INHIBITION OF MEDIATOR RELEASE IN ALLERGIC RHINITIS BY PRETREATMENT WITH TOPICAL GLUCOCORTICOSTEROIDS

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Abstract Patients with allergic rhinitis often have immediate symptoms after antigen challenge (the early-phase response), followed several hours later by a recurrence of symptoms (the late-phase response). Systemic glucocorticosteroids are known to inhibit the late-phase but not the early-phase response. We studied the effect of one week of pretreatment with topical (rather than systemic) glucocorticosteroids on the response to nasal challenge with antigen in a double-blind, randomized, placebo-controlled crossover study of 13 allergic patients who had previously had a dual response to nasal challenge. The patients were challenged with three 10-fold increments of allergen, producing an early response, and were then followed for 11 hours, encompassing the late response, before they were rechallenged with the lowest dose of allergen. We monitored their responses by means of symptom scores and measurements of the levels of histamine, tosyl-L-arginine methyl ester (TAME)-esterase activity, and kinins in nasal lavages.

Topical glucocorticosteroids significantly reduced both the symptoms and the levels of histamine, TAME-esterase activity, and kinins in the early, late, and rechallenge allergic reactions. The fact that, in contrast to treatment with systemic glucocorticosteroids, prolonged pretreatment with topical glucocorticosteroids inhibited the early-phase response to antigen suggests that the route and duration of administration affect the mechanisms of action of the steroids.

We conclude that inhibition of the early-phase as well as the late-phase response by topical glucocorticosteroids may provide an advantage over treatment with systemic glucocorticosteroids in patients with allergic rhinitis. (N Engl J Med 1987; 316:1506-10.)

SYSTEMICALLY administered glucocorticosteroids, which are potent agents in the treatment of allergic disease, affect the late but not the early phase of the biphasic response to antigen challenge, as shown in numerous studies involving the lungs and skin in animals and humans.[1-4] For example, in a nasal-challenge study, we recently found that pretreatment with systemic prednisone (20 mg three times a day for 48 hours) had no effect during the early reaction on either the symptoms reported or the levels of histamine, prostaglandin D_2, tosyl-L-arginine methyl ester (TAME)-esterase activity, or albumin recovered in nasal secretions.[5] During the late reaction, however, prednisone reduced the symptoms reported and the mediators recovered in lavages. In addition, when antigen was presented again 11 hours after the early reaction, prednisone reduced the usual augmented response ("rechallenge reaction"). Combining these data with the observation that overnight culture of human lung mast cells with glucocorticosteroids does not inhibit IgE-mediated histamine release,[6] we speculated that prednisone had no effect on nasal mast cells but affected the inflammatory events that occur after the early reaction.

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Topical glucocorticosteroids effectively and safely treat allergic rhinitis,[7,8] although their mode of action remains obscure. Several clinical studies suggest that pretreatment with topical glucocorticosteroids inhibits the early nasal and bronchial allergic reaction,[9-12] although some have not observed this inhibition.[13,14] If the former results are valid, the mechanism of glucocorticosteroid activity could be one or more of the following: a direct effect on mast-cell mediator release, an effect on end-organ sensitivity after mediator release, a change in the metabolism of mediators, or an effect on the status of the mucosa before antigen challenge.[15-17] Our investigation was designed to ascertain whether pretreatment with topical glucocorticosteroids would inhibit the symptoms of the early nasal allergic reaction, as well as the late and rechallenge reactions, and whether a reduction in symptoms correlated with a reduction in mast-cell mediator release.[18]

METHODS

Study Design

We performed a double-blind, randomized, placebo-controlled crossover study of asymptomatic subjects in the pollen-free winter months. Nasal challenges with antigen were performed after one week's treatment with 200 μg of flunisolide spray or placebo spray per day. A one-month washout period separated the two challenges.

Subjects

We selected 13 patients, 4 women and 9 men 20 to 36 years of age (mean age, 30.5), with seasonal allergic rhinitis due to grass or ragweed pollens. All subjects had a positive intradermal skin test to 10 PNU (protein nitrogen units) or less of antigen extract, and all had previously had a dual response (early and late) to nasal challenge with antigen. The study was approved by the Joint Committee on Clinical Investigation of Johns Hopkins University, and in-

formed consent was obtained from the patients before their participation in the study. All 13 patients completed the study, and none were omitted from the analysis.

Reagents

Ragweed and mixed-grass pollen extracts (timothy, orchard, June, and meadow grass in a ratio of 3:2:3:2) were purchased from Greer Laboratories (Lenoir, N.C.); lactated Ringer's solution and oxymetazoline hydrochloride (Afrin, Schering, Kenilworth, N.J.) were purchased from the hospital pharmacy.

