

Topical Glucocorticoid Reduces the Topical Decongestant–Induced Histologic Changes in an Animal Model Nasal Mucosa

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Objectives/Hypothesis: To investigate the histologic consequences of simultaneous nasal glucocorticosteroid and xylometazoline HCl administration in the rabbit nasal mucosa.

Study Design: Prospective randomized study.

Methods: Twenty New Zealand male rabbits were randomly placed into three groups: group I, control (n = 6); group II, xylometazoline HCl (n = 8); or group III, xylometazoline HCl-fluticasone furoate (n = 6). Group I received no treatment. Groups II and III received two intranasal puffs of xylometazoline HCl 0.5 mg/mL twice daily or two puffs of xylometazoline HCl 0.5 mg/mL twice daily plus one puff of 27.5 µg fluticasone furoate twice daily to each nostril (110 µg), respectively. At the end of 3 weeks, the rabbits were sacrificed. The mucosa of the nasal cavities was excised. Specimen sections (5 µm) were stained with hematoxylin and eosin, mucicarmine, and Gomori one-step trichrome and were examined under a light microscope. The presence of edema, congestion, inflammatory cell infiltration, nasociliary loss, epithelial and nerve-ending degeneration, and goblet cell increase were evaluated semiquantitatively (grades 0–3).

Results: Statistically significant differences were detected between groups II and III in terms of edema, congestion, inflammatory cell infiltration, nasociliary loss, and epithelial degeneration ($P = .006$, $P = .049$, $P = .015$, $P = .014$, and $P = .049$, respectively). Nerve-ending degeneration, goblet cell increase, and quantitative goblet and neutrophil cell counts did not yield statistically significant differences between groups II and III ($P = .137$, $P = .580$, $P = .770$, and $P = .616$, respectively).

Conclusions: The combined simultaneous intranasal administration of xylometazoline HCl and fluticasone furoate appears to be beneficial in minimizing the long-term usage-associated congestion, edema, inflammatory cell infiltration, epithelial degeneration, and nasociliary loss in the rabbit model nasal mucosa.

Key Words: Nasal mucosa, rhinitis medicamentosa, congestion, decongestant, nasal steroid, rebound, edema.

Level of Evidence: 2c.

Laryngoscope, 122:741–746, 2012

INTRODUCTION

Nasal topical decongestants are widely prescribed medications, mainly for the symptomatic relief of nasal congestion in allergic, vasomotor, or viral rhinitis, rhinitis associated with pregnancy, acute and chronic sinusitis, and nasal polyposis.^{1,2} They fall into two categories: sympathomimetic amines and imidazolines. Imidazolines include oxymetazoline, naphazoline, xylometazoline, and clonidine.^{1,3} The imidazolines primarily act postsynapti-

cally on sympathetic nerves, cause vasoconstriction through decreasing the blood flow, and subsequently, decongestion.^{4,5} Xylometazoline, a nonselective α_1 and α_2 adrenergic receptor agonist and endothelial postsynaptic α_2 receptor agonist, provides instant nasal vasoconstriction with subsequent nasal decongestion.^{6,7}

Histologic changes, including edema, congestion, inflammatory cell infiltration, epithelial degeneration, goblet cell hyperplasia, squamous cell metaplasia, and nasociliary loss with subsequent mucociliary dysfunction are associated with prolonged xylometazoline exposure. The most prominent feature of prolonged use (overuse) is rebound nasal congestion,^{3,8} which results in a reduction in treatment efficacy (tachyphylaxis) defined as rhinitis medicamentosa.^{2,8,9}

Rhinitis medicamentosa is managed with different regimens, such as discontinuation of nasal decongestants or systemic decongestant and nasal corticosteroid usage. Activation of prostaglandin synthesis, stimulation of β -adrenergic receptors, and inhibition of endothelial leukocyte adherence have been proposed as mechanisms of action.^{2,3,8,10}

A few previous animal and clinical studies have investigated the efficacy of nasal corticosteroids administered following the use of nasal decongestants in the

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Editor's Note: This Manuscript was accepted for publication December 22, 2011.

Presented at the 7th Turkish Rhinology Congress, Antalya, Turkey, May 19–22, 2011, and awarded third place in the oral presentation category.

