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# Histological Effects of Inhaled Corticosteroids and $\beta_2$ -agonists on Laryngeal Mucosa in an Allergic Rat Model

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## Abstract

**Objective.** To constitute an animal model of laryngeal allergy and evaluate the laryngeal effects of inhaled corticosteroids and  $\beta_2$ -agonists on the laryngeal mucosa in an allergic rat model.

**Study Design.** Prospective randomized.

**Setting.** The Experimental Medical Research Institute (DETAE) at Istanbul University.

**Subjects and Methods.** Wistar Albino rats ( $n = 32$ ) were sensitized with ovalbumin. Unsensitized rats ( $n = 8$ ) served as controls. The rats were exposed to aerosolized ovalbumin (1%). On days 28 through 42, every 2 days preceding ovalbumin exposure, rats were further exposed to aerosolized phosphate buffered saline ( $n = 8$ ), fluticasone propionate ( $n = 8$ ), salbutamol ( $n = 8$ ), and combined salbutamol+fluticasone propionate ( $n = 8$ ). Inflammatory cell infiltration was graded semi-quantitatively. The quantitative data included mast cell count and degranulation. Ultrathin sections were investigated under transmission electron microscope.

**Results.** The simultaneous and pairwise comparison of groups (Kruskal-Wallis) revealed statistically significant difference among groups at supraglottic level (critical  $P < .05$ ,  $< .01$ ) and no difference at glottic level. In ovalbumin+phosphate buffered saline exposed rats, the light microscopy of supraglottic mucosa revealed regular epithelium with severe inflammatory cell infiltration and increased mast cell count. Electron microscopy revealed increased mast cell degranulation. Increased inflammatory cell infiltration was detected along with reduced mast cell count among fluticasone propionate treated rats. Mild inflammatory cell infiltration was encountered in combined salbutamol+fluticasone propionate treated rats.

**Conclusion.** This study supported the presence of localized allergic reaction in the supraglottic laryngeal mucosa through the observation of increased mast cell number and degranulation. It

was also shown that inhaled corticosteroids increase inflammation whereas combined inhaled corticosteroids and  $\beta_2$ -agonists minimize allergic and inflammatory reactions in supraglottic laryngeal mucosa providing a safer therapeutic option.

## Keywords

laryngeal mucosa, laryngeal allergy, inhaled corticosteroids,  $\beta_2$ -agonists, mast cells, degranulation, inflammation

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## Introduction

Due to its location between upper and lower airways, the larynx is exposed to environmental challenges, including inhaled house dust, pollens, and chemicals.<sup>1</sup> Despite intensive studies of allergic rhinitis, chronic laryngeal allergy is a rarely investigated phenomenon, and the effects of allergen exposure on the laryngeal mucosa are unknown.<sup>2,3</sup> Allergic laryngitis is considered a component of allergic rhinitis and asthma rather than an independent disease process.<sup>4</sup> The literature supporting direct laryngeal mucosal effect by allergens emphasizes laryngeal symptoms and improvement following antiallergic therapy.<sup>5</sup>

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Laryngeal allergic reactions have acute and chronic forms. The respiratory mucosa is consistent throughout the nasal cavity, larynx, trachea, and bronchi and triggers a type I IgE mediated laryngeal hypersensitivity reaction, which is similar to the response of the nasal mucosa upon allergen exposure. The acute allergic response of the larynx is associated with severe and rapid onset edema.<sup>2</sup> However, the previous study results concerning subsites of larynx where edema is encountered have been contradictory. The laryngeal edema was reported to involve the supraglottis and subglottis in 2 previous studies,<sup>6,7</sup> whereas another study reported that the type I hypersensitivity reaction elicits supraglottic edema rather than subglottic edema.<sup>8</sup>

The mechanisms associated with supraglottic and subglottic edema in the human larynx are not clearly understood. In a previous study reporting that the human laryngeal mucosa has the potential to induce type I allergic reaction, the distribution of mast cell phenotypes in the human laryngeal mucosa was studied, and a large number of mast cells was detected in the superficial layer of the subepithelial connective tissue of the epiglottis, arytenoid, and subglottis.<sup>6</sup> Another study reported connective tissue mast cell-derived histamine to be responsible for edema in the epiglottis following mast cell activation.<sup>9</sup>

Mast cells play a major role in inflammatory and allergic reactions, resulting in a subsequent increase at sites of inflammation.<sup>10</sup> They are found in mucosal tissues and provide the release of inflammatory mediators, including histamine, chemotactic factors, proteases, cytokines, and arachidonic acid metabolites acting on the vasculature, smooth muscle, connective tissue, mucous glands, and inflammatory cells.<sup>11</sup>

Corticosteroids are the most efficacious anti-inflammatory agents in the treatment of allergic diseases.<sup>12</sup> Inhaled corticosteroids are currently the most widely used anti-inflammatory agents for the long-term management of mild and moderate asthma in combination with  $\beta_2$ -agonists.<sup>13</sup> They reduce airway inflammation and hyperresponsiveness. The long-term use of inhaled corticosteroids has been reported to decrease allergen response and reduce mast cells in the bronchial mucosa.<sup>14</sup> The dose, regimen, formulation, and type of inhaled corticosteroid play major roles in the emergence of local as well as systemic side effects.<sup>15</sup> Although inhaled corticosteroids have anti-allergic and anti-inflammatory effects in systemic and intranasal topical form, they may cause irritation and histological inflammation, especially at the primary sites of contact, including the laryngopharyngeal mucosa.<sup>16</sup>  $\beta_2$ -agonists, on the other hand, have been shown to have anti-inflammatory effects through the inhibition of inflammatory cell activation, chemotaxis, and mediator release, in addition to their relaxation effect on the smooth muscle of the airway.<sup>17</sup>  $\beta_2$ -agonists have also been reported to enhance the effects of corticosteroids through glucocorticoid receptor activation.<sup>18</sup>

