EFFECT OF BOTULINUM TOXIN ON SURGICAL WOUND HEALING AND SCAR FORMATION (AN EXPERIMENTAL STUDY)

Doaa A. Abd El-Gawad[1]* BDS, Nevein S. Abdallah[2] PhD,
Hanaa S. Raslan[3] PhD, Noha Y. Dessoky[4] PhD

ABSTRACT

INTRODUCTION: The process of wound healing is complicated. Despite research, hypertrophic scars still occur and can pose functional and aesthetic issues. Improvement for hypertrophic scars has been attained using a variety of treatment modalities. The strain from the underlying muscles working on the wound edge throughout the healing process is a critical factor in shaping the scar's final appearance. Since botulinum toxin type A (BTA) causes total muscle paralysis, it was suggested as a possible treatment.

OBJECTIVES: To evaluate the effect of BTA injection on the final appearance of the surgical scar.

MATERIALS AND METHODS: Thirty-six mature male New Zealand rabbits weighing 3.5–4 kg were studied experimentally (one year of age). They were divided into two groups: One received BTA injections into the cheek muscles surrounding a Y-shaped surgical incision. The other group received no further treatment after the incision. A follow-up was performed after 2, 4, and 6 weeks for the assessment of the scar parameters (wound width and Vancouver scar scale (VSS), along with clinical photographs). After each period, the sacrifice group of rabbits was done. Samples were prepared for histological and histomorphometric analysis by being dissected.

RESULTS: When compared to the control group, the BTA-treated group showed an improvement in the appearance of scars, VSS and a reduced increase in wound width. Histological and histomorphometric results indicate that the BTA group had a better layout and less collagen deposition than the control group.

CONCLUSIONS: BTA injection effectively reduced collagen fibril production and improved hypertrophic scar appearance.

KEYWORDS: Botulinum toxin, Surgical scars, Wound healing, Muscle paralysis, Rabbit model.

RUNNING TITLE: Effect of BTA on surgical scar appearance.

1-BDS, 2016, Faculty of Dentistry, Faculty of Dentistry, Alexandria University, Alexandria, Egypt

2-Professor of Oral and Maxillofacial Surgery, Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt

3-Professor of Oral Pathology, Oral Pathology Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt

4-Lecturer of Oral and Maxillofacial Surgery, Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt

Corresponding author
doaagawad91@gmail.com

INTRODUCTION

Scars on the face or neck can be especially upsetting for patients, especially a hypertrophic scar (1), which is both aesthetically and functionally undesirable (2).

It is a common and unfavorable consequence of surgical incisions (3).

Several therapy techniques have been offered for the treatment of hypertrophic scars; however, there is no effective treatment for removing scars, likely due to a lack of knowledge of the complicated mechanisms underlying the severe scarring process (4, 5).

The wound-healing process consists of four interconnected and overlapping phases. Numerous factors may affect one or more stages of this process, impairing or preventing effective wound healing (6). One of these elements is the strain that generates at the wound edges during healing (7).

The main distracting tensile force is caused by mechanical factors, such as adjacent muscle contraction and skin elasticity. Wide hypertrophic scars have been an issue, but various solutions have been proposed; however, none of them prevent the pathologic process that causes wide hypertrophic scars, which is the distracting force of muscular pull. Since BTA toxin, which is generated by Clostridium botulinum, is a powerful neurotoxin that causes muscle paralysis by blocking acetylcholine release at the neuromuscular junction, it can be applied to reduce all tensile stresses coming from a surgical wound's underlying muscles (8, 9).

The objective of the study was to investigate the usefulness of BTA using qualitative and quantitative measures to confirm its beneficial effects on facial scarring. The null hypothesis of this study that there was no change in the final cosmetic appearance seen between group that received Botulinum Toxin type A injections and the control group.

MATERIALS AND METHODS
Study design
This was an experimental study.
Materials
Experimental Animals
After receiving approval from the Research Ethics Committee of the Faculty of Dentistry at Alexandria University, the study was conducted.

Thirty-six full-grown adult white male New Zealand rabbits weighing 3.5–4 kg were used in the study (approximately one year of age). The two groups were created by random assignment according to the material used for injection.

Group A (study): received injections of BTA in the muscles around the sutured wound on the same day of surgery.

Group B (control): received no further treatment.

Botulinum Toxin Type A
Nabota botox (Daewoong Pharmaceutical Co., Ltd., Korea) was used, it is a purified botulinum toxin type A. Each vial contained 100 units (U) of type A Clostridium botulinum neurotoxin.

Methods
The experimental procedure was held in an animal lab. Rabbits were housed at 22–25 °C in a ventilated laboratory throughout the trial. Rabbits were housed in cages and fed standardized, suitable food and tap water (10).

Presurgical phase
Botox preparation
The syringes used were microfine, 1.0-ml insulin syringes with a 30-G needle. The dosages of the medications were based on biological activity, which is expressed in biological units (U). There were 100 units in the vial, that had been reconstituted with 2.0 mL of sodium chloride injection solution at 9 mg/mL (0.9%). As a result, a transparent liquid containing 100 U of the active ingredient was obtained. At a final concentration of 1 unit/0.01 ml, the reconstitution was carried out following good clinical practice. Particularly concerning is sepsis within 15 days of reconstitution (11, 12).

