Effects of High-Dose Inhaled Fluticasone Propionate via Spacer on Cell-Mediated Immunity in Healthy Volunteers*

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**Background:** Systemic corticosteroids are known to alter cell-mediated immunity (CMI). However, the effects of inhaled steroids on CMI are unclear. We therefore sought to assess CMI following high-dose inhaled steroids in healthy subjects.

**Methods:** Ten healthy nonasthmatic subjects self-administered fluticasone propionate (FP), 440 μg bid, with a spacer device. CMI was assessed by delayed hypersensitivity skin testing to multiple antigens and in vitro by phytohemagglutinin (PHA) stimulation of peripheral blood T lymphocytes. Percentages of CD3+, CD4+, and CD3+CD8+ cells expressing CD69+ were determined by three-color flow cytometry. Studies were conducted before and after 4 weeks of FP treatment.

**Results:** After 4 weeks of FP treatment, two of nine subjects became anergic, whereas six of nine subjects had reduced skin responses (one subject was excluded). Mean total skin test score fell from 18.4 ± 10.9 to 9.1 ± 7.2 mm (p = 0.02). There was a decline in tuberculin responses in all four subjects who were positive prior to FP treatment. Following FP treatment, the percentage of unstimulated (from control subjects receiving saline solution) CD3+CD4+CD69+ cells declined from 14.8 ± 4.2% to 8.5 ± 4.6% (p = 0.02) and the CD3+CD8+CD69+ cells decreased from 29.7 ± 12.7% to 17.1 ± 5.0% (p = 0.007). PHA stimulation produced significant increases in the percentage of CD3+CD4+CD69+ cells before and after FP treatment (67.0 ± 9.1%, p < 0.02 before FP; 55.4 ± 17.0%, p < 0.02 after FP), and in the percentage of CD3+CD8+CD69+ cells before and after treatment (79.7 ± 9.3%, p < 0.03 before FP; 71.2 ± 11.4%, p = 0.008 after FP).

**Conclusions:** High doses of FP suppress the proportion of activated circulating T cells but do not affect the ability of T cells to respond to direct stimulation with PHA. However, depression of skin test responses to antigens following treatment with FP suggests an impairment of in vivo clinical manifestations of T-cell activation by a mechanism that requires further investigation.

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**Key words:** adrenal function; asthma; cell-mediated immunity; inhaled steroids

**Abbreviations:** ACTH = adrenocorticotropic hormone; BDP = beclomethasone dipropionate; CMI = cell-mediated immunity; DCH = delayed cutaneous hypersensitivity; FP = fluticasone propionate; PHA = phytohemagglutinin
mediated immunity (CMI) and thus increase an individual’s susceptibility for acquiring infection. The mechanisms underlying the suppressive effects of steroids on CMI are not completely understood. Tuberculosis has probably been the best studied pathogen, but patients receiving pharmacologic doses of oral steroids are susceptible to all types of facultative intracellular infections. It is unknown whether high doses of inhaled steroids can exert such effects, although their use may be associated with topical infections such as oral candidiasis, which is thought to be secondary to suppression of local cellular immunity. To our knowledge, there is no documented evidence that the incidence or the course of acute viral or bacterial respiratory infections is affected by the use of conventional doses of inhaled steroids in immune-competent hosts, and only occasional cases of reactivation of tuberculosis have been reported with the use of inhaled steroids. However, studies to date have been limited to low-dose inhaled steroid preparations, with no data available (to our knowledge) using formulations delivering up to five times the previous standard dose.

Given the widespread adoption of inhaled steroids for the treatment of asthma and the sparse literature documenting their effects on immune responses, we sought to determine whether recommended doses of inhaled steroids for the treatment of moderate-to-severe persistent asthma alter CMI. We chose to examine delayed cutaneous hypersensitivity (DCH) and lymphocyte proliferative responses to mitogen as markers of CMI.

**Materials and Methods**

**Study Design**

Ten healthy nonasthmatic volunteers between the ages of 18 and 50 years were selected for this open-label prospective study. Subjects with a recent history of infections, use of steroids (including nasal steroids) in the previous 6 months, and diseases or medications that could possibly influence CMI were excluded. The protocol was approved by the local Institutional Review Board, and informed consent was obtained from all subjects prior to entry into the study. Phthiocene propionate (FP), 220 μg at two puffs bid with a spacer device, was self-administered for 4 weeks. Subjects were instructed to rinse their mouth with water and discard the water after using the inhaler.