The updated treatment regimens for moderate and severe persistent asthma include a combination of inhaled corticosteroids and  $\beta_2$ -agonists.<sup>19</sup> Previous studies of this combined therapy have reported better clinical improvement with lower corticosteroid doses.<sup>20–22</sup>

Few histological studies have investigated the laryngeal mucosal allergic response and the effects of inhaled corticosteroids and  $\beta_2$ -agonists on allergen-sensitized laryngeal mucosa. The purpose of this study was to investigate the supraglottic and glottic laryngeal mucosal response to inhaled allergens and evaluate the histological effects of an inhaled corticosteroid (fluticasone propionate) and a  $\beta_2$ -agonist (salbutamol) on supraglottic and glottic laryngeal mucosa of an allergic rat model.

The present study tested the following hypotheses:

*Hypothesis 1:* The allergic stimuli may trigger a direct reaction in sensitized rat supraglottic and glottic laryngeal mucosa.

*Hypothesis 2:* Inhaled corticosteroids can cause increased laryngeal mucosal inflammation at the supraglottic and glottic level in an animal model of laryngeal allergy.

*Hypothesis 3:* Combined inhaled corticosteroids and  $\beta_2$ -agonists might decrease mucosal inflammation while providing an equivalent anti-allergic effect.

## Methods

All procedures with rats were approved by the animal research committee and performed in the Experimental Medical Research Institute (DETAE) at Istanbul University. Wistar albino rats ( $n = 32$ ) were sensitized intraperitoneally on days 0 and 7 with 1 mg ovalbumin (Sigma Aldrich, St. Louis, Missouri) and 200  $\mu$ g aluminium hydroxide ( $\text{Al}(\text{OH})_3$ ) (Merck, Germany). The protocol used widely for the induction of allergic airway involves the use of  $\text{Al}(\text{OH})_3$  as adjuvant for the allergen (ovalbumin) in the intraperitoneal phase of sensitization.<sup>23</sup>

Eight additional rats served as controls. Aerosolization was achieved through a nebulizer (Omron CompAIR NE-C28-E) in a  $70 \times 70 \times 50$  cm chamber with manipulation caps.

Every 2 days from day 14 to day 28, the rats were exposed to aerosolized ovalbumin (1%) for 30 minutes. On days 28 to 42 ovalbumin exposure was continued in sensitized rats ( $n = 32$ ); 30 minutes before each ovalbumin exposure, rats (32) in 4 separate groups ( $n = 8$ ) were administered aerosolized (nebulized) phosphate buffered saline (PBS) ( $n = 8$ ), fluticasone propionate (Flixotide, GlaxoSmithKline, Australia; 2 mg fluticasone propionate in 2 ml tamponaded isotonic saline solution) ( $n = 8$ ), salbutamol (Ventolin, GlaxoSmithKline; 2.5 mg salbutamol sulfate in buffered 2.5 ml isotonic saline solution, 100  $\mu$ g/dose) ( $n = 8$ ), and combined salbutamol+fluticasone propionate ( $n = 8$ ) consequently (**Table I**). Serum levels of ovalbumin-specific IgE were measured both in controls and ovalbumin-sensitized rats. On day

**Table 1.** Study groups.

| Group                                    | Day 1 and 7             | Day 14-28 (every 2 days) | Day 28-42 (every 2 days)          |          |
|------------------------------------------|-------------------------|--------------------------|-----------------------------------|----------|
|                                          | Exposure                | Exposure                 | Therapy (30 min before exposure)  | Exposure |
| 1. Control                               | —                       | —                        | —                                 | —        |
| 2. OVA+PBS                               | OVA-Al(OH) <sub>3</sub> | OVA                      | PBS                               | OVA      |
| 3. OVA+fluticasone propionate            | OVA-Al(OH) <sub>3</sub> | OVA                      | Fluticasone propionate            | OVA      |
| 4. OVA+salbutamol                        | OVA-Al(OH) <sub>3</sub> | OVA                      | Salbutamol                        | OVA      |
| 5. OVA+fluticasone propionate-salbutamol | OVA-Al(OH) <sub>3</sub> | OVA                      | Fluticasone propionate-salbutamol | OVA      |

Abbreviations: OVA, ovalbumin; PBS, phosphate buffered saline.

42, rats were sacrificed and the laryngeal mucosa were harvested at the glottic and supraglottic level. Specimens were fixed in formaldehyde, and paraffin blocks were prepared. Sections of 5 mm were cut and stained with hematoxylin and eosin (H&E) and toluidine blue. Excised laryngeal tissue (3 mm<sup>3</sup>) was fixed with glutaraldehyde in PBS, postfixed in osmium tetroxide in PBS, and embedded in Epon 812 resin (Fluka, Sigma-Aldrich Chemical, Steinheim, Switzerland) polymerized at 60°C. The Epon blocks were sectioned with Ultracut R microtome (Wien, Austria). The ultrathin sections (60 nm) were collected on 200 naked mesh copper grids and stained with uranyl acetate and lead citrate.