Anesthesia
All surgical procedures were done under general anesthesia, with 0.05 ml of xylazine hydrochloride (Xyla-ject) (ADWIA, Egypt) and 0.1 ml of ketamine hydrochloride (Aneket) (NEON Pharmaceutical, UK) administered intramuscularly for every 100 grammes of body weight (13).

Animals preparation
Before any surgery, the rabbits' cheeks were shaved, and to prevent contamination, the skin was washed and cleansed with povidone-iodine (Betadine) (El-Nile Co., Egypt) (14). And because maximal precision was mandatory, definite dimensions (2 cm in length) were drawn using a ruler to determine the outline of the incision.

Surgical phase
Using a surgical blade number 10, a y-shaped incision of 2 cm was established perpendicular to relaxed skin tension lines on the right side of the cheeks of all rabbits. Standard surgical methods were used to remove the skin and subcutaneous tissue, and the masseter muscle was maintained at the defect's base. By exerting pressure, hemostasis was achieved, and each wound was individually sutured using a multiple non-resorbable surgical silk suture (5-0; Ethicon silk suture, Johnson & Johnson, USA) in a continuous manner (Figure 1). By randomization, one group of rabbits was used as the study group (Group A), and the other group became the control group. (Group B).

Group (A) Study
Injection points were determined by a skin marker.

Using an insulin syringe with a 1-ml, 30-gauge needle, with the needle prick placed roughly 5 mm from the borders of the wound.

The mimetic musculature underlying the incision was injected under direct vision. Every point was injected with 1 unit of Nabota botox in 0.9% saline (1 unit = 1 ml), with total 7 point for each rabbit.

There were 126 units used throughout the entire study, divided into 42 units for each group of rabbits.

Group (B) control
Received no further treatment.

Post-surgical phase (14)
1. All rabbits received antibiotics in the form of ampicillin (Epicocillin, Epico, Cairo, Egypt) 1 g IM immediately post-operatively.
2. Animals were observed to determine whether an infection or wound dehiscence was present or not.
3. On postoperative day 5, all sutures were removed.

Follow-up and wound assessment
A through follow- up was performed after 2, 4, and 6 weeks for the assessment of the surgical scar. Assessment included measurement of wound width and Vancouver scar scale, along with surgical scars photographs

Photographic observation of hypertrophic scar appearance
Revealed that the overall scar appearance in BTA- treated group was better than the control group regarding erythema and edema of the surround skin.

Evaluation of scar characteristics using VSS and a digital caliper
After the photographs were taken, VSS was applied to quantify scar appearance at the follow-up phases (2, 4, and 6 weeks). Also a digital caliper was used to measure the width of the rabbits' forehead wounds (Figure 2). Immediately after taking photographs and measurements each time, the animals were sacrificed.

Animal sacrifice
Rabbits were sacrificed at each of the experimental periods (2, 4, and 6 weeks postoperatively; 12 rabbits each time) by giving them an overdose of the

anaesthetic ketamine (NEON Pharmaceutical, UK) (15).

Following successful euthanasia, a scalpel (Aesculap, Inc., USA) was used to remove representative scar sections, and care was taken to involve the deep layers of the skin. All specimens were labelled and fixed for one day in 10% neutral buffered formalin, after which segments that contained the surgical sites were dissected and left for five days in the same fixation (16).

Histological procedures (13)

Following fixation, specimens were cleaned, dehydrated in ethanol solutions with increasing ethanol concentrations, cleared with xylene, infiltrated, and embedded in paraffin wax. A rotatory microtome was used to cut thin sections in serial that were 2-5 m thick. The sections were stained with Harris hematoxylin and eosin for analysis (H&E) and mounted on clear glass slides covered with a thin layer of albumin adhesive.

Histomorphometric evaluation (5)

Each sample's collagen density in scar tissue was measured. For each sample, the area with the highest collagen concentration was chosen at low power (40). Then, the Image J software quantitatively measured the proportion of collagen (NIH, USA).

Steps for measuring the percentage of the formed collagen fiber

Each sample's collagen density was calculated in the two types of scar tissue. For each sample, the collagen area with the highest concentration was chosen at low power (40). Then a camera took a high-power image (400) of this area. It was done to make the histological image in black and white. Then, using an image J software program, by the aid of automated equation in the software, a quantitative calculation of the collagen proportion was made (NIH, USA).

Statistical methods

Data were entered into the computer and analyzed using IBM SPSS version 20.0 software. (Armonk, New York: IBM Corporation). The Shapiro-Wilk test is employed to confirm the distribution's normality. Range (minimum and maximum), mean, and standard deviation were used to characterize quantitative data. The significance threshold of the collected results was determined to be 5%.

The tests used were:

1.A student t-test was used for normally distributed quantitative variables to compare the two studied groups.

