Effects of Intracordal Estradiol and Dexamethasone Injection on Wound Healing in Vocal Fold Injuries

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Summary: Objective. The aim of this study was to investigate the effects of intracordal estradiol and dexamethasone injection on wound healing in vocal fold injuries.
Study Design. A prospective controlled animal study was carried out.
Setting. This study was conducted at a tertiary center.
Subjects-Methods. Ten rabbits were randomly divided into two groups. As surgical procedure, cordotomy technique was performed in the middle third of the vocal folds bilaterally. In the first group, 0.1 mL of dexamethasone was injected into the right side, and 0.1 mL of saline was injected into the left side. In the second group, 0.1 mL of estradiol was injected into the right side, and 0.1 mL of saline was injected into the left side. Animals were sacrificed after 1 month and laryngeal specimens were evaluated histopathologically.
Results. No statistically significant difference was observed in terms of inflammatory response, epithelial thickness, type I and III collagen, and hyaluronic acid parameters in dexamethasone and estradiol injections compared to the saline injection. In terms of elastin level, estradiol injection demonstrated statistically higher values compared to the saline injection. Elastin level of dexamethasone injected vocal folds was not statistically different compared to the saline injection. No significant differences were observed in terms of inflammatory response, epithelial thickness, type I and III collagen, and hyaluronic acid parameters between the estradiol and dexamethasone injected vocal folds.
Conclusion. It is thought that the effects of estradiol or dexamethasone injections may have similar effects on wound healing in vocal fold injuries. Intracordal estradiol injection has positive effects on tissue elastin levels.
Key Words: Dexamethasone−Estradiol−Injection−Rabbit−Vocal fold scarring.

INTRODUCTION

With the advances in phonosurgery, many types of vocal fold (VF) pathology have become curable. However, the most important factor affecting the functional consequence is the development of the scar tissue.[1] The main reasons of VF scarring are trauma, radiation, iatrogenic reasons, infection, and inflammations secondary to airway injuries.[1,2] The most common reason of dysphonia observed following phonosurgery is VF scarring.[2]

VF scarring is characterized by fibrosis of the epithelium and lamina propria.[3] In VF injuries, various extracellular matrix (ECM) components are synthesized to form the tissue structure.[4] In VF scar tissues, increase in the density of collagen, decrease in the density of elastin, and reduction in the density of hyaluronic acid (HA) have been observed.[5,6] Changes in the ECM components disrupt the normal mucosal wave formation and cause a permanent deterioration in VF function.[5] To optimally regenerate the wounded VF, it is important to understand changes in ECM synthesis as a result of injury and to develop therapies that address these changes. Many treatments have been developed, including surgical implantation, speech therapy, or injection of various substrates such as autologous fat, fascia, collagen, HA, hydroxyapatite, and platelet-rich plasma.[7,8] However, the effectiveness of these treatments is usually limited because these approaches do not restore a normal distribution of ECM components.

In laryngeal surgery, corticosteroids are widely used to prevent formation of scar tissue and enable a better voice quality.[9] Corticosteroids have significant anti-inflammatory and immune modulator effects. During the inflammatory processes, they inactivate multiple inflammatory genes encoding cytokines, chemokines, adhesion molecules, inflammatory enzymes, receptors and proteins, and form anti-inflammatory effects.[9] In addition, they suppress the fibroblast functions and impede the wound-healing pattern.[9] They also inhibit collagen synthesis induced by transforming growth factor-beta and suppress the enzymes related to ECM cycle.[10]

Estrogen, which is a steroid hormone, acts as a key regulator in target tissues during the wound-healing process.[11] Estrogens have a protective effect on wound healing, and these effects are mediated with G protein-coupled estrogen receptor 1 (GPER1).[12,13] Through this receptor, the estrogen creates rapid signal responses and regulates the inflammatory response.[14] During the wound-healing process; estrogens inhibit the production of inflammatory cytokines and chemokines, and reduce the expression of Toll-like receptor.[15,16] They suppress the fibroblast functions and arrange ECM components to maintain the structural frame.[17,18]

Development of preventive and treatment options for VF scarring is a commonly investigated subject. No currently

Accepted for publication January 23, 2018.
This article was presented at the 38th National Otolaryngology Congress, October 26−30, 2016, Antalya, Turkey, and won the first prize.
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Journal of Voice, Vol. 33, No. 5, pp. 759−766
0892-1997

https://doi.org/10.1016/j.jvoice.2018.01.020

available therapeutic molecule is effective in the treatment of VF scarring. Despite the fact that the beneficial effects of corticosteroids on VF wound healing were previously demonstrated, there were possible complications.[19] Although estrogen has beneficial effects on various tissue injuries, its effects on VF scarring are unknown. In this study, we aimed to investigate histologic changes of VFs and to analyze the possible complications after VF dexamethasone and estradiol injections in a rabbit model.