## Microscopic Examination

The specimens of supraglottic and glottic laryngeal mucosa were evaluated for general morphology, including inflammatory cell infiltration, epithelial thickness, submucosal gland hypertrophy, and mucous production as well as mature submucosal granulated and degranulated mast cells under light microscope (BX51, Olympus, Tokyo, Japan). The grade of inflammatory cell infiltration was determined through microscopic evaluation of the polymorphonuclear leucocytes, including neutrophils, eosinophils, and basophils. Inflammatory cell infiltration was graded semi-quantitatively to determine the percentage of histological changes as described in previous studies (grade 0 = no change; grade 1 = mild change, 0%-33%; grade 2 = moderate change, 34%-66%; grade 3 = severe change, 67%-100%).<sup>24</sup> The quantitative data included mast cell count and degranulation. Cells containing metachromatic granules were identified and counted as mast cells under 400× magnification with the aid of a graticule (0.0785mm<sup>2</sup>) in 5 consequent mucosal areas and expressed as the cell number/unit area. The ultrathin sections were investigated under transmission electron microscope (JEOL 1200EXII, JEOL Ltd, Tokyo, Japan) at 80 kV accelerating voltage to observe mast cell degranulation and photographed with a side-mounted digital camera (Morada Soft Imaging System, Olympus, Washington). The sections were analyzed microscopically by a histologist who was blind to the study

groups. The analysis was repeated for all specimens to determine intraobserver variability.

## Statistical Analysis

The statistical analysis was performed with the SAS System (version 9.2, Cary, North Carolina). The normality assumption of the data was tested using the Saphiro-Wilk method. Since the normality assumption was violated and measurements were in rankings, multiple comparisons of groups were performed with nonparametric ANOVA,  $P < .05$ . The pairwise comparison of groups was accomplished using the Kruskal-Wallis test. The critical  $P$  value for pairwise comparisons was identified as .01 after Bonferroni correction.

## Results

The mean  $\pm$  standard deviation, median, and interquartile range values of histological quantitative and semi-quantitative data for each group, including mast cell count, degranulation, and inflammation at the supraglottic and glottic levels, are summarized in **Table 2** and **Table 3**, respectively. The graphic distribution of overall data among the groups for the supraglottic and glottic levels is presented in **Figure 1**.

Simultaneous multiple comparisons with nonparametric ANOVA (Kruskal-Wallis) revealed statistically significant differences among the 5 groups for each of the 3 variables—mast cell count, degranulation, and inflammation—at the supraglottic level, but not at the glottic level ( $P < .05$ ) (**Table 4**). The subsequent pairwise comparison with Kruskal-Wallis for the supraglottic level revealed statistically significant differences among the groups. The critical  $P$  value was identified as .01 for pairwise comparisons after Bonferroni correction (**Table 5**). The differences in serum levels of ovalbumin-specific IgE among the controls and the ovalbumin sensitized rats were statistically significant ( $P < .01$ ).

Fluticasone propionate increased inflammation significantly ( $P = .003$ ) in the supraglottic mucosa of the ovalbumin sensitized rats (**Tables 2** and **5**, **Figure 1**). Fluticasone propionate+salbutamol was found to decrease inflammation, mast cell count, and mast cell degranulation

**Table 2.** The mean  $\pm$  standard deviation values of included parameters at supraglottic level in 5 groups.

| Supraglottic level<br>Group              | Mast cell number |        |      | Degranulation   |        |      | Inflammation    |        |      |
|------------------------------------------|------------------|--------|------|-----------------|--------|------|-----------------|--------|------|
|                                          | Mean $\pm$ SD    | Median | IQR  | Mean $\pm$ SD   | Median | IQR  | Mean $\pm$ SD   | Median | IQR  |
| 1. Control                               | 1.50 $\pm$ 0.55  | 1.50   | 1.00 | 0.50 $\pm$ 0.84 | 0.00   | 1.00 | 0.30 $\pm$ 0.50 | 1.00   | 0.50 |
| 2. OVA+PBS                               | 5.00 $\pm$ 1.41  | 5.00   | 2.00 | 3.00 $\pm$ 1.26 | 2.50   | 2.00 | 2.00 $\pm$ 3.00 | 1.00   | 3.00 |
| 3. OVA+fluticasone propionate            | 4.17 $\pm$ 1.47  | 4.00   | 1.00 | 2.83 $\pm$ 1.17 | 2.50   | 1.00 | 3.17 $\pm$ 1.33 | 0.00   | 3.00 |
| 4. OVA+salbutamol                        | 3.17 $\pm$ 0.75  | 3.00   | 1.00 | 1.67 $\pm$ 0.82 | 1.50   | 1.00 | 2.00 $\pm$ 0.63 | 1.00   | 2.50 |
| 5. OVA+fluticasone propionate-salbutamol | 1.67 $\pm$ 0.84  | 1.50   | 1.00 | 0.83 $\pm$ 0.75 | 1.00   | 1.00 | 1.00 $\pm$ 0.84 | 1.00   | 1.00 |

Abbreviations: OVA, ovalbumin; PBS, phosphate buffered saline; SD, standard deviation; IQR, interquartile range.

