High-Dose Inhaled Fluticasone and Delayed Hypersensitivity Skin Testing*

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Introduction: Systemic steroids have been associated with anergy. Treatment with high-dose inhaled steroids has many documented systemic side effects, including adrenal suppression, reduction in growth velocity, and increased bone metabolism; however, little is known about their effect on delayed-type hypersensitivity (DTH).

Study objectives: The purpose of this study was to determine if a 28-day course of high-dose inhaled fluticasone suppresses DTH to a standard panel of antigens.

Methods: Forty-five healthy, steroid-naı̂ve subjects volunteered for this randomized, double-blinded, placebo-controlled trial. All subjects had baseline DTH assessed by intradermal skin testing to a standard panel of antigens (tetanus, candida, mumps, and tuberculin) read 72 h after placement. Subjects were then randomized to receive placebo or high-dose inhaled fluticasone (880 μg/d) for 28 days, after which a second DTH panel was performed. A third DTH panel was performed after a 30-day washout period.

Measurements and results: Of the 45 enrolled subjects, 38 subjects completed the study, including 20 subjects in the placebo group and 18 subjects in the drug group. There was no significant difference in the amount of induration between drug and placebo groups for any of the three periods tested.

Conclusion: Twenty-eight days of treatment with high-dose inhaled fluticasone did not suppress DTH in healthy volunteers. (CHEST 2003; 123:1014–1017)

Key words: anergy; delayed-type hypersensitivity; inhaled corticosteroids; purified protein derivative testing

Abbreviations: DTH = delayed-type hypersensitivity; PPD = purified protein derivative

Systemic steroids have been associated with anergy since the 1950s. In 1958, the effect of systemic steroids on tuberculin skin testing was studied in Thailand in 70 patients with positive delayed-type hypersensitivity (DTH) to purified protein derivative (PPD).1 PPD testing was repeated every 72 h. All subjects were treated with prednisone, 10 mg po qid. Prednisone completely suppressed the PPD skin test in 68 of the 70 patients, with an average rate of reversion to negative PPD of 13 ± 10 days (mean ± SD) after starting the prednisone. The average rate of reconversion to positive PPD after stopping prednisone was 6 ± 3 days. Articles addressing this topic have been sparse since these initial studies were done.

Treatments with high-dose inhaled steroids has been shown to have systemic effects, including adrenal suppression,2-3 reduction in growth velocity,4-5 and increased bone metabolism.6-7 A study by Sharma et al8 suggested that DTH reactions may also be suppressed by high-dose inhaled steroids. In that study, 10 healthy volunteers were recruited for a small, nonplacebo-controlled, prospective study. All subjects received fluticasone, 880 μg/d, for 30 days. The Multitest CMI (Connaught Laboratories; Swiftwater, PA) was performed before and after inhaled
steroid treatment as well as after a 6-week washout period to assess DTH. Eight of nine subjects were reported to have a reduction in cumulative skin score on the DTH panel, and two of nine subjects also acquired anergy (i.e., reduction in reactivity of < 3 mm for all tested antigens) after inhaled steroid treatment. One of four subjects with a history of a positive PPD had no reaction to tuberculin after fluticasone therapy. The purpose of this study was to prospectively assess whether 28 days of high-dose inhaled fluticasone was able to suppress DTH in a steroid-naïve population, compared to placebo.

MATERIALS AND METHODS

Study Design

This was a randomized, double-blinded, placebo-controlled trial. Healthy volunteers (age range, 18 to 50 years) recruited largely from employees at a military hospital gave informed consent and were enrolled in the study as approved by the local institutional review board. Exclusion criteria included systemic or inhaled steroids within the past 6 months, nasal steroids within the past month, pregnancy, or chronic illnesses associated with anergy, such as malignancy, chronic infection, or autoimmune disease. Baseline DTH responses were measured on all subjects on entry into the study. Subjects were then randomly assigned to receive placebo or inhaled fluticasone, 880 μg/d (Flovent; GlaxoWellcome; Research Triangle Park, NC) for 28 days, after which a second DTH panel was measured. A third DTH panel was performed after a 4-week washout period.