**Protocol**

CBC count, adrenocorticotropic hormone (ACTH) stimulation tests, and lymphocyte proliferation studies were performed on day 1 (prior to the first dose of medication) and on day 29. DCH testing to multiple antigens (Multitest CMI; Connaught Laboratories; Swiftwater, PA) was performed 5 days prior to starting FP treatment and on day 27 of treatment with readings 48 h after placement. An additional Multitest was performed 6 weeks after the last dose of FP but was not part of the initial protocol. All testing was performed in the morning.

**Lymphocyte Activation Assay**

Lymphocyte activation was assessed by monitoring the expression of an early activation marker (CD69) in whole blood after stimulation with saline solution or phytohemagglutinin (PHA) for 6 h at 37°C using a three-color flow cytometric assay system (FastImmune; Becton Dickinson; San Jose, CA). This assay uses small blood samples, does not require lymphocyte isolation or purification that may alter lymphocyte function, and allows the assessment of lymphocyte subset analysis. Previous studies have shown a good correlation between CD69 expression and H-thymidine incorporation in lymphocytes following PHA stimulation. After incubation of heparinized whole blood with either saline solution or PHA (10 μg/mL), the samples were stained with monoclonal antibody (CD4 FITC/CD69 PE/CD3 PerCP or CDS FITC/CD69 PE/CD3 PerCP or appropriate isotype control antibodies). After lysis of the RBCs (FACS Lysing Solution; Becton Dickinson), the samples were assayed on a flow cytometer that was calibrated for three-channel fluorescence compensation using Cali-BRITE beads and AutoCOMP software (Becton Dickinson). Data were acquired using LYSIS II software (Becton Dickinson) using fluorescence triggering in the FL3 channel (CD3 PerCP) to gate on the CD3+ lymphocyte population. Following acquisition, the data were displayed as two-color dot plots of FL1 vs FL2, and the percentage of CD4+ or CD8+ lymphocytes expressing CD69 were determined using WinList software (Verity Software House; Topsham, MA). Samples stained with isotype control antibodies were used to define nonspecific staining.

**The Multitest CMI Skin Test**

One investigator (K.C.S.) was designated to administer and interpret the Multitest CMI skin test. The system consists of a plastic disposable multipuncture device that simultaneously administers eight test materials. A battery of seven glycerinated antigens (old tuberculin, tetanus toxoid, diphtheria toxoid, streptococcus, candida, trichophyton, and proteus antigens) and a glycerinated control have been standardized in this system. Briefly, the test is performed on the volar surface of the forearm. Firm pressure of the loaded device on the stretched skin causes simultaneous intradermal application of the antigens and the glycerin control at eight sites in a standardized and reproducible fashion. The test CMI was performed on the initially tested forearm with the opposite arm to avoid retest phenomenon. The third Multitest was performed 6 weeks after placement. An additional Multitest was performed 6 weeks after the last dose of FP but was not part of the initial protocol. All testing was performed in the morning.

**Adrenal Function Tests**

A short ACTH stimulation test was performed on day 1 and day 29. A baseline 5-mL blood specimen was taken between 8 AM and 9 AM during the patient’s first visit. Synthetic ACTH, 250 μg, was injected IV, and another 5 mL of blood for serum cortisol was collected at 30 min. The test was considered abnormal if (1) the fasting serum cortisol level was < 5.5 μg/dL, or (2) the 30-min
increment in serum cortisol level was < 20 µg/dL, or (3) the final 30-min serum cortisol level was less than twice the reference baseline level.

Data Analysis

Data are presented as means ± SD. For the lymphocyte studies, Wilcoxon signed ranks test was utilized to assess for differences before and after treatment with FP. The paired t test was used to assess differences in total skin test scores at baseline, after 4 weeks of FP treatment, and then after a 6-week washout period. Post hoc analysis in the presence of statistical differences in skin test scores at the various time points was performed utilizing Wilcoxon signed ranks test. Statistical significance was considered at < 0.05.

Results

Ten subjects (6 men and 4 women) completed the study. Subject age ranged from 27 to 44 years (mean, 34.3 ± 6.2 years). All subjects had normal blood cell counts and normal WBC differential counts. Four subjects reported sore throat and intermittent dysphonia that did not require treatment or discontinuation of treatment with FP. One individual developed oropharyngeal candidiasis requiring topical antifungal treatment but was able to continue FP treatment for the duration of the study.