MATERIALS AND METHODS

The study was approved by the Animal Research Review Committee of Bezmi Alem University School of Medicine, Istanbul, Turkey (July 17, 2014; No. 147). The principles of the Declaration of Helsinki for laboratory animals were followed.

All animals were cared for under a protocol approved by our institutional animal care group, which was in accordance with the experimental ethical principles and animal protection laws according to the rules and regulations in Turkey. A total of 10 adult male New Zealand rabbits, weighing 3−4 kg, were included in the study conducted between September 2015 and March 2016.

Surgical technique

The surgical procedure was performed on all rabbits by the same surgeon. Animals were anesthetized with intramuscular ketamine hydrochloride (40 mg/kg), xylazine hydrochloride (5 mg/kg), and then they were placed on the surgical table in supine position with the front limbs parallel to the body. The larynx was visualized by pediatric laryngoscope with a 75-mm premature size laryngeal blade (Miller blade size 0, San Antonio, TX). VF intervention was performed under visual guidance using a 4-mm, 30-degree Hopkins telescope (Karl Storz, Tuttlingen, Germany). The instrument (Bahadir Medical, Istanbul, Turkey) used to produce the surgical injury was a spear-shaped knife (2-mm wide, 2-mm deep at its central end, and 1-mm deep at its borders) with a transverse shield, which restricted the depth of penetration into the VF to 2 mm in the center of the lesion. A puncture wound was made with the surgical knife in the upper surface of each VF in the midpoint of its membranous portion. The dimensions of the injury were standardized and were similar to the tip of the knife. The rabbits were randomly divided into two groups. A 0.1-mL dexamethasone (Dekort 4 mg/mL, Deva Holding, Istanbul, Turkey) was injected into the right VF (lateral to incision) of the first group, and 0.1-mL estradiol (Progynon Depot 10 mg/mL, Bayer, Cadila Healthcare, Gujarat, India) was injected into right the VF (lateral to incision) of the second group by a 27-gauge needle. Left VFs of both groups were subjected to the same surgical operation and 0.1 mL of saline injection was administered, lateral to the incision. Each rabbit was euthanized via potassium chloride injection after 1 month. Laryngectomy was performed in all animals and VFs were removed.

Pathologic examination

Hematoxylin-eosin (H&E) staining was used for evaluation of the inflammatory response and epithelial thickness (Figure 1). Changes in type I collagen, type III collagen, elastin, and HA were examined with the immunohistochemical method (Figure 2). One pathologist observed the tissue sections by light microscope in a blinded fashion. Primarily, dexamethasone and estradiol that were injected into the right VFs of the first and second group were compared to saline injected into the left VFs of the corresponding group. Next, dexamethasone and estradiol injected into the right VFs of the two groups were compared.

(a) H&E Staining

The larynx was removed from each rabbit and then fixed in 10% buffered formaldehyde solution and embedded in paraffin. The specimens were sectioned in the coronal plane at 4- to 5-μm thickness and stained with H&E for assessing inflammatory response and epithelial thickness. A semiquantitative staging system, ranging from 1 to 4, was used for comparison of damaged and undamaged parts of the

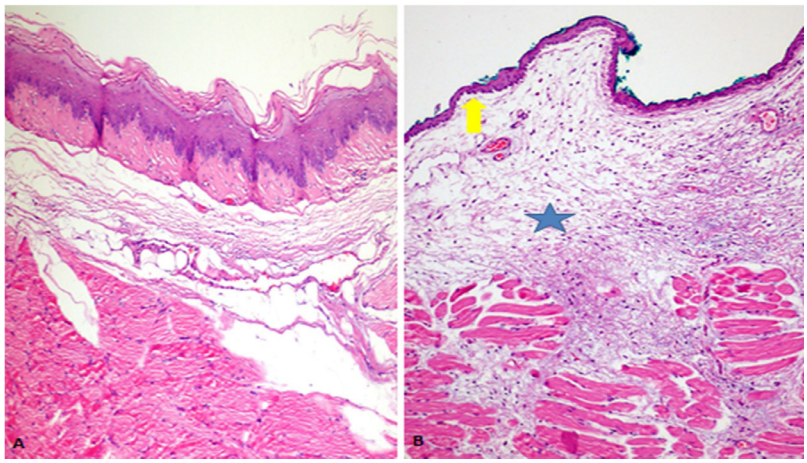

FIGURE 1. **A.** Normal vocal fold. **B.** Scarred vocal fold. Increase in the inflammatory response (blue star) and thinning of the epithelial layer (yellow arrow) are observed (H&E ×100). H&E, hematoxylin and eosin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

