Airway Remodeling in a Guinea Pig Model of Chronic Asthma: Its Influence on Airway Responsiveness and Pharmacological Properties of Airway Smooth Muscle, and Its Prevention by Corticosteroids

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SUMMARY

Airway remodeling in bronchial asthma occurs as a result of continuance or repetition of eosinophilic airway inflammation. It has the potential to affect airway responsiveness to nonspecific stimuli and the pharmacological properties of airway smooth muscle, but the direct evidence of this is sparse. It is also unknown whether corticosteroids can prevent the development of airway remodeling. To assess these questions, we made a guinea pig model of chronic asthma that consistently showed airway remodeling and analyzed it not only pathologically but also physiologically and pharmacologically. The effects of the corticosteroid treatment on the development of airway remodeling were also examined. Guinea pigs were sensitized by repeated exposure to aerosolized ovalbumin (OVA) and challenged once a week with the inhalation of the same antigen over a 24 week period. Five days after the last antigen challenge, airway responsiveness to inhaled histamine was determined, the animals were then sacrificed, and the lungs and tracheae were removed. Airway responsiveness in this model was significantly increased compared with that in control animals that were challenged with saline. Pharmacological experiments using extracted tracheal and bronchial smooth muscle revealed that carbamylcholine -induced tracheal muscle contraction was significantly greater in the remodeling model than in the control animals. In the contrast, isoproterenol -induced tracheal muscle relaxation was significantly lower in the remodeling model than in the control animals. The treatment with intraperitoneal injection of 20 mg/kg or 40 mg/kg triamcinolone before each OVA challenge significantly blocked the development of airway remodeling, an increase in airway hyperresponsiveness and changes in pharmacological properties of airway smooth muscle. Thus, the present results indicate that airway remodeling enhances an airway responsiveness to nonspecific stimuli and alters contractile and relaxing responses of airway smooth muscle, and these changes can be prevented by corticosteroids together with structural changes of airways.

Key Words: airway remodeling, bronchial asthma, airway smooth muscle, airway hyperresponsiveness, corticosteroids

INTRODUCTION

The current concept of asthma pathogenesis is that a characteristic inflammatory process involving the airway wall causes the development of airflow limitation and increased airway responsiveness, the latter of which pre-
disposes the airway to narrow in response to a variety of stimuli. Characteristic features of the airway inflammation are increased numbers of activated eosinophils, mast cells, macrophages, and T lymphocytes in the airway mucosa and lumen. In addition to these changes, structural alterations, referred to as "airway remodeling", are observed in the bronchi of patients with asthma, especially in those with chronic and severe asthma.

Airway remodeling is a collective term that encompasses the changes in structural cells and tissues in the asthmatic, as opposed to the normal, airways. They include mucus metaplasia, subepithelial fibrosis (basement membrane thickening), smooth muscle hypertrophy and hyperplasia and thickening of the peribronchial adventitia. Overall, the airways in asthma display an increase in wall thickness. The clinical consequences of airway wall remodeling are incompletely understood. However, a number of studies suggest that physiologic abnormalities observed in many asthmatics even when they are asymptomatic and under symptomatic control with anti-inflammatory treatment may be due to the airway remodeling. For example, significant correlations have been reported between the basement membrane thickness measured in bronchial biopsy specimens and FEV1, a marker of airway obstruction, measured during a forced expiratory maneuver using a spirometer. The irreversible component of the airway obstruction is more prominent in patients with thickened airway walls measured by high-resolution computed tomography (HRCT). Moreover, several studies have reported significant correlations between the thickness of basement membrane and degree of airway hyperresponsiveness determined in asymptomatic patients, which is clinically the most relevant physiological abnormality in asthma. Thus, there are a number of reports on the physiologic consequences of airway remodeling in asthma. However, to our knowledge, there is no reported study which investigated the pharmacologic consequences of airway remodeling. In asthmatics, β2-agonists are extremely effective in improvement of airway obstruction. However, some asthmatics, in particular those with chronic severe disease, have evidence of poor response to inhaled β2-agonist therapy. Although the underlying mechanisms are unknown, this may occur associated with remodeling process of airway smooth muscle.

The prevention of airway remodeling is thought to be the most effective therapeutic approach to inhibit asthma being chronic and severe. Despite that, the effects of anti-inflammatory drugs in the prevention of airway remodeling have not been studied enough. Leukotriene modifiers such as montelukast and pranlukast have been reported to effective in preventing the increase in airway smooth muscle mass and airway mucosal fibrosis in animal models of chronic asthma. Corticosteroids are key drugs in the long-term management of asthma and used most widely. However, no studies have been conducted to date to determine if corticosteroids can prevent the development of airway remodeling.

