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# Intralesional treatments for hypertrophic scars: comparison among corticosteroid, 5-fluorouracil and botulinum toxin in rabbit ear hypertrophic scar model

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**Abstract. – OBJECTIVE: Different treatment modalities have been used either alone, or in combination to achieve an optimum improvement for hypertrophic scars. Intralesional injections of corticosteroids and 5-fluorouracil are among the most commonly used treatments. Recently, botulinum toxin is proposed as a new treatment option. In this study, it is aimed to compare the efficacies of intralesional triamcinolone acetonide, 5-fluorouracil and botulinum toxin-A for hypertrophic scars. In order to minimize the variables affecting scar formation, standardized wounds in rabbit ear hypertrophic scar model was used.**

**MATERIALS AND METHODS: Four surgical wounds were created on both ears of eight rabbits. Injections to be compared (triamcinolone acetonide, 5-fluorouracil, botulinum toxin-A and control) are administered intralesionally to established scars on day 30. Scars were harvested on day 60 for morphometric analysis including hypertrophic index, fibroblast density, and relative collagen density.**

**RESULTS: Triamcinolone acetonide and 5-fluorouracil injections decreased hypertrophic indexes significantly compared to botulinum toxin-A and control group. However, only 5-fluorouracil was effective to reduce fibroblast counts significantly. No statistically significant differences were found between the treatment groups in terms of collagen index.**

**CONCLUSIONS: According to the results of our study, triamcinolone acetonide and 5-fluorouracil are comparatively effective as monotherapy, but botulinum toxin-A was not effective on established hypertrophic scars.**

*Key Words:*
Animal model, Botulinum toxin-A, Scar treatment, Triamcinolone acetonide, 5-fluorouracil.

## Introduction

Hypertrophic scars (HS) and keloids are functionally and cosmetically important concerns both for patients and dermatologists. Both entities are characterized by pathologically excessive dermal fibrosis and aberrant wound healing which results from abnormal wound healing responses to trauma, inflammation, surgery, or burn in predisposed individuals[1]. Various therapeutic modalities including topical agents, pressure dressings, intralesional injections, radiotherapy, cryosurgery, laser applications, surgical interventions and combinations of these techniques have been suggested for the treatment of hypertrophic scars and keloids with variable results[2-4].

Intralesional injection, mainly triamcinolone acetonide (TA) and 5-fluorouracil (5-FU) are the most widely used intralesional drugs[5-8] and in recent years, botulinum toxin A (BTA) is proposed as a new treatment option for established HSs and keloids[9,10]. Although efficacies of these treatments have been shown in various clinical studies[3-5], these clinical trials have some limitations. A large number of variables affecting the severity of the scarring, such as genetic and ethnic background, skin type and immunity, in addition to susceptibility of certain anatomic sites to scarring especially in young adults make an objective assessment of comparative studies difficult[1-3].

In this respect, although they are not perfect representatives of human scars, animal studies are valuable for some reasons. In addition to standardized wounds within the same individual,

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predetermination of treatment time in relation to the phase of wound healing process make animal models unique for comparative studies.

We were unable to find any studies comparing the efficacy of TA and 5-FU in animal models. In addition we could not detect any studies comparing BTA with these relatively more established treatments.

Thus, we designed a study to objectively compare the efficacies of TA, 5-FU and BTA injections on established HSs. In order to achieve more reliable comparisons with the evaluated agents, we created standardized wounds on rabbit ear model and to exclude interindividual differences, all treatments were administered on the same rabbit ear.

## Material and Methods

The study was carried out with the approval of Animal Experimentation Ethics Committee of our institution (13/36; 2013). Eight young male New Zealand white rabbits (2500 to 3500 g) were used. The animals were kept under standardized conditions following the guidelines of the ethics committee.