The authors have no funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lary.23207

treatment of rhinitis medicamentosa, but there has been no study investigating the histologic consequences of simultaneous usage.^{11–13}

The objective of this study was to investigate the histologic consequences of simultaneous nasal decongestant and corticosteroid usage in the nasal mucosa of an animal model and to test whether the simultaneous usage may minimize the mucosal histologic changes and provide novel data for prolonged usage in clinical practice. We hypothesized that the combined simultaneous intranasal administration of xylometazoline HCl and fluticasone furoate would reduce and/or delay xylometazoline HCl-dependent rebound congestion, edema, inflammatory cell infiltration epithelial degeneration, and nasociliary loss in the rabbit model nasal mucosa.

MATERIALS AND METHODS

Animal model

Researchers performed the procedures with rabbits in a Marmara University School of Medicine animal research laboratory. The surgical procedures for harvesting mucosal specimens from the animals were performed with maximum care, providing effective anesthesia to avoid pain and stress; all procedures were approved by the animal research committee. Twenty New Zealand male rabbits with body weights of 1,250 to 1,500 g (mean, 1,350 g) were grouped randomly as follows: group I, control (n = 6); group II, xylometazoline hydrochloride (n = 8); and group III, xylometazoline hydrochloride–fluticasone furoate (n = 6). The preservatives included in the administered drugs were benzalkonium chloride, disodium edetate, sodium dihydrogen phosphate, disodium phosphate, sodium chloride, and purified water (Otrivine; Novartis, Basel, Switzerland); and glucose (dry), suspendable cellulose, polysorbate 80, benzalkonium chloride, disodium edetate, and purified water (Avamys; Glaxo Operations, Durham, UK).

Group I received no treatment. Group II received two puffs of xylometazoline HCl 0.5 mg/mL twice daily, and group III received two puffs of xylometazoline HCl 0.5 mg/mL twice daily plus one puff of 27.5 µg of fluticasone furoate twice daily. The drug solutions in the original spray package were sprayed into each nostril of nonsedated rabbits with the heads held in an upright position.

Two rabbits from group II were sacrificed at the end of 2 weeks to verify the xylometazoline-dependent histologic changes. At the end of 3 weeks, all rabbits were sacrificed with combined intraperitoneal ketamine (50 mg/kg) and xylazine (3 mg/kg) anesthesia to provide pain control. Fixation was by cardiac perfusion with 0.9% NaCl solution followed by 4% paraformaldehyde.

The dorsal nasal region was shaved and cleansed with 10% povidone iodine solution. The nasal cavities were opened using sterile surgical instruments through a dorsal midline incision, and the nasal bone was split. The mucosa of, presumably, the most exposed parts, including the anterior part of the septum, the inferior conchal edge, and the lateral nasal wall were excised bilaterally to prepare the specimens. Identical parts of the mucosa were excised from all animals. The specimens from each rabbit were postfixed for 4 hours in a 4% paraformaldehyde solution. The right-sided specimens were used, and the left sides were reserved for future immunohistochemical investigation in the continuum of the research project. After macroscopic examination, the specimens were embedded in paraffin. Sections of 5-µm thickness were cut from the paraffin

blocks and stained with hematoxylin and eosin (H&E), mucicarmine, and the Gomori one-step trichrome method.

Microscopic Examination

The sections were analyzed under a light microscope (BX51; Olympus, Tokyo, Japan) by a histologist who was blinded to the study groups. The analysis was repeated for all specimens to determine intraobserver variability.

Outcome Measures

The presence of congestion, edema, inflammatory cell infiltration, epithelial and nerve-ending degeneration, epithelial metaplasia, nasociliary loss, and goblet cell increases were the parameters investigated and graded semiquantitatively by determining the percentage of the histologic changes as described in previous studies¹² (grade 0 = no change, grade 1 = mild change (0%–33%), grade 2 = moderate change (34%–66%), grade 3 = severe change (67%–100%).

The congestion was determined histologically in the presence of dilated tissue capillaries. The average scores from the anterior septum, the inferior conchal edge, and the lateral nasal wall specimens were calculated and converted to a single value to simplify the statistical analysis. The grade of inflammatory cell infiltration was determined through microscopic evaluation of the polymorphonuclear leucocytes, including neutrophils, eosinophils, and basophils.

