

Experimental Study of Injetable Corticoid in Laryngeal Microsurgery

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SUMMARY

- Objective:** Evaluate the presence of synechiae and comparatively quantify collagen fibers deposition in vocal folds that underwent excision of the mucosa fragment with cold instrument, with or without using local injected corticoid.
- Type:** Controlled experimental.
- Method:** 12 larger white pigs were sedated and underwent excision of mucosa fragment of right vocal fold free edge with posterior division in two groups: control, which did not receive injected corticoid, with surgical procedure in right vocal fold; experimental, animals with corticoid injection in right vocal fold before surgical procedure. Thirty days after the experiment, the animals underwent euthanasia to collect samples of vocal folds and to stain using the picosirius red technique with polarization to quantify the total collagen deposition.
- Results:** The presence of synechiae in the anterior third of the vocal folds post-surgery was not observed in the studied group. In the control group, the average area of collagen deposition in left vocal folds without surgery was 3,116.33 square micrometers and in right vocal folds was of 2,353.28 square micrometers. In the experimental group, the average of left vocal folds without surgery was 3,526.05 square micrometers and in the right vocal folds with surgery and corticoid was 2,167.92 square micrometers. The corticoid injected in the vocal fold that underwent surgery did not show decrease in collagen deposition ($p=0.1320$).
- Conclusion:** Synechiae were not observed in experimental model. Using injected corticoid in vocal folds lamina propria has promoted a non-significant decrease of collagen deposition, when compared to the control group with surgery and without corticoid.
- Key words:** adrenal cortex hormones, healing, vocal fold, pigs.

INTRODUCTION

The lamina propria helps viscosity, contractility and formation of mucous wave of vocal fold. Its good performance is essential for the beginning and maintenance of vibration supported by Bernoulli's principle (1). Humans can produce sound within an ample band of frequency and intensity, varying in tone quality. To do that, they have to use their vocal folds, which hold histological and structure features that are suitable for phonation activity. From viewpoint of phonation, vocal fold forms a structure of multiple layers with different mechanical properties. Vocal folds do not work as a single structure vibrator like a musical instrument string, but as a vibrator of different multiple layers. Such laminar structure can be adjusted on frequency and intensity of human voice (2). FRIEDRICH et al (1993) support the idea the complexity of vocal fold, which is the base of understanding of human vocal capacity (3).

Microflap techniques were developed to help dissection and removal of mature cysts, polyps and nodules on the superficial layer of lamina propria while there is a preservation of adjacent normal mucosa layer and subjacent vocal ligament (4,5,6). It aims scars development and hardening with synechiae formation on mucosa covering that is responsible for damages on vocal fold vibration.

Clinical studies aiming to evaluate effects of injected corticoid use on lamina propria of vocal fold with the help of a fiberscope on larynx surgery under local anesthesia without causing injury on the structure reported its useful effect on therapy of phonotraumatic injury, such as vocal nodules and Reinke's oedema, leading to vocal quality improvement, reduction on phonotherapy and on injuries, in most of cases (7,8).

There is also improvement report on glottic closure and vocal quality after corticoid injection followed by microflap technique (9). However, a study on dogs, did not relate corticoid injection before microflap technique with scar reduction on experimental group, or did not reveal difference on vocal fold activity measured by videostroboscopy (10).

Scars on lamina propria layer of vocal folds produce an abnormal relation between body and mucous covering, affecting its sliding and then leading to a reduction on mucosa wave. In case scar gets worse, segment with no mucous wave gets greater and dysphonia gets inflamed. Some report this is the most common reason of dysphonia (35%) followed by endolarynx surgery (11).

Systemic or injected corticosteroids on vocal folds

are usually associated with laryngeal microflap in order to prevent scars, then assuring vocal quality.

Systemic corticosteroids delay cicatrisation leading to a better organization of scar tissue; therefore, their effects over vocal folds are unclear (10).

Other types of therapy have been under observation in order to adjust scar process on vocal fold after removing its mucous covering. Systemic or topic (12) corticosteroids, cooling of the vocal cord before the surgery, fibrin adhesive and other methods have been used in order to improve cicatrisation of mucous covering after phonosurgery procedure (13).

OBJECTIVE

Having revising the literature aimed the following:

- a. To macroscopically observe the presence of synechiae on vocal cords after mucous portion removal by using cold instrument and with no injected corticoid on vocal fold.
- b. To comparatively quantify total deposition of collagen fibers on pig vocal fold submitted to mucous portion removal with and without using injected corticotherapy.

METHOD

This study was carried at *Fazenda Experimental da Universidade Federal do Paraná – UFPR* (Experimental Farm of the Federal University) after being approved by the Research Ethics Committee of the *Hospital Angelina Caron* – protocol #008-2005. Then, it was followed the principles of the *Colégio Brasileiro de Experimentação Experimental-COBEA* (Brazilian Institution of Experiment) and recommendations of euthanasia for experimental animals recommended by Close and by Internacional Comitee on Veterinary Gross Anatomical Nomenclature.