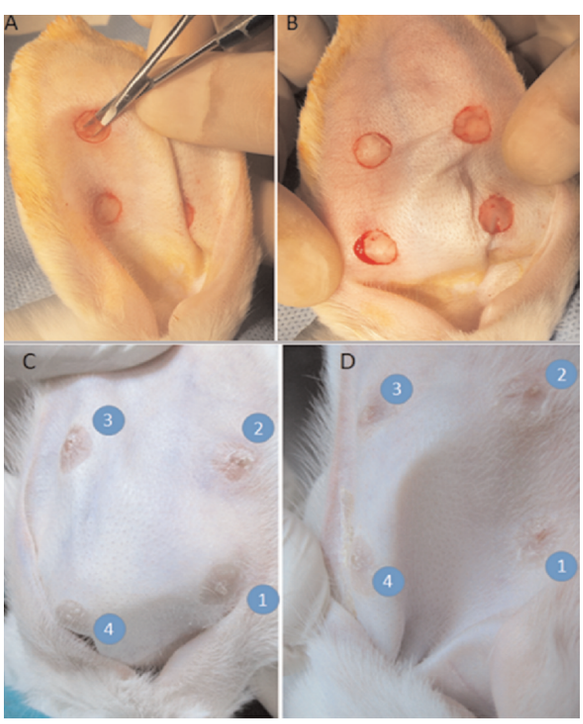


**Figure 1.** Macroscopic appearance of treatment areas on rabbit ear. ***(A)*** surgical wounds created with biopsy punch and perichondrium is dissected from the underlying ear cartilage. ***(B)*** Appearance of the created four punch defects immediately after the surgical procedure. ***(C)*** Wounds healed with hypertrophic scarring. ***(D)*** The eventual appearance of treatment sites which will be evaluated histologically. Note; injection sites are numbered depending on the treatment group.

### Surgical Procedure

Rabbits were anesthetized with intramuscular injection of ketamine (45 mg/kg) and xylazine (5 mg/kg). Surgical wounds were performed on day 0 with an 8-mm biopsy punch (Figure 1-A). Four wounds were created meticulously on the ventral surface of each ear down to cartilage. The perichondrium was removed with the aid of a magnifying loupe (Looks®, Xenosys Co, Korea) (Figure 1-B). After the hemostasis has been achieved with manual pressure, wounds were covered with sterile gauze for 1 day. At the end of the procedure, 64 wounds were created on 8 rabbits.

### Treatment groups

Treatment groups to be administered were as follows; control (0.1 ml of 0.9% saline), TA (4 mg/0.1 ml), 5-FU (5 mg/0.1 ml) and BTA (2 U/0.1 ml). Injection volumes were adjusted to 0.1 ml for each agent. On postoperative day 30, the wounds to be treated were numbered from 1 to 4 for each ear (Figure 1-C). Determined numbers were rotated clockwise once for each subsequent rabbit. All treatments were administered intralesionally to the center of the scar by using 29 gauge needles. On day 60, the eventual scars were obtained (Figure 1-D). The animals were sacrificed and scars were harvested with more than 5 mm margin of adjacent skin.

### Histological Evaluation

After fixation with 10% buffered formaldehyde solution, the samples were put into a buffered formic acid solution for decalcification. The samples yielded 5 μm sections which were embedded into paraffin after processing. The sections were stained with haematoxylin-eosin and Masson-trichrome stain.

Hypertrophic index (HI), fibroblast density, and relative collagen density were used for morphometric analysis. HI index is the ratio of the highest vertical height of scar area between perichondrium and skin surface (Figure 2-A) to the highest vertical height of normal area around the scar between perichondrium and skin surface. In order to establish fibroblast density, a hotspot in high-power field within the scar was determined and fibroblasts were counted in 1 $mm^2$ (Figure 2-B). Collagen content within the newly formed

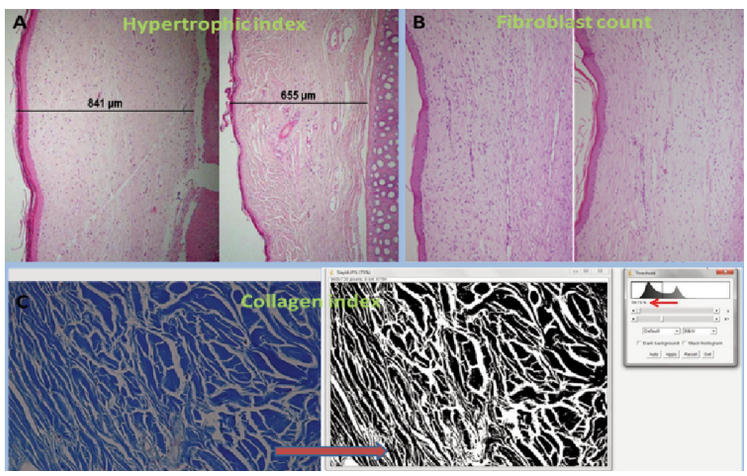


**Figure 2.** Methodology of the pathologic evaluation. ***(A)*** Hypertrophic index is obtained with the measurement of highest vertical height from perichondrium to epidermal surface in scar area (HE x100) and normal tissue around the scar (HE x100). ***(B)*** The distribution of fibroblasts in scar areas. A sample from BTA group (left) and another sample from 5-FU group (right) (HE x400). ***(C)*** Collagen density measurement: a high power view of scar area (lower left image) (Masson Trichrome stain x400) is converted to black and white format (lower middle image) and numerical value is obtained with a software (lower right image).

dermal architecture may be affected by the variations of underlying cartilaginous tissue. To exclude such probable effects we evaluated collagen density relative to the adjacent unwounded tissue, namely collagen index (CI). Collagen density in scar and normal tissue was calculated for each sample. The greatest concentration of collagen area was selected at low power view (x40) for each sample. Then, an image of this area was taken at high power view (x400) by a camera. The histological image was converted to black-and-white format. Then, the proportion of collagen was quantitatively calculated by *ImageJ* software program (NIH, USA) (Figure 2-C). CI was obtained by normalizing the collagen density of treatment area to the unwounded adjacent skin.

