

Influence of treatment with intranasal corticosteroids on the nasal mucosa, weight, and corticosteroid concentration in rats

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ABSTRACT

Background: The effect of intranasal corticosteroids on the nasal epithelium mucosa is an important parameter of treatment safety. This study was designed to examine whether treatment with topical corticosteroids in patients with allergic rhinitis causes atrophic nasal mucosal changes, when compared with systemic corticosteroids, in rats.

Methods: Male Wistar rats were treated daily during 7 weeks with topical administration with 10 microliters of normal saline (control group), 10 microliters of mometasone furoate group, 10 microliters of triamcinolone acetonide (T group), and 8 mg/kg of daily subcutaneous injections of methylprednisolone sodium succinate (MP group). Body weight was evaluated weekly. At the end of the treatment, rats were killed by decapitation to collect blood for determination of corticosterone levels and nasal cavities were prepared for histological descriptive analyses.

Results: Treatment with T and MP decreased body weight. Plasma corticosterone concentration was significantly reduced by MP treatment and presented a clear tendency to decrease after T treatment. Histological changes observed in group T included ripples, cell vacuolization, increase in the number of nuclei, and decrease in the number of cilia in the epithelial cells.

Conclusion: Growth and corticosterone concentration were impaired by T and MP at the same proportion, suggesting a role of this hormone in body gain. With the exception of T, intranasal or systemic treatment with the corticosteroids evaluated in this study did not affect nasal mucosa.

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Since 1949 corticosteroids have been used for systemic therapy because of their potent anti-inflammatory and immunosuppressive effects. Currently, these agents are available in different formulations that may be administered by several routes (oral, i.v., by inhalation, intranasal, and dermatologic) to treat a wide variety of diseases such as rheumatologic,¹ allergic,^{2,3} hematologic,⁴ renal,⁵ and neoplastic disorders.^{6,7} To minimize potential side effects associated with the use of these drugs and to decrease the total dose required to achieve the desired effect, corticosteroids have increasingly been administered topically.⁸ Consequently, this reasoning has led to the development of intranasal corticosteroids for allergic rhinitis, which have earned an important role in the management of this disease.⁹

Allergic rhinitis is characterized by inflammation of the nasal mucosa, which is induced by exposure to allergens that trigger an inflammatory response mediated by immunoglobulin E. The symptoms of allergic rhinitis may be chronic or recurrent and include rhinorrhea, nasal obstruction, sneezing, and ocular manifestations (pruritus and conjunctival hyperemia), which resolve spontaneously or with treatment.¹⁰ Intranasal glucocorticoids are among the most commonly prescribed and effective medications used to treat allergic rhinitis.¹¹

The intranasal corticosteroids currently available in Brazil include beclomethasone dipropionate, triamcinolone acetonide (T), budesonide, fluticasone propionate, and mometasone furoate (M).¹² These drugs down-regulate the recruitment and influx of inflammatory cells and inhibit the secretion of proinflammatory mediators during the late phase of the inflammatory response.¹³ Patients with allergic rhinitis who are treated with intranasal corticosteroids typically experience improvement of all nasal symptoms, indicating that these drugs are effective in the early phase and late-phase reactions of allergic inflammation.^{14–17} The safety and efficacy of these drugs have been

well established in multiple clinical trials; of note, intranasal glucocorticoids have not been associated with substantial untoward adverse events.¹⁸ Side effects associated with the use of intranasal corticosteroids can be local or systemic; more commonly, local adverse effects are seen and include nasal crusting and dryness, with epistaxis being less frequent. Local fungal infections and perforations of the nasal septum have been rarely reported.¹⁴ Effects on the hypothalamic-pituitary-adrenal (HPA) axis, mineral metabolism, childhood growth, and ocular function are rare systemic adverse effects of intranasal corticosteroids.¹⁹

Although histological and functional data generated from long-term studies that investigated the local effects of several intranasal corticosteroids have shown no evidence of atrophy,^{20–22} the effect of these drugs on the nasal epithelium appears to be an important parameter to be measured.^{20,21} The most obvious indicators of local damage are morphological and functional changes in the ciliated surface of the nasal mucosa; in addition, measurable indicators of atrophy include factors such as collagen deposition, presence of elastin, basal lamina duplication, epithelial layer thickness, mucosal edema, and cellular inflammation. To our knowledge, there have been no published studies on the effect of M on the normal nasal mucosa in animals. Thus, this study was designed to examine whether treatment with topical corticosteroids causes atrophic nasal mucosal changes when compared with systemic corticosteroids.