**Table 3.** The mean  $\pm$  standard deviation, median, and interquartile range data of included variables at glottic level in 5 groups.

| Glottic level<br>Group                   | Mast cell number |        |      | Degranulation   |        |      | Inflammation    |        |      |
|------------------------------------------|------------------|--------|------|-----------------|--------|------|-----------------|--------|------|
|                                          | Mean $\pm$ SD    | Median | IQR  | Mean $\pm$ SD   | Median | IQR  | Mean $\pm$ SD   | Median | IQR  |
| 1. Control                               | 1.00 $\pm$ 0.71  | 1.00   | 0.00 | 0.37 $\pm$ 0.52 | 0.00   | 1.00 | 0.38 $\pm$ 0.74 | 0.00   | 0.50 |
| 2. OVA+PBS                               | 1.60 $\pm$ 0.55  | 1.50   | 1.00 | 0.75 $\pm$ 0.70 | 1.00   | 1.00 | 0.88 $\pm$ 1.12 | 0.50   | 1.50 |
| 3. OVA+fluticasone propionate            | 1.25 $\pm$ 0.50  | 1.00   | 2.00 | 0.50 $\pm$ 0.75 | 0.00   | 1.00 | 2.00 $\pm$ 2.20 | 1.50   | 3.50 |
| 4. OVA+salbutamol                        | 0.40 $\pm$ 0.55  | 0.00   | 0.00 | 0.37 $\pm$ 0.74 | 0.00   | 0.5  | 1.33 $\pm$ 1.46 | 0.50   | 2.00 |
| 5. OVA+fluticasone propionate+salbutamol | 0.80 $\pm$ 0.50  | 0.00   | 1.00 | 0.83 $\pm$ 0.76 | 0.00   | 1.00 | 1.00 $\pm$ 1.19 | 0.50   | 2.00 |

Abbreviations: OVA, ovalbumin; PBS, phosphate buffered saline; SD, standard deviation; IQR, interquartile range.

significantly ( $P = .006$ ,  $P = .004$ ,  $P = .006$ , respectively) in the supraglottic mucosa of the ovalbumin sensitized rats (Table 5).

In pairwise comparisons among the control and treatment groups, no statistically significant differences were encountered in terms of inflammation, mast cell count, or degranulation ( $P = .456$ ,  $P = .093$ ,  $P = .734$ , respectively) in the glottic mucosa of the ovalbumin sensitized rats (Tables 3 and 4, Figure 1).

## Microscopy Findings

Light microscopy of the supraglottic laryngeal specimens from the ovalbumin+PBS exposed rats revealed a normal epithelium with severe inflammatory cell infiltration and increased granulated and degranulated mast cell counts compared to normal supraglottic mucosa, with a small number of inflammatory cells and granulated mast cells observed in the controls. Electron microscopy revealed prominent mast cell degranulation in the supraglottic mucosa of the ovalbumin+PBS exposed rats (Figures 2 and 3). Regional epithelial desquamation and thinning, increased inflammatory cell infiltration, and mucosal bleeding were detected, along with reduced granulated/degranulated mast cell counts, in the ovalbumin+fluticasone propionate exposed rats' supraglottic mucosa (Figure 4). Mild inflammatory cell infiltration, decreased mast cell count, and degranulation were encountered in the supraglottic mucosa of

the ovalbumin+fluticasone propionate+salbutamol exposed rats (Figure 5).

Light microscopy of the glottic mucosa revealed occasional mast cells and a small number of inflammatory cells in the ovalbumin sensitized rats. Electron microscopy of the glottic mucosa revealed occasional mast cell degranulation in all groups, including ovalbumin+PBS exposed (sensitized) rats.

## Discussion

Allergic reactions akin to those of nasal and bronchial mucosa may be encountered at the laryngeal level.<sup>3,4</sup> However, the existing data regarding laryngeal mucosal response to allergy are limited and contradictory. A previous clinical study with allergic singers reported the rapid induction of laryngeal complaints following allergen provocation and during pollen season.<sup>1</sup>

In this study, a statistically significant increase in mast cell number and degranulation was detected in rat supraglottic laryngeal mucosal specimens upon allergen exposure. In addition, increased inflammatory cell infiltration in allergen-sensitized rat supraglottic laryngeal specimens was associated with inhaled corticosteroids. The mechanisms of local laryngeal response to inhaled corticosteroid seem to be complex and multifactorial. The associated mechanisms of increased inflammation may be either inhaled corticosteroid or mucosal condition dependent. Despite their anti-allergic and anti-inflammatory effects in systemic and intranasal topical form,