We have previously reported that guinea pigs sensitized by repeated exposure to aerosolized ovalbumin and received weekly inhaled-antigen challenge over a 24-week period displayed airway remodeling which mimics that of patients with chronic asthma. In the present study, using this animal model, we investigated whether airway remodeling exaggerates the airway responsiveness to histamine, whether remodeling of airway smooth muscle alters its pharmacologic properties, and whether corticosteroids have the ability to prevent airway remodeling.

**MATERIAL AND METHODS**

*sensitization and antigen challenge of guinea pigs (Figure 1)*

Male Hartley guinea pigs weighing 250 to 300 g (Japan SLC, Inc, Tokyo, Japan) were used. Guinea pigs were placed in a plastic antigen inhaler and sensitized by exposure for 10 min to aerosolized ovalbumin (OVA) (Grade V, Sigma Chemical Co., Mo., USA) 10 mg/ml in saline, given through an ultrasonic nebulizer (NEU-U 12, Omron, Tokyo, Japan). The procedure was repeated once a day for 10 days. The sensitized guinea pigs were allocated to the following four groups. The remodeling group animals were challenged once a week over a 24-week period with the aerosolized OVA (10 mg/ml). Each challenge was performed in a same fashion as the sensitization. The control group received challenge with saline instead of OVA. The corticosteroids treatment group animals received an intraperitoneal injection of 20 mg/kg or 40 mg/kg triamcinolone 60 min before each OVA challenge (S20 group and S40 group, respectively). To prevent death from excessive bronchoconstriction,
Sensitization and antigen challenge of guinea pigs. Guinea pigs were sensitized by 10 min exposure to aerosolized ovalbumin once a day for 10 days. The animals were then allocated to the following four groups. The remodeling group animals were challenged once a week over a 24-week period with the aerosolized OVA (10 mg/ml). The control group received challenge with saline instead of OVA. Rhamaphorinone treatment group received an intraperitoneal injection of 20 mg/kg or 40 mg/kg triamcinolone 60 min before each OVA challenge. To prevent death from excessive bronchoconstriction, chlorpheniramine maleate was intraperitoneally injected immediately before each exposure to aerosolized OVA in the latter half of the sensitization period and throughout the challenge period. Airway responsiveness to inhaled histamine was measured 5 days after the last challenge. The animals were then sacrificed, and the lungs and tracheae were removed. A part of them were used for the pharmacological experiments and the remainings were fixed with formalin for histologic examination.

**Measurement of airway hyperresponsiveness**

After baseline measurements of respiratory resistance (Rrs) were made, the guinea pigs were provoked with stepwise incremental concentrations of aerosolized histamine (Wako Shinyaku, Tokyo, Japan). successively doubling from 78 to 20,000 µg/ml, until Rrs increased to 0.5 cm H2O/ml/sec or more. The threshold was defined as the concentration at which Rrs was ≥ 0.5, when calculated according to the following formula: Rrs = 0.2 x b (pressure)/a (flow) cm H2O/ml/sec.

Rrs was measured by a forced oscillation techniques following the modified Mead’s oscillation method. Briefly, the guinea pig was placed inside a body plethysmograph, and 18 Hz sine wave oscillation was applied to its body surfaces. Oscillating pressure was obtained with a 25-inch loudspeaker driven by a sine wave generator and power amplifier. A plastic mask with a 400-mesh screen was applied to the face in order to measure the flow rate.

**Preparation of histological specimens**

The tissue was fixed in formalin solution (9%), embedded in paraffin, sliced into thin sections, and stained with Giemsa, hematoxylin and elastica Van Gieson stains.

**Quantification of eosinophil infiltration in the airway mucosa**

Eosinophils were counted in the mucosa on the hematoxylin and eosin-stained sections using an Olympus BHS microscope at a magnification of x400. The total area of mucosa examined was calculated by delineating the
area of the section on the Video Micro Meter System (VM-31) (Olympus Optical Co., Tokyo, Japan). The results were expressed as the number of eosinophils per square millimeter of the mucosa.

**Morphometric analysis of airway cross-sectional area**

Morphometric analysis was performed according to the method described by James et al. Briefly, measurements were made by projecting images onto a computer-assisted graphics tablet. The measurements included the following. 1) The perimeter of the luminal border (Pi) and airway area (Ai). 2) The area enclosed by the inner border of the smooth muscle (Aim). 3) The area enclosed by the outer border of the smooth muscle (Aem). From the measured dimensions, the total wall area (Aw) was calculated by subtracting airway area from the area enclosed by the outer border of the smooth muscle (Aw = Aem - Ai). Similarly, smooth muscle layer area (Asm) was given by (Asm = Aem - Aim), and mucosal layer area (Amuco) given by (Amuco = Aim - Ai). To allow comparison of the airways of different size, data for individual airways were standardized by dividing each area by Air. The Air is the theoretical fully dilated internal area (airway) given by Pi^2/4π, which is calculated based on the assumptions that Pi does not change and that Pi is circular in the relaxed and dilated state. The results were expressed as indices of dimensions.

