

Original article

Clinical and pathologic methods to assess the long-term safety of nasal corticosteroids

Background: The main objective of this long-term prospective local safety study was to evaluate endoscopic and histologic changes in nasal epithelium after 6-month treatment with triamcinolone acetonide (TAA). We describe here a method to measure quantitatively epithelium thickness. Results were compared with those seen with the use of cetirizine (an antihistamine) and another oral intranasal corticosteroid, beclomethasone dipropionate (BDP).

Methods: Patients were examined by an ENT specialist who first performed an endoscopic evaluation of the nasal cavities, assessing any morphologic abnormalities and the aspect of the mucosa. Biopsies were taken from the inferior turbinate before and after 24 weeks of treatment. Biopsies were immediately fixed in cold acetone (-20°C) and embedded in glycolmethacrylate; sections of 2 μm were cut on an ultramicrotome. Morphometric evaluations were done in a blinded fashion by computerized image analysis to measure an epithelial area over a minimum length of 50 μm . The thickness was ascertained by the ratio of area to length.

Results:

- 1) For all three treatment groups, the nasal epithelium thickness decreased slightly from pretreatment to the end of treatment.
- 2) No statistically significant differences between the three treatment groups were found in epithelium thickness.
- 3) Macroscopically, nasal tissues in all treated groups were normal.

Conclusions: These results clearly indicate that long-term treatment with TAA has no atrophic effect on nasal mucosa.

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Key words: corticosteroids safety; morphometric analysis; nasal mucosa; perennial allergic rhinitis; triamcinolone acetonide.

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Accepted for publication 3 April 2000

Topical corticosteroids represent the first-line treatment of perennial allergic rhinitis (PAR) and are often used for a long period of time (1). Although there is a general consensus that these drugs are highly effective and usually safe, the long-term safety of each should be assessed (2, 3).

Triamcinolone acetonide (TAA) is an intranasal corticosteroid (4–8). Clinical trials have shown that once-daily TAA 110–220 μg reduces symptoms of allergic rhinitis within the first day of administration and reduces nasal eosinophil influx. Furthermore, TAA 220 μg per day was significantly more effective at reducing the nasal symptoms of allergic rhinitis than oral H_1 -blockers (9). However, intranasal TAA local long-term safety has not been fully characterized.

The local long-term safety of topical corticosteroids can be assessed clinically, but an objective biologic method assessing the drug effect on the nasal mucosa is of great importance. In particular, the effect of the drugs on the nasal epithelium appears to be an important

parameter to be measured (2, 3). The measurement of epithelial thickness is always a problem in nasal or bronchial specimens because of the difficulty in orienting the biopsies at the embedding step, to obtain sections perpendicular to the basement membrane, leading to artifactual variations in the measured thickness of the epithelium. Thus, repeated measures should be studied.

In the present study, we report a method to measure quantitatively epithelium thickness by computerized image analysis in patients with PAR before any treatment and after 6-month continuous treatment with either cetirizine (10 mg o.d.), TAA (220 $\mu\text{g}/\text{day}$), or beclomethasone dipropionate (BDP) (400 $\mu\text{g}/\text{day}$).

Material and methods

Patients

Ninety-two patients with perennial rhinitis were

included in the study. They were selected on the following criteria. Patients (men or women who were not pregnant or breast-feeding) aged 18–65 years had had symptoms of perennial rhinitis for at least 1 year and a positive skin test to house-dust mites (*Dermatophagoides pteronyssinus*, Der p) and/or the presence of serum Der p-specific IgE (CAP System[®], Pharmacia Upjohn).

Patients who had taken one of the following drugs were excluded: oral or nasal antihistamines (7 days), astemizole (3 months), nasal cromoglycate (7 days), corticosteroids in any form (4 weeks), and parenterally administered corticosteroids (3 months).

Design of the study

The trial was a multicenter, randomized, parallel-group, open study, involving eight centers scattered around France. The morphologic analysis was done in a single center by two investigators blinded to the treatments received by the patients.

The study was not placebo-controlled, since it would have been unethical to maintain patients with moderate or severe symptoms untreated for 6 months, and because of the probable high rate of dropouts. Cetirizine was chosen as a noncorticosteroid comparator since it has been shown to be effective in PAR (10–13), and was unlikely to be a confounding factor for the primary variable outcome. BDP was used as a corticosteroid comparator since it is the most commonly used nasal corticosteroid (14–16). No patient blinding was performed during the study, but ENT doctors performing the nasal endoscopy and the staff analyzing the biopsy were not aware of the treatments given to the patients.

