Budesonide affects allergic mucociliary dysfunction

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Budesonide affects allergic mucociliary dysfunction. J. Appl. Physiol. 85(3): 1086–1091, 1998. Airway inflammation characterized by neutrophils and free elastase contributes to allergic mucociliary dysfunction. Glucocorticosteroids are the most important anti-inflammatory agents used in the treatment of asthma, but their effect on allergic mucociliary dysfunction is not known. Therefore, we assessed both the prophylactic and therapeutic effects of the glucocorticosteroid budesonide on antigen-induced mucociliary dysfunction in sheep. Tracheal mucus velocity (TMV), a marker of mucociliary clearance, was measured by using a roentgenographic technique. When budesonide was administered either 30 min before or 1 h after antigen challenge, the time courses of the protective effects were different: budesonide gave a more immediate response, improving TMV by 8 h after challenge, whereas the effect of $\alpha_1$-protease inhibitor was seen only at 24 h after challenge. These results were similar to previous findings with $\alpha_1$-PI (18). However, when budesonide and $\alpha_1$-PI were administered 6 h after antigen challenge, the time courses of the protective effects were different: budesonide gave a more immediate response, improving TMV by 8 h after challenge, whereas the effect of $\alpha_1$-PI was seen only at 24 h after challenge.

METHODS

Measurement of TMV. Adult ewes (median weight 30 kg) were restrained in an upright position, with their heads immobilized, in a specialized body harness within a modified shopping cart. They were nasally intubated with an endotracheal tube (7.5 mm ID; Mallinckrodt Medical, St. Louis, MO) which had been shortened by 6 cm. The cuff of the tube was placed just below the vocal cords (as verified by fluoroscopy) to allow for maximal exposure of tracheal surface. The inspired air was warmed and humidified by using a Bennett Humidifier (Puritan-Bennett, Lenexa, KS). To minimize possible impairment of TMV caused by inflated cuffs, the endotracheal

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IN RECENT YEARS, guidelines for asthma treatment have placed increasing emphasis on topical anti-inflammatory therapy. Aerosolized anti-inflammatory agents can alleviate symptoms, reduce airway hyperresponsiveness, and improve biochemical and histological indexes of bronchial inflammation (14, 17). Asthma is associated with mucociliary dysfunction (22) in addition to airway obstruction and airway hyperresponsiveness. Antigen challenge decreases tracheal mucus velocity (TMV) in asthmatic patients (16) and in allergic animals (4, 23). Although the mechanisms underlying this abnormality are unknown, airway inflammation is thought to contribute to this pathophysiological response.

In support of this hypothesis, we found that, in allergic sheep, antigen-induced mucociliary dysfunction was associated with an influx of neutrophils and free elastase in bronchoalveolar lavage fluid (16) and that this decreased clearance could be prevented both by pretreatment and by treatment 1 h after antigen challenge with $\alpha_1$-protease inhibitor ($\alpha_1$-PI) and ICI-200,355, a synthetic inhibitor of human neutrophil elastase (18). Although these data indicate that antiprotease agents have the potential to prevent or interrupt the inflammatory cascade that contributes to mucociliary dysfunction in experimental asthma, the effects of other anti-inflammatory therapies on this pathophysiological endpoint have not been completely evaluated. For example, glucocorticosteroids are the mainstay of anti-inflammatory therapy for clinical asthma, yet relatively little is known about the effects of glucocorticosteroids on allergic mucociliary dysfunction. In one study (19), a single aerosolized dose of the glucocorticosteroid beclomethasone did not appear to have any direct effect on tracheal mucociliary clearance in unchallenged allergic sheep. Other investigators reported that systemic glucocorticosteroid therapy administered for 4 wk may improve mucociliary clearance in stable chronic asthma (3).

