Absence of Nasal Mucosal Atrophy With Fluticasone Aqueous Nasal Spray

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Objective: To evaluate whether 1 year of continuous treatment with intranasal fluticasone propionate would lead to atrophy in the nasal mucosa compared with an active control, oral terfenadine.

Design: Prospective, randomized, multicenter, open-label, parallel-group study.

Setting: Two tertiary care academic institutions.

Patients: Seventy-five subjects older than 18 years with perennial allergic rhinitis.

Interventions: Patients received either fluticasone propionate aqueous nasal spray, 200 µg once daily, or terfenadine, 60 mg twice daily, for 1 year. Nasal biopsy specimens were obtained before and after 1 year of treatment and were evaluated for evidence of atrophy.

Main Outcome Measures: Epithelial and collagen layer thickness of the nasal mucosa as assessed by light microscopy and the presence and degree of edema, and regularity of collagen fibrils as assessed by electron microscopy. Analyses were performed without knowledge of subject identity or treatment assignment.

Results: Neither fluticasone nor terfenadine treatment led to atrophy in the nasal mucosa by clinical or histologic observation. No significant changes from baseline were observed for any assessment of atrophy. In contrast to what would have been expected if atrophy were to occur, mean epithelial layer thickness in the fluticasone group significantly increased compared with terfenadine treatment (P = .03).

Conclusions: Treatment with intranasal fluticasone for 1 year increases the thickness of the nasal epithelium as compared with a year's treatment with terfenadine and does not lead to atrophy in the nasal mucosa. The increased thickness in the fluticasone treatment may represent repair from epithelial damage caused by chronic allergic inflammation.


Allergic rhinitis affects more than 40 million Americans and costs society more than $3 billion each year. A major characteristic of allergic rhinitis is inflammation. The inflammatory process stimulates the glands, blood vessels, and nerves of the nasal mucosa, creating the symptoms of the disease. Whether this inflammation causes changes to matrix structures, such as the epithelium, the basement membrane, and the collagen matrix, is not known.

Intranasal corticosteroids are among the most commonly prescribed and effective medications used to treat this disease. The safety and efficacy of intranasal corticosteroids have been well established in multiple clinical trials. Furthermore, the widespread clinical use of these compounds during the past 25 years has not been associated with substantial untoward adverse events. Intranasal corticosteroids exert a potent topical anti-inflammatory effect. They inhibit the recruitment of inflammatory cells and prevent the release of inflammatory mediators, resulting in a decrease in symptoms and the blocking of both the early- and late-phase allergic reactions.

Appropriate treatment options must be evaluated over the long term for potential adverse sequelae. This is particularly true for patients with perennial allergic rhinitis who may require long-term treatment over several years to control their disease. Long-term use of oral corticosteroids and topical dermatologic corticosteroids (ie, creams and ointments) have been observed to lead to atrophic changes in the skin. Although there is no clinical or histologic evidence that intranasal corticosteroid treatment leads...
SUBJECTS AND METHODS

SUBJECTS

Men and nonpregnant, nonlactating women 18 years of age or older were recruited from The Johns Hopkins University Hospital, Baltimore, Md, and the University of Chicago Hospitals, Chicago, Ill, to participate in this study. All subjects had to have a diagnosis of perennial allergic rhinitis and a skin test positive for dust mites. Eligible subjects also had to have nasal symptoms for more than 1 hour per day on most days for which they used at least 1 antihistamine medication during the 12 months before the study. Subjects with a marked (≥50%) physical obstruction in the nose, previous nasal septal surgery or perforation, or viral or bacterial infection within 30 days of screening were excluded. Medications that could affect rhinitis symptoms or allergic inflammation, such as topical and systemic glucocorticoid therapy, intranasal cromolyn sodium, and antihistamines, were not permitted for at least 1 month before or during the study. None of the subjects was taking immunotherapy during the study, and those who had received previous immunotherapy had to have stopped taking it at least 2 years before participation. Before initiation of the study, the protocol and informed consent document were reviewed and approved by the institutional review board governing research at each site. All subjects provided written informed consent before participation.