MATERIALS AND METHODS

Experimental Animals

Eighty male Wistar rats, obtained from a local facility at the University of São Paulo (Campus of Ribeirão Preto), at 28–78 days of age, were used in this study. The initial body weight ranged between 90 and 140 g. The animals were maintained at $24 \pm 0.5^\circ\text{C}$ on a 12:12 hour light–dark cycle (lights on at 6:00 A.M.), with free access to tap water and food. All animals were handled at 11:00 A.M. All experimental procedures were approved by the local Research Ethics Committee (protocol 097/2007) and developed in conformity with our institutional guidelines and with international standards for manipulation and care of laboratory animals.

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Experimental Groups

Rats were distributed into four groups: control group (C), receiving 10 μ L of sterile 0.9% saline in each nasal cavity daily; M group (M), receiving 10 μ L (5.5 μ g; Nasonex; Schering-Plough, Heist-op-Berg, Belgium) in each nasal cavity daily; T group (T), receiving 10 μ L (6.1 μ g; Nascort; Sanofi-Aventis, Holmes Chapel Crewe, Cheshire, England) each nasal cavity daily; and methylprednisolone sodium succinate group (MP), receiving daily subcutaneous injections of 8 mg/kg of this agent (Solu-Medrol; Pfizer, Puurs, Belgium).²³ All groups received normal saline or active drugs for 7 weeks and had their body weight measured weekly. At the end of the treatment, all rats were decapitated. One week before the death, all animals were anesthetized with 0.8 mL of ketamine and 0.7 mL of xylazine for implantation of an orthodontic device for the study of tooth movement; the results of such study will be published separately.

Histological Preparation and Analysis

After decapitation, the animals' nasal cavities were postfixed for 48 hours in 10% formaldehyde for histological studies. Tissue demineralization was prepared for 10 days in sodium citrate and formic acid solution, which was changed every 2 days. After demineralization, the tissue was washed in tap water during 30 minutes for removal of the demineralizing solution and remained submerged for 15 minutes under agitation in solution of sodium sulfate 5%. The tissue was washed again in tap water for 3 hours, dehydrated in increasing series of alcohols, cleared in xylol, and embedded in paraffin. After demineralization, the nasal septum was removed from the nasal cavity for exposition of the nasal conchae. Semiserial transverse slices of 6 μ m were performed. Hematoxylin and eosin was used for histological staining.

Histological descriptive analysis was performed for six animals in each group, with both right and left nasal epithelia being examined. The morphological features were described using an optical microscope (Axioskop 2 plus; Carl Zeiss, Thornwood, NY) connected to a system for image analysis (Axiovision 3.1; Carl Zeiss).

Plasma Corticosterone Determination

On decapitation, trunk blood was collected to determine plasma concentrations of corticosterone (with sodium heparin used as anticoagulant). Plasma samples were extracted using ethyl ethanol and corticosterone concentration was quantified in the resuspended pellet by radioimmunoassay.²⁴ To avoid interassay error, all samples were assayed in one assay. The primary antibody and standard were provided by Sigma, Inc. (St. Louis, MO) and the ³H-labeled hormone was obtained from Amersham (Piscataway, NJ). The lower limit for detection was 21.4 ng/mL and the intra-assay error was 3.9%.

Statistical Analysis

Variables of interest were summarized by means and SDs. Data were analyzed for normality distribution by the Kolmogorov-Smirnov test. Body weight was measured during all of the study and compared within and between groups using analysis of variance followed by Tukey's test to control for type I (false positive) error. Plasma corticosterone concentration was compared using the nonparametric Kruskal-Wallis test followed by Dunn's test, to adjust for multiple comparisons. In each test, the null hypothesis was rejected at values of $p < 0.05$.