The quantitative parameters, including counts of neutrophils and goblet cells, were also determined. Neutrophils and goblet cells from three different randomly chosen areas of each specimen were counted per millimeter epithelium under 400× magnification. The total cell number from the three different areas of each specimen was calculated, and the averages of the total cell numbers from the anterior septum, the inferior conchal edge, and the lateral nasal wall specimens were determined and statistically compared.

Statistical Analysis

The statistical analysis was performed with the SAS Package (version 9.2; Cary, NC) with a 95% confidence interval ($P < .05$). The normality assumption of the data was tested with Shapiro-Wilk. The normality assumption was not satisfied in any of the parameters included ($P < .05$); therefore, nonparametric tests were utilized in subsequent analyses. The statistical pairwise comparisons of groups I, II, and III were performed with the Kruskal-Wallis test.

RESULTS

The microscopic, histologic evaluation of the groups with H&E and the Gomori trichrome stain revealed the normal nasal mucosal epithelium and the subepithelium in the control group. Compared to group I, group II revealed increased inflammatory cell infiltration, edema, congestion, and epithelial and nerve-ending degeneration. In group III, the inflammatory cell infiltration, congestion, edema, and epithelial degeneration were decreased, relative to group II. However, continuing vasocongestion was evident (Fig. 1 and Fig. 2). The histologic evaluation of the three groups with the mucicarmine stain revealed pseudostratified, ciliated, columnar respiratory epithelium with goblet cells in all three groups. Groups II and III showed an increased number of goblet cells on the surface epithelium compared to group I (Fig. 3).