DESIGN

This was a randomized, open-label, parallel-group study in 75 subjects with perennial allergic rhinitis that compared the effects on the nasal mucosa of 1 year of treatment with fluticasone propionate aqueous nasal spray, 200 µg once daily, vs an active control, oral terfenadine, 60-mg tablet twice daily. At the time this study was conducted in the mid-1990s, terfenadine was a commonly prescribed medication for the treatment of allergic rhinitis and was considered a suitable therapeutic alternative to intranasal corticosteroid therapy.

The study consisted of a 21-day screening phase and a 12-month randomized, open-label treatment phase. During the screening phase, eligibility for the study was confirmed and baseline rhinoscopy and nasal biopsy were performed. After healing of the nasal biopsy site (approximately 1 week later), subjects were randomly assigned to receive 1 of the following study treatments for 1 year: intranasal fluticasone propionate once daily (2 sprays of 50 µg per spray in each nostril in the morning) or terfenadine, 60-mg tablet orally twice daily. Subjects were allowed to take only pseudoephedrine as needed for the relief of breakthrough symptoms during the study and were allowed to take other medications that did not interfere with allergic inflammation, such as analgesics. Subjects returned to the clinic at monthly intervals to reinforce medication adherence, receive additional medication, and assess their clinical status. After 12 months of treatment, another nasal biopsy specimen was obtained from the opposite nostril to avoid the confounding effects of scar formation at the site of the first biopsy specimen.

NASAL BIOPSY

After local anesthesia was induced with 1% lidocaine hydrochloride with epinephrine 1:100,000, biopsy samples were obtained with punch forceps from the anterior tip of the inferior turbinate by standard methods. This biopsy site was selected because it is the expected site of drug impact with aqueous nasal spray medications and a major site for allergen deposition. Each biopsy specimen was divided into sections to enable light and electron microscopic evaluation of atrophy and labeled such that subject to atrophy of the nasal mucosa, we undertook this comparative nasal biopsy study to investigate the effects of 2 treatment options for allergic rhinitis. We compared the structural characteristics of nasal mucosal biopsy specimens from subjects allergic to dust mites who were treated with either fluticasone propionate aqueous nasal spray or oral terfenadine, the active control. Nasal mucosal atrophy was assessed qualitatively and quantitatively by means of light and electron microscopy. Thus, this study was designed to examine whether 1 year of treatment with a topical corticosteroid (fluticasone) causes atrophic nasal mucosal changes when compared with an oral antihistamine (terfenadine).

RESULTS

SUBJECT DISPOSITION AND DEMOGRAPHICS

Seventy-five subjects (38 in the fluticasone group and 37 in the terfenadine group) were enrolled in the study (Table 2). The treatment groups were similar with respect to age, sex distribution, ethnic origin, and number of subjects withdrawn early. The most common reason for attrition during the 12-month study period was that subjects failed to return to the clinic. Four subjects were withdrawn from the study because of nonserious adverse events: 3 in the fluticasone group (epistaxis, nasal dryness, and exacerbation of asthma) and 1 in the terfenadine group (exacerbation of uveitis). The adverse event profile was similar to that observed in previous studies. There were no serious or unusual events.

BIOPSY RESULTS

All evaluable nasal biopsy specimens from subjects with both baseline and study end biopsy samples were included in the analyses of atrophy (n=52). This included samples from the 51 subjects who completed the study and 1 subject who was discontinued from the study after 8 months of treatment because of relocation.