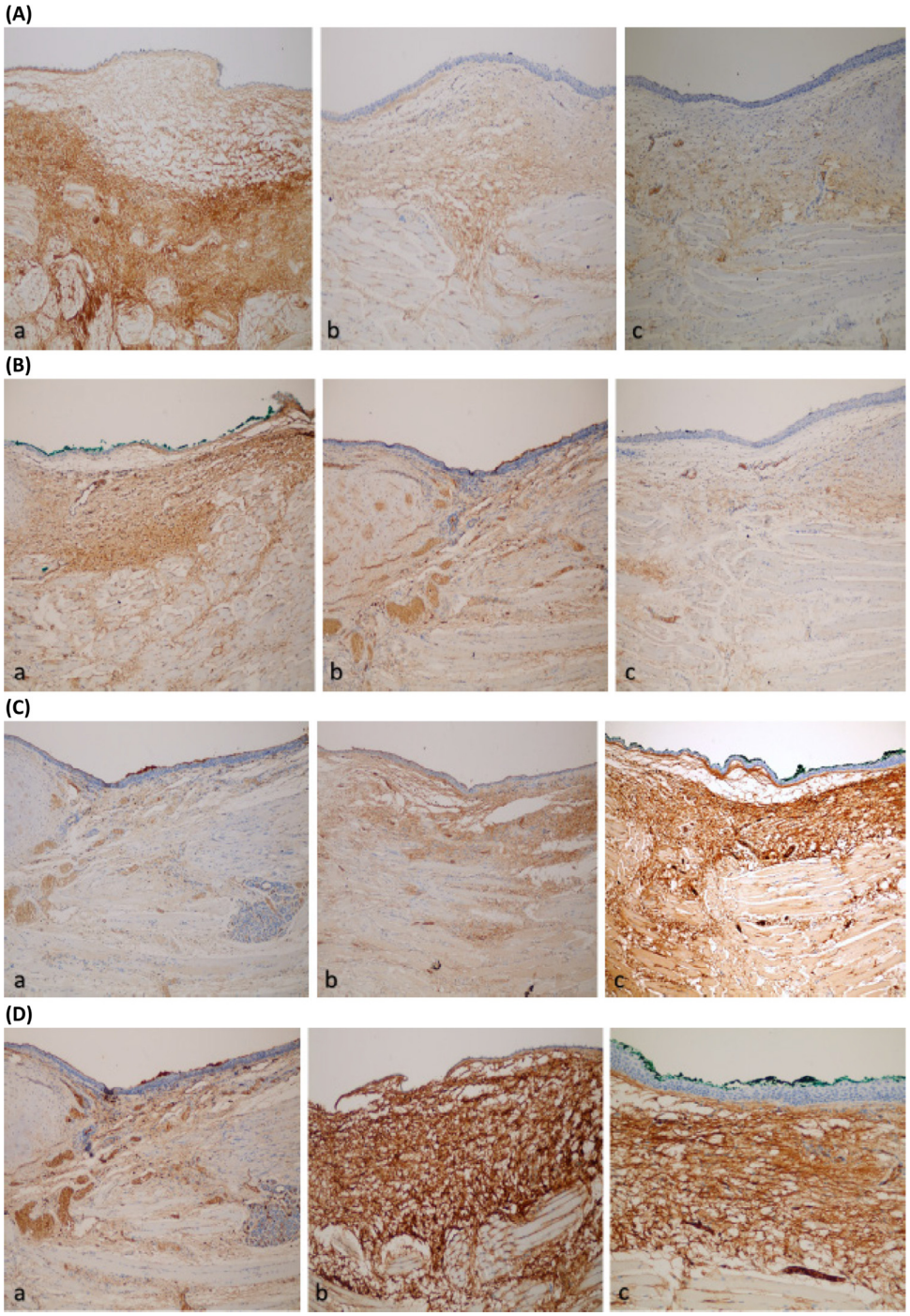

FIGURE 2. Immunohistochemically stained coronal section of the (**a**) saline-injected vocal folds, (**b**) dexamethasone-injected vocal folds, and (**c**) estradiol-injected vocal folds (×100). (**A**) Collagen type I, (**B**) collagen type III, (**C**) elastin, and (**D**) hyaluronic acid.

VF (1 = same, 2 = slight difference, 3 = intermediate difference, and 4 = severe difference).