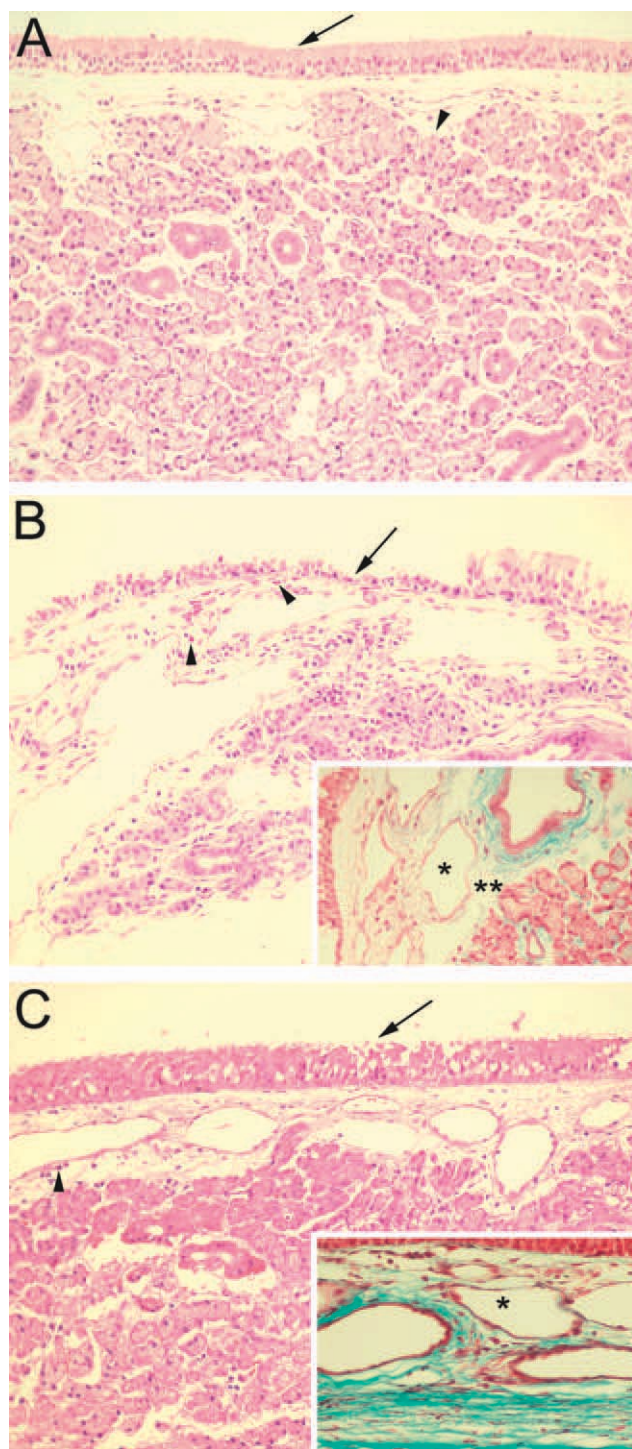


Fig. 1. The histologic evaluation of the nasal mucosa in three groups (with hematoxylin and eosin [H&E] and Gomori one-step trichrome stains, inset). (A) Group I revealed the normal nasal mucosa of the rabbit showing pseudostratified, ciliated, columnar epithelium (arrow) and subepithelium with serous and mucous glands (arrowhead). (B) Group II showed marked degeneration of the epithelium (arrow) and severe infiltration of neutrophils (arrowheads) in the subepithelial zone. Inset: * = vasocongestion; ** = edema in the subepithelium. (C) Group III showed mild cell infiltration (arrowhead). Mild to moderate degeneration of epithelium (arrow) was apparent. Inset: * = continuing vasocongestion and decreased edema in the subepithelium. H&E, $\times 200$, original magnification. Insets: Gomori one-step trichrome stain, $\times 400$, original magnification

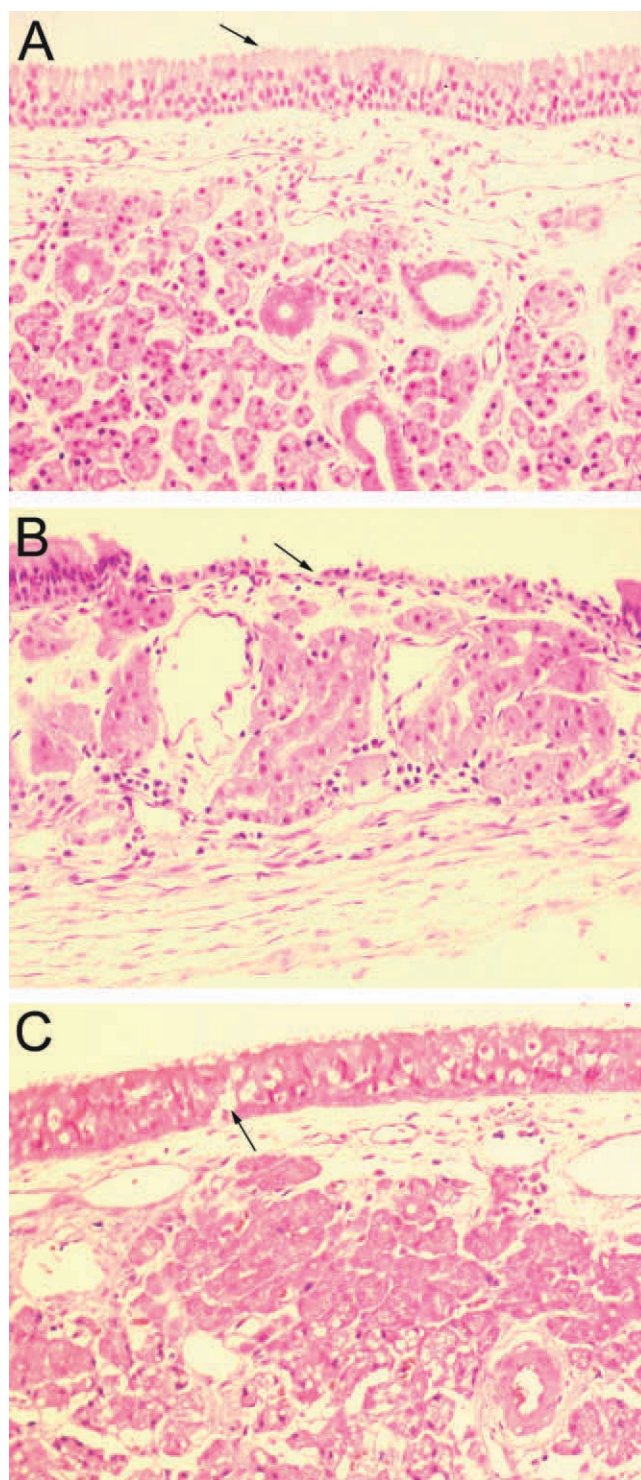


Fig. 2. The histologic changes of nasal epithelium in three groups. (A) Pseudostratified ciliated columnar epithelium (arrow) and subepithelium observed in group I. (B) Severe degeneration of the epithelium (arrow) evident in group II. (C) Ongoing degeneration (arrow) in some areas of the epithelium observed in group III. Hematoxylin and eosin, $\times 400$, original magnification.