In the present study, we used the sheep model of antigen-induced mucociliary dysfunction to determine whether inhaled corticosteroids could modify this event. We tested the hypothesis that corticosteroids may prevent or reverse antigen-induced impairment of mucociliary clearance. We thought this to be a reasonable hypothesis because corticosteroids have been shown to decrease chemotaxis of neutrophils to the pulmonary microcirculation as well their adhesion to and migration through the vascular endothelium (5–7). Thus inhibiting the extravasation of activated neutrophils into the bronchial mucosa could protect against the allergen-induced increase in free elastase and the subsequent reduction in mucociliary clearance. Our results showed that budesonide given either before or 1 h after antigen challenge prevented the antigen-induced fall in TMV at 6 h. These results were similar to previous findings with $\alpha_1$-PI (18). However, when budesonide and $\alpha_1$-PI were administered 6 h after antigen challenge, the time courses of the protective effects were different: budesonide gave a more immediate response, improving TMV by 8 h after challenge, whereas the effect of $\alpha_1$-PI was seen only at 24 h after challenge.
to be measured over a 1-min period by using videotaped fluoroscopy in a manner previously described (19). A collar containing radiopaque markers of known length was applied to the exterior of the animals and used as a standard to convert distance traversed by the particles on the video screen to actual distance traveled.

Administration of aerosols. Aerosols were generated by a jet nebulizer (Raindrop Nebulizer, Puritan-Bennett), operated at a flow rate of 6 l/min, that produced a droplet mass median aerodynamic diameter of 3.6 μm, with a geometric standard deviation of 1.9, as measured by a cascade impactor (Anderson, Atlanta, GA). To control the aerosol delivery, a dosimetry system was used, consisting of a solenoid valve and a source of compressed air (at 20 pounds/in.²), which was activated for 1 s at the onset of the inspiratory cycle by a piston respirator (Harvard Apparatus, South Natick, MA). The ventilator was set at a tidal volume of 500 ml, an inspiratory-to-expiratory ratio of 1:1, and a rate of 20 breaths/min.

Agents. Ascaris suum extract (Greer Diagnostics, Lenoir, NC) was diluted in PBS to a concentration of 82,000 protein nitrogen units/ml and delivered as an aerosol (20 breaths/min for 20 min). The Ascaris suum extract has an endotoxin level of 50 EU/ml, a concentration which did not alter airway resistance in previous studies in this model (1, 12). The dose of budesonide (Sigma Chemical, St. Louis, MO) was 1 mg in 3 ml of ethanol-PBS suspension (0.2 ml ethanol/3 ml PBS). α1-PI was delivered as an aerosol (10 mg of Prolastin; Miles, Elkhart, IN) in 5 ml of bacteriostatic water. The dose of budesonide was based on a previous study in allergic sheep (2); the study demonstrated that these doses blocked the late bronchomotor response to antigen and reduced antigen-induced hyperresponsiveness. The dose of α1-PI was based on a recent study of the effects of this agent on antigen-induced changes in TMV (18).

Study protocol. Two separate series of experiments were conducted. The groups of animals for each series were different. For the first series of studies, conducted over 6 h, animals received antigen alone (n = 13), PBS alone (n = 7), budesonide alone (n = 6), budesonide before antigen (n = 6), and budesonide 1 h after antigen (n = 6). The order of the treatments was randomized. Each animal received antigen alone and, on average, two of the other treatments. At least 14 days elapsed between antigen challenges. We have previously demonstrated that this is an adequate interval to allow TMV to return to baseline (4). On different experimental days, TMV was measured before and then at 30 min and 1, 2, 4, and 6 h after antigen challenge. Similar measurements were made after challenge with PBS or budesonide alone to check for nonspecific or time-related effects of these agents on TMV. When budesonide was given 30 min before antigen challenge, TMV was measured before and after the drug treatment, and then at the designated times after antigen challenge (30 min and 1, 2, 4, and 6 h). For the experiments in which budesonide was given after antigen challenge, the measurements were obtained at baseline, 30 min, and 1 h after challenge; then the animals were treated with budesonide. TMV was then measured at the remaining time intervals described above. The time points chosen for this first series of studies were based on our previous study that used α1-PI (18).

In the second series of studies, a second group of animals was used to follow the antigen-induced depression in TMV for up to 24 h. Using this model, we administered budesonide (n = 6) and α1-PI (n = 5) 6 h after antigen challenge, and the TMV responses were compared with antigen alone at 8 and 24 h after challenge. We chose to administer treatments at 6 h and to evaluate the response at 8 and 24 h after challenge to better understand the therapeutic potential of each agent.

Finally, to rule out the possibility that the antigen-induced effects on TMV resulted from endotoxin contamination of the Ascaris suum extract, the sheep were challenged with 400 breaths of a nebulized solution containing 50 EU/ml of lipopolysaccharide (LPS, Escherichia coli serotype 0.26:B6; Difco Laboratories, Detroit, MI), and the effects on TMV were monitored for 6 h.