2.An ANOVA with repeated measures was used for normally distributed quantitative variables to compare more than two periods or stages.

Figure (1): A) A Y-shaped incision with a length of 2 cm is made on the cheek. B) Suturing of the wound using multiple non-resorbable surgical silk sutures (5-0) in a continuous manner. C) Determining the injection points using a black skin marker. D) One insulin syringe containing 7 units of botulinum toxin after reconstitution with 0.9% saline.
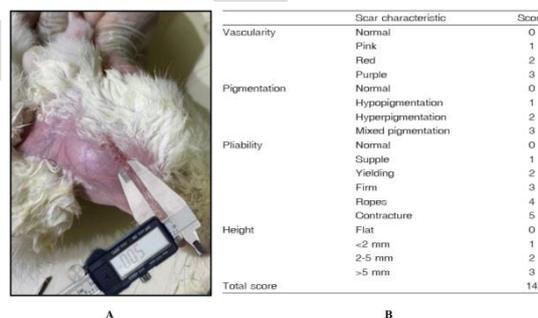
Figure (2): A) Measuring wound width using a digital Vernier caliper. B) VSS.

RESULTS

All rabbits included in this study were clinically healthy and tolerated the surgical procedures well. No adverse reactions such as allergies or post-operative infections were seen after surgery. Also, no skin dehiscence was detected.

Photographic observation of hypertrophic scar appearance (Figure 3)

The following photographs show that the scar appearance improved significantly in the study group treated with BTA compared to the non-treated control group by macroscopic observation throughout the study. (2, 4, and 6 week intervals)

Two weeks after surgery, both scars were not completely healed, with the appearance of well-defined areas with erythema and edema of the surrounding skin, which was more prominent in the control group.

Four weeks post-surgically, less erythema and improved scar quality are seen. In the model with botulinum toxin injection, while erythematous scarring continues to be problematic in the other model without botulinum toxin injection.

Six weeks post-surgically, after complete healing in both groups, the scar in the control group

had a hypertrophic appearance. The scar in the study group, however, was nearly undetectable because it was the same color as the surrounding skin. A scar was considered normal when the injury healed without becoming red, raised, or rigid when compared to normal skin. In order to evaluate the final appearance of the scar in the study group, the head had to be decapitated in order to cut the blood supply and see the scar.

Evaluation of scar characteristics using VSS and a digital caliper [Table 1]

Comparable results were seen in both groups at each of the three observational periods, according to the data from the study of VSS and wound width, with higher total values for the control group. An analysis comparing the three observational periods revealed that after six weeks, the evaluated parameter (VSS), in both the control group and the study group decreased significantly from the beginning to the end of the study (p<0.001).

Regarding the wound width [Table 2]

In Table 2, the average wound width was compared between the study groups and the control group at 2, 4, and 6 weeks. An analysis comparing the three observational periods revealed that after six weeks, the evaluated parameters (wound width) in both the control group and the study group decreased significantly from the beginning to the end of the study.

Histological results

Scar tissue histological examination of both the control and study groups (injected with BTA) was hematoxylin and eosin stained and investigated under a light microscope for 6 weeks postoperatively and revealed some epidermal and dermal changes.

The scar underwent examination, which revealed mature collagen fibers and bundles with no indications of inflammation or continuing remodeling. All of the sections examined were stained with hematoxylin and eosin and investigated under a light microscope.

Two weeks postoperatively, the epidermal layer of both the control and study groups revealed normal contours with the normal organization of the basal cell layer and proliferation of the rete pegs in their normal organizations. The dermis (connective tissue) in the control group demonstrated delicate proliferating fibroblasts arranged in a disarrayed manner and haphazardly distributed subepithelial. While in the study group, more organized, proliferating cellular fibroblasts and collagen fibers, which are organized in a wavy direction and occupy the upper reticular dermis layer, were observed (Figure 4).

At four and six weeks postoperatively, the epidermal layer in the control group showed flattening of the epithelial layer and loss of rete pegs. While the epidermal layer in the study group showed its normal contour, with its rete pegs

arranged in normal orientation and organization (Figure 5).

The dermal connective tissue revealed more mature cellular collagen fibers and bundles arranged in a defined wavy and loose pattern parallel to the scar surface in the study group. While the dermal layer in the control group revealed dense, unorganized collagen bundles. Also, fibrosis and some thick collagen bundles are seen in the deeper layers and in between the muscle tissues (Figure 6).