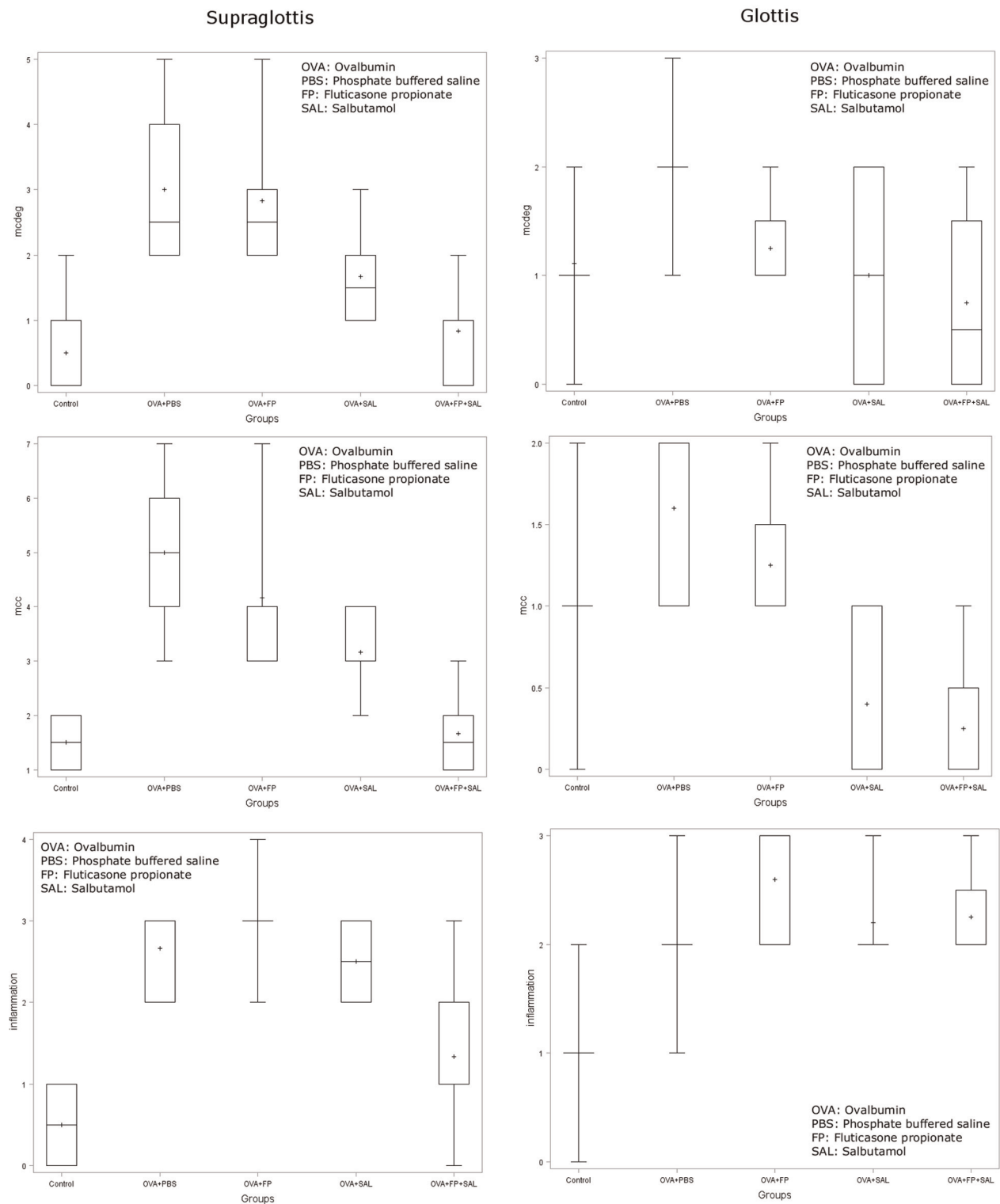


**Figure 1.** The distribution of mast cell count (mcc), mast cell degranulation (mcdeg), and inflammation grade among the groups, supraglottic and glottic level.

**Table 4.** The *P* values for simultaneous multiple comparisons.

| Variable         | Supraglottic level * <i>P</i> values | Glottic level * <i>P</i> values |
|------------------|--------------------------------------|---------------------------------|
| Mast cell number | .002                                 | .093                            |
| Degranulation    | .012                                 | .734                            |
| Inflammation     | .0007                                | .456                            |

\*Nonparametric ANOVA (Kruskal-Wallis), critical *P* value = .05.

**Table 5.** The *P* values for pairwise comparisons of supraglottic level.<sup>a</sup>

| Groups                                                | * <i>P</i> value |               |              |
|-------------------------------------------------------|------------------|---------------|--------------|
|                                                       | Mast cell count  | Degranulation | Inflammation |
| Control, OVA+PBS                                      | .003             | .006          | .003         |
| Control, OVA+fluticasone prop                         | .003             | .006          | .002         |
| Control, OVA+salbutamol                               | .006             | .036          | .002         |
| Control, OVA+fluticasone prop+salbutamol              | .789             | .382          | .003         |
| OVA+PBS, OVA+fluticasone prop                         | .248             | .862          | .003         |
| OVA+PBS, OVA+salbutamol                               | .026             | .053          | .335         |
| OVA+PBS, OVA+fluticasone prop+salbutamol              | .004             | .006          | .006         |
| OVA+fluticasone prop, OVA+fluticasone prop+salbutamol | .005             | .006          | .007         |

Abbreviations: OVA, ovalbumin; PBS, phosphate buffered saline; fluticasone prop, fluticasone propionate.

<sup>a</sup>Bolded values are statistically significant.

\*Kruskal-Wallis, the critical *P* value after Benferroni correction is 0.01.

inhaled corticosteroids may lead to irritation and histological inflammation, especially at the primary sites of contact, including the laryngopharyngeal mucosa.<sup>25</sup> The intrinsic inflammation in allergen-sensitized laryngeal mucosa may be enhanced through laryngeal deposition of inhaled corticosteroids. The reduction in mast cell number might not be evident, and expected anti-allergic effects might not be manifested, due to the combined effect of an increase in allergen-induced mast cell number and an increase in corticosteroid-associated inflammation. Future studies investigating the nature of inflammatory response at the receptor and mediator levels are essential.