12 large white pigs (*Suideo Sus*) were used for such experiment. There were seven males and five females, aging from 27 to 31 years, weighing between 8.7 Kg and 11.5 kg (average: 10.1kg), and divided into two equally numbered groups.

Control group underwent surgery on vocal fold with no injected corticoid. Surgery of mucous portion removal from the right vocal fold was performed with laryngeal scissors, by beginning from prehension of the mucosa of the anterior third of the freebord, of the right vocal fold with Bouchayer's tweezers (Microfrance®). Afterwards 2mm of mucosa was cut with laryngeal scissors to the left. Left vocal fold was not touched.

Graphic 1. Animal division in groups.

Groups	Corticoid Application	Vocal Fold and Procedure
Control Group (n = 6)	No Corticoid Injection Group - in Vocal Fold	Left Vocal Fold: with no surgery Right Vocal Fold. Removal of free edge fragment.
Experimental Group (n = 6)	Corticoid Application Group - Injected in Vocal Fold	Left Vocal Fold: with no surgery Right Vocal Fold. Removal of free edge fragment.

Experimental group underwent injection of 0.1ml of sodium phosphate salt form of dexamethasone by using a 23g Venescalp needle on each focal fold, guided by Bouchayer's tweezers (Microfrance®) before removal surgery of the mucous portion on right vocal fold. Venescalp was introduced in the anterior third of right and left vocal folds in their superficial layers of the lamina propria. Removal of mucous portion of right vocal fold was done with laryngeal scissors after corticoid injection with prehension of the mucosa from the anterior third of the freebord of right vocal fold, by using Bouchayer Microfrance® prehension tweezers. Afterwards, it was performed a 2mm cut of the apprehend mucosa with laryngeal scissors to the left. Mucous fragment was not removed from the left vocal fold, which was only corticoid injected.

Euthanasia was carried 30 days after surgery in the slaughterhouse at *Fazenda Experimental da UFPR*, followed by total larynx removal, so vocal folds could be analyzed. Macroscopic analysis of the glottis on the anterior third of vocal folds aimed researching presence and absence of postoperative synechiae; after that, a ventral longitudinal incision was performed in the larynx with #15 razor scapel to expose vocal fold, to analyze macroscopically the presence or absence of postoperative synechiae for the second time and another removal of mucous portion of vocal fold near thyroid cartilage.

The laminae analysis was done through Pro-image-plus 4.5® for Windows® in a Pentium IV® attached to a Olympus® BX50 camera and Sony® video (Picture 12), calibrated in micrometers in 20 times zoom lenses. Such program read picosirius red stained tissues, by starting from the analysis of microscopic features of tissues as epithelium and submucosa. The same program performed image polarization to analyze collagen deposition through tissue colors, and then measures were transferred to Windows Excel®.

Fibroplasy was also measured in square micrometers, which consists of collagen deposition on surface of mucosa from scar process.

Statistical analysis was done through paired comparison (right and left side) with the help of nonparametric Wilcoxon test. When comparing results from groups, parametric Mann-Whitney test was applied. In all comparisons, null hypothesis met the same results from paired groups and alternative hypothesis met different ones ($p \leq 0.05$).

RESULTS

Macroscopic analysis showed absent synechiae on the larynx mucous after surgery in both groups.

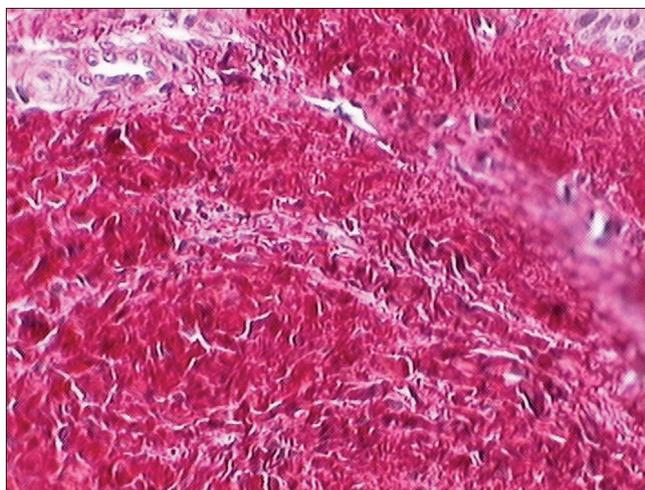
Once the image polarization is performed, the collagenous tissue which has been presently studied may be differentiated from the non-collagenous tissue, through the observation of the collagen birefringence and of its orange and red coloring, when compared to the non-collagenous substance which has a darken color (Picture 1 and 2).