Histomorphometric results [Table 3]

Comparable results were found from the histomorphometric study of collagen density in both groups during the three observational periods, with the control group having higher values overall.
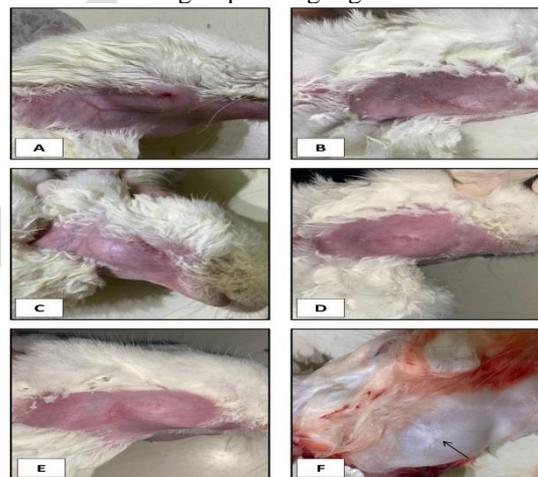
Figure (3): A, B) Represent the hypertrophic scar in a rabbit model without BTA and with BTA injection, respectively, after two weeks post-surgically. C, D) Represent the hypertrophic scar in a rabbit model without BTA and with BTA injection, respectively, after four weeks post-surgically. Improved scar quality and erythema are noted in the model with botulinum toxin injection, while erythematous scarring continues to be problematic in the other model without botulinum toxin injection. E) Six weeks after surgery, represent the scar in a rabbit model without BTA. The scar appears to be erythematous. F) After head decapitation, the black arrow indicates the scar in a rabbit model with BTA injection after six weeks, represented as a normal scar.
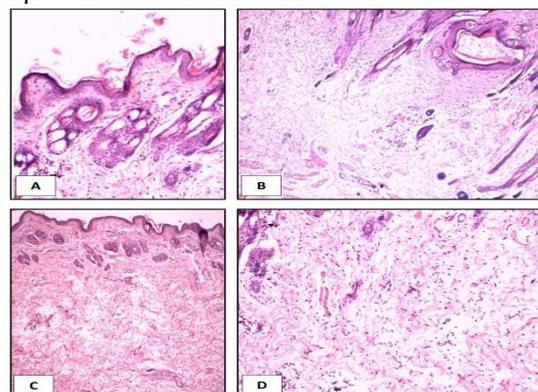
Fig. (4): A) A photomicrograph of a scar tissue (control group) after two weeks showing the regular and normal organization of the basal cell layer which is correlated with the presence of rete pegs and normal contour of the epidermal layer (H&E x 200). B) A photomicrograph of another case from the control group after two weeks showed the scanty, unorganized, delicately proliferating fibroblasts and collagen fibers. (H&E x 100). C) A photomicrograph of a study group (injected with botulinum toxin) after two weeks shows loose, delicately proliferating cellular fibroblasts. (H&E X 100). D) A higher magnification of a section of the previous slide shows the loose and wavy arrangement of the collagen fibers. (H&E x 200).
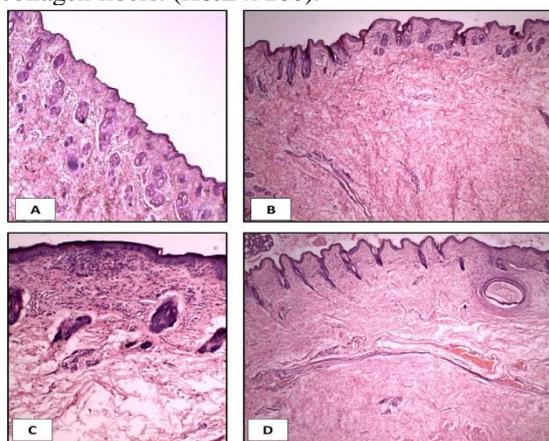
Figure (5): A) A four-week photomicrograph of a control group demonstrating straightening of the lining epithelium and the absence of rete pegs. Note that some scattered, diffuse, chronic inflammatory cells are distributed subepithelially. (H&E x 200). B) A photomicrograph of a study group (injected with botulinum toxin) after four weeks shows the normal contour of the epithelial layer and mature collagen fibers and bundles subepithelial (H&E x 100). C) A photomicrograph of a control group after six weeks, showing straightening of the lining epithelium and absence of the rete pegs. Note the subepithelial chronic inflammatory cells. (H&E x 200). D) A photomicrograph of a study group injected with botulinum toxin after six weeks, showing the normal contour of the epidermal cell layer, the presence of rete pegs, and the normal organization of the basal cell layer. (H&E x 100).
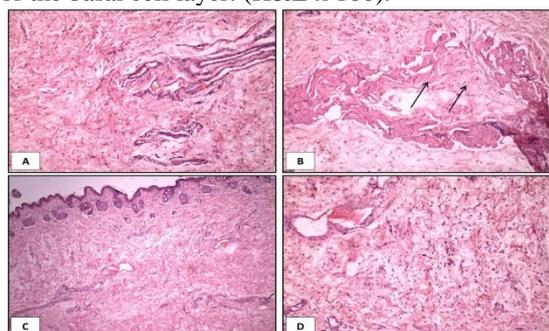
Figure (6): A) A photo micrograph of a control group after six weeks showing dense collagen bundles and fibrosis (H&E x100). B) Another section of the previous slide showing the formation of dense collagen fibers and bundles occupying a deeper layer between muscle tissue. (black arrow). (H&E x200). C) A photomicrograph of another case of a study group injected with botulinum toxin after six weeks shows the loose orientation of the collagen bundles in the deeper layer of the dermis. (H&E x 100). D) A higher magnification of the previous slide, showing the loose deposition of mature collagen fibers in a wavy pattern. (H&E x 200).