Multitest

Six subjects reported significant pruritus associated with the initial skin testing punctures. Pruritus resolved in five of the six subjects following 4 weeks of daily administration of FP. The results of Multitest CMI are shown in Tables 1, 2. One subject (subject 2) was unavailable for measurement of the skin test response; therefore, this subject has not been included in the skin test score results. A second subject required one reading by a coinvestigator (D.S.). The mean total skin test scores fell from

Table 1—Frequency of Positive Skin Responses (≥ 2 mm) to Individual Antigens in Nine Healthy Subjects at Baseline, After Inhalation of FP (Post-FP) for 4 Weeks, and 6 Weeks After Cessation of FP Treatment (Recovery)*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Baseline</th>
<th>Post-FP</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus</td>
<td>6/9</td>
<td>5/9</td>
<td>5/9</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>6/9</td>
<td>3/9</td>
<td>4/9</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>3/9</td>
<td>2/9</td>
<td>5/9</td>
</tr>
<tr>
<td>Tuberculin</td>
<td>4/9</td>
<td>3/9</td>
<td>4/9</td>
</tr>
<tr>
<td>Glycerine</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Candida</td>
<td>4/9</td>
<td>1/9</td>
<td>1/9</td>
</tr>
<tr>
<td>Trichophyton</td>
<td>3/9</td>
<td>1/9</td>
<td>1/9</td>
</tr>
<tr>
<td>Proteus</td>
<td>2/9</td>
<td>1/9</td>
<td>1/9</td>
</tr>
</tbody>
</table>

*Data are presented as No./total subjects.

Table 2—Multitest CMI Total Skin Scores and the Number of Positive Test Results (≥ 2 mm) in Nine Healthy Subjects at Baseline, After Inhalation of FP (Post-FP) for 4 Weeks, and 6 Weeks After Cessation of FP Treatment (Recovery)*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Multitest CMI Total Skin Score, mm</th>
<th>Multitest CMI Positive Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-FP</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>16</td>
</tr>
</tbody>
</table>

*Data are presented as No./total subjects unless otherwise indicated.

18.4 ± 10.9 at baseline to 9.1 ± 7.2 after FP treatment (p = 0.004; 95% confidence interval, 4.0 to 14.5). Eight of nine subjects had a fall in the cumulative skin test responses after inhaling FP for 4 weeks, as shown in Table 2. The other subject had an increase from 6.0 to 6.5 mm. Two individuals developed anergy (no response to any skin test antigen). One of four subjects with a previously positive tuberculin test result had no reaction to tuberculin after FP administration. There was a decline in tuberculin responses in all four subjects who were skin-test positive prior to FP treatment. All four tests changed > 3 mm, with a mean change of 5.1 mm and range of 4 to 8 mm. The baseline cumulative tuberculin skin test score of 42 mm was reduced to 27.5 mm after FP treatment. Five subjects had previously received bacille Calmette-Guérin vaccine. An additional Multitest CMI was repeated in all subjects 6 weeks after the last dose of FP was administered. Eight of nine subjects showed an increase in total skin test score. Although the mean score did not return to baseline, there was no statistical difference, compared to baseline (p = 0.7).

Lymphocyte Proliferative Responses

The proportion of unstimulated (ie, saline solution control) CD3+ CD4+ CD69+ lymphocytes declined from 14.8 ± 4.2% to 8.5 ± 4.6% (p = 0.02) following FP treatment, and CD3+ CD8+ CD69+ lymphocytes decreased from 29.7 ± 12.1% to 17.1 ± 5.2% (p = 0.007) following FP treatment (Fig 1, 2). PHA stimulation significantly increased the proportion of CD3+ CD4+ CD69+ lymphocytes from 14.8 ± 4.2% to 67.0 ± 9.1% (p < 0.02) and significantly increased the proportion of CD3+ CD8+ CD69+ lymphocytes from 29.7 ± 12.7% to 79.7 ± 9.3 (p < 0.03). Although
there was a reduction in the percentage of CD69+ lymphocytes in response to PHA stimulation after FP treatment, this was not statistically significant (CD3+CD4+CD69+, p = 0.13; CD3+CD8+CD69+, p = 0.25; Fig 1, 2). However, technical difficulties in three CD4+ and four CD8+ samples precluded analysis of PHA stimulation.