(b) Immunohistochemical Staining

The VFs were fixed in 10% formalin then embedded in paraffin for sectioning. Successive 4- to 5-μm sections of the VF specimens were prepared. For immunohistochemical examination, an antigen retrieval procedure was performed by incubating the slides at 98°C for 15 minutes with a 0.01-M citric acid solution. After blocking for 1 hour at room temperature (5% normal goat serum and 0.1% Triton-X in phosphate buffered saline), sections were incubated in primary antibody solution at 20°C overnight. After being washed, sections were incubated for 1 hour at room temperature with secondary antibodies and 4′,6′-diamidino-2-phenylindole dihydrochloride for nuclear staining. The primary antibodies used in this study were as follows: mouse anti-collagen type I antibody (ab90395; Abcam, Cambridge, MA) at 1:2000; mouse anti-collagen type III antibody (ab6310; Abcam) at 1:4000; mouse anti-elastin antibody

TABLE 1.
The Immunoreactive Score (IRS)

A (Percentage of Positive Cells)	B (Intensity of Staining)	IRS Score (Multiplication of A and B)
0 = No positive cells	0 = No color reaction	0–1 = 0 (Negative)
1 = <10% of positive cells	1 = Mild reaction	2–3 = 1+ (Mild)
2 = 10–50% positive cells	2 = Moderate reaction	4–8 = 2+ (Moderate)
3 = 51–80% positive cells	3 = Intense reaction	9–12 = 3+ (Strongly positive)
4 = >80% positive cells	Final IRS score (A × B): 0–12	

(ab6519; Abcam) at 1:200; and sheep anti-HA antibody (ab53842; Abcam) at 1:300. The secondary antibodies used in this study were as follows: goat anti-mouse immunoglobulin G (ab205719; Abcam) at 1:10,000 and donkey anti-sheep immunoglobulin G (ab97123; Abcam) at 1:500. Primary and secondary antibodies were diluted in 1% normal goat serum and 0.1% Triton-X in phosphate buffered saline. Negative controls, for which samples were exposed to the secondary antibody in the absence of the primary antibody, revealed no immunostain.

The evaluation of the immunohistochemical staining was performed by using Remmeles immunoreactivity score such that the extent (percentage of positive cells) and the intensity of color reaction were taken into account.[20,21] The extent of the immunoreactivity was scored as 0 for absence of the positive cells, +1 for less than 10% of positive cells, +2 for 11%–50% positive cells, +3 for 51%–80% of positive cells, and +4 for more than 80% of positive cells. The intensity of the immunoreactivity was scored as 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. The final score of the staining for each case was obtained by multiplying the extent of the immunoreactivity score with the intensity score. Cases with final score 0 were considered negative, with final score 1–3 were considered weakly positive, with final score 4–8 were considered moderately positive, and with final score 9–12 were considered strongly positive (Table 1).

Statistical analysis

Statistical analysis was made using the SPSS 20.0 for Windows program (SPSS, IBM Corp, Armonk, NY). Average, standard deviation, median, and the lowest and highest values were used for the descriptive statistics of the data. Range of the variables was measured by Kolmogorov-Smirnov test. For the analysis of independent groups, Mann-Whitney *U* test was used. Analyses of the dependent variables were made by Wilcoxon test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

First (dexamethasone) group

In terms of inflammatory response, epithelial thickness, type I and type III collagen, elastin, and HA parameters, no statistically significant difference was observed in dexamethasone-injected VFs, compared to saline injection ($P = 0.083$, $P = 1.0$, $P = 0.450$, $P = 0.317$, $P = 0.083$, $P = 1.0$, and $P = 0.317$) (Table 2, Figure 3).

Second (estradiol) group

In terms of inflammatory response, epithelial thickness, type I and type III collagen, and HA parameters, no statistically significant difference was observed in estradiol-injected VFs, compared to saline injection ($P = 0.102$, $P = 0.317$, $P = 1.0$, $P = 0.317$, $P = 0.102$, and $P = 0.157$) (Table 3, Figure 4). In terms of elastin level, estradiol injection demonstrated statistically higher values compared to saline injection ($P = 0.046$) (Table 3, Figure 4).