Table (1): Comparison between the two studied groups according to VSS through the three observation period and between the two studied groups from the beginning to the end of the study.

VSS	Study (n = 6)	Control (n = 6)	t	p
2weeks Min. – Max.	6.0 – 9.0	7.0 – 10.0	1.414	0.188
Mean ± SD.	7.50 ± 1.05	8.50 ± 1.38		
4weeks Min. – Max.	2.0 – 5.0	4.0 – 8.0	3.162*	0.010*
Mean ± SD.	3.50 ± 1.05	5.83 ± 1.47		
6weeks Min. – Max.	0.39 – 0.53	0.12 – 0.21	11.743*	<0.001*
Mean ± SD.	0.47 ± 0.05	0.17 ± 0.04		
Decrease 2weeks / 6 weeks Min. – Max.	5.51 – 8.53	6.79 – 9.88	1.811	0.100
Mean ± SD.	7.03 ± 1.08	8.34 ± 1.40		

SD: Standard deviation   t: Student t-test
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

Table (2): Comparison between the two studied groups according to wound width through the three observation period and between the two studied groups from the beginning to the end of the study.

Wound width	Study (n = 6)	Control (n = 6)	t	p
2weeks Min. – Max.	0.37 – 0.50	0.57 – 0.67	6.825*	<0.001*
Mean ± SD.	0.43 ± 0.05	0.61 ± 0.03		
4weeks Min. – Max.	0.20 – 0.38	0.47 – 0.58	7.860*	<0.001*
Mean ± SD.	0.27 ± 0.07	0.52 ± 0.04		
6weeks Min. – Max.	0.12 – 0.21	0.39 – 0.53	11.743*	<0.001*
Mean ± SD.	0.17 ± 0.04	0.47 ± 0.05		
Decrease 2weeks / 6 weeks Min. – Max.	0.16 – 0.37	0.07 – 0.28	2.920*	0.015*
Mean ± SD.	0.27 ± 0.07	0.14 ± 0.08		

SD: Standard deviation   t: Student t-test
p: p value for comparing between the two studied groups
*: Statistically significant at p ≤ 0.05

Table (3): Comparison between the two studied groups regarding the collagen density through the three observation period and between the two studied groups from the beginning to the end of the study.

Area fraction of collagen density	Study (n = 6)	Control (n = 6)	t	p
2weeks Min. – Max.	17.05 – 19.63	28.57 – 32.21	17.604*	<0.001*
Mean ± SD.	18.75 ± 0.99	30.36 ± 1.28		
4weeks Min. – Max.	17.54 – 25.16	32.57 – 36.05	11.781*	<0.001*
Mean ± SD.	19.77 ± 2.74	34.58 ± 1.40		
6weeks Min. – Max.	17.54 – 25.87	37.87 – 41.22	14.491*	<0.001*
Mean ± SD.	19.89 ± 3.02	39.31 ± 1.28		
Increase 2weeks / 6 weeks Min. – Max.	-0.01 – 6.35	5.66 – 12.65	5.522*	<0.001*
Mean ± SD.	1.14 ± 2.56	8.96 ± 2.34		

SD: **Standard deviation   t: Student t-test**
p: p value for comparing between the two studied groups
*: Statistically significant at p ≤ 0.05

DISCUSSION

Hypertrophic scars are caused by benign hyperproliferative dermal collagen growth. Major physical and psychological issues are frequently experienced by patients with hypertrophic scars. The treatment of hypertrophic scars has always been a source of contention because the etiology of their formation has not been fully identified (2, 17).

Numerous techniques have been proposed over the years to improve scars (18). Reducing the amount of reactive suture material, executing a high-quality closure, utilizing occlusive or semi-occlusive dressings, minimizing sun exposure, and using various scar care products are routinely employed measures to promote favorable healing. Reducing strain on the wound margins is more important than the modalities indicated above (11).

A fundamental therapeutic idea in wound healing is immobilization. Different methods are used to lessen excessive tension on incisions. However, these techniques minimize the stress impacting the healing wound, but not completely. A novel method to ease the strain on the healing wound's edges is to temporarily paralyze the muscle underneath the wound, which can be achieved by botulinum toxin injection (7).

Clostridium botulinum produces BTA, a potent neurotoxin. It causes striated muscle flaccid paralysis by inhibiting acetylcholine secretion at the nerve terminals (19).

The benefits of BTA may result in temporary denervation and may be effective in producing the intended result of reducing muscle pulls. Some studies indicated that the mechanism by which BTA treated hypertrophic scars involved releasing the tension brought on by nearby muscles (20). Also, some researchers found that BTA may inhibit neuromuscular junction release and reduce muscular tension (21).

In this study, to ascertain the botulinum toxin's effects other than muscle paralysis, we investigated its effect on collagen deposition during the healing of a surgical scar, which affects its width and degree of hyperplasia.