Treatment

Identical-looking nasal sprays of flunisolide and placebo were provided by Syntex Laboratories (Palo Alto, Calif.). The patients were asked to spray each nostril twice two times a day, starting one week before the day of the challenge. The investigators also administered a dose of the spray one hour before the challenge and again two hours after the antigen was administered. Each use of the flunisolide spray delivered 25 μg of the drug, giving the patients 200 μg daily. In order to be sure that the intended dose was actually received by the patient, the bottles were weighed before and after treatment. The measurements suggested that all patients had complied with the instructions for taking the medications. No other medications were allowed during the study.

Procedure for Nasal Challenge

The technique used for the nasal challenge has previously been described in detail.[18,19] Briefly, the protocol involved four prechallenge nasal lavages with lactated Ringer's solution to reduce the levels of cell-free mediators that are typically present in nasal secretions. Then oxymetazoline hydrochloride was sprayed into the nose (two sprays per nostril) to prevent mucosal congestion, which would interfere with the collection of nasal secretions. It has been shown previously that this dose of oxymetazoline does not affect histamine release during the early reaction to antigen.[19] Subsequently, two challenges with the diluent for the pollen extracts were used to control for nonspecific effects of the diluent or the delivery system. Thereafter, serial challenges with 10, 100, and 1000 PNU of antigen were undertaken. Nasal lavages with lactated Ringer's solution were performed 10 minutes after each of the procedures described above. In addition, lavages were performed 20 minutes after the last antigen challenge and every hour for the next 11 hours. After a second dose of oxymetazoline, we repeated the challenge with only the 10-PNU antigen dose. Lavages were performed 10 and 20 minutes later.

Mediator Assays

The assays for histamine, TAME-esterase activity, and kinins made use of techniques previously described in detail.[19,20] In brief, histamine concentrations in individual samples, at one dilution, were determined by an automated fluorometric technique that was sensitive to 1 ng per milliliter and accurate to within ±5 percent.[21] Enzymes displaying arginine esterase activity were measured as individual samples at one dilution in duplicate by the method of Imanari et al.,[22] in which ^{3}H-labeled methanol is liberated from the synthetic substrate [^{3}H]TAME. The appearance of TAME-esterase activity in nasal lavage fluids correlates with the acute response to nasal antigen challenge.[19] TAME-esterase activity detected in the immediate response to antigen challenge represents approximately 75 percent plasma kallikrein complexed to α_2-macroglobulin, 25 percent mast-cell tryptase, and a small amount of glandular kallikrein.[23,24] EDTA was added to the samples for the measurement of kinins to make the samples 40 mM with respect to this agent, in order to prevent enzymatic degradation by kininases. Kinins were assayed in duplicate at two or three dilutions by a radioimmunoassay that is sensitive to 20 pg per milliliter with an intraassay and interassay variation of less than 5 percent.[20]

Assessment of Symptoms

The patients maintained a symptom score sheet during the challenge procedure. In addition to the number of sneezes, a six-point scale from 0 to 5 (with 0 equal to no symptoms and 5 equal to severe symptoms) was used to assess nasal secretion, blockage, and itching. The degree of blockage is, of course, underestimated on these score sheets because of the pretreatment with oxymetazoline hydrochloride. The presence or absence of symptoms correlates with the presence or absence of mediators during the late reaction.[18]

Statistical Analysis

To analyze the results, we defined base lines and time intervals as follows. The early response was assessed 10 minutes after each of the 10-, 100-, and 1000-PNU antigen challenges and 20 minutes after the 1000-PNU challenge. The late response was defined as the response occurring 2 through 11 hours after challenge, and the rechallenge reaction was defined as the response occurring 20 minutes after the second 10-PNU challenge. The base line for the early response was the average of the two diluent challenges, whereas the base line for the late reaction was the lowest level during hours 2 through 4. The base line for the rechallenge reaction was the lavage preceding the antigen rechallenge.