### Statistical Analysis

All data are expressed in mean ± SD. SPSS for Mac 20.0 package program (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation. Kolmogorov-Smirnov test was used for analyzing the distribution pattern of data and normally distributed continuous variables were expressed as mean ± standard deviation. The parametric values were compared with ANOVA test for normally distributed groups. Data were analyzed using the analysis of variance Tukey-Kramer multicomparison test to compare the means between study groups. The level of significance was set to $p$-values < 0.05.

## Results

All wounds demonstrated histologic features of matured scarring. The mean HIs of the groups (mean±SD) were 1.41± 0.17, 1.02± 0.22, 0.98± 0.3 and 1.31± 0.16 in control, TA, 5-FU and BTA groups respectively (Figure 3). HIs were significantly lower with TA and 5-FU treatments in comparison to BTA and control groups ($p$=0.001). HIs of TA and 5-FU groups were not statistically different ($p$=0.91).

Fibroblast counts within the groups were 633.3± 174.7, 562.7± 140.2, 474.6± 147.7 and 556.8± 160.2 in control, TA, 5-FU and BTA groups respectively. Fibroblast counts in the 5-FU group were significantly lower than other treatments ($p$=0.028) (Figure 3).

There were not statistically significant differences between the groups in terms of collagen index ($p$=0.63) (Figure 3).

## Discussion

Early preventive approaches of proper surgical technique and optimal after-care to promote wound healing are much easier and effective for excessive scarring[3]. However, the majority of the patients are in need of remodeling treatments for their matured scars. Among many treatments, intralesional injections are very useful for clinical practice and, therefore, many physicians prefer this approach primarily[5]. In this work, we comparatively evaluated the efficacies of intralesional TA and 5-FU as mostly preferred approaches, and BTA as a recently proposed agent for hypertrophic scars.

Steroid injections are one of the most common approaches for decades and despite relatively few randomized controlled trials, intralesional TA is generally considered as first-line therapy in clinical practice[2,5,11]. 5-FU is another anti-mi-

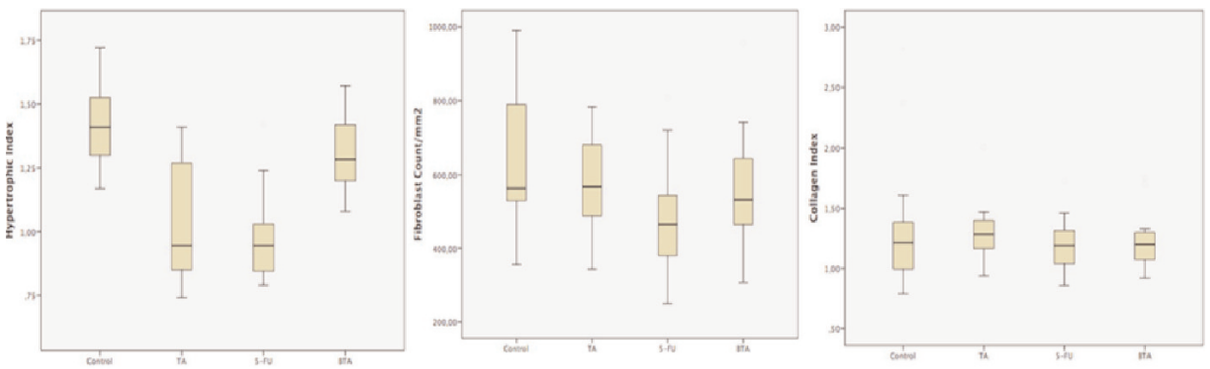


**Figure 3.** Comparison of treatment groups for hypertrophic index, fibroblast count and collagen index.

totic drug as an intralesional injection. In 1999, Fitzpatrick was the first to report the effectiveness of this agent for HSs either alone or in combinations[12]. Since then, several clinical studies were conducted to compare both agents in different settings[6,7]. More recently, some reports suggested the use of BTA for HSs and keloids[9,10]. However, others did not verify these favorable results of BTA for established scars[13,14]. Therefore, the efficacy of BTA on HS seems controversial.

