, the rate of epithelialization of the Botox lidocaine group was significant compared to that of the lidocaine group (p = 0.020) (Table 1).

Discussion

Treatment of abdominal wall hernias is a rapidly evolving field in surgery. Similarly, there has been a dramatic increase in the writing of articles in this field. There are many reasons for this spread: a considerable increase in the number of laparotomies, advancement of anesthesia, an increase in older patients with weak connective tissue, an increase in patients with risk factors for herniation.[19] All of the above indicate the need to use various methods to repair and prevent recurrence of abdominal defects. Owing to the challenge of reopening the hernia area or rupture of the abdominal wall muscles after surgery in humans, particularly in heavy animals, the need to provide practical solutions will be highly important. Therefore, the present study aimed to investigate the effect of botulinum toxin type A (Botox) alone or in combination with lidocaine on repairing the experimental defect of abdominal wall muscles in a rat model.

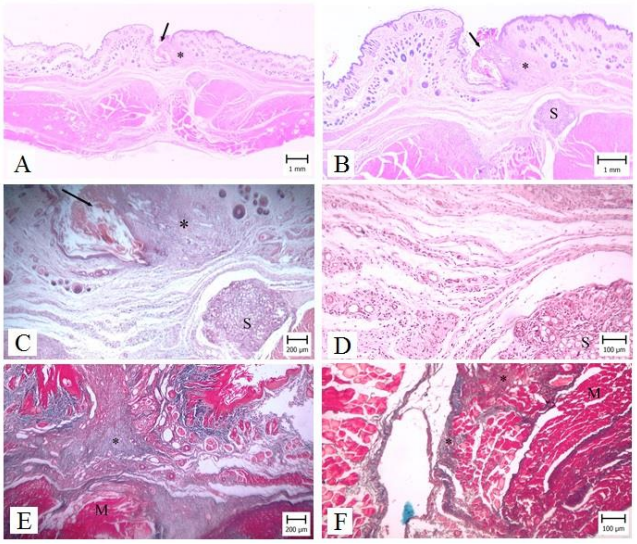

Figure 7. Botox group. Day 12. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the decreased size of the granulation tissue area at the incision site and around the suture section in comparison with Figures 5 and 6 (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

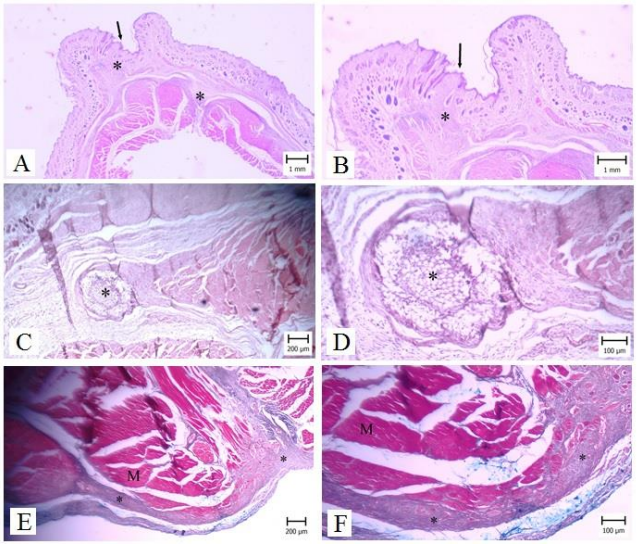

Figure 8. Botox and lidocaine group. Day 12. Cross section of the incision site. Cutting place: Arrow. Granulation tissue and collagen fibers: Asterisk. Suture section: S. Muscle fibers: M. Note the decreased size of the granulation tissue area at the incision site and around the suture section in comparison with Figures 5 and 6 (A-D: Hematoxylin and Eosin staining. E & F: Masson's Trichrome).

Thus far, several methods have been proposed, such as the use of different suture patterns, Botox, and local anesthetics in the incision line to reduce the likelihood of hernia opening or muscle defect.[4] Deerenberg *et al*. (2020) used Botox® diluted in 100 ml of normal saline in 140 human patients with large abdominal hernias using 200 units of botulinum toxin type A.[20] They distributed the prepared solution in 12 separate 10 ml syringes with 8 ml solution per syringe (containing 16 units of BTX). Capece *et al*. (2021) began a preliminary closure by studying a 33-week-old twin born with a large umbilical cord mass.[21] For this purpose, two weeks before surgery, 8 units of Botox were injected into the abdominal muscles through ultrasonography. The results showed that the use of BTA could help to repair muscle defects without the use of mesh.[21] One study conducted by Çakmak *et al*. (2006) on the effect of botulinum toxin A-induced abdominal muscle paralysis on intra-abdominal pressure showed that intra-abdominal pressure was significantly lower in the group using Botox than in the control group.[22]

Table 1. Medium (Minimum - Maximum) Scoring of Inflammation, granulation tissue formation and epithelialization at the surgical site following closure of experimental muscle defect in Rats.