During the study, patients were not allowed to take any other medication for allergic rhinitis. They were instructed that if their nasal symptoms were severe enough to warrant additional treatment, they should return immediately for consultation in order to be withdrawn from the trial or to receive a prescription for an additional treatment if they were suffering from an acute nasal disease.

Compliance with the treatment was assessed at the end of the study from the data supplied on the test drug management form and the patient's diary cards. Each bottle of TAA and BDP was weighed before delivery and after return by the patients. The number of cetirizine tablets was counted. If compliance with a treatment was under 70%, the patient was excluded from the analysis.

The study was approved by the ethics committee of the Montpellier hospital, and it was conducted in accordance with the Declaration of Helsinki and after written informed consent was obtained from the patients.

Nasal endoscopy and biopsy

Patients were examined by an ENT specialist, who first performed an endoscopic evaluation of the nasal

cavities, assessing any morphologic abnormalities and the aspect of the mucosa. The inferior and middle turbinates as well as the nasal septum were examined.

Biopsies were obtained from the posterior part of the inferior turbinate with a Microfrance forceps (2 mm) after local anesthesia with a spray of xylocaine and epinephrine. Bleeding was controlled with a silver nitrate stick if necessary.

Tissue preparation

Biopsies were immediately fixed at -20°C in acetone containing the protease inhibitors iodoacetamide and phenylmethylsulfonyl (PMSF), by the method of Delsol et al. (17). The specimens were kept at -20°C and immediately shipped to the central laboratory in dry ice by express mail.

Within 24 h after the biopsy procedure, the specimens were processed and embedded in the water-soluble resin glycolmethacrylate (GMA, JB4 Kit, Park Science Ltd, Northampton, UK) by the technique of Britten et al. (18). GMA was chosen because the morphology of resin-embedded tissue sections is superior to that of frozen and paraffin-embedded tissue. Sections of 2 μm were cut from the resin-embedded specimen with a LKB Nova ultramicrotome, and floated on 0.2% volume of ammonia solution for 1.5 min. The sections were then collected, in pairs, on silane-coated glass slides.

Morphologic analysis

All slides were observed using a $\times 20$ SPlan Apo objective on a BHS microscope (Olympus, Japan) equipped with a Sony DXC-950P 3CCD color video camera (Sony, Japan). All computed images were analyzed by SIS 2.01 Software for image analysis (Soft-Imaging Software GmbH, Germany).

For the morphologic measurements, we did not use repeated measures, but the epithelium thickness was evaluated by a measurement of area over a minimum length of 50 μm (Fig. 1) from the internal side of the epithelium ("area method"). The ratio of area to length gives the thickness. The accuracy of the method was assessed by calculating coefficients of variation (CV) of five measures of area/length from seven biopsies, and comparing them with repeated linear measures of the thickness in the same biopsies.

The factors considered in the choice of "area method" for morphometric analysis were primarily theoretic. For analysis, the following features had to be present: a longitudinal section of pseudostratified epithelium, and equal thickness of the basement membrane (as defined by light microscopy) all along the measured area, correlated with a transverse section of the neighboring glands in the submucosa (Fig. 2). Moreover, the specimens were analyzed only if these conditions were met for the two biopsies studied for each patient. To ensure accuracy, the measurements were performed on the two

biopsies in areas which were as similar as possible in epithelium structure, because the mucosa of the anterior turbinate can display three different types of epithelium (19).

Statistical analysis

The study was powered in order to get 72 sets of patient data. For all statistical analyses, all comparisons were two-tailed, all hypothetic normalities of variables were verified by the Kolmogorov test, and the Bartlett test was used to test the equivalence of variances. The demographic criteria were evaluated by analysis of variance and Fisher’s exact test. The primary and secondary end-point criteria were analyzed by a combination of covariance models and the Wilcoxon W test.

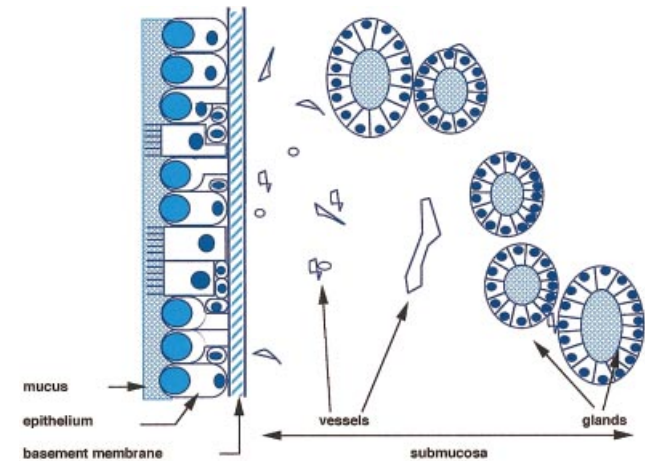


Figure 2. Schematic representation of nasal mucosa: organization of components required in choice of measurement area.