RESULTS

Body Weight

All experimental groups showed similar body weight at the 1st week of treatment. After 2 weeks, although not statistically significant, the animals treated with T and those treated with MP showed a trend toward a decreased body weight gain and remained with a clear tendency to be lower during all of the treatment, when compared with

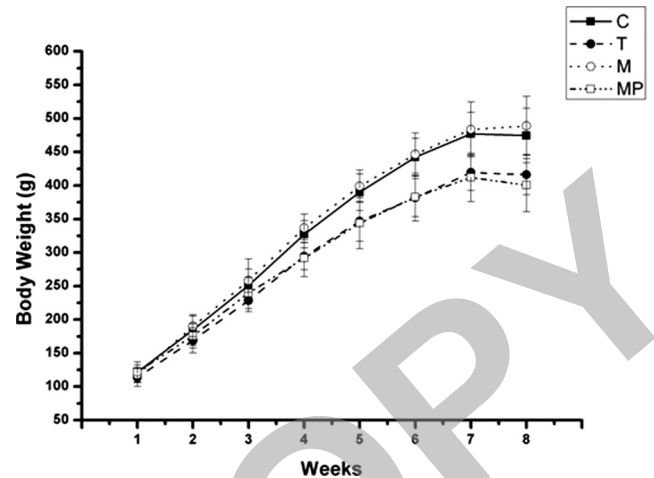


Figure 1. Body weight (g) during treatment with intranasal sterile saline (control group [C]), mometasone furoate group (M), triamcinolone acetonide group (T), and subcutaneous methylprednisolone sodium succinate group (MP). Data are expressed by mean $\pm$ SEM. Data of body weight were analyzed by two-way analysis of variance. * $p < 0.05$; MP group versus C and M groups after 7 weeks of treatment. [†] $p < 0.05$; M group versus T after 7 weeks of treatment.

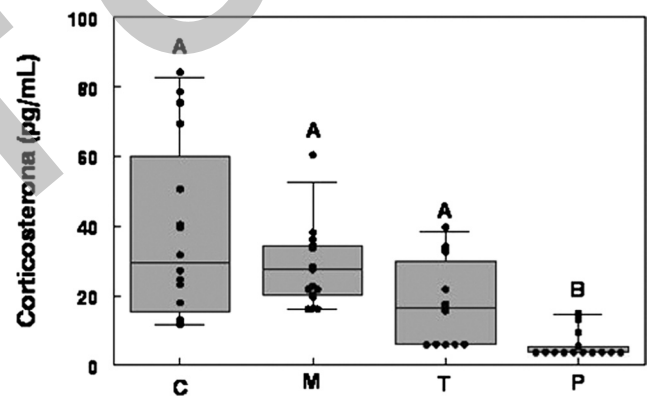


Figure 2. Plasma corticosterone (median and interquartile range) in control rats (C), treated with mometasone (M), triamcinolone (T), or methylprednisolone (P). Data were analyzed by Kruskal-Wallis test. Different letters indicate significant differences between groups: A $\neq$ B, $p < 0.0001$.

groups C and M. At the end of the experimental period, body weight of group M was similar to that of C group, and that of group T was similar to that of the MP group, both clearly lower than M and C groups. However, statistical significance was found only between groups M and T as well as between C and MP. Nevertheless, it is evident the tendency for group T to be different from the C group (Fig. 1).

Plasma Corticosterone Concentration

Plasma corticosterone concentration was lower in the MP group, when compared with other treatment groups, as shown in Fig. 2. Although not statistically significant, there was a clear tendency for corticosterone of group T to be lower than that of the C group.

Epithelial Features

In the C group the nasal epithelium, the basal membrane, and the lamina propria, as expected, showed a normal aspect with a pseudostratified ciliated columnar epithelium. The epithelium was characterized by a single layer of cells covered with cilia and nuclei in different positions. In some animals, a mild inflammatory infiltrate was observed in the epithelium (Fig. 3). Treatment with M appeared

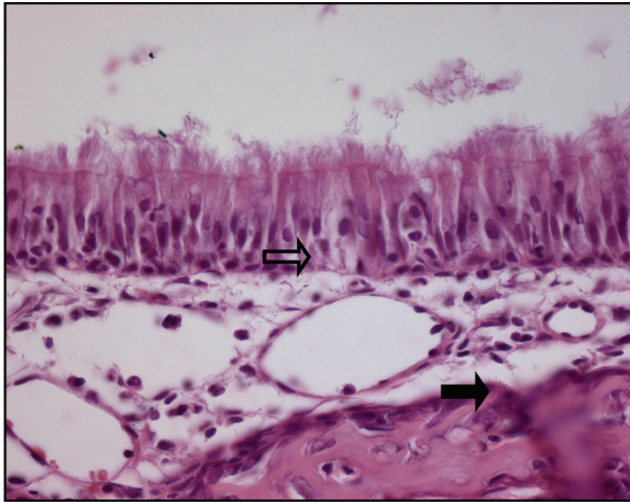


Figure 3. Normal epithelium (open arrow) and lamina propria (solid arrow) observed in animals treated with intranasal sterile saline for 7 weeks (light microscopy, hematoxylin and eosin [H&E] staining, 400× magnification).