Statistics. Statistical analysis was performed by using a commercially available program (Systat for Windows, version 5; Systat, Evanston, IL). Comparisons of baseline TMV measurements were made with Kruskal-Wallis analysis of variance (25). For each experiment or trial (within-group analysis), data were analyzed across time by using Friedman’s two-way analysis of variance. If the null hypothesis was rejected, then pairwise comparisons were made by using Wilcoxon signed rank tests. Comparisons of treatments at specific time intervals were first evaluated by using Friedman’s test, followed by a Wilcoxon signed rank test for paired differences or the Kruskal-Wallis test, followed by the Mann-Whitney test for data with unequal sample sizes. A value of P ≤ 0.05 was considered significant, using two-tailed analysis. All values in the text, tables, and figures are reported as means ± SE.

RESULTS

The baseline TMV values for the different treatment protocols are provided in Table 1 (6-h studies) and in Table 2 (24-h studies). Despite some variation among the groups, there were no significant differences detected.

Figure 1 illustrates the changes in TMV after airway challenge with PBS alone, with budesonide alone, with antigen alone, and the effect of antigen challenge after pretreatment with budesonide. There was no significant difference at 6 h among the TMV values (expressed as a percentage of baseline TMV value) for PBS (79.8 ± 9.5%) and budesonide (98.5 ± 7.0%). In contrast, the TMV declined significantly after antigen challenge, with values at 6 h being only 51.9 ± 4.4% of baseline (P < 0.05 vs. all other trials). Pretreatment with budesonide attenuated the antigen-induced fall in TMV. For those animals treated with budesonide, the
TMV was 91.5 ± 12.0% of baseline at 6 h (P < 0.003 vs. antigen alone).

The protective effect of budesonide on the antigen-induced fall in TMV could also be observed if it was given later than 1 h after antigen challenge (Fig. 2). When the budesonide was given after antigen challenge, the TMV value at 6 h was 115.7 ± 19.9% of baseline, which was significantly different from antigen alone (P = 0.003).

In the second series of studies, we assessed the ability of budesonide and \( \alpha_1 \)-PI to reverse antigen-induced mucociliary dysfunction. In these studies, the fall in TMV that was seen after antigen challenge was maintained for up to 24 h (Figs. 3 and 4). When these sheep were treated 6 h after antigen challenge, the results varied with the agent used. In the animals receiving budesonide, TMV in the treated group showed a significant increase over control at 8 h after antigen challenge (50.2 ± 1.2 vs. 65.7 ± 4.8%, control vs. budesonide-treated, respectively; P = 0.03). This effect was short-lived, because there was no significant difference between the two trials at 24 h (51.3 ± 1.2 vs. 59.7 ± 4.3%, control vs. budesonide-treated, respectively; P = not significant). When \( \alpha_1 \)-PI was administered 6 h after antigen, there were no significant dif-

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**Table 2. Baseline TMV values for 24-h treatment studies**

<table>
<thead>
<tr>
<th></th>
<th>Budesonide (n = 6)</th>
<th>( \alpha_1 )-Protease Inhibitor (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>Mean: 12.4</td>
<td>Mean: 13.4</td>
</tr>
<tr>
<td></td>
<td>SE: 1.2</td>
<td>SE: 1.9</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Mean: 10.7</td>
<td>Mean: 15.4</td>
</tr>
<tr>
<td></td>
<td>SE: 1.8</td>
<td>SE: 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in mm/min; n, no. of sheep.

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**Fig. 1.** Changes in tracheal mucus velocity (TMV), expressed as % baseline, after airway challenge with PBS (n = 7 sheep), after challenge with budesonide alone (n = 6 sheep), after challenge with Ascaris suum antigen (Antigen; n = 13 sheep), and after antigen challenge when animals were pretreated with budesonide (n = 6 sheep). Challenge with antigen produced significant decrease in TMV at 6 h. Pretreatment with budesonide significantly reduced the antigen-induced fall in TMV. This effect was specific for budesonide, because neither PBS alone nor budesonide alone had a significant effect on TMV. Inhalation challenge with lipopolysaccharide (LPS) equal to that contained in Ascaris suum extract did not affect TMV (data not shown). Values are means ± SE. *Significant difference vs. all others, P < 0.05.