In the control group, the deposition area average on submucosa of left vocal folds which were not submitted to surgery was 3116.33 square micrometers; and the one of the right vocal folds submitted to surgery was 2353.29 square micrometers (Table 1).

In the experimental group the same areas were 3526.05 square micrometers and 2167.92 square micrometers respectively (Table 1).

$P = 0.0464$ was found when comparing collagen deposition of control group with no injury X control group with injury, and that showed the difference between total quantity of collagen in the two groups was statistically significant (Graphic 2).

The comparison between control group with injury X experimental group with injury showed $P=0.0043$, also showing that total quantity of collagen on the vocal folds was statistically significant (Graphic 2). When comparing experimental group with no injury X control group with



Picture 1. Example of animal laminae (sample/E-D3) colored by Picrosirius Red technique showing conjunctive tissue of the lamina propria before polarization (400x larger).



Picture 2. Example of animal laminae (sample/E-D3) colored by Picrosirius Red technique showing conjunctive tissue of the lamina propria before polarization (400x larger).

Table I. Amount of deposition of collagen on submucosa of evaluated groups.

Groups	Vocal folds and Procedures	Deposition area of total collagen per square micrometers				
		Minimum	Maximum	Median	Average	Standard Deviation
Control Group (n = 6)	Vocal folds non-submitted to surgery without using Corticoid	2283.06	3684.88	3173.61	3116.33	542.10
	Vocal folds submitted to surgery without using Corticoid	2116.09	2587.57	2353.08	2353.29	164.13
Experimental Group (n = 6)	Vocal folds non-submitted to surgery with the use of Corticoid	3113.89	3067.24	2707.95	3526.05	283.49
	Vocal folds non-submitted to surgery with the use of Corticoid	1885.56	2350.84	2183.90	2167.92	159.39

injury, $P = 0.0065$; comparing experimental group with no injury X experimental group with injury, $p = 0.0277$, what showed statistical significance, or that, there was a difference between total collagen quantity in the two groups (Graphic 2).

DISCUSSION

Only one paper, during literature revision, has compared endolaryngeal surgery in animals. It compared

vocal folds in dogs, pigs and apes by observing quantity of collagen deposition, elastin fibers and amorphous tissues, concluding that pig's and dog's vocal folds present a larger amount of elastic fibers and collagen in profound layers of lamina propria, which is similar to histological features of human vocal folds, than ape's. Dissection process of microflap on dog's and pig's vocal folds are very similar to the human's (14).

It was also found two papers reporting the use of corticoid on vocal folds injuries. A clinical study reported

Graphic 2. Comparison between p values of the statistical tests.

Groups under Comparison			p Values
Control with no injury	versus	Experiment with no injury	0.8182
Control with no injury	versus	Control with injury	0.0464*
Control with no injury	versus	Experiment with injury	0.0043*
Experiment with no injury	versus	Control with injury	0.0065*
Experiment with no injury	versus	Experiment with injury	0.0277*
Control with injury	versus	Experiment with injury	0.1320
(Statistical Significant $p \leq 0.05$)			

improvement on glottic closure and on vocal quality after corticoid injection followed by microflap on vocal folds. And an experimental study on dogs, in which corticosteroids injection was used before microflap, concluded that, despite the fact that the corticoids delay scar process in both inflammatory infiltrate and neovascularization, and that did not cause quantity improvement at stroboscopic analysis of the larynx (9,10).

Systemic corticoids delay cicatrisation process by providing a better organization on scar tissue and they are also often used in association with laryngeal microflap in order to prevent scars, providing then a better vocal quality. There is still very little information on the use of injected corticoids on vocal fold in the literature (10).

This study reports less deposition of total collagen in the group operated with corticoid than in the one operated with no corticoid, though this difference was not expressive according to the literature (10).

An expressive difference could be seen when comparing groups regarding surgery with or without injury, which was observed in both groups the experimental and control ones. In the group with injury, there was a lesser deposition of total collagen.

The lack of statistical importance on total collagen decrease in the corticoid-operated group makes one aware of the need of more experimental studies with the purpose of evaluating the activity of such substances in scar areas of the vocal folds, however many clinical studies confirm its use by vocal fold improvement in patients with phonotraumatic injuries (vocal nodules, polyps and Reinke oedema), with no scar injuries (removable ones) (7,8).

CONCLUSION

Before the results from this study, one might conclude that:

- Presence of synechiae was not observed in experimental examples of mucosa fragment removal on the freebord with cold instrument with or without injected corticoid use on vocal fold at the macroscopic analysis.
- The use of injected corticoid on lamina propria of the vocal folds which were submitted to mucosa fragment removal with cold instrument provide a non-expressive difference of total collagen deposition on submucosa which was quantified by optical microscopy assisted by *Pro-image-plus 4.5*[®] program.

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