As would be expected because of the location of the biopsy (anterior tip of the inferior turbinate), all specimens but 1 had nonciliated squamous epithelium present at baseline. In 2 specimens, both respiratory and squamous epithelium were seen, and in 1 specimen, only respiratory epithelium was seen in the areas examined.
identity, treatment, and date of biopsy were not apparent to those evaluating the specimens. Biopsy samples were prepared as follows:

1. Section 1 of the biopsy specimen was fixed in formalin, embedded in paraffin, sectioned into 3-µm sections, and stained with hematoxylin-eosin. These samples were evaluated by means of light microscopy.

2. Section 2 of the biopsy specimen was fixed in 2.5% glutaraldehyde in Millonig buffer (1.7% monobasic sodium phosphate + 0.3% sodium hydroxide in distilled water with pH 7.3–7.4) at 4°C for 24 hours, then transferred and stored in Millonig buffer at 4°C until postfixing in 1% osmium tetroxide, dehydrating, infiltrating, and embedding in epoxy resin. The epoxy resin–embedded tissue was then sectioned into 50- to 70-nm sections, placed on copper grids, and contrast stained with 1.5% uranyl acetate aqueous solution and Reynold lead citrate solution and examined by transmission electron microscopy (Philips CM-10; Philips Inc, Mahway, NJ).

ASSESSMENT OF ATROPHY

Atrophy was assessed by means of 4 objective and quantitative measures based on light and transmission electron microscopy (Table 1). In addition, qualitative evaluations including the presence of squamous and respiratory epithelium, elastin, basal lamina duplication, and collagen type were performed with electron microscopy. Specimens were visualized at ×1000 to examine the epithelium and underlying stroma (Figure 1), ×8000 to evaluate the superficial vessels, ×10000 for high-power views of the basal lamina and the epithelium, and ×14000 for evaluation of collagen. Prints at each of these magnifications were unidentified as to subject name and treatment received and were examined by 2 investigators (C.C.C. and E.S.).

The thickness of the collagen and epithelium was measured in micrometers at 5 points along the specimen and that of the epithelium at 3 points along the specimen. The average value of these respective measurements was used for analysis.

The presence of edema (presence of lucent spaces) and regularity of collagen fibrils were graded on a scoring system described in Table 1. Micrographs representative of the low and high ends of these scales are provided in Figure 2 and Figure 3, respectively. Figure 2 shows the electron-lucent spaces between the collagen fibrils. Figure 2A (×15 500) demonstrates the lack of lucent spaces (0 or none) between the collagen fibrils, and Figure 2B (×14 500) demonstrates a large number of electron-lucent spaces (3 or severe). Figures 3A and 3B show low (+1, least regular) and high (+3, most regular) regularity, respectively, of collagen fibrils observed at ×11 500 magnification.

STATISTICAL ANALYSES

Atrophy of the nasal mucosa was assessed by the evaluation of epithelial and collagen layer thickness, presence of edema (lucent spaces), and the regularity of collagen fibrils. Treatment groups were compared in data sets that included all subjects who had both baseline and end-of-treatment biopsy data available. Small sample size and distributional properties required that nonparametric statistical tests be used for within- and between-treatment comparisons for both light and electron microscopy data.

Two analyses were conducted: a main-effects analysis using a Wilcoxon rank sum test and a rank analysis of covariance. Within treatment group, changes from baseline to study end were also analyzed with a Wilcoxon signed rank test.

The nasal biopsy specimens taken at the end of the study similarly included squamous epithelium, although 2 specimens contained both respiratory and squamous epithelial cells. At both baseline and study end, the collagen was type I, basal lamina duplication was rare, and no elastin fibers were seen, providing reassurance that severely damaged nasal mucosa had not been observed.