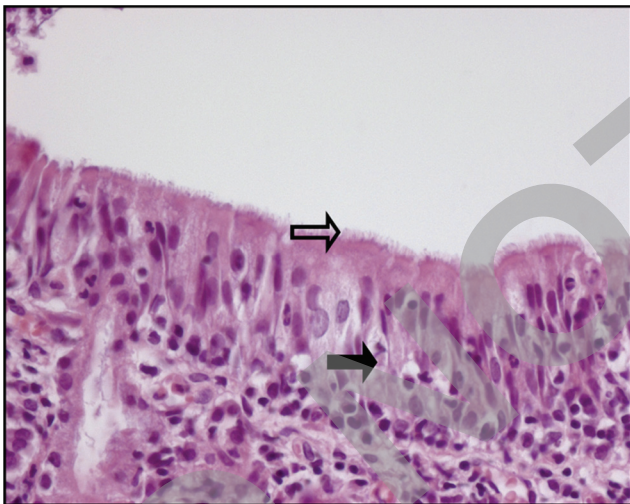


Figure 4. Normal epithelium (open arrow) with slight leukocyte exocytosis (solid arrow) observed in animals treated with intranasal mometasone furoate for 7 weeks (light microscopy, hematoxylin and eosin [H&E] staining, 400× magnification).

to cause no changes in the nasal epithelium, which showed a normal aspect and displayed the ciliary layer in all samples analyzed (Fig. 4). Similar result were observed in group MP, where all animals presented normal nasal epithelium characterized by a single layer of goblet cells with nuclei in various positions and covered by a layer of cilia. In this group, most samples appeared to show a slight paucity of neutrophils in the lamina propria (Fig. 5).

The nasal epithelium in group T presented ripples, cell vacuolization, and a slight increase in the number of nuclei, some of which were hyperchromatic. In this group, the epithelial cells had an important decrease in the number of cilia and only one animal showed a normal mucosa. In most samples from group T, chronic mild inflammation was observed, with neutrophilic exocytosis present in the lamina propria (Fig. 6).

DISCUSSION

To evaluate the effect of treatment with glucocorticoids during childhood, animals used in this study were 28 days old at the beginning and 78 days old at the end of the experiment. This period was chosen because it was shown that our rats enter puberty between 40

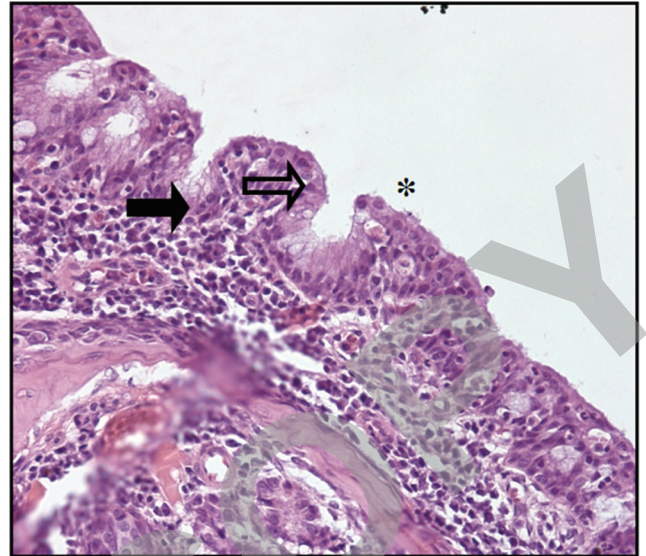


Figure 5. Epithelium with reactive changes (open arrow), mild chronic inflammation (solid arrow), and absence of cilia (*) observed in animals treated with intranasal triamcinolone acetonide for 7 weeks (light microscopy, hematoxylin and eosin [H&E] staining, 400× magnification).