Either alone or in combination with TA, 5-FU is reported to be at least comparable to TA with fewer side effects[6,7,15]. In a study by Manuskiatti and Fitzpatrick[6], intralesional TA alone, TA/5-FU combination, 5-FU alone and pulsed-dye laser were evaluated and clinical improvements were statistically comparable. However, more adverse reactions were observed in the steroid-treated patients. Similarly, Darougheh et al[15] reported that the overall efficacy of TA+5-FU was comparable with TA, but the TA+5-FU combination was more acceptable to the patients. In another study by Davison et al[7] 5-FU/steroid combination with excision was superior to steroid injection with excision treatment (92% *vs*. 73%) retrospectively. Differences in complication rates were not statistically significant between these treatments. According to HI scores in our study, TA and 5-FU were comparatively effective, whereas BTA was not effective on hypertrophic scars. Our findings are consistent with the previous studies reporting similar efficacy with TA and 5-FU. However, we did not observe beneficial effects of BTA on HSs, as did Gauglitz et al[13] and on contrary to studies by Xiao et al[9] and Zhibo and Miaobo[10].

Mechanisms of action of intralesional therapeutics for HSs are generally accepted to be due to decreasing collagen and glycosaminoglycan synthesis and fibroblast proliferation[5,8,16]. We also evaluated histological differences with these treatments in relation to fibroblast count and collagen index. In regard to fibroblast count in our study, only 5-FU treatment decreased fibroblast density, but there was no statistical difference in TA and BTA groups compared to control. In addition, we did not determine any difference between treatment groups according to CI scores.

Despite general acceptance of steroids to decrease fibroblast proliferation[8,12,17], Carroll et al[18] stated that TA did not alter the proliferation of fibroblasts, but increases the production of bFGF and decreases the production of TGF-β1. Teot and Roques[16] suggested that the inhibition of fibroblast proliferation by corticosteroids may be dose dependent and may not be observed in lower concentrations.

5-FU's mechanism of action on excessive scarring is also attributed to antimetabolite activity on rapidly proliferating fibroblasts[5,8,11,19]. In a study by Hendricks et al[20], 5-FU did not reduce fibroblast proliferation and collagen synthesis for cultured skin fibroblasts. However, if the fibroblasts were cultured under stimulated conditions with the presence of TGF-β as in excessive scarring, collagen synthesis was significantly inhibited by 5-FU. In another study, Huang et al[8] evaluated the effects of TA, 5-FU and their combination on keloid fibroblasts. In that study, even though TA alone significantly suppressed fibroblast proliferation, it did not induce apoptosis. The authors stated a greater inhibition in cell proliferation induced by TA/5-FU combination when compared to TA alone but a comparable efficacy to 5-FU alone in the long term. Our findings are consistent with that study indicating a decrease in the fibroblast density with 5-FU treatment.

Favorable outcomes reported in clinical practice with BTA[9,10] has prompted the *in vitro* studies with this agent. BTA is stated to be effective in inhibiting fibroblast proliferation and TGF-β1 expression[21,22]. In another study[23] the authors verified the effects of BTA to inhibit collagen deposition on HS rabbit model. In comparison to control group, collagen fibers were observed to be orderly arranged and thinner. However other studies did not support the above stated beneficial effects of BTA on excessive scarring. Gauglitz et al[13] reported that clinical improvement for the keloid tissue was not noted. Collagen synthesis, TGF-β and other ECM markers studied was not different from the control group. In addition, cellular metabolism and proliferation of fibroblast were not affected. In another study, Haubner et al[14] evaluated microvascular endothelial cells in addition to fibroblasts to display metabolic modifications of scar tissue in response to BTA. However, neither cell proliferation nor cytokines and growth factors were affected. We did not determine any differences in the BTA group from control wounds in parallel with the studies by Gauglitz et al[13] and Haubner et al[14].

However, BTA should not be disregarded in the treatment of scars. Mechanical force reduction during wound healing is very important to prevent excessive scarring. BTA has the potential to reduce tensile strength across the wound due to its local paralyzing effect. Favorable results of BTA in conjunction with primary closure have been previously reported by Gassner et al[24,25]. But, in order to overcome the negative impacts of BTA related to wound size enlargement, combining BTA with wound closure is essential.

## Conclusions

The intralesional TA and 5-FU injections are comparatively effective as monotherapy for HSs and 5-FU was the only agent reducing the fibroblast density in our study. However, BTA was ineffective for established HSs for all evaluated parameters. Molecular mechanisms affecting the obtained outcomes with these agents were not clarified in this study and further studies are needed.

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### Conflict of Interest

The Authors declare that they have no conflict of interests.

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