Biopsy results for epithelial layer thickness, collagen layer thickness, lucent spaces score, and regularity of collagen score are summarized in Table 3. At baseline, the mean epithelial layer thicknesses in the fluticasone group (27.77 µm) and the terfenadine group (28.55 µm) were not significantly different. After 1 year of treatment, the mean epithelial layer thickness had increased to 35.17 µm (+7.40 µm) in the fluticasone group whereas it had decreased to 20.91 µm (−7.64 µm) in the terfenadine group. These changes did not represent a significant change from baseline for either group as tested by the Wilcoxon signed rank test (for fluticasone, P = .37; for terfenadine, P = .23). However, when the treatment groups were compared at study end, significant differences were observed for both the main-effects analysis based on the Wilcoxon rank sum test (P = .05) and when baseline effects were included as a covariate in the model (P = .03, rank analysis of covariance). This was a significantly higher epithelial thickness at the end of treatment in the fluticasone group (35.17 µm) compared with the terfenadine group (20.91 µm).

Mean collagen layer thickness was not significantly different before or after 1 year’s treatment with either fluticasone (P = .68) or terfenadine (P = .47). At baseline, the mean collagen layer thickness was 11.45 µm in the fluticasone group and 11.71 µm in the terfenadine group. At study end, mean collagen layer thickness values remained essentially unchanged (11.97 µm in the fluticasone group and 12.46 µm in the terfenadine group).

A lucent spaces score, graded on a 4-point scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe), was created to quantify the degree of tissue edema observed. The mean scores at baseline were similar: 1.19 for the fluticasone group and 1.09 for the terfenadine group, indicating mild edema. No significant within- or between-treatment group differences were observed after 1 year of treatment. The mean score at study end was essentially unchanged for both treatment groups (1.05 for the fluticasone group and 1.14 for the terfenadine group).
The regularity of collagen was graded on a 3-point scale of +1 (least regular) to +3 (most regular). The mean scores at baseline were not significantly different: 1.83 for the fluticasone group and 1.75 for the terfenadine group, indicating a midrange level of regularity. After 1 year of treatment, the regularity scores increased slightly in both groups (2.05 in the fluticasone group and 1.85 in the terfenadine group). No significant within-treatment group changes were observed, and no significant differences were observed when the treatment groups were compared.

**COMMENT**

The primary objective of this study was to evaluate the effect of fluticasone on the nasal mucosa and determine whether atrophy would be observed after continuous treatment for 1 year. The presence of atrophy was primarily based on 4 objective evaluations, including epithelial and basement membrane (collagen layer) thicknesses, the degree of edema (lucent spaces), and the regularity of the collagen fibrils, as well as clinical observation of the nasal mucosa during the monthly visits.

Our results showed that there was no evidence that treatment with fluticasone propionate aqueous nasal spray, 200 µg once daily, caused nasal mucosal atrophy as compared with oral terfenadine, the active control. None of the expected quantitative changes that could have indicated atrophy were observed, and qualitative assessments confirmed that elastin was not present, type I collagen was not replaced, and basal lamina duplication did not increase. Furthermore, clinical observations during the monthly visits showed no abnormalities on rhinoscopy.

When the treatment groups were compared, no significant differences between treatment groups were observed with the exception of epithelial layer thickness, which showed a significant difference between treatments. The fluticasone group experienced a mean increase in the epithelial layer thickness at study end, while the terfenadine group experienced a mean decrease. This result, coupled with the qualitative assessments based on visualization of nasal mucosal tissue, suggests that the integrity of the nasal mucosa was not affected adversely during treatment with fluticasone in subjects with perennial allergic rhinitis. Furthermore, the increase in epithelial thickness after fluticasone treatment may represent repair of epithelial damage associated with the chronic inflammation that is characteristic of perennial allergic disease.

Our findings are consistent with the existing literature and confirm what has been the clinical experience for decades, ie, that long-term use of intranasal corticosteroid therapy does not lead to atrophy or other severe adverse sequelae in the nasal mucosa. Moreover, our study is the first, to our knowledge, to use objective and quantitative assessment of the nasal mucosa by transmission electron microscopy and in which lack of change in these objective criteria were used to conclude that fluticasone treatment did not have deleterious effects on the nasal mucosa. Several other intranasal biopsy studies have similarly failed to find an atrophic effect of other intranasal corticosteroids or fluticasone.