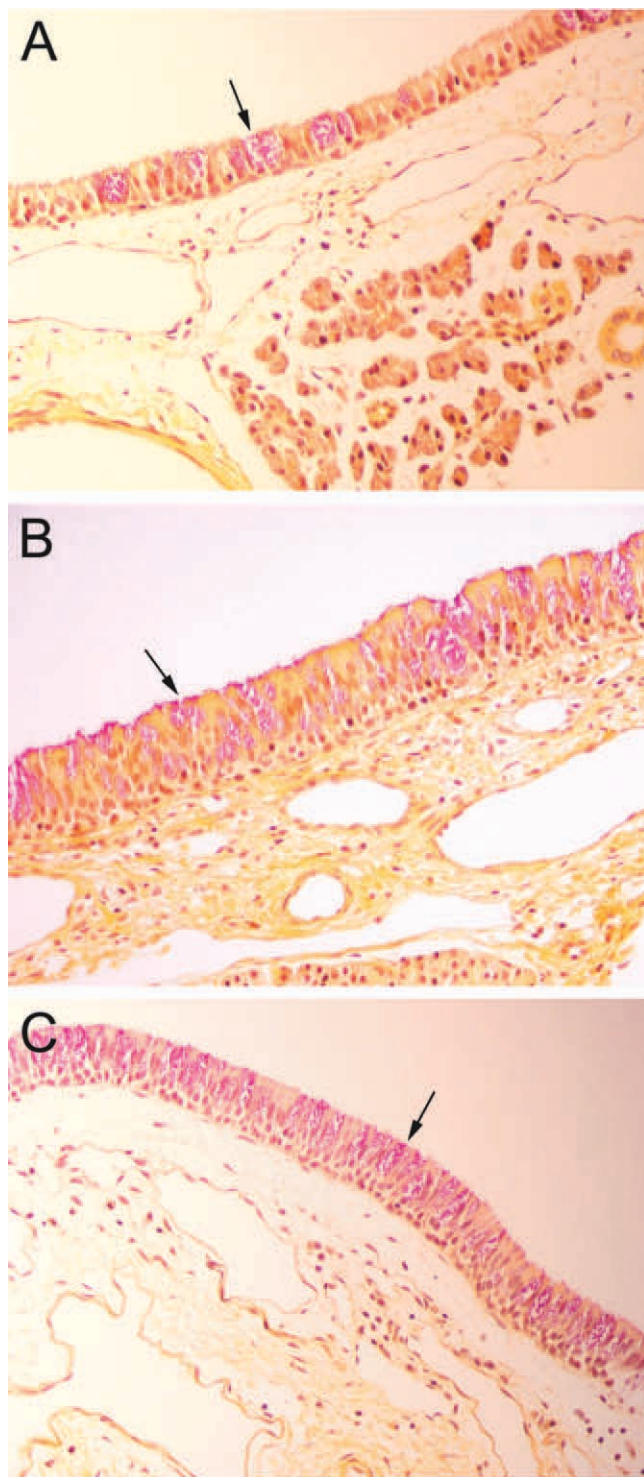


Fig. 3. The histologic evaluation of the nasal mucosa in three groups (with mucicarmine stain). (A) Group I showed pseudostratified, ciliated, columnar respiratory epithelium with goblet cells (arrow). (B) Group II showed an increased number of goblet cells (arrow) on the surface epithelium. (C) The increased number of goblet cells (arrow) on the surface epithelium is evident in group III. Mucicarmine stain, $\times 400$, original magnification.