According to some previous studies, botulinum toxin type A injections are most efficient in the early stages of wound healing (22); So, in this study, we chose to inject botulinum toxin immediately following primary closure.

Our attempt at early injection of botulinum toxin was consistent with a number of studies, as suggested by Sheta et al. (9) who suggested that injection of botulinum toxin in the first 5 days after wound closure could improve forehead scar appearance. Additionally, Kim et al. (23) reported successful results for thyroidectomy scars after 6 days of injection of botulinum toxin type A.

Because the healing procedure in rabbits is physiologically similar to a person's, this study used a rabbit as a model (24). There are obvious

ethical and practical reasons to test the safety and efficacy of the obtained results before applying them to humans, in addition to closely simulating the influence of muscle activity on facial skin wounds in humans. Animal models are exceptional for comparative studies because of the predetermination of treatment time concerning the wound healing process. For this reason, clinical research involving human subjects has traditionally been thought to require the use of animal experimental models. All the rabbits in the current study were male to exclude any variations that might be caused by female hormonal changes (5, 13).

Surprisingly, BTA improved scar appearance in the three phases of follow-up, the discoloration, texture, and wound width were found to be better in the study group (injected with botulinum toxin) when compared to the control group.

Our results were consistent with a number of studies by Gassner et al., who discovered that BTA could lessen scarring's proliferation and enhance its appearance (7). Also, in some clinical trials, injections of BTA into dermal tissue to reduce hypertrophic scarring have yielded promising results (25).

Furthermore, another author determined the usage of botulinum toxin to treat a group of patients who were at high risk of experiencing complications from slowed wound healing. These patients had undergone blepharoplasty and experienced no complications as a result of the procedure (8).

Zimbler et al. (26) discovered that laser skin resurfacing in the face had a longer-lasting impact when the affected skin was made immobile with botulinum toxin.

The findings of this study showed that the parameters of wound assessment were different between the botulinum toxin group and the control group, which were significantly superior in the group treated with BTA. The results were based on assessment measures (VSS score and wound width) and the results of the histological healing process, which demonstrated that the scars in the group receiving botulinum toxin were always smaller than those of the control group.

Our findings supported several studies, including Ziade et al.(2013) (27) reports' of comparable outcomes for the treatment of facial wounds with BTA. Wilson (28) discovered that the results of BTA injection during revision surgery for facial scars were highly satisfactory in terms of surgical scars.

When Chang et al. (29) and Li et al. (30) examined scar width information, they discovered that the outcomes in the BTA and control groups differed statistically significantly.

Other papers reported that individuals with hypertrophic scars also scored highly on the outcomes related to appearance. 90% of patients, according to Wilson (28), were pleased with the reduced scarring.

Some researchers discovered that the width was not significantly better than the patient's features before surgery (30).

Chang et al. (29) also discovered that botulinum toxin type A injections produced more beautiful and narrower cheiloplasty scars at the 6-month follow-up assessment.

The use of botulinum toxin improved scar pigmentation, as evidenced by a considerable drop in the overall VSS score in comparison with the control group.

Many significant cellular mediators in the inflammatory response to cutaneous injuries have a variety of effects on melanocytes and melanogenesis: During an inflammatory response, nitric oxide, histamine, p53, and transforming growth factor 1 (TGF-1) are all released, and they are all known to promote melanogenesis and skin discoloration (32).

Our findings were in line with several studies, such as those by Lee et al. (33), who found that BTA reduced inflammation-related cell invasions, fibrosis, and TGF-b1 expression in a rat model of a surgical wound compared to controls. Additionally, according to Ward et al. (34) studies, BTA injection significantly reduced the invasion of CD4 T cells and dermal dendritic cells in a KC-Tie2 mouse model, resulting in significantly reduced skin inflammation.

Histological evaluation in the present study was carried out at two-, three-, and six-week intervals. The results revealed increased cellularity, fibroblasts, and deposition of collagen bundles in the control group compared to the botulinum toxin group. While collagen in the botulinum toxin group was observed to be thinner and more orderly arranged.

Reduced blood vessel density and apoptosis in both fibroblasts and myofibroblasts during the regeneration phase of wound healing both contribute to the development of scar tissue. Therefore, mature scar tissue hardly has any fibroblasts. But in pathological settings like hypertrophic scars, myofibroblasts continue to exist. This may be because extracellular matrix synthesis by fibroblasts is excessive and extracellular matrix breakdown by collagenases, proteoglycanases, and proteases is reduced.

Recently, it was discovered that botulinum toxin suppresses TGF-1 expression, cell division, and fibroblast proliferation (35).

Our results were in agreement with previous published literature. We went over a few of them to clarify the reasons behind how BTA affects collagen fiber bundles. The BTA-induced reduction in tension alters the functional state of local fibroblasts over time by causing them to proliferate

slowly and create less extracellular matrix, including collagen.