**Fig. 2.** Effect of budesonide (n = 6 sheep), administered 1 h after antigen challenge, on antigen-induced fall in TMV (Antigen; n = 13 sheep). Budesonide reversed antigen-induced fall in TMV. Values are means ± SE. *Significant difference, trials with antigen alone vs. trials with budesonide given 1 h after antigen, P < 0.05.

**Fig. 3.** Effect of budesonide (n = 6 sheep), administered 6 h after antigen challenge, on antigen-induced fall in TMV. Budesonide significantly improved clearance at 8 h but had no effect at 24 h after antigen. Values are means ± SE. *Significant difference vs. antigen alone, P < 0.05.
The results of this study indicate that 1) a single dose of the topical glucocorticosteroid budesonide, administered 30 min before or 1 h after antigen challenge, can prevent or reverse the antigen-induced reduction in TMV; 2) treatment with a single dose of budesonide, 6 h after antigen challenge, resulted in a transient increase in TMV at 8 h after challenge, but TMV was not significantly improved at 24 h after challenge; and 3) a single dose of α1-PI, administered 6 h after antigen challenge, can significantly attenuate the severity of mucociliary dysfunction present 24 h after antigen challenge; this indicates that the protease inhibitor had a more sustained effect than did the corticosteroid.

The choice of treatment times used for the first series of experiments allowed us to maintain consistency with our previous study, which demonstrated the protective effects of α1-PI and ICI-200,355 on allergen-induced mucociliary dysfunction (18). The demonstration that both glucocorticosteroids and elastase inhibitors, when given 1 h after antigen challenge, could ameliorate mucociliary dysfunction suggests that the mechanism of action by which these agents affect mucociliary dysfunction is by the inhibition of secondary inflammatory cells and mediators rather than by the stabilization of mast cells. However, at 1 h after challenge, neither the cellular influx nor the changes in mucociliary dysfunction have become fully established (18). Therefore, in the second series of experiments, we decided to treat animals when the inflammatory response and the reduction in mucociliary clearance were much more severe. Our findings demonstrate, for the first time, that administration of a single dose of a glucocorticoid or of an elastase inhibitor, 6 h after antigen challenge, can improve mucociliary clearance even transiently (budesonide) or in a more sustained manner (α1-PI) at a time when the cellular influx and mucociliary dysfunction are known to be fully established.

Our previous study (18) indicated that neutrophil elastase contributes to the allergen-induced depression in TMV in allergic sheep. Although there is some controversy concerning the role of the neutrophil in asthma, more recent data indicate that the neutrophil may participate in acute exacerbations of the disease. The neutrophil was the predominant inflammatory cell found in the airways of some patients dying suddenly of status asthmaticus (21). More importantly, neutrophil elastase has been identified in secretions from patients during exacerbations of asthma (8). Furthermore, bronchoscopic evaluation of asthmatics showed a twofold higher concentration of neutrophils in lavage in those dependent on high doses of corticosteroids compared with either persons with mild-to-moderate asthma or with normal controls (24). Collectively, these observations suggest that neutrophil elastase might contribute to the pathophysiology of asthma, including the mucociliary dysfunction associated with the disease.

Although glucocorticosteroids have multifactorial anti-inflammatory effects, given the observations above, one could speculate that the mechanism by which budesonide prevented antigen-induced mucociliary dysfunction in the present study is related, in part, to its action on neutrophils. In clinical studies, corticosteroid therapy prevents allergen-induced neutrophil influx in allergic rhinitis (5). Furthermore, in vitro experiments show that corticosteroids alter the expression of adhesion molecules on both neutrophils and endothelial cells (6) and reduce the production of inflammatory cytokines that affect neutrophil migration and activation (7). Corticosteroids may also reduce the release of elastase from intracellular granules, although in vitro data suggest that this effect may require significantly higher concentrations than are required for inhibition of adhesion and chemotaxis (7). On the basis of these findings, then, it is possible that the protective effects of budesonide could be, in part, explained by a reduction in neutrophil movement and/or activation. This conclusion, however, is speculative because this hypothesis was not tested directly in this study.