Intrarater agreement was assessed by an interclass correlation coefficient indicating strong agreement between measurements ($r = 0.894$). Considering the strong agreement, the means of the determined histologic grades were included as the final measurements for the analyses.

The means, medians, and 25-75 interquartile range values of the histologic grades for the included parameters and cell counts in all groups are summarized in Table I. The P values for the statistical pairwise comparisons were determined (Table II). Statistically significant differences were detected between groups II and III in terms of edema, congestion, inflammatory cell infiltration, nasociliary loss, and epithelial degeneration ($P = .006$, $P = .049$, $P = .015$, $P = .014$, and $P = .049$, respectively). Nerve-ending degeneration, goblet cell increase, goblet cell count, and neutrophil cell count did not yield statistically significant differences between groups II and III ($P = .137$, $P = .580$, $P = .770$, and $P = .616$, respectively) (Table II).

DISCUSSION

Decreased production of endogenous sympathetic norepinephrine through a negative feedback mechanism is considered to be responsible for the rebound of nasal congestion and the edema in the nasal epithelium and underlying stroma following prolonged xylometazoline HCl usage.^{2,14,15} The disappearance of vasoconstrictive action with subsequent vasodilation results in nasal mucosal congestion (swelling). Previous studies reported increases in edema in the second week and cellular damage with prominent production of mucous along with decreases in the number of cilia on the epithelial layer in the third week. Reports concerning the period of rhinitis medicamentosa development have been limited and controversial. One study reported rebound congestion after 8 weeks of topical decongestant use,¹⁶ and others reported rebound congestion following 3 to 10 days of use.^{1,17} Additional studies reported no rebound congestion (Graf and Juto) before 10 days of use in healthy volunteers.^{18,19} Although definitive evidence concerning the onset of adverse effects is lacking, the use of topical decongestants beyond 10 days is not recommended.

The present study investigated the histologic mucosal changes in response to simultaneous long-term use of xylometazoline and fluticasone furoate in rabbits to evaluate whether xylometazoline HCl-dependent adverse effects would be prohibited or delayed.

Topical steroids are known to reduce the sensitivity of irritant receptors and the irritation effect of the drug on the nasal mucosa.²⁰ Glucocorticoids inhibit the functions of infiltrating inflammatory cells and their recruitment into the nasal mucosa. They change vascular permeability indirectly through the inhibition of the cellular inflammatory processes rather than by directly affecting the vascular endothelial cells.^{10,21} Fluticasone furoate is an enhanced-affinity glucocorticoid used in the treatment of allergic rhinitis. In a recent clinical study, the addition of oxymetazoline to fluticasone furoate in the treatment of perennial allergic rhinitis was reported to increase its

TABLE I.
Mean, Median, 25–75 Interquartile Range Values of Histologic Grades for the Included Parameters in Group I, II, and III.

Variable	Group I (Control)				Group II (XY)				Group III (XY + FF)			
	Mean	Median	25–75 Interquartile Range		Mean	Median	25–75 Interquartile Range		Mean	Median	25–75 Interquartile Range	
Edema	0.00	0.00	0.00	0.00	2.25	2.50	1.50	3.00	0.67	1.00	0.00	1.00
Congestion	0.00	0.00	0.00	0.00	1.50	1.50	1.00	2.00	0.83	1.00	0.00	1.00
ICI	0.00	0.00	0.00	0.00	2.25	3.00	1.50	3.00	0.50	0.00	0.00	1.00
NCL	0.00	0.00	0.00	0.00	1.29	1.00	1.00	2.00	0.17	0.00	0.00	0.00
ED	0.00	0.00	0.00	0.00	2.50	3.00	2.50	3.00	1.33	1.00	1.00	2.00
NED	0.00	0.00	0.00	0.00	1.86	2.00	1.00	2.00	1.00	1.00	0.00	2.00
GCI	0.00	0.00	0.00	0.00	2.38	2.50	2.00	3.00	2.00	2.00	1.00	3.00
GCC	33.80	24.00	13.00	39.00	65.25	59.50	26.00	102.5	58.80	33.00	22.00	101.00
NCC	0.20	0.00	0.00	0.00	63.71	21.00	11.00	164.0	34.33	25.00	3.00	31.00

XY = xylometazoline; XY + FF = xylometazoline + fluticasone furoate; ICI = inflammatory cell infiltration; NCL = nasociliary loss; ED = epithelial degeneration; NED = nerve-ending degeneration; GCI = goblet cell increase; GCC = goblet cell count; NCC = neutrophil cell count.

efficacy.¹⁴ Previous animal and clinical studies reported decreases in the interstitial edema associated with rhinitis medicamentosa following treatment with intranasal steroids.¹² An experimental animal model study investigating the simultaneous use of the two compounds and its consequences has not been reported previously.