Inhaled corticosteroid-associated laryngitis is a recently defined clinical entity. It is characterized by laryngeal changes, including mucosal edema, erythema, and thickening, which are all similar to laryngopharyngeal reflux.<sup>26</sup> Full recovery requires cessation of inhaled corticosteroids. In a recent study on corticosteroid inhalers, inhaled corticosteroids were indicated to predispose subjects to pharyngitis through the induction of inflammation.<sup>27</sup> Although the exact mechanism of increased inflammatory response following inhaled corticosteroids is not evident, direct mucosal contact and allergen-sensitized laryngeal mucosa might be contributing factors, due to particle size and direct mucosal contact.<sup>25,26,28</sup>

Fluticasone propionate and some other inhaled corticosteroids are inhaled in active form with laryngeal mucosal deposition. The direct laryngeal mucosal contact of the active form is associated with higher rates of local side effects presenting clinically, with symptoms of dysphonia,

cough, and pharyngolaryngeal candidiasis.<sup>13</sup> Inhalation in inactive form and subsequent activation in the target tissue lessen the side effects associated with direct mucosal contact.<sup>28</sup>

The present study did not reveal significantly decreased inflammation, mast cell number, or degranulation in the  $\beta_2$ -agonist administered group. In this study, the combined use of an inhaled steroid and a  $\beta_2$ -agonist was found to decrease mast cell number, mast cell degranulation, and mucosal inflammation significantly in sensitized rat supraglottic laryngeal mucosa. The combined use might contribute to corticosteroid-induced  $\beta_2$ -receptor expression of inflammatory cells. The increase in  $\beta_2$ -receptor expression of inflammatory cells might have a more intense anti-inflammatory effect.

One limitation of this study is that the subglottic laryngeal mucosa were not evaluated. Moreover, the investigation of additional parameters associated with structural airway changes (remodeling), including collagen, goblet cells, and fibronectin, were not incorporated. There is a variety of allergic animal models, each of which has limitations and advantages. The pitfalls might be mostly, although not entirely, eliminated through the choice of a practical and well-established method, as has been accomplished in the present study.

## Conclusion

This study supported the presence of localized allergic reaction in the supraglottic laryngeal mucosa through the

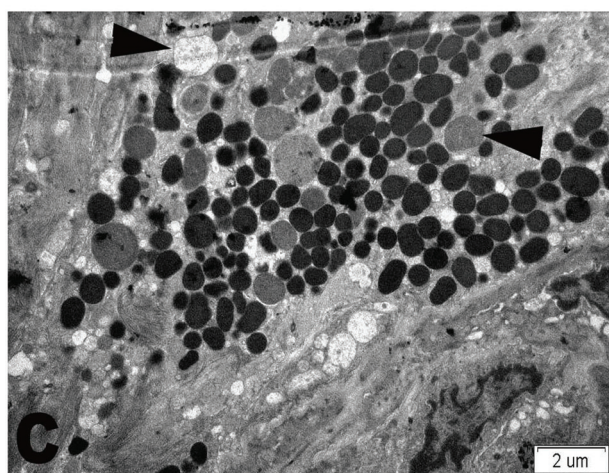
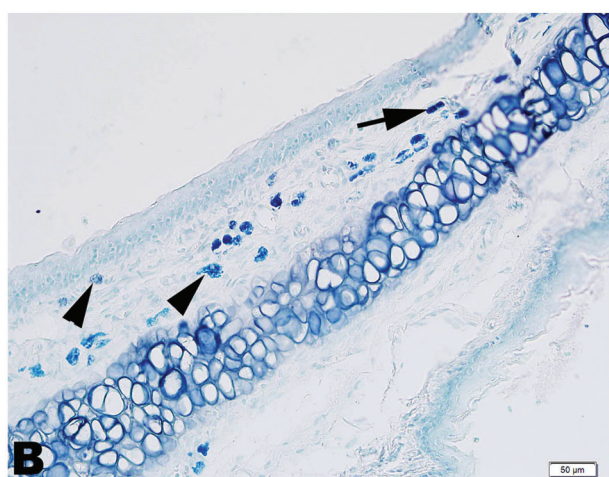
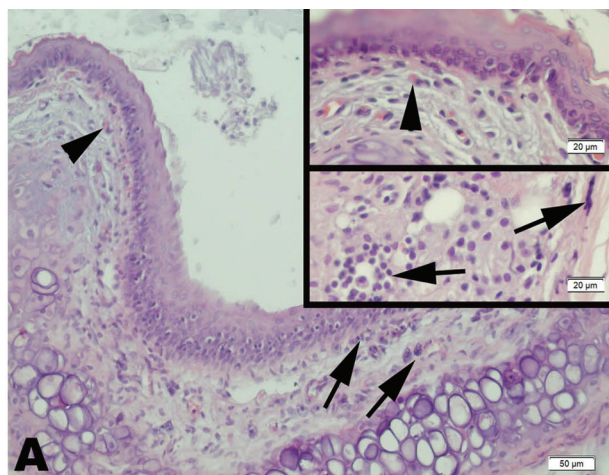


**Figure 2.** Increased vasculogenesis (arrow head), inflammatory cells (arrow) (A), increased granulated (arrow) mast cells (B), degranulated mast cell (arrow head) under electron microscopy (EM) (C). A: hemotoxiylen-eosin (H&E), B: Toludine blue (TB), C: electron micrograph, sensitized rats.