Table 1. Assessment of Atrophy

<table>
<thead>
<tr>
<th>Measure of Atrophy</th>
<th>Expected Changes From Baseline If Atrophy Occurred*</th>
<th>How Measured</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium layer thickness (light microscopy)</td>
<td>↓</td>
<td>Thickness measured in micrometers at 3 points along specimen; mean value analyzed</td>
<td>Decrease in thickness suggestive of tissue atrophy</td>
</tr>
<tr>
<td>Collagen layer thickness (light microscopy)</td>
<td>↓</td>
<td>Thickness measured in micrometers at 5 points along specimen; mean value analyzed</td>
<td>Decrease in thickness suggestive of tissue atrophy</td>
</tr>
<tr>
<td>Presence of edema (electron microscopy)</td>
<td>↑</td>
<td>4-Point scale for lucent spaces: 0 (none), 1 (mild), 2 (moderate), 3 (severe)</td>
<td>The higher the score, the more edema; suggestive of tissue damage</td>
</tr>
<tr>
<td>Regularity of collagen fibrils (electron microscopy)</td>
<td>↑</td>
<td>3-Point scale: +1 (least regular) to +3 (most regular)</td>
<td>Highest degree of regularity (+3) indicated tissue damage</td>
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* Downward arrow indicates decreased; upward arrow, increased.

Figure 1. Low-power transmission electron micrograph of the nasal mucosa demonstrating, from top to bottom, the epithelium, collagen layer, lamina propria, and the glandular layer (original magnification × 1200).
Among the findings of interest, Sørensen et al\textsuperscript{13} in 1976 performed biopsies of polyps after treatment with beclomethasone dipropionate aerosol for 1 year and observed decreased interstitial fluid within the tissue by light microscopy. Furthermore, there were no consistent changes of the surface epithelium by transmission electron microscopy. Quantitative data or controls were not presented. In 1977, Poyntner\textsuperscript{14} observed that no mucosal atrophy occurred in patients treated with beclomethasone for 2.5 to 3.5 years. In 1978, Mygind et al\textsuperscript{15} re-
ported that biopsy specimens of nasal polyps in patients receiving beclomethasone treatment for 3 years showed no changes in epithelial metaplasia or ciliary structure. In 1982, Holopainen and colleagues performed biopsies in 6 patients after 6 years of treatment with beclomethasone and observed no evidence of atrophy. No quantitative data were presented. In 1983, Knight and Kolin performed biopsies in 9 patients with perennial rhinitis with or without allergy after the patients had received beclomethasone for 48 weeks in an open, noncomparative study. They reported no evidence of atrophy or scarring as assessed by light microscopy, but few details were presented. In 1988, Pipkorn et al reported on a multicenter, open longitudinal study of 24 patients receiving budesonide. Biopsy specimens obtained at entry and after 1 year (5 patients) and between 2.5 and 5.5 years (5 patients) of use showed no significant change. In a more recent study, Minshall and colleagues evaluated nasal biopsy specimens from an open study of 52 subjects with perennial rhinitis before and after 1 year of treatment with mometasone furoate by light microscopy and compared the results with those from 24 healthy subjects who underwent biopsy at baseline and after 1 year without treatment. They found no change in epithelial thickness, no signs of atrophy, and a decrease in focal metaplasia.

In 1991, Orgel and colleagues evaluated 41 paired nasal biopsy specimens from an open-label study of fluticasone butyl. They reported no evidence of basement membrane thickening and, similar to our findings, observed a tendency for improvement of the epithelium toward columnar. The authors concluded that intranasal corticosteroids improve epithelial injury secondary to chronic inflammation.

Holm and colleagues performed a double-blind, placebo-controlled biopsy study with fluticasone with the use of light microscopy in patients with perennial allergic rhinitis. Twenty-eight sets of paired biopsy data were able to be evaluated before and after 1 year of treatment. No differences between the groups were observed before or after treatment. The biopsy site in the Holm et al study (inferior edge of the inferior turbinate) differed from that in ours (anterior tip of the inferior turbinate) but demonstrated that a year's treatment with fluticasone did not alter the mucosa in the ciliated epithelium.