Furthermore, fibroblasts that are under weak tension secrete fewer biologically active mediators, which prevents them from rapidly proliferating and producing a lot of extracellular matrix. These factors led to enhanced hypertrophic scars (20).

Using a representation of a rabbit's ear, Xiao and Qu (2) investigated the impact of BTA on fibroblasts derived from hypertrophic scars. And they discovered that using BTA might reduce tension, prevent collagen deposition, and improve collagen fiber arrangement. Furthermore, TGF-1 protein expression in hypertrophic scar fibroblasts could be inhibited by BTA in vitro experiments (36).

In addition to controlling collagen deposition, TGF-1 has been recognized as a significant cytokine that is intimately linked to the development and hypertrophic scar formation. These findings led to the hypothesis that BTA's effects on the collagen of hypertrophic scars were caused by its suppression of TGF-1 secretion in vivo (2). Unlike Haubner et al. (37) whose reports contradicted our findings. He concluded that BTA provided no discernible therapeutic benefit to fibroblasts or skin wound healing.

Other studies disproved the BTA's advantageous effects on excessive scarring. According to Gauglitz et al. (4), the keloid tissue showed no clinical improvement. TGF-, collagen synthesis, and the other ECM markers were comparable to the control group. Additionally, fibroblast proliferation and metabolism were unaffected.

However, favorable outcomes were reported in our study. BTA injections into areas close to suture sites improve the wound healing procedure. Also, it is stated to be efficient in inhibiting collagen deposition in hypertrophic scars and enhancing the cosmetic appearance of surgical scars.

This research may have some limitations. There was no immediate feedback on the extent of the resulting muscle paralysis, where treatment side effects can include neurotoxin diffusion to adjacent muscle groups. Also, there was no specific determination of collagen type, whether it was type I or type III.

CONCLUSION

The purpose of this study was to evaluate the effect of BTA injection on the overall cosmetic appearance of surgical scars. We concluded that, after wound closure, the group of rabbits which received BTA injections in the underlying muscles around a performed surgical incision, showed better scar discoloration and overall appearance. Also, better qualitative measures regarding VSS and wound width were noted in the BTA-treated group. The histological results of the study group demonstrated less fibroblast production and collagen synthesis compared to the control group.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Gassner HG, Brissett AE, Otley CC, Boahene DK, Boggust AJ, Weaver AL, et al. Botulinum toxin to improve facial wound healing: A prospective, blinded, placebo-controlled study. Mayo Clin Proc. 2006;81:1023-8.

2. Xiao Z, Qu G. Effects of botulinum toxin type a on collagen deposition in hypertrophic scars. Molecules. 2012;17:2169-77.

3. Atkinson JA, McKenna KT, Barnett AG, McGrath DJ, Rudd M. A randomized, controlled trial to determine the efficacy of paper tape in preventing hypertrophic scar formation in surgical incisions that traverse Langer's skin tension lines. Plast Reconstr Surg. 2005;116:1648-56; discussion 57-8.

4. Gauglitz GG, Bureik D, Dombrowski Y, Pavicic T, Ruzicka T, Schauber J. Botulinum toxin A for the treatment of keloids. Skin Pharmacol Physiol. 2012;25:313-8.

5. Çalışkan E, Gamsızkan M, Açıkgöz G, Durmuş M, Toklu S, Doğrul A, et al. Intralesional treatments for hypertrophic scars: comparison among corticosteroid, 5-fluorouracil and botulinum toxin in rabbit ear hypertrophic scar model. Eur Rev Med Pharmacol Sci. 2016;20:1603-8.

6. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010;89:219-29.

7. Gassner HG, Sherris DA, Otley CC. Treatment of facial wounds with botulinum toxin A improves cosmetic outcome in primates. Plast Reconstr Surg. 2000;105:1948-53; discussion 54-5.

8. Al-Qattan MM, Al-Shanawani BN, Alshomer F. Botulinum toxin type A: implications in wound healing, facial cutaneous scarring, and cleft lip repair. Ann Saudi Med. 2013;33:482-8.

9. Sheta OA, Soliman MM, Dessoky NY. Clinical evaluation of the efficacy of botulinum toxin a for improving facial scars (randomized clinical trial). Alex Dent J. 2022;47:33-41.

10. National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academy of Sciences.; 2011.

11. Lee SH, Min HJ, Kim YW, Cheon YW. The efficacy and safety of early postoperative

botulinum toxin A injection for facial scars. Aesthetic Plast Surg. 2018;42:530-7.

12. Hexsel D, Rutowitsch MS, de Castro LCM, do Prado DZ, Lima MM. Blind multicenter study of the efficacy and safety of injections of a commercial preparation of botulinum toxin type A reconstituted up to 15 days before injection. Dermatol Surg. 2009;35:933-40.

13. Zohery AA, Nour ZM, Abd El Rehim SS, Mady MI. Histomorphometric analysis of bone regeneration after use of propolis versus nanobone graft materials for the management of class ii furcation defects in dogs. Alex Dent J. 2017;42:198-203.