DTH Skin Testing and Interpretation

Using the standard Mantoux technique, intradermal injections of 0.1 mL of candida (Candin; Allermed Laboratories; San Diego, CA), mumps (Mumps Skin Test Antigen USP; Aventis Pasteur; Swiftwater, PA), tetanus (Tetanus Toxoid USP; Aventis Pasteur;), and PPD (Tuberculin Purified Protein Derivative [Mantoux] Tubersol; Aventis Pasteur) were placed on the volar aspect of the forearm. Panels were placed on alternating arms to prevent local effects from the previous test. The induration at each site was measured, using the ballpoint pen technique, 5–10 h after placement by two independent observers who were blinded to the treatment group. Orthogonal diameters of the induration were measured for each reaction, and the sum of these diameters was recorded. The pen mark was washed off using alcohol between the readings of each observer. If there was a difference > 5 mm between the sum of the diameters between the readings of the two observers, a third independent reading was obtained. The average of the sum of orthogonal diameters of the two closest readings was reported as the outcome variable.

Medication Blinding and Dosing

A pharmacy representative blinded the drug (fluticasone, 220 μg/puff) and placebo inhalers by peeling off the labels, applying study labels to all canisters, and inserting all blinded canisters into fluticasone (Flovent) actuators. Instructions were provided on proper use of a metered-dose inhaler with a spacer (Ellipse; GlaxoWellcome), including rinsing the mouth following administration of the study drug. All subjects were instructed to take two puffs bid from the blinded inhaler.

Statistical Analysis

A Pearson χ² test was employed to ensure baseline comparability between the demographics of the two groups. A student t test was used to compare mean ages of the two groups. A repeated-measures analysis of variance was calculated to compare the mean diameters of the two groups for the three DTH panels (i.e., presteroids, poststeroids, and after washout period). A Pearson χ² test was performed to determine difference between groups with regard to compliance.

RESULTS

Subjects

Forty-five subjects were enrolled from April to November 2002. Thirty-eight subjects completed the study, including 20 subjects who received placebo and 18 subjects who received active drug. Seven subjects withdrew: four subjects were unable to comply with the study schedule, one subject desired to discontinue the study after testing positive to PPD on the first test, one subject in the fluticasone group acquired hoarseness, and one subject in the placebo group acquired an upper respiratory tract infection.

The subjects were predominately white and male (mean age, 33 years; Table 1). There was no significant significance found between treatment groups with regard to previous use of systemic steroids, inhaled steroids, and nasal steroids ever or within the past 6 months.

DTH Testing

No patient who completed the study had a positive PPD test result. Depicted in light gray, Figure 1 shows individual measurements of the sum of orthogonal diameters for each of the three candida, mumps, and tetanus DTH skin tests comparing drug and placebo groups. Each graph depicts the mean with corresponding SD error bars as solid black lines for each of these subgroups. No statistical signifi-

Table 1—Demographics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SEM), yr</td>
<td>33.60 (1.42)</td>
<td>33.78 (1.53)</td>
</tr>
<tr>
<td>Male gender</td>
<td>15/20 (75)</td>
<td>16/18 (88)</td>
</tr>
<tr>
<td>White race</td>
<td>18/20 (90)</td>
<td>13/18 (72)</td>
</tr>
<tr>
<td>Systemic steroids ever</td>
<td>3/20 (15)</td>
<td>2/18 (11)</td>
</tr>
<tr>
<td>Inhaled steroids ever</td>
<td>2/20 (10)</td>
<td>2/18 (11)</td>
</tr>
<tr>
<td>Nasal steroids ever</td>
<td>8/20 (40)</td>
<td>6/18 (33)</td>
</tr>
<tr>
<td>Nasal steroids past 6 mo</td>
<td>6/20 (30)</td>
<td>3/18 (17)</td>
</tr>
</tbody>
</table>