Comparing dexamethasone and estradiol groups

In terms of inflammatory response, epithelial thickness, type I and type III collagen, elastin, and HA parameters, no statistically significant difference was observed in dexamethasone-injected VFs, compared to estradiol injection ($P = 0.050$,

TABLE 2.
Comparison of Collagen Type I, Collagen Type III, Elastin, Hyaluronic Acid, Inflammatory Response, and Epithelial Thickness in Groups Subject to Saline or Dexamethasone Injection

	Saline		Dexamethasone		
	Mean ± SD	Med (Min–Max)	Mean ± SD	Med (Min–Max)	*P*
Collagen I	3.0 ± 0.0	3.0 (3.0–3.0)	2.8 ± 0.4	3.0 (2.0–3.0)	0.317
Collagen III	3.0 ± 0.0	3.0 (3.0–3.0)	2.4 ± 0.5	2.0 (2.0–3.0)	0.083
Elastin	2.6 ± 0.5	3.0 (2.0–3.0)	2.6 ± 0.5	3.0 (2.0–3.0)	1.000
Hyaluronic acid	2.4 ± 0.5	2.0 (2.0–3.0)	2.8 ± 0.4	3.0 (2.0–3.0)	0.317
Inflammatory response	1.6 ± 0.5	2.0 (1.0–2.0)	1.0 ± 0.0	1.0 (1.0–1.0)	0.083
Epithelial thickness	1.4 ± 0.9	1.0 (1.0–3.0)	2.0 ± 1.0	2.0 (1.0–3.0)	0.450

Wilcoxon test.
Abbreviations: SD, standard deviation; Med, median; Min, minimum; Max, maximum.

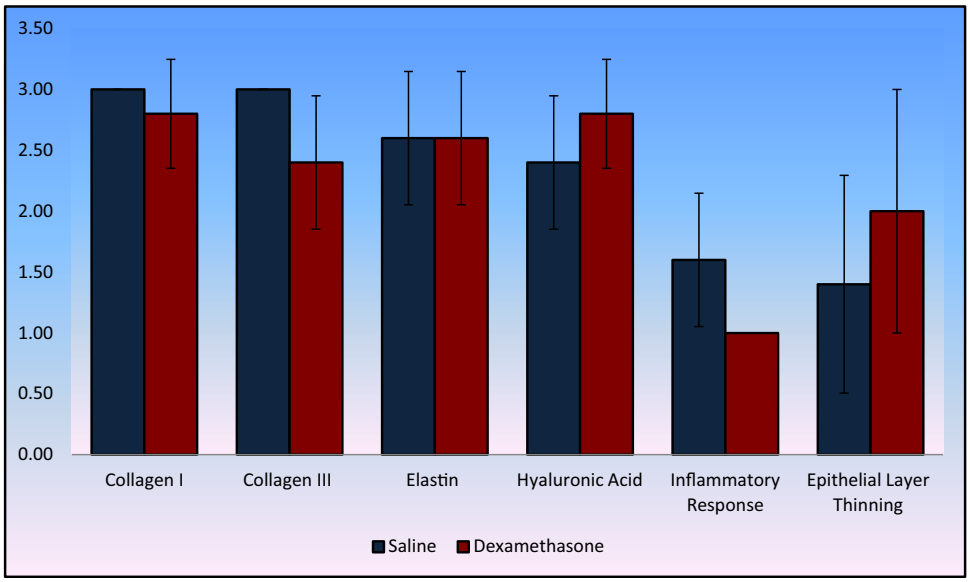

FIGURE 3. Comparison of collagen type I, collagen type III, elastin, hyaluronic acid, inflammatory response, and epithelial thickness in groups subject to saline or dexamethasone injection.

$P = 0.155$, $P = 0.513$, $P = 0.729$, $P = 0.549$, and $P = 1.0$) (Table 4, Figure 5).

DISCUSSION

VFs have a specific structure, based on a specific distribution of the various cellular population and the different constituents of the ECM.[22] The ECM of the VF lamina propria includes fibrous proteins and interstitial constituents.[23] Collagen and elastin are two of the fibrous proteins found in the VF lamina propria, which contribute to tissue strength and flexibility.[24,25] The interstitial constituents of the VF lamina propria consist of glycosaminoglycans that occupy the spaces between fibrous proteins. HA is the most important glycosaminoglycan, which plays an important role in creating the optimal tissue biomechanical properties and contributing to tissue viscosity.[26]

When there is a trauma on the VF, a high-intensity inflammatory process takes place to restore the structure and the function of the VF. After the intense inflammatory response occurs, the healing process can lead to the formation of a scar tissue.[1] This scar tissue mostly located in the lamina propria of the VF adds great stiffness and interferes with the normal vibratory properties of the VF by losing the structure of the lamina propria.[5] Scarring is characterized by alterations in the organization and distribution of ECM components.[27] In VF scarring models, Procollagen I (a precursor to collagen) was significantly increased, elastin was significantly decreased, and no differences in density of HA in scarred samples during an early (2 months postoperatively) phase of VF wound repair were found.[5] Elastin density was significantly decreased, procollagen I and HA density were similar in scarred samples compared to control samples during a later (6 months postoperatively) phase of VF wound repair.[28] As a result, it has been shown that reducing collagen and increasing elastin and HA in the early phase of VF wound repair have positive effects on VF wound healing.[5,28]