Adrenal Function Studies

The adrenal function data from one subject (subject 2) was not included in the analysis because of no baseline post-ACTH cortisol level, but the rest of the subjects had normal responses to ACTH prior to FP treatment, with a mean of 31.5 ± 10.4 µg/dL (range, 21.2 to 53.7 µg/dL). After FP treatment, baseline ACTH values were 8.7 ± 6.7 µg/dL (range, 0.8 to 18.2 µg/dL) and post-ACTH values were 19.1 ± 8.8 µg/dL (Table 3). Depressed ACTH stimulation tests were noted in three subjects (subjects 3, 6, and 9) after FP administration for 4 weeks. Normal adrenal function was found on retesting these three subjects 6 weeks after FP therapy was discontinued. Only one of these subjects had a positive tuberculin skin test response prior to FP treatment, with a measurement of 10 mm and a post-FP reading of 5 mm. Their skin test responses varied similar to those of the remaining subjects, with two subjects developing a decreased total skin score and essentially no change in the total skin score of the other subject (Table 2).

Discussion

Systemic glucocorticoids are known to cause immune suppression, leading to an increased incidence of opportunistic infections. Inhaled preparations have been widely used reportedly without an increase in the incidence of bacterial or viral infections. Although rare, reactivation of tuberculosis and severe varicella infection has been reported in patients using inhaled steroid preparations. The US Food and Drug Administration has directed the manufacturers of oral, injectable, and inhaled corticosteroids to warn physicians about the increased risk of tuberculosis or severe viral infections in patients using these preparations. Interestingly, this directive is not applicable to topical dermatologic preparations. This warning seems to be unwarranted, according to a position statement issued by the American Academy of Allergy and Immunology. These contradictory positions led us...
to evaluate the effects of inhaled steroids on CMI. After 4 weeks of regular administration of inhaled FP, 880 µg daily, to 10 healthy volunteers, we observed suppression in the proportion of proliferating T cells, but this did not affect the ability of T cells to respond to direct stimulation with PHA. However, the mean skin tests scores declined significantly and two subjects became anergic. One of four subjects who were initially tuberculin positive became nonreactive to tuberculin on repeat skin testing after FP treatment, whereas the others had a depressed tuberculin response. These results should be confirmed with standard intradermal Mantoux testing, since the type and dose of tuberculin delivered in the Multitest are not necessarily the same as with standard Mantoux testing. These results still suggest that inhaled FP can suppress CMI.

Systemic steroid administration is followed by a rapid (within 4 to 6 h), transient (lasting 24 to 48 h) increase in the levels of blood neutrophils and decreases in monocytes, eosinophils, helper cells (CD4+), and total circulating lymphocytes while suppressor (CD8+) lymphocyte levels remain unchanged. Similar changes, but to a lesser extent, have been described with the use of inhaled steroids. Single doses of beclomethasone dipropionate (BDP), either 400 µg or 1,600 µg, had similar effects; and BDP, 800 µg over 24 h in divided doses, showed a similar trend, although the differences at 0 h and 24 h were not statistically significant. There was a 10 to 20% fall in lymphocyte count in patients taking a single dose of BDP, 400 µg or 1,600 µg, while the count increased by 25% in the control group. The effect of BDP, 800 µg over 24 h, showed a decrease

**Table 3—Baseline Cortisol and Cortisol Responses to Synthetic ACTH at Baseline and After Inhalation of FP (Post-FP) for 4 Weeks**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline, µg/mL</th>
<th>Post-ACTH</th>
<th>Baseline, µg/mL</th>
<th>Post-ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.2</td>
<td>27.4</td>
<td>5.1</td>
<td>20.1</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>32.6</td>
<td>14.9</td>
<td>29.3</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>29.2</td>
<td>1.4†</td>
<td>10.4</td>
</tr>
<tr>
<td>5</td>
<td>13.4</td>
<td>21.2</td>
<td>15.0</td>
<td>24.6</td>
</tr>
<tr>
<td>6</td>
<td>14.0</td>
<td>42.6</td>
<td>10.5</td>
<td>23.2</td>
</tr>
<tr>
<td>7</td>
<td>42.6</td>
<td>53.7</td>
<td>0.8†</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>13.4</td>
<td>24.4</td>
<td>18.2</td>
<td>25.6</td>
</tr>
<tr>
<td>9</td>
<td>17.8</td>
<td>25.9</td>
<td>11.2</td>
<td>21.1</td>
</tr>
<tr>
<td>10</td>
<td>12.7</td>
<td>27.6</td>
<td>1.0†</td>
<td>9.8</td>
</tr>
</tbody>
</table>

*NA = data not available.
†Abnormal ACTH response.
in the percentage of E-rosette positive cells and total T cells, but these changes were not significant. The residual blood lymphocytes appear to be less stimulated by some mitogens, possibly because of the decreased CD4+ and monocyte levels.