*Data are presented as No. of subjects/total subjects unless otherwise indicated. No statistical significance was found between the groups for any of the categories. No subjects received nasal steroids for at least 1 month prior to the start of the study.
cance was found between drug and placebo groups comparing the baseline skin test with the postdrug skin test for candida, mumps, and tetanus. A subanalysis comparing those who received nasal steroids 2 to 6 months prior to initiation of the study to those who had not received nasal steroids showed no statistical difference between groups.

Anergy (defined as induration < 3 mm to a selected panel of DTH antigens) was not seen in any of the subjects before or immediately following use of the study drug (i.e., tests 1 and 2). However, three subjects were anergic following the washout period (i.e., test 3). Two of the subjects with anergic responses were in the placebo group, and one subject was in the drug group. None of these subjects had received nasal steroids or any form of steroids previously, including nasal steroids.

Compliance and Side Effect Surveys

Compliance surveys were completed by 37 of 38 subjects, demonstrating no statistically significant difference between active and placebo groups (placebo, 2.16 doses missed per week; fluticasone, 1.93 doses missed per week). Few side effects were reported in either group with regard to inhaler use, including sore throat, hoarseness, cotton-mouth, and dysgeusia. Local side effects from DTH skin testing, including pruritus, pain, erythema, edema, and discoloration of skin, were not statistically significant between the groups.

DISCUSSION

Studies in the 1950s directly linked systemic steroids to anergy. Since these initial studies, several
retrospective chart reviews of hospitalized patients have verified that patients receiving systemic steroids are at increased risk for anergy.\textsuperscript{14–16} It has been suggested that inhaled steroids may also cause anergy.\textsuperscript{5} However, in our study, use of high-dose inhaled fluticasone for 28 days did not suppress DTH reactions when compared to placebo. Equally important, no subjects in the drug or placebo groups had anergic responses after the first two DTH panels.

There is only one previous study addressing the effect of inhaled steroids on DTH, reporting suppression of DTH in healthy subjects receiving high-dose inhaled steroids.\textsuperscript{5} This study only examined nine healthy adults without a placebo control and employed the Multitest CMI to assess DTH. The Multitest CMI uses a multipuncture device that is dipped into wells of a battery of seven recall antigens: tuberculin, tetanus, diphtheria, streptococcus, candida, trichophyton, and proteus. The most recent guidelines for PPD placement by the American Thoracic Society do not recommend multipuncture devices for PPD screening due to concern about delivery of consistent amounts of antigen.\textsuperscript{17} Due to these concerns, we believed it was important to use the standard Mantoux technique in our study.

The strength of this study includes use of a placebo-controlled design as well as the blinding of skin test readers, investigators, and subjects. Limitations include interobserver variability, as the same person could not read all tests in our clinic. Attempts were made to minimize this variability by taking an average of the two closest readings. Presstudy power analysis revealed that a sample size of 20 per group provided an 89% chance of detecting large differences (ie, 0.8 to 1.0 SD) between groups. The large variability of individual DTH responses was an unforeseen finding. This variability may have limited the ability to detect differences between the study groups. Another limitation was that our subjects were predominately men recruited from employees of a military hospital. In addition, previous use of various forms of steroids may have been higher than expected in our subjects compared to the general population. This may be explained by recruitment of military health-care workers who have free access to medical care. However, despite these limitations, no subjects in the drug group acquired anergy after 28 days of high-dose inhaled steroid treatment.

**CONCLUSION**

Use of high-dose inhaled fluticasone for 28 days compared to placebo did not suppress DTH reaction to candida, mumps, and tetanus in healthy, steroid-naïve volunteers. Further study may be warranted in subjects who have a history of a positive PPD in addition to conditions such as asthma or COPD requiring the use of inhaled steroids.