Skin atrophy from topically applied corticosteroids can be detected within time frames varying from 1 to 4 months of administration. Why the nasal mucosa acts differently from the skin is not known. There are several possible explanations. One explanation is that, while the nose contains only type I collagen, the skin contains elastin and type IV collagen. The latter elements may be more glucocorticoid sensitive. Another explanation may relate to residence time of drug at the application site. With dermatologic preparations, the creams or ointments are applied to a specific site and left in situ, sometimes under occluded conditions, for up to 24 hours. With an intranasal spray, drug is removed within a few hours from the airways by mucociliary transport.

An open-label study, although not generally optimal, was specifically selected as the design for this study. Any attempt to double-blind this study, given the differences in the formulations of the test medications (intranasal spray vs oral tablets), would have required a double-dummy design that would have necessitated that all subjects take both oral medication and intranasal spray. Although not expected to have detrimental effects on the nasal mucosa, a placebo spray or the insertion of the device into the nostrils to deliver the nasal spray could potentially have confounded the biopsy data. To obviate this problem, all biopsy evaluations were performed by clinicians who were otherwise uninvolved in the care of subjects and who were blinded to the subject's treatment and identity. This eliminated potential bias and ensured the validity of the nasal mucosa assessments, which were the primary safety assessments in this study.

This study was not designed to compare the efficacy of these compounds in the treatment of allergic rhinitis. Fluticasone and terfenadine have been compared in well-controlled clinical trials by others. However,
subjects were examined in this study on a monthly basis to demonstrate that therapeutic doses of both treatment regimens were being taken by the subjects and to reinforce medication adherence. During the study, the treatment groups experienced reductions from baseline in their rhinitis symptom scores as assessed by both clinicians and subjects. Thus, it appeared from these assessments that study medication adherence was sufficient to derive therapeutic benefit.

Study treatments were well tolerated during this year-long study. The adverse event profile was similar between treatments and to that observed in numerous other studies. No serious or unusual events were observed. Only 1 subject withdrew from the fluticasone group because of localized bleeding. Whether this patient would have shown changes on a nasal biopsy specimen consistent with mucosal damage is unknown. Our findings, however, support the clinical impression that, if patients are tolerating intranasal corticosteroids, they can safely continue them. It is therefore prudent to reinforce the clinical practice of examining patients within 2 to 4 weeks of initiation of intranasal corticosteroid treatment with careful attention to the nasal mucosa. If bothersome bleeding is reported by the patient and the nasal mucosa shows evidence of injury, it is prudent to decrease the dose of administered corticosteroids or even discontinue them.

The results of this study show that the use of intranasal fluticasone propionate aqueous nasal spray, 200 μg once daily for 1 year, does not lead to atrophy in the nasal mucosa. Conversely, this study shows that treatment with fluticasone leads to a significant increase in epithelial thickness compared with the posttreatment epithelial thickness in the terfenadine-treated group. This suggests a positive effect of intranasal fluticasone on the nasal epithelium. Our study has extended the previous work of others with nasal biopsy by using quantitative objective measures of atrophy from light and electron microscopy in a larger number of subjects and comparing the results with those of biopsy specimens from subjects using a suitable therapeutic alternative. In compilation, these studies all speak to the lack of histologic damage induced by intranasal corticosteroids.

In summary, our study should make clinicians feel more secure in their use of intranasal corticosteroids to treat allergic rhinitis. Using light and electron microscopy, we performed a detailed and controlled investigation of the nasal mucosa and found no evidence of structural damage after 1 year of use. Combining these observations with the established efficacy of intranasal corticosteroids in treating nasal symptoms argues that they should be a first-line treatment in patients with perennial allergic rhinitis.

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