Results

Number of subjects

Among the 92 patients initially included, one discontinued the study, one underwent nasal surgery, and three did not comply with at least 70% of treatment. Seven patients received concomitant nasal therapy during the treatment period for acute nasal symptoms, but this treatment was administered at least 1 month before the second biopsy, and the patients were not excluded. Nineteen patients were not evaluable because of the size or quality of the biopsy sample. At the end, 70 patients remained in the study: 21 patients were evaluable in the TAA group, 26 in the BDP group, and 23 in the cetirizine group.

Table 1. Patient demographics (evaluable population)

Variable	TAA (n = 21)	BDP (n = 26)	Cetirizine (n = 23)
Sex			
Male	11	14	17
Female	10	12	6
Age (years)			
Mean	27	26.4	28
Median	23	22.5	25
SD	9.15	9.9	8.65
Minimum	19	18	20
Maximum	54	62	52
Race			
White	21	24	23
Black	0	2	0
Oriental	0	0	0
Child-bearing status?			
No	1	0	0
Yes	9	12	6
Duration of PAR (years)			
Mean	11.7	8.5	11.2
Median	11	8.5	11
SD	5.4	5.81	6.81
Minimum	2.9	1.3	1.8
Maximum	21.3	22.9	27.9

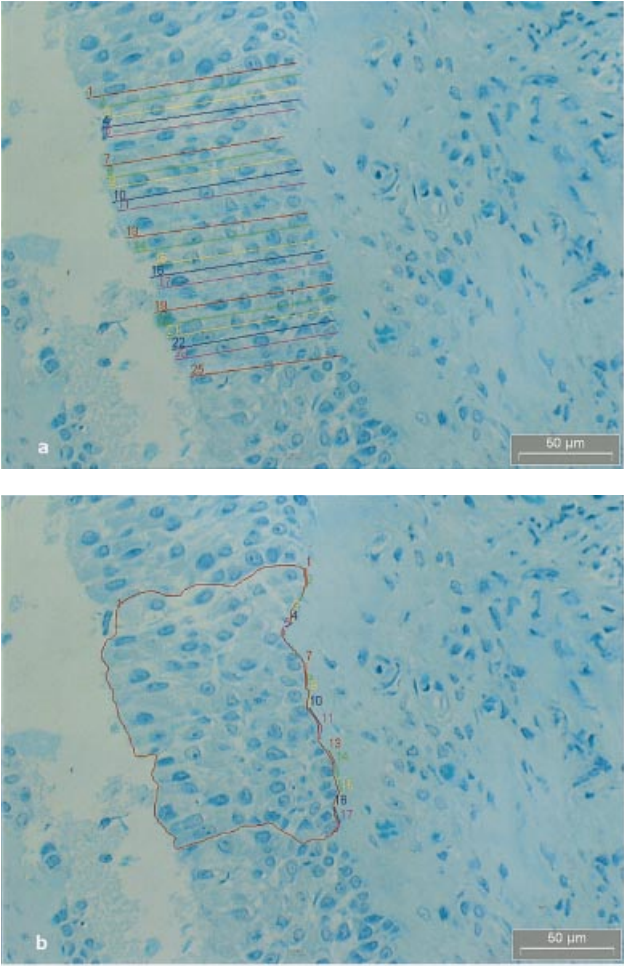


Figure 1. Computerized measurement of epithelial thickness. a) Many measurements of distance. Note difficulty in determining true perpendicular to outermost edge of epithelium. Twenty-five measurements were made, numbered 1–25. b) Area method. On right, nos. 1–17 give length corresponding to area measured.

Table 2. Nasal biopsy before treatment (visit 1 = V1)

	Epithelium thickness (μm) (evaluable population)			
	TAA (n=21)	BDP (n=26)	Cetirizine (n=23)	Total (n=70)
Mean	108.8	103.4	102.7	104.8
Median	112.3	88.3	106.3	105.5
SD*	22.52	35.43	24.52	28.29
Minimum	65.7	50.2	62.1	50.2
Maximum	140.9	172.8	138.0	172.8

* SD: standard deviation.

Demographic characteristics of the patients

The demographic characteristics of the patients were similar in the three patient groups. The proportion of male to female patients was approximately equal in the evaluable population for TAA and BDP, and, although there were still a disproportionate number of men, the difference in the male-to-female ratio in the cetirizine group was much less than in the intent to treat population.

The mean age was also similar between treatment groups, 27.0 years (± 9.15) for TAA, 26.4 years (± 9.90) for BDP, and 28.0 years (± 8.65) for cetirizine patients.