The data were analyzed in several different ways. The threshold

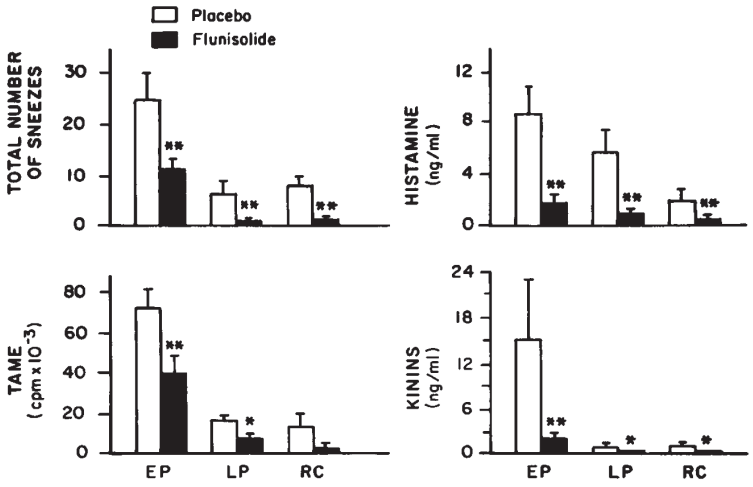

Figure 1. Mean Net Increases in Mediator Levels (±SEM) in Lavage Fluids and the Total Number of Sneezes in 13 Patients.

For each time period, the sum of the net increases (base-line levels subtracted) is presented. In the early phase (EP), the lavage samples were those obtained 10 minutes after each of the three antigen challenges plus the lavage samples obtained 20 minutes after the third antigen challenge; in the late phase (LP), the hourly samples during hours 2 through 11; and in the rechallenge (RC), the two samples obtained after antigen exposure. The base line for the early reaction was the mean level of the two diluent challenges; for the late reaction, the lowest value during hours 2 through 4; and for the rechallenge, the value in the lavage fluid immediately before rechallenge. All variables except TAME-esterase activity during rechallenge were significantly reduced by pretreatment with glucocorticosteroid. * indicates $P<0.05$; ** indicates $P<0.01$.

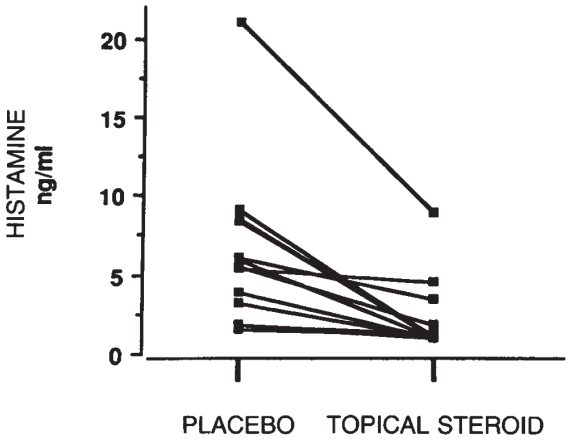

Figure 2. Comparison of the Maximal Level of Histamine during the Early Response to Antigen after Pretreatment with Placebo or Topical Glucocorticosteroid (Steroid).

The significant reduction ($P<0.01$) after topical glucocorticosteroid pretreatment also occurred with symptoms and levels of TAME-esterase activity and kinins (data not shown).

for the early response was the first dose of antigen that led to a twofold increase over the base-line value. The maximum response was the highest level of mediators in the lavage fluids collected. The net increase in the level of mediators was the sum of the levels of the mediators above the base-line value in the lavage fluids collected during a particular time period.

The statistical evaluation was performed on an Apple Macintosh microcomputer with use of the Statfast statistical software package. The nonparametric Wilcoxon signed rank-sum test was used to evaluate differences between placebo and active treatment. No crossover effect was observed.

Results

In general, after pretreatment with the placebo, increasing doses of antigen caused an immediate increase in symptoms associated with an increase in recovered nasal lavage fluids of the levels of histamine, TAME-esterase activity, and kinins (early response). The response dissipated over the next several hours and was typically followed by a spontaneous recurrence of symptoms and mediators (late reaction). Rechallenge with the lowest dose of antigen (10 PNU) used 11 hours previously induced another immediate response. The patients' individual response patterns after pretreatment with placebo were similar to those in our previous study[18] in that the number of peaks and the timing of peaks varied. These results contrast with the response after pretreatment with topical glucocorticosteroids, which, like prednisone, dramatically reduced both the symptoms and the expected increase in the mediators measured in the late and rechallenge reaction. Topical flunisolide, unlike systemic prednisone,[5] also reduced the early response. Not only did symptoms and mediators decrease absolutely, but the dose–response curves appeared to be shifted approximately 10-fold.