Corticosteroids have potent anti-inflammatory and antifibrotic properties. They are frequently used in laryngeal microsurgery of the VFs to prevent scar formation and, consequently, to ensure better voice quality.[29] Corticosteroids delay the healing process, permitting a better organization of

TABLE 3.
Comparison of Collagen Type I, Collagen Type III, Elastin, Hyaluronic Acid, Inflammatory Response, and Epithelial Thickness in Groups Subject to Saline or Estradiol Injection

	Saline		Estradiol		
	Mean ± SD	Med (Min–Max)	Mean ± SD	Med (Min–Max)	*P*
Collagen I	2.8 ± 0.4	3.0 (2.0–3.0)	2.6 ± 0.5	3.0 (2.0–3.0)	0.317
Collagen III	3.0 ± 0.0	3.0 (3.0–3.0)	2.2 ± 0.8	2.0 (1.0–3.0)	0.102
Elastin	1.6 ± 0.5	2.0 (1.0–2.0)	2.4 ± 0.5	2.0 (2.0–3.0)	**0.046**
Hyaluronic acid	2.4 ± 0.5	2.0 (2.0–3.0)	2.8 ± 0.4	3.0 (2.0–3.0)	0.157
Inflammatory response	2.6 ± 0.9	2.0 (2.0–4.0)	1.6 ± 0.5	2.0 (1.0–2.0)	0.102
Epithelial thickness	2.8 ± 0.8	3.0 (2.0–4.0)	2.8 ± 0.4	3.0 (2.0–3.0)	1.000

Wilcoxon test.
P value in bold indicates statistical significance.
Abbreviations: SD, standard deviation; Med, median; min, minimum; max, maximum.

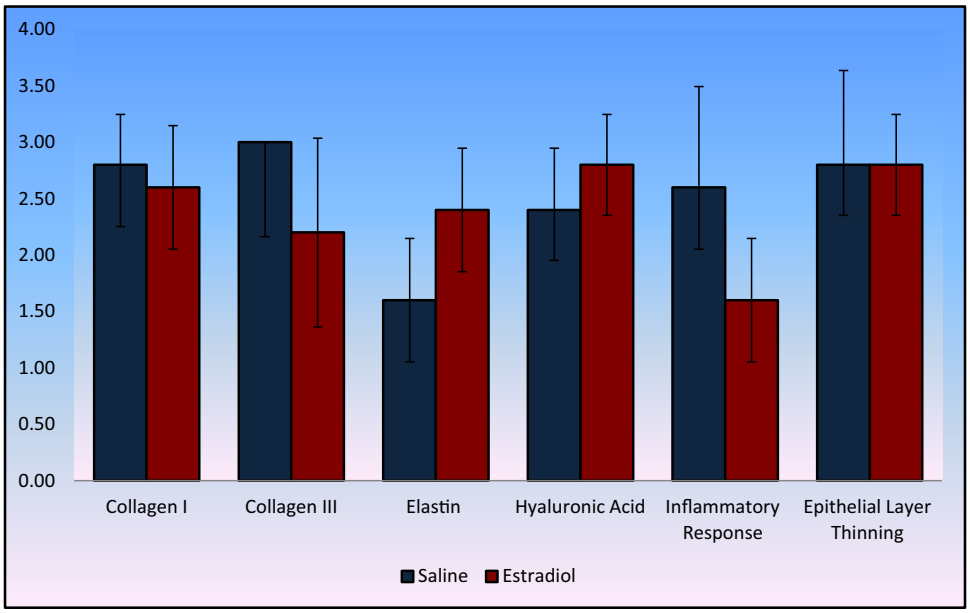

FIGURE 4. Comparison of collagen type I, collagen type III, elastin, hyaluronic acid, inflammatory response, and epithelial thickness in groups subject to saline or estradiol injection.

scar tissue.[9] Campagnolo et al demonstrated that dexamethasone resulted in reduced accumulation of collagen during the acute (3−7 days postoperatively) inflammatory phase of rabbit VF damage.[30] In another study, there was no difference in collagen deposition between the triamcinolone acetonide and saline injections into rabbit VF from 2 to 12 weeks postoperatively.[19] Our study is important in terms of being the first to immunohistochemically examine the scar-healing parameters following dexamethasone injection into the VFs. In the present study, no statistically significant differences were observed in terms of type I and type III collagen, elastin, and HA levels between the dexamethasone group compared to saline injection.