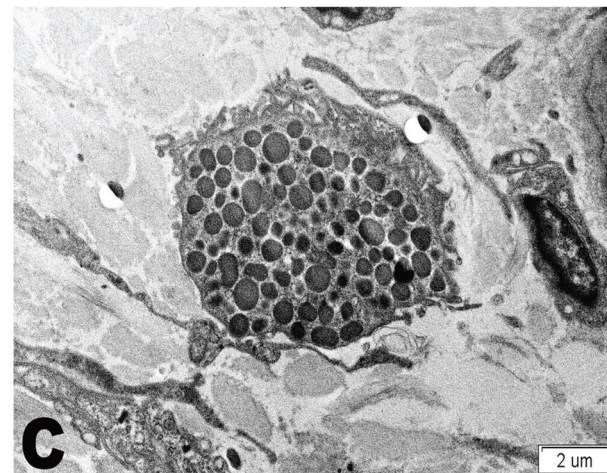
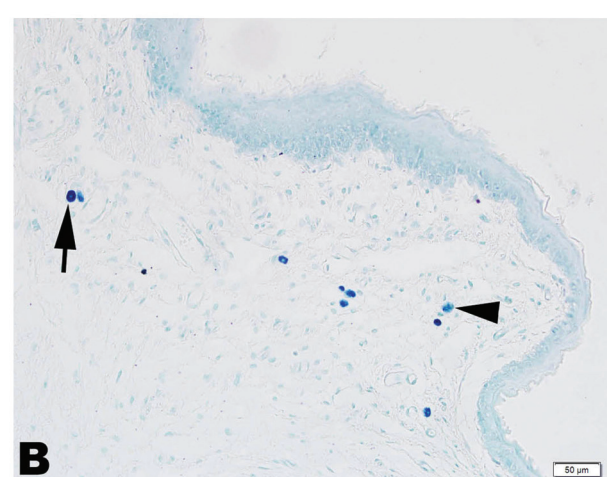
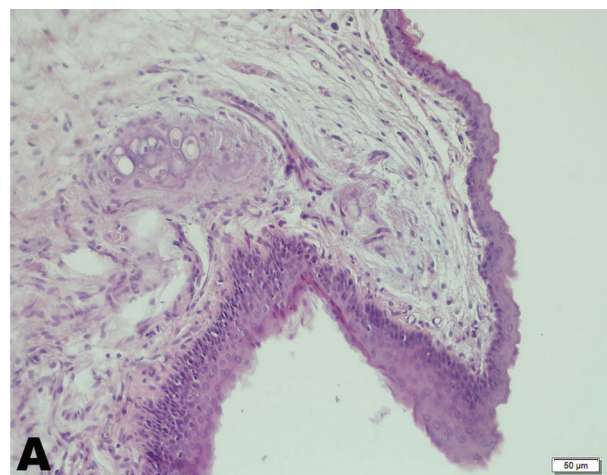


**Figure 3.** Regular supraglottic larynx mucosa (A), fewer granulated (arrow) and degranulated (arrow head) mast cells (B), granulated mast cell under electron microscopy (EM) in (C). A: hemotoxiylen-eosin (H&E), B: Toludine blue (TB), C: electron micrograph, controls.

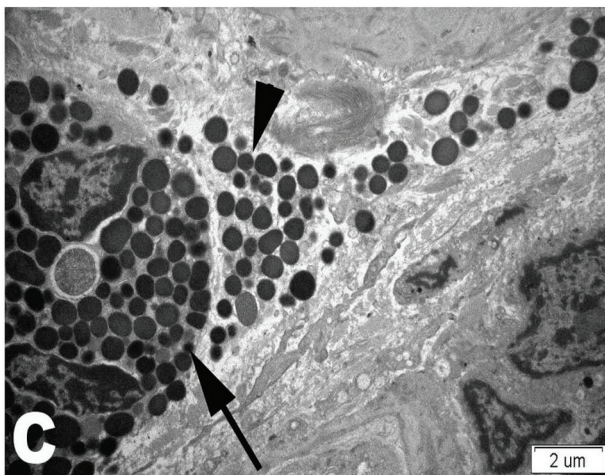
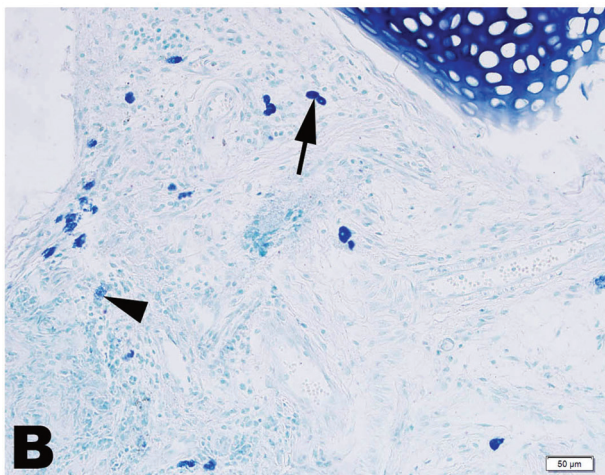
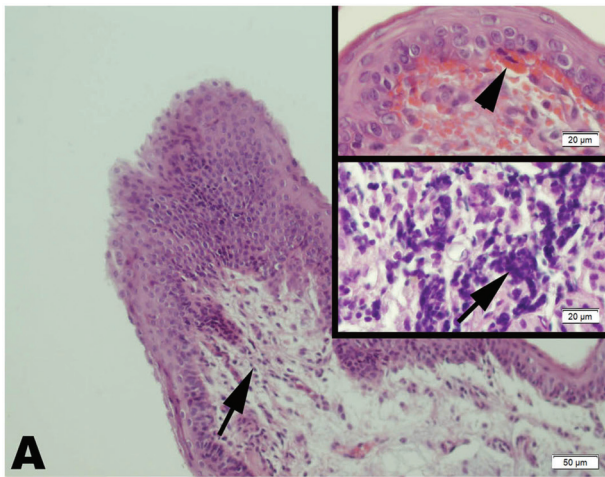