Group data for the early, late, and rechallenge responses are shown in Figure 1. During the early reaction, both indexes of clinical response — sneezes and symptom score (data not shown) — were decreased significantly by topical glucocorticosteroid treatment ($P<0.01$). The net increase in the amount of histamine released during the entire early response was decreased by about 75 percent ($P<0.01$), as was the amount of histamine released during the maximal response to antigen ($P<0.01$) (Fig. 2). Kinin levels during the early response were decreased about 80 percent ($P<0.01$). The decrease in TAME-esterase activity, though less dramatic (55 percent), was significant ($P<0.01$). There was also an increase in the threshold dose for a positive response to antigen (i.e., the dose required to cause a twofold or greater increase in the level of mediators above base line) in 9 of the 13 patients ($P<0.01$) (Fig. 3).

The decrease in clinical indexes after pretreatment with glucocorticosteroids, as compared with placebo, was, as expected, more striking during the late phase than during the early phase ($P<0.01$). The same pertained to the levels of histamine ($P<0.01$), TAME-esterase activity ($P<0.01$), and kinins ($P<0.05$). Furthermore, the response to 10 PNU of antigen at 11 hours was reduced by pretreatment with topical glucocorticosteroids. This was also true for symptoms and the net increase above the base-line value (lavage before rechallenge) for histamine and kinins. The increase in sensitivity is examined in Figure 4. After pretreatment with placebo, rechallenge with the lowest dose of antigen (10 PNU) caused increases in the levels of histamine, TAME-esterase activity, and kinins to levels equivalent to those seen after a 10-fold higher dose of antigen (100 PNU) given 11 hours before ($P<0.01$ for each comparison). After pretreatment with topical glucocorticosteroids, the levels of histamine, kinins, and TAME-esterase activity and

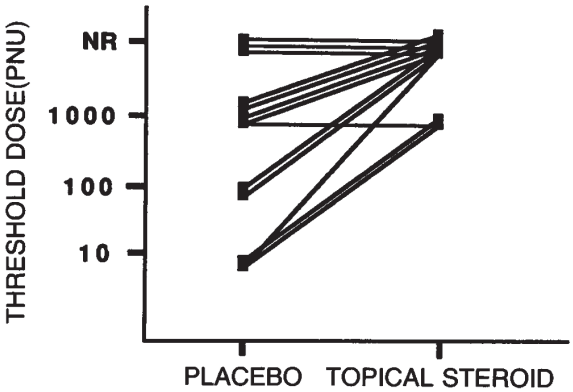

Figure 3. The Threshold Dose for a Positive Histamine Response.

The threshold dose (the first dose of antigen that caused a twofold or greater increase in the level of histamine relative to the diluent challenges) increased significantly ($P<0.01$) with pretreatment with topical glucocorticosteroids. Similar results were seen for symptoms and the threshold levels of TAME-esterase activity and kinins. NR denotes no response (i.e., a threshold was not reached). Steroid denotes glucocorticosteroid.