The present study results indicate that the effect of fluticasone furoate on nasal congestion seems to be associated with the inhibition of inflammatory cell infiltration rather than with the downregulation of goblet cells or neutrophils through the avoidance of recruitment to the nasal mucosa. The goblet cells contribute to nasal congestion through mucin production caused by the upregulation in their numbers.

Persistent nasal decongestion is an ongoing issue in the management of patients with chronic rhinitis, both allergic and nonallergic. The option of simultaneous usage of nasal glucocorticoids and decongestants may provide safer and longer decongestion, thus providing symptomatic relief that is more efficient in the clinical management of these patients. The data provided through the study may contribute to reducing limitations in the use of decongestants and establish novel treatment options.

In this study, the length of delivery of xylometazoline and xylometazoline plus fluticasone furoate was 3 weeks. The rationale came from the previous studies that reported the onset of rhinitis medicamentosa and the subsequent appearance of the most critical histopathologic changes in the first 3 weeks. Three weeks seemed most appropriate to observe simultaneously the most prominent xylometazoline-encountered histopathologic changes and to identify the extended usage (beyond 10 days) without adverse effects following the addition of fluticasone furoate. On the other hand, it is a short period for the assessment of safety beyond 3 weeks. Studies with long-term follow-up may provide additional insights.

Previous reports have indicated that benzalkonium chloride, a quaternary ammonium compound used as a preservative in most nasal sprays to prevent bacterial

contamination, may increase the risk of rhinitis medicamentosa. Benzalkonium-free nasal decongestants have been recommended.²² However, no previous evidence exists concerning the increased nasal congestion produced by nasal corticosteroid sprays containing benzalkonium chloride.²³ Although benzalkonium chloride was present in the content of the nasal decongestant and the corticosteroid compounds used in this study, its histologic consequences were considered to be beyond the scope of the present study's objectives.

The small sample size was a limitation of the present study. A larger sample size could not be provided because of ethical concerns and the restrictions of the animal research committee regarding the number of animals used. A larger sample size, through minimizing the type II error risk, could possibly provide detection of significant statistical differences in the parameters that did not yield statistical significance in the

TABLE II.
P Values of Statistical Pairwise Comparisons Among Three Groups.

Variable	Kruskal-Wallis Test P Values		
	Group II (XY) vs. Group III (XY + FF)	Group I (Control) vs. Group II (XY)	Group I (Control) vs. Group III (XY + FF)
Edema	.006*	.001*	.019*
Congestion	.049*	.001*	.021*
ICI	.015*	.003*	.140
NCL	.014*	.005*	.317
ED	.049*	.002*	.007*
NED	.137	.001*	.040*
GCI	.580	.003*	.018*
GCC	.770	.142	.347
NCC	.616	.004*	.005*

*P < .05.

XY = xylometazoline; XY + FF = xylometazoline + fluticasone furoate; ICI = inflammatory cell infiltration; NCL = nasociliary loss; ED = epithelial degeneration; NED = nerve-ending degeneration; GCI = goblet cell increase; GCC = goblet cell count; NCC = neutrophil cell count.

comparison of groups II and III in this study. Future studies with larger sample sizes may provide further information.

CONCLUSION

The combined simultaneous intranasal administration of xylometazoline HCl and fluticasone furoate reduced xylometazoline HCl-dependent rebound congestion, edema, and inflammatory cell infiltration in the rabbit model. The encountered epithelial degeneration and nasociliary loss were limited in extent. The combination of fluticasone furoate and xylometazoline seemed to be beneficial primarily in terms of limiting histologic adverse effects. These findings may provide a basis for novel perspectives on the duration and content of rhinitis treatments, and thus, on treatment protocols in clinical practice.

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