DCH is a type IV reaction to an intradermal injection of a recall antigen to which the lymphocytes have been sensitized, and represents the most sensitive in vitro reflection of CMI. Oral steroids are known to affect DCH. In different studies, anergy (absence of DCH reaction) has been reported in 3 to 6% of normal individuals. Pharmacologic doses of systemic steroids can variably depress DCH responses generally after days to weeks of therapy. The suppression of DCH probably results from inhibition of the migration of lymphocytes to the sites of antigen challenge, inhibition of production of lymphocyte growth, and activating factors including interleukin-1, interleukin-2, and γ-interferon with consequent inhibition of lymphocyte proliferation. Skin reactivity returns to pretreatment levels several weeks after cessation of therapy.

Our study in healthy nonasthmatic individuals demonstrates that although inhaled steroids suppress activated T-helper and suppressor subsets, their response to PHA stimulation are not affected. This suggests that although the absolute number of cells available may be altered, the actual responses to antigenic stimulation is not suppressed in subjects receiving recommended doses of inhaled steroids. In a study performed on asthmatics, a single dose of 400 μg, 1,600 μg, or 800 μg of inhaled BDP in divided doses did not significantly suppress total lymphocyte count; however, the investigators did not extend their study beyond 24 h. Levy et al demonstrated no effect of inhaled BDP on CMI (assessed by tritiated thymidine incorporation) in asthmatic children receiving BDP for a mean period of 22.6 months. The dose of inhaled steroid used in this study was comparatively small (from 200 to 600 μg/d), and no baseline studies were performed. Additionally, no healthy control subjects were studied for comparison. This may be relevant, as some symptomatic asthmatics may have activated T cells. However, it is not necessarily true that our findings in healthy individuals would occur in people with asthma, as systemic absorption of inhaled steroids in an asthmatic lung may not be the same as in normal volunteer subjects.

The occurrence of adrenal suppression in three of nine subjects could be due to the use of spacer device and supposedly normal airway caliber, promoting better drug deposition and thus increased bioavailability. The adrenal suppression found in our study resolved 6 weeks after stopping FP treatment.

Our study is limited due to a small sample size and the use of only a single agent (FP) at one dose. Additional studies with larger populations, differing doses, and different preparations are necessary to further investigate the potential effects of inhaled steroids on CMI. Although it is unlikely that a placebo effect would alter our testing of CMI, it would be prudent to incorporate a randomized placebo-controlled study design in future investigations. Nevertheless, to our knowledge, there are no previously published reports that have evaluated CMI in healthy volunteers receiving prolonged inhaled steroid therapy. In addition, the lymphocyte proliferation assay was employed and the monitoring of expression of an early activation marker (CD69+) in whole blood after stimulation with various mitogenic and antigenic stimuli provide additional insights to the influence or lack of influence of inhaled steroids on CMI.

In conclusion, 4 weeks of high-dose inhaled FP treatment in healthy subjects appears to influence CMI. Although the absolute number of activated lymphocytes appears to be diminished, it is reassuring to note that their response to PHA was normal. Our study also suggests that DCH response to antigens is diminished after administration of FP. It is possible that CMI, as measured by the Multitest CMI, may not parallel in vitro lymphocyte proliferation to PHA. The mechanism involved is not apparent at present. Perhaps the most disconcerting observation is the decrease in tuberculin response. We fully recognize that our data must be viewed as preliminary and that the standard Mantoux test was not utilized. Nevertheless, given the widespread use of inhaled steroids in moderate-to-high doses and the continued public health concerns of tuberculosis, further investigations specifically directed at tuberculin-positive subjects appear to be indicated.

REFERENCES

1 National Heart, Lung, and Blood Institute, National Institute of Health. International consensus report on diagnosis and
4 Wilson AM, McFarlane LC, Lipworth BJ. Dose response effect for adrenal suppression with repeated twice daily inhaled fluticasone propionate and triamcinolone acetonide in adult asthmatics. Am J Respir Crit Care Med 1997; 156:1274–1277
13 Frank A, Dash CH. Inhaled beclomethasone dipropionate in acute infections of the respiratory tract. Respiration 1985; 48:122–126
26 FDA Medical Bulletin, December 1991; 21:3
28 Reichman LB. Tuberculin skin testing: the state of the art. Chest 1979; 76:764–779