The mean duration of PAR was also similar between groups, 11.7 years (± 5.4) for TAA, 8.5 years (± 5.81) for BDP, and 11.2 years (± 6.81) for cetirizine patients.

The patient demographic profile for the evaluable population is displayed in Table 1.

Endoscopic evaluation

Macroscopically, nasal tissues in all treated groups were normal.

Epithelial thickness

The “area method” for thickness evaluation was found to be the quickest way of measurement. Moreover, we found a significantly ($P < 0.005$, Mann–Whitney U test) better coefficient of variation for the measurement by the “area method” (CV, median and 25–75th percentiles: 1.9; 1.8–3.1%) than by repeated linear measures (CV: 6.2; 6–8.1%).

The results of the quantitative analysis first revealed a great individual variation in epithelium thickness at the visit before treatment (V1) (Table 2) but revealed no relevant change ($P = 0.88$) in the epithelium thickness measured in specimens after 6 months of treatment at visit 4 (V4), compared with these features in the pretreatment biopsy specimens (V1). Moreover, the morphometric analysis of all patient data revealed no significant difference in epithelium thickness between each of the three treatment groups ($P = 0.06$) (Table 3).

When examining the epithelial appearance, we observed only rare cases of metaplasia, the major part of sections (at V1 and V4) exhibiting pseudostratified respiratory epithelium with goblet cells, ciliated cells,

Table 3. Nasal mucosa epithelium thickness (μm) before (visit 1) and after (visit 2) treatment (evaluable population)

	Adjusted mean at visit 4	Mean at visit 1
Epithelium thickness		
TAA	97.3	108.8
BDP	98.7	103.4
Cetirizine	95.2	102.7
SD (d.f.)	23.8 (66)	
P value	0.88	

d.f.: degree of freedom.

and basal cells. Qualitative analysis showed that there was no striking change in the histologic features of individual patients between biopsies taken before and after treatment. Moreover, we never found epithelium destruction in the biopsies studied here.

Discussion

The method used in this study is interesting on two levels: first, in the assessment of the safety of topical steroids; second, in the ability to interpret the individual variability of epithelium and submucosa in the nasal mucosa of patients with PAR.

Efficacy was not the primary end point of this study. Thus, in order to assess safety, we used a design similar to that for study of fluticasone propionate and mometasone furoate (2, 3).

The safety of topical corticosteroids may be assessed with different outcome measures. In the present study, we used a primary end point, epithelial layer, as previously proposed.

While data have been reported elsewhere on measurement of epithelium thickness, there is no agreement on how many measurements should be made to obtain a reliable estimate of this thickness. In a recent paper, Sullivan et al. (20) reported that many measurements should be made when measuring basement-membrane thickness in bronchial biopsies. They recommended that this thickness should be measured at 20-μm intervals over a 1-mm length! This should be extrapolated to epithelium thickness measurement in nasal mucosa. Nevertheless, measurement over a 1-mm length is difficult because of the size of biopsies. Here we propose the alternative solution of using the ratio of epithelial cross-sectional area to the length of the basement membrane to measure epithelial layer thickness. Moreover, to improve accuracy, we always used together three complementary criteria to determine the measured zone:

- 1) strict longitudinal cutting of the epithelium
- 2) constant thickness of the basement membrane
- 3) transversal cutting of mucoserous glands in the submucosa.

Finally, when paired biopsies were examined, we took care to make measures on the same epithelial type.

The joint use of all these criteria allows us to avoid drawbacks due to the different methods used in nasal biopsy studies – endoscopy, inclusion, and cutting – and thus to ascertain precisely histologic features due only to pathologic or individual variations.

Quantitative analysis of biopsies taken from patients before treatment shows individual variations. There are several explanations for this: individual morphologic difference, natural fluctuation of the disease, or variation in antigen exposure. We did not find epithelial destruction as described in bronchial asthma. This observation is consistent with the observations of Lim et al. (19).

In conclusion, by the method here described, we have

demonstrated that a 6-month treatment with TAA, BDP, or cetirizine did not induce any significant change in the size of the epithelium, and that long-term nasal treatment with TAA does not cause epithelium atrophy.

Like other, comparative topical steroids, TAA does not affect epithelial thickness (2, 3). Thus, in accordance with the recommendations of the International Rhinitis Management Working Group on the use of nasal glucocorticoids, and their overall safety (1), TAA may be considered a first-line therapy in patients with PAR.

Acknowledgments

We thank Nicolas Rouyère for statistical analysis. We are especially grateful to the participants of the multicenter study group. This study was supported by Rhône Poulenc Rorer, France.

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