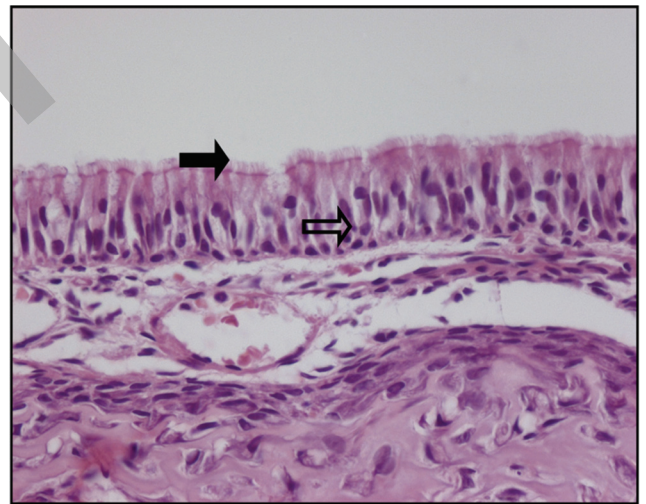


Figure 6. Normal epithelium (open arrow) and lamina propria (solid arrow) observed in animals treated with subcutaneous methylprednisolone sodium succinate for 7 weeks (light microscopy, hematoxylin and eosin [H&E] staining, 400× magnification).

and 50 days of age and become fully mature between 83 and 97 days.²⁵ Thus, we have studied rats during their childhood, including the puberty period, but before adulthood. It was shown in this study that treatment with MP significantly decreased the body weight gain as well as the corticosterone plasma concentration. In addition, there was a clear tendency of the T group body weight gain to be lower than controls, and the same tendency was observed in the corticosterone plasma levels of this group. Thus, corticosterone plasma levels seem to be correlated to the body weight gain.

Among the concerns with systemic effects resulting from the use of intranasal corticosteroids are the potential for growth suppression and the potential for HPA axis suppression. In fact, intranasal corticosteroids have potential systemic adverse effects, because the administration of some corticosteroids to a highly vascular nasal mu-

cosa might be associated with systemic bioavailability. In addition, the respiratory system does not provide first-pass inactivation, and the absorbed drug would retain its native high potency in the systemic circulation.²⁶ Drug absorption across the nasal mucosa into the circulation also varies significantly with different agents; *e.g.*, the estimated absolute bioavailability is less than 1% of the dose after intranasal administration of M.²⁷ This low bioavailability appears to be associated with no systemic effects as seen in our study and in studies with adults²⁸ and children.²⁹ For MP administered *i.v.*, a bioavailability of 100% is expected. This may explain the impairment on growth rate and the decrease of corticosterone release observed in the group treated systemically with this drug. Likewise, a relatively high bioavailability (46%²¹) for intranasal T can explain the similar effects observed in the group treated with this drug.

Clinical trials evaluating the effects on growth rate in children have generally found that intranasal corticosteroids do not affect growth. One exception was observed in a study reported in 2003 in which the mean urine cortisol/creatinine ratio was significantly lower during treatment with fluticasone propionate, 200 µg/day, than with T, 110 or 220 µg/day, or placebo.³⁰ In our study, a lower body weight was observed in the animals treated with C and T, which may be related to the decreased corticosterone plasma concentration of these animals, notwithstanding the findings observed in humans. A possible explanation for the discrepancy between our results and those from the literature in humans could be that primates differ significantly from rodents in multiple aspects involving the HPA axis, such as a different cortisol/corticosterone ratio.³¹

Compared with C, rats treated with M showed no significant changes neither on the nasal epithelium nor on the basement membrane and lamina propria. We could not find any study in the literature about the effect of M on the nasal mucosa in animals. However, careful analyses of the tissue sections of the animals treated with T have shown that a normal nasal epithelium was observed only in one rat. In all other samples, several alterations such as a reduction of ciliated cells, the absence of cilia, goblet cell vacuolization, and moderate chronic inflammation were observed.

Our results in combination with data found in the literature lead us to believe that T, unlike M, does not seem to be safe for the treatment of rhinitis in children because of the changes observed in the nasal epithelium, which can be credited to the use of this drug, and to a possible influence on growth, as suggested by the significant decrease in weight gain in animals treated with this drug.

CONCLUSIONS

Our results indicate that intranasal M and systemic C do not affect the nasal mucosa. These results corroborate the currently prevailing view in the literature that treatment of allergic rhinitis with most intranasal corticosteroids is safe. To our knowledge, our study is the first to examine the effect of M on the normal nasal mucosa in animals.

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