It is unlikely that the protective effects on mucociliary clearance, seen with budesonide in the present study, are caused by direct ciliary stimulation, because budesonide given to unprovoked animals did not have a...
direct stimulatory effect on TMV. This finding is consistent with that of a previous study in this model with another topical glucocorticosteroid, beclomethasone (19). Our finding that glucocorticosteroids can protect against experimentally induced allergic mucociliary dysfunction is consistent with clinical observations that suggest that the anti-inflammatory effects of glucocorticosteroids may improve mucociliary dysfunction in asthma. Treatment of asthmatic patients with oral prednisolone (15 mg for 2 wk, followed by 30 mg for a further 2 wk) resulted in some improvement in mucociliary clearance (3). In another study of patients with status asthmaticus who were treated with high-dose systemic glucocorticosteroids, significant improvement in mucociliary clearance occurred after hospital discharge compared with clearance measured during their acute exacerbation (15). On the basis of these findings, the authors of that study speculated that the glucocorticosteroids may have reduced the airway inflammation, thereby resulting in improved mucociliary clearance.

The anti-inflammatory effects of $\alpha_{1}$-PI on the airway are much more specific than those of glucocorticosteroids and raise the possibility of a more targeted therapeutic approach to antigen-induced mucociliary dysfunction. As is the case with budesonide, $\alpha_{1}$-PI does not appear to directly stimulate mucociliary clearance (18). $\alpha_{1}$-PI has several anti-inflammatory effects, including inhibition of neutrophil elastase and tissue kallikrein (11). However, the inhibition of neutrophil elastase is the most likely mechanism contributing to its protective effect observed in these studies, because neutrophil elastase itself can decrease TMV and, like the antigen-induced impairment, this effect that can be prevented by pretreatment with $\alpha_{1}$-PI and the synthetic elastase inhibitor ICI-200,355 (18). The mechanism by which neutrophil elastase decreases mucociliary clearance is unknown, but it could be related to its direct effect on cilia (22) or through its potent secretagogue properties (9, 22). Of note, studies on tracheal explants have demonstrated that elastase inhibition by either ICI-200,355 (9) or by another inhibitor of neutrophil elastase, secretory leukoprotease inhibitor (20), can inhibit the elastase-induced secretagogue effects.

The difference between the results observed at 24 h with budesonide and those with $\alpha_{1}$-P may have several possible explanations. The most likely explanation, however, is that the drugs have different pharmacokinetic profiles. For example, in the sheep model, the duration of action of $\alpha_{1}$-PI in reducing airway hyperresponsiveness is usually >24 h (11), whereas with budesonide, it is usually significantly <24 h (2). Another explanation can be advanced if the mechanism by which corticosteroids ameliorate antigen-induced mucociliary dysfunction is through the inhibition of neutrophil chemotaxis, adhesion, and migration, because such a mechanism may be more effective early in the inflammatory cascade, whereas $\alpha_{1}$-PI, by acting directly on neutrophil elastase, may be effective at a later stage of the inflammatory process.

The extract of Ascaris suum that was used in this and previous studies contained endotoxin at a level of 50 EU/ml (1). Although our previous studies (18) indicate that antigen-induced mast cell degranulation, which leads to an influx of secondary cells, especially neutrophils, is primarily responsible for the slowing of TMV after Ascaris infection, it is important to rule out a possible contribution by endotoxin. Endotoxin contamination of ragweed extract instilled into the airways of asthmatic patients has been reported to cause neutrophilia and increased elastase activity in bronchoalveolar lavage (13) Therefore, in the present study, we determined whether an equivalent dose of endotoxin given to sheep would cause a fall in TMV; we found that TMV was not affected by endotoxin challenge. These results are consistent with our previous studies, which showed that inhalation of ragweed extract, which also presumably contains endotoxin and was used as a control in sheep sensitive to Ascaris suum, did not cause a fall in TMV (4). Therefore, these data indicate that it is the antigen itself that initiates the processes that cause the fall in TMV.

In conclusion, the finding that a single dose of $\alpha_{1}$-PI, administered 6 h after antigen challenge, can attenuate experimentally induced mucociliary dysfunction suggests that elastase inhibition may be worthy of further study as a therapeutic approach to mucociliary dysfunction in asthma. Finally, our observation that a topical corticosteroid can prevent or ameliorate antigen-induced mucociliary dysfunction suggests that this may be yet another mechanism by which corticosteroids contribute to the control of asthma.

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