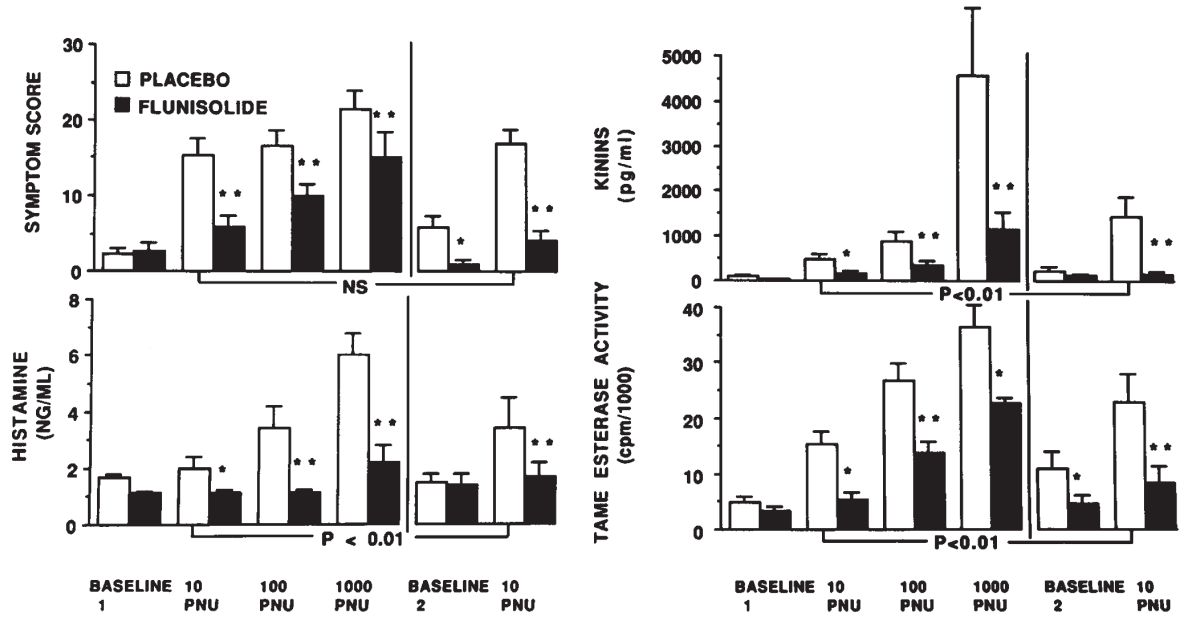

Figure 4. Levels of Histamine, TAME-Esterase Activity, and Kinins and Symptom Scores (Includes Number of Sneezes) 10 Minutes after Each Challenge (Means ±SEM).

Statistical comparison of results after placebo and topical glucocorticosteroid pretreatment are indicated (* = $P<0.05$; ** = $P<0.01$; NS = not significant). The vertical line indicates 11 hours' separation in time between challenges. The connecting line indicates the comparison between the two 10-PNU challenges on the placebo day. The second challenge, 11 hours after the first, is augmented and is not statistically different from the initial 100-PNU challenge.

the symptom scores were significantly reduced after each antigen challenge, as compared with placebo, and the increased sensitivity following rechallenge was no longer evident.

Discussion

Our results clearly demonstrate that one week of pretreatment with topical glucocorticosteroids inhibits both the symptoms and the release of histamine and other inflammatory mediators during not only the late and rechallenge reactions to nasal challenge with antigen but also the early response. These experiments thus demonstrate inhibition of mediator release by glucocorticosteroids during the immediate phase of an IgE-mediated anaphylactic reaction in humans. The early response to nasal challenge is believed to reflect mast-cell activation on the basis of the following observations: (1) biopsy specimens of the nasal mucosa after antigen challenge show an increased number of degranulated mast cells,[25] (2) prostaglandin D_2, the major cyclooxygenase product of mast cells (but not made by basophils), increases during the early response,[19] (3) topical administration of azatadine, a drug that inhibits IgE-mediated mast-cell histamine release in vitro, also inhibits histamine release in vivo,[26] and (4) pretreatment with aspirin inhibits the release of prostaglandin D_2 without augmenting the release of leukotriene C_4 or histamine — a pattern similar to that seen after pretreatment of human lung mast cells with nonsteroidal antiinflammatory drugs.[27] In vitro studies have shown that human lung mast cells, unlike basophils, are not inhibited by pretreatment with glucocorticosteroids.[6,28] The lack of effect of a high dose of prednisone on antigen-induced histamine release in the nose supported this notion, and the medical literature is quite clear in showing a lack of effect of systemic glucocorticosteroids on the early response to antigen.[1-5] Our unexpected results indicate that glucocorticosteroids affect the pathophysiology of nasal challenge between antigen presentation and histamine release, and thus give rise to several hypotheses. High doses of steroids may reduce the numbers of mast cells in the nasal mucosa,[29] a change that could markedly alter the early response. Another likely possibility is that direct application of soluble glucocorticosteroids to the nasal mucosa may increase the dose to local cells sufficiently to bring out glucocorticosteroid effects not detected during systemic administration or in vitro. Or the increased time of exposure to glucocorticosteroids (seven versus two days) may explain the difference between the inhaled and orally administered drug. It is also possible that the epithelial barrier may have been altered to reduce antigen presentation to mast cells. Our data do not differentiate among these alternatives and several others.

The reduction in the late and rechallenge reactions may be the mechanism by which this class of drugs

achieves clinical efficacy. Unfortunately, our experiments do not indicate whether this is a direct effect on the cells and mediators involved in the late reaction or an indirect effect related to the reduction of the early reaction.

Since these drugs take several days to achieve an effect, clinicians usually recommend beginning their use several days before the pollen season occurs. Our data provide a strong, scientific rationale for this practice. When one can anticipate seasonal exposure, premedication with glucocorticosteroids reduces the early response to antigen, in addition to suppressing inflammation associated with more chronic aspects of allergic rhinitis.

In summary, the present study shows that prolonged pretreatment with topical glucocorticosteroids reduces symptoms and the release of mediators not only during the late reaction but also during the early allergic reaction in the nose. This effect is at least partly due to a reduction in the generation of inflammatory mediators. The decrease in the late reaction may be either a direct effect of the treatment with glucocorticosteroids on components of that reaction or an indirect effect of a decrease in the early reaction. This study points to differences between the mechanisms of action of topical and systemic glucocorticosteroids and suggests additional reasons for preferring topical administration.

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