Estrogens create rapid signal responses and regulate the inflammatory response through GPER1.[14] It was observed that GPER1 receptors and estrogens reduced the level of myocardial inflammation in a heart ischemia-reperfusion model in rats and also reduced the magnitude of the damage in the myocardium.[18] The trauma-hemorrhage model conducted on rats showed that the estrogens had a protective effect on hepatic injuries and that these effects were mediated with G protein related receptors.[31] The GPER1 receptor analogue model conducted on rats showed that the estrogen analogues reduced both the inflammatory cytokines and also the magnitude of infarction after experimental stroke.[32]

GPER1 receptor was detected in many tissues and recently demonstrated in the VF.[33] In our study, we applied estradiol injection for VF injury for the first time in the literature, and the tissue elastin level was found significantly higher in the estradiol group compared to saline injection; no statistically significant differences were observed in terms of type I and type III collagen, HA, and inflammatory response levels between the estradiol group compared to saline injection. Elastin, which is highly concentrated in the lamina propria of the normal VF, is central to the viscoelasticity of the VF.[23,25] It has been suggested that the loss of normal tissue elasticity might be related to decreased elastin fibers.[5,6,25] Findings are consistent with research that has shown a progressive quantitative decline in the amount of elastin from atrophic to normal scars.[34] Therefore, we conclude that a higher level of elastin in

TABLE 4.
Comparison of Collagen Type I, Collagen Type III, Elastin, Hyaluronic Acid, Inflammatory Response, and Epithelial Thickness in Groups Subject to Dexamethasone or Estradiol Injection

	Dexamethasone		Estradiol		
	Mean ± SD	Med (Min−Max)	Mean ± SD	Med (Min−Max)	*P*
Collagen I	2.8 ± 0.4	3.0 (2.0−3.0)	2.6 ± 0.5	3.0 (2.0−3.0)	0.513
Collagen III	2.4 ± 0.5	2.0 (2.0−3.0)	2.2 ± 0.8	2.0 (1.0−3.0)	0.729
Elastin	2.6 ± 0.5	3.0 (2.0−3.0)	2.4 ± 0.5	2.0 (2.0−3.0)	0.549
Hyaluronic acid	2.8 ± 0.4	3.0 (2.0−3.0)	2.8 ± 0.4	3.0 (2.0−3.0)	1.000
Inflammatory response	1.0 ± 0.0	1.0 (1.0−1.0)	1.6 ± 0.5	2.0 (1.0−2.0)	0.050
Epithelial thickness	2.0 ± 1.0	2.0 (1.0−3.0)	2.8 ± 0.4	3.0 (2.0−3.0)	0.155

Mann-Whitney *U* test.
Abbreviations: SD, standard deviation; Med, median; min, minimum; max, maximum.

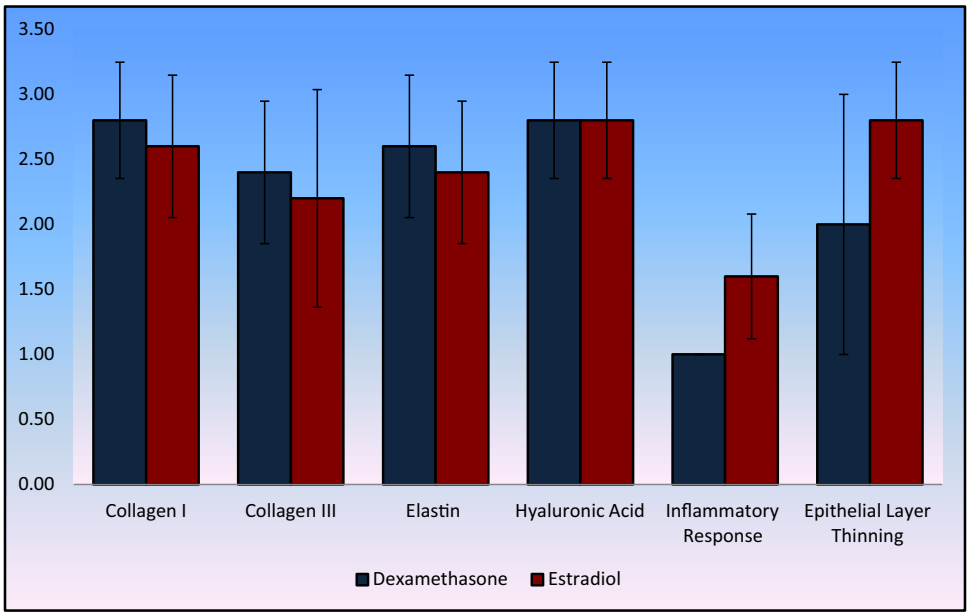

FIGURE 5. Comparison of collagen type I, collagen type III, elastin, hyaluronic acid, inflammatory response, and epithelial thickness in groups subject to dexamethasone or estradiol injection.

the estradiol group may have positive effects on VF wound healing.