14. Ghanem MA, Sharara AA, Sweedan AO, Khalil NM. Evaluation of using mineralized plasmatic matrix in osseous regeneration (experimental and histomorphometric study on rabbits). Alex Dent J. 2018;43:53-9.

15. Leary SL, Underwood W, Anthony R, Cartner S, Corey D, Grandin T, et al. AVMA guidelines for the euthanasia of animals. American Veterinary Medical Association Schaumburg, IL. 2013.

16. Ghanem MA, Sharara AA, Sweedan AO, Khalil NM. Evaluation of using mineralized plasmatic matrix in osseous regeneration (experimental and histomorphometric study on rabbits). Alex Dent J. 2018;43:53-9.

17. Zhang DZ, Liu XY, Xiao WL, Xu YX. Botulinum Toxin Type A and the Prevention of Hypertrophic Scars on the Maxillofacial Area and Neck: A Meta-Analysis of Randomized Controlled Trials. PLoS One. 2016;11:e0151627.

18. Liu A, Moy RL, Ozog DM. Current methods employed in the prevention and minimization of surgical scars. Dermatol Surg. 2011;37:1740-6.

19. Lam SM. The basic science of botulinum toxin. Facial Plast Surg Clin North Am. 2003;11:431-8.

20. Sherris DA, Gassner HG. Botulinum toxin to minimize facial scarring. Facial Plast Surg. 2002;18:35-9.

21. Ahmed K, Oas KH, Mack KJ, Garza I. Experience with botulinum toxin type A in medically intractable pediatric chronic daily headache. Pediatr Neurol. 2010;43:316-9.

22. Gassner HG, Sherris DA. Chemoimmobilization: improving predictability in the treatment of facial scars. Plast Reconstr Surg. 2003;112:1464-6.

23. Kim YS, Lee HJ, Cho SH, Lee JD, Kim HS. Early postoperative treatment of thyroidectomy scars using botulinum toxin: a split-scar, double-blind randomized controlled trial. Wound Repair Regen. 2014;22:605-12.

24. Kamaruzaman NA, Kardia E, Kamaldin N', Latahir AZ, Yahaya BH. The rabbit as a model for studying lung disease and stem cell therapy. Biomed Res Int. 2013;2013:691830.

25. Jeong HS, Lee BH, Sung HM, Park SY, Ahn DK, Jung MS, et al. Effect of Botulinum Toxin Type A on Differentiation of Fibroblasts Derived from Scar Tissue. Plast Reconstr Surg. 2015;136:171e-8e.

26. Zimbler MS, Holds JB, Kokoska MS, Glaser DA, Prendiville S, Hollenbeak CS, et al. Effect of botulinum toxin pretreatment on laser resurfacing results: a prospective, randomized, blinded trial. Arch Facial Plast Surg. 2001;3:165-9.

27. Ziade M, Domergue S, Batifol D, Jreige R, Sebbane M, Goudot P, et al. Use of botulinum toxin type A to improve treatment of facial wounds: a prospective randomised study. J Plast Reconstr Aesthet Surg. 2013;66:209-14.

28. Wilson AM. Use of botulinum toxin type A to prevent widening of facial scars. Plast Reconstr Surg. 2006;117:1758-66; discussion 67-8.

29. Chang CS, Wallace CG, Hsiao YC, Chang CJ, Chen PK. Botulinum toxin to improve results in cleft lip repair. Plast Reconstr Surg. 2014;134:511-6.

30. Li W, Gao Y, Sun Z. Application of Botox A in the repair of facial linear scar. Chin J Aesth Plast Surg. 2014;25:426-9.

31. Xiao Z, Zhang F, Cui Z. Treatment of hypertrophic scars with intralesional botulinum toxin type A injections: a preliminary report. Aesthetic Plast Surg. 2009;33:409-12.

32. Chadwick S, Heath R, Shah M. Abnormal pigmentation within cutaneous scars: A complication of wound healing. Indian J Plast Surg. 2012;45:403-11.

33. Lee BJ, Jeong JH, Wang SG, Lee JC, Goh EK, Kim HW. Effect of botulinum toxin type a on a rat surgical wound model. Clin Exp Otorhinolaryngol. 2009;2:20-7.

34. Ward NL, Kavlick KD, Diaconu D, Dawes SM, Michaels KA, Gilbert E. Botulinum neurotoxin A decreases infiltrating cutaneous lymphocytes and improves acanthosis in the KC-Tie2 mouse model. J Invest Dermatol. 2012;132:1927-30.

35. Spyrou GE, Naylor IL. The effect of basic fibroblast growth factor on scarring. Br J Plast Surg. 2002;55:275-82.

36. Zhibo X, Miaobo Z. Botulinum toxin type A affects cell cycle distribution of fibroblasts derived from hypertrophic scar. J Plast Reconstr Aesthet Surg. 2008;61:1128-9.

37. Haubner F, Leyh M, Ohmann E, Sadick H, Gassner HG. Effects of botulinum toxin A on patient-specific keloid fibroblasts in vitro. Laryngoscope. 2014;124:1344-51.