**Figure 4.** Mucosal bleeding (arrow), inflammatory cell infiltration (arrow) (A), increased granulated (arrow)-degranulated (arrow head) mast cells (B), granulated (arrow) and degranulated mast cell (arrow head) under electron microscopy (EM) (C). A: hemotoxylin-eosin (H&E), B: Toluidine blue (TB), C: electron micrograph, fluticasone propionate group.

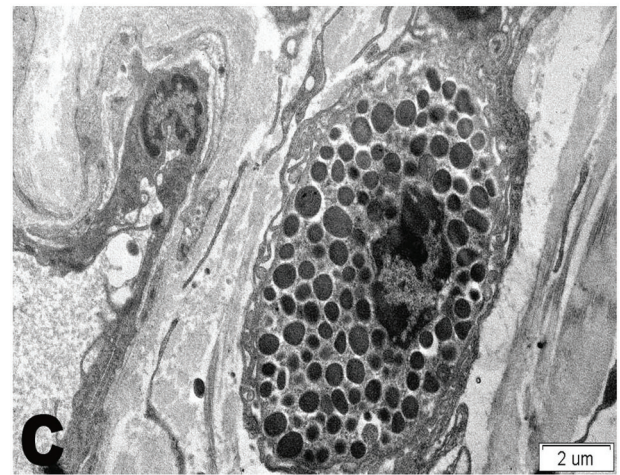
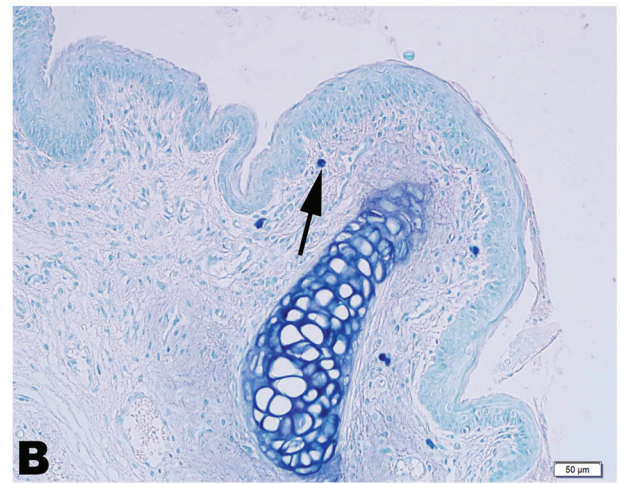
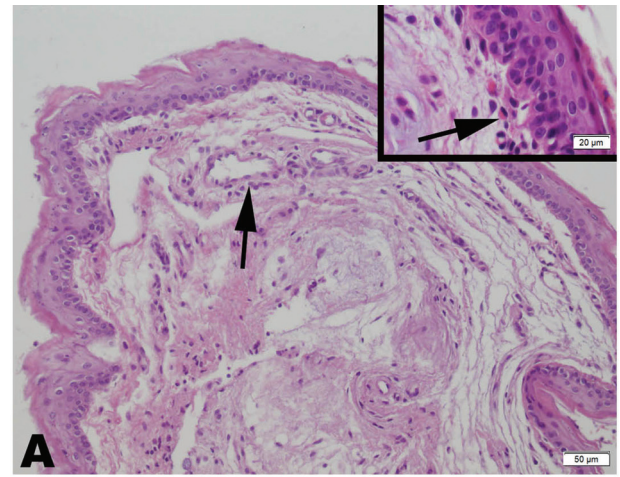


**Figure 5.** Inflammatory cell infiltration (arrow) (A), few number of granulated mast cells (arrow) (B), and granulated (arrow) mast cells under electron microscopy (EM) (C). A: hemotoxylin-eosin (H&E), B: Toluidine blue (TB), C: electron micrograph, fluticasone propionate + salbutamol treated group.

observation of increased mast cell numbers and degranulation. It also showed that inhaled corticosteroids increase supraglottic laryngeal inflammation and epithelial desquamation, possibly as a result of direct mucosal contact, whereas combined inhaled corticosteroids and  $\beta_2$ -agonists minimize allergic and inflammatory reactions in the supraglottic laryngeal mucosa, thereby providing a safer therapeutic option. To contribute to the development of novel diagnostic and therapeutic approaches, further research should identify the underlying mechanisms mediating localized allergic responses in the larynx and inhaled corticosteroid laryngitis.

### Author Contributions

**Meltem Esen Akpınar**, conception and design, analysis of data, drafting, revising, final approval; **Nihal Seden Tekke**, acquisition of data, drafting, final approval; **Ozgur Yigit**, analysis of data, drafting, revising, final approval; **Feriha Ercan**, conception and design, analysis of data, drafting, revising, final approval; **Yusuf Durna**, acquisition of data, drafting, final approval; **Demir Kiran**, acquisition of data, drafting, final approval.

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