Mukudai et al demonstrated the expression levels of ECM genes in cell culture from the VF after steroid hormone administration.[35] In this study, mRNA levels for procollagen I, procollagen III, elastin, and Hyaluronic Acid Synthetase I (HAS I) were examined by quantitative real-time polymerase chain reaction 48 hours after estradiol or corticosteroid administration. Procollagen I-III, elastin, and HAS I mRNA were significantly reduced in cell cultures from the VF after estradiol administration. Similarly, Procollagen I-III, elastin, and HAS I mRNA was significantly reduced in cell cultures from the VF after corticosteroid administration. That study emphasized that effects of estradiol and corticosteroid at the cellular level were similar. In our study, we applied estradiol and corticosteroid injection for VF injury and compared effects of those molecules on tissue. Similarly, no statistically significant differences were observed in terms of type 1−3 collagen, elastin, and HA levels between the dexamethasone group compared to estradiol injection in our study. When these findings are considered, it is thought that the antifibrotic characteristics of both estradiol and dexamethasone injections applied in VF damages might be similar.

Residual inflammation is one of the most common causes of VF scarring; all of the surgical procedures are aggressive and trigger local inflammation. Both steroid and estrogen effects are multifactorial, which have anti-inflammatory effects for efficient wound repair. Campagnolo et al demonstrated that there was no quantitative difference in the inflammatory response between VFs injected with the dexamethasone and control VFs during the acute inflammatory phase of rabbit VF damage.[30] Jin et al demonstrated that there was no difference in the inflammatory response between the triamcinolone acetonide and saline injections into rabbit VF from 2 to 12 weeks postoperatively.[19] In the present study, no statistically significant differences were observed in the inflammatory response between the estradiol or dexamethasone injected VFs compared to saline injection. Estrogen and corticosteroids, with the potential for anti-inflammatory effects in different tissues, consist of complex mechanisms. To examine the anti-inflammatory effects of corticosteroids and estradiol against VF scarring in detail, further studies are required.

It was previously demonstrated that a certain amount of epithelial thinning might be observed in the VFs of rabbits after triamcinolone injection from 2 to 12 weeks.[19] In our study, the parameters of epithelial thickness were examined, and no statistically significant difference was observed between the estradiol- or dexamethasone-injected VFs compared to saline injection.

In laryngeal surgery, corticosteroids are widely used to prevent formation of scar tissue and enable a better voice quality.[36] A meta-analysis demonstrated significant improvements after intracordal steroid injection for benign VF disorders from both objective and subjective measurements.[36] The use of intracordal corticosteroids has been shown to be beneficial in clinical outcomes, although the efficacy of preventing VF scarring has not yet been demonstrated histologically and immunohistochemically in this study and other related studies. Intranasal form of estradiol has been shown to improve the voice quality in surgically induced menopausal women.[37] The estradiol not only has positive effects on wound healing in various tissues, but it also increases VF elastin levels, so intracordal estradiol injection may be used as a new treatment modality in the prevention of VF scarring.

This study has several limitations. Our main limitation was the small number of subjects enrolled, and the scoring systems used to judge treatment differences were simple, semiquantitative, and subjective. In this study, we evaluated the effects of estradiol and dexamethasone on wound healing of VF injuries at 1 month after injury. Although that was a reasonable time to

evaluate the wound healing, it was not representative of the entire healing process. Further studies are required to investigate the short- and long-term effects of dexamethasone or estradiol injections on the viscoelastic characteristics and the changes in the histopathologic structure of the VF. It would be more effective if further studies were conducted using different doses and dose ranges of estradiol and dexamethasone in local applications. Additional studies including rheologic measurements and biomechanical tests are needed to demonstrate the effects of histologic changes at the tissue level on the phonation.

CONCLUSION

In this study, we applied intracordal estradiol and dexamethasone injection in VF injuries without causing any significant side effects and we did not find any significant difference in wound-healing parameters (except elastin) compared to saline-administered controls. We conclude that the effects of injectable estradiol and dexamethasone might be similar on wound healing in VF injuries. Intracordal estradiol injection increases the level of elastin during the VF wound healing but still requires further investigation to achieve stronger conclusion to further consider clinical applications.

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