Parameters	Days/Groups	Normal saline (N)	Botox (B)	Lidocaine (L)	Botox + Lidocaine (BL)
Inflammation	6	3 (3-3) BL	1 (1-3)	2 (2-3) BL	2 (1-2) N, L
	12	2 (1-2) B, BL	1 (1-2) N	1 (1-2)	1 (1-1) N
Granulation tissue	6	3 (1-3) BL	2 (1-3)	3 (2-3) BL	1 (1-2) N, L
	12	3 (2-3) B, BL	1 (1-2) N, L	2 (1-3) B	2 (1-2) N
Epithelialization	6	2 (1-3)	2 (1-2)	2 (1-3)	2 (1-3)
	12	3 (1-3)	2 (2-3)	2 (1-3) BL	3 (3-3) L

The group name at the top of each data indicates a significant difference with that group ($p \leq 0.05$).

One study conducted by Blaha *et al*. (2020) found that the use of Botox during hernia repair surgery in the abdominal wall muscles reduced the need for opioids as well as the postoperative pain.[23] El Shaer *et al*. (2019) by studying the effect of botulinum toxin A and lidocaine on the survival of skin flaps, demonstrated that the survival of flaps was higher in the group receiving Botox than in the lidocaine group.[24] In one study conducted by Lien *et al*. (2015) on three groups of rats, one group received no laparotomy, one group received normal saline in the laparotomy incision line, and one group received Botox.[13] In a study conducted on the 14th day after surgery, Hernia, adhesion and muscle tension were examined; they significantly reduced the occurrence of hernia and muscle tension in the Botox group receiving saline, being consistent with the present study results.

Lee *et al*. (2009) examined the effect of botulinum toxin A on wound healing in a rat model of wound size, degree of fibrosis and inflammation, blood vessel permeability, wound thickness and expression (TGF) -β1.[25] There was a significant difference in the wound size in the third and fourth weeks between the Botox and control groups. The Botox group showed significantly fewer inflammatory cells than the control group did, being similar to the present study results. The Botox group exhibited less fibroblasts and fibrosis than the control group did in the fourth week, being similar to the present study results on day 12. The Botox group showed a higher density of collagen than that of the control group in the eighth week, in the immunohistochemical study. They revealed that the level of (TGF) -β1 in the Botox group was lower than that in the control group in the fourth week. Lien *et al*. examined the effect of Botox on abdominal wall muscle contraction compared to normal saline in a shear hernia model in rats.[13] Researchers have studied the effect of Botox diluted in lidocaine containing epinephrine on skin rejuvenation. Given that lidocaine causes immediate muscle relaxation and epinephrine causes vascular contraction and long-term absorption of the drug from the site, it may exert a longer lasting effect and lead to better performance of Botox.[17] In the present study, the combination of Botox and lidocaine showed amleptable results. Moreover, in one study conducted by Kucukkaya *et al*. on histological examinations, in Botox injection groups, there was a significant increase in the amount of collagen as well as connective tissue cells, and the collagen structure showed fibril formation.[26] In the Botox groups, new angiogenesis was significant.

In Botox groups, the upper capillary diameter was larger. Regarding regurgitation, a statistically significant increase was observed in Botox groups. Fibroblast density was increased in Botox groups, and the difference between groups was statistically significant.[26] These results are similar to the present study results on day 12 in Botox and Botox-lidocaine groups. Gassner *et al*. showed that addition of a local anesthetic enhanced the expected effects of botulinum toxin type A.[18] Botox causes muscle paralysis and muscle relaxation by blocking nerve-damaging nerves. In this double-blind study, they added lidocaine to botulinum toxin type A to rapidly exert a paralytic effect on the muscle into which it was injected, and they used epinephrine to reduce the release of the solution into adjacent muscles. Amlordingly, it was observed that muscle relaxation was faster and better compared to the group that was injected with Botox diluted in normal saline,[18] which was also used in the present study.

In one study conducted Jalalipour *et al*. (2013) on the effect of Botox on wound healing in horses, no significant difference was observed in wound size, number of inflammatory cells and fibroblasts, being inconsistent the results of the present study on day

12.[27] However, in one study conducted by Jalalipour et *et al*. (2013) the mean number of vessels and epithelization on day 5 after surgery showed a significant increase, being in line with the results presented on day 12 for Botox and Botox-lidocaine groups.[27]

According to the present study results and clinical and histopathological evidences, it appear that local administration of Botox-lidocaine can be a good solution to reduce suture line tension and to prevent rupture of abdominal sutures. It is recommended that further empirical and case studies be conducted in this regard before clinical use of this method.

Acknowledgment

The project was approved by the local Committee of the Institutional Animal Care and Use of Shahid Chamran University of Ahvaz, Iran. The authors would like to thank members of surgery division, veterinary hospital, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.

Conflict of Interest

The authors declare no conflict of interest related to this report.

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