The thickness of the basal laminae was determined in membranous airways on sections stained with elstica van Gieson’s stain. Five estimates were made for each section and mean values were calculated.

**Pharmacological studies of tracheal and bronchial smooth muscle**

To pharmacologically study contraction and relaxation of tracheal and bronchial smooth muscle, the trachea and bronchi were removed from the guinea pigs and cut into rings. The rings were suspended in an organ bath containing Krebs solution. Contraction was induced in a stepwise fashion by exposure to increasing concentrations of carbamylcholine chloride (Carbachol, Sigma Chemical Company), and the results were recorded, thus obtaining concentration-response curves. Fifty percent corrected values were determined and compared. To assess relaxation, maximal contraction was similarly induced by 2 × 10^-3 M histamine, and relaxation was induced in a stepwise fashion by exposure to increasing concentrations of isoproterenol (Sigma Chemical Company). The pEC_{50} values were determined and compared. Treatment-induced contraction and relaxation were measured with an isometric transducer (TB-651T, Nihon Kohden, Tokyo, Japan) and an isometric amplifier (EF-601G, Nihon Kohden). The trachea and bronchus were separately evaluated, and the results were compared.

**Statistical analysis**

Results were expressed as mean ± SEM. Statistical analysis was done with Student’s unpaired t-test. P values of less than 0.05 were considered to indicate statistical significance. Alternatively, one-way analysis of variance was performed, and P values of less than 0.05 were considered to indicate statistical significance on multiple comparison tests done after confirming that the data were normally distributed (only when p values where less than 0.05 on F test).

**RESULTS**

**Determination of optimal doses of corticosteroids**

In preliminary study, we examined the effect of various doses of triamcinolone (5, 20, 40 mg/kg) on the eosinophil infiltration in the tracheae of guinea pigs received single antigen challenge after the establishment of sensitization. The numbers of eosinophils per square millimeter (mean ± SEM, n = 5) in the animals without triamcinolone treatment and those received 0, 5, 20, 40 mg/kg of triamcinolone 60 min before the antigen challenge were 972 ± 70, 368 ± 88, 191 ± 90, 70 ± 15, respectively. Although the eosinophil infiltration was significantly inhibited by any dosage of triamcinolone, 20 and 40 mg/kg were considered the optimal doses for the present study because these doses of triamcinolone were able to inhibit antigen-induced eosinophil infiltration up to less than 20% of that of non-treated animals. And these dosage are no effect for systemic problems.

**Eosinophil infiltration in the mucosa of trachea and bronchi** (Fig. 2)

Repeated exposure to antigen may dampen the sensitization status to the antigen and consequently, the intensity of antigen-induced airway inflammation may weaken. To confirm that eosinophilic airway inflammation was
induced even after repeated antigen challenge, the degree of eosinophil infiltration was examined in the tracheal and bronchial mucosa. In the control animals received repeated challenge with aerosolized saline, no eosinophil infiltration was observed. In the animals of the remodeling group, the numbers of eosinophils per square millimeter were 143 ± 35 for tracheae and 1.8 ± 1.2 for bronchi. These data indicated that eosinophilic airway inflammation, though less prominent as compared with that observed in first challenge, occurred even after repeated antigen challenge. The treatment with 40 mg/kg triamcinolone significantly inhibited eosinophil infiltration both in the trachea and bronchi. However, 20 mg/kg of triamcinolone blocked eosinophil infiltration only in the bronchial mucosa.

Pathohistological findings

Fig. 3 shows membranous bronchioles from a control animal challenged with saline, from an animal challenged with aerosolized OVA once a week over 24 weeks, from an animal received intraperitoneal injection of 40 mg/kg triamcinolone 60 min before each OVA challenge. The airway wall from a treated animal is markedly thickened. Airway wall thickening was obviously prevented in the animal received triamcinolone treatment.

Fig. 3 Membranous bronchioles from a control animal challenged with saline (a), from an animal challenged with aerosolized OVA once a week over 24 weeks (b), from an animal received intraperitoneal injection of 40 mg/kg triamcinolone 60 min before each OVA challenge. The airway wall from a treated animal is markedly thickened. Airway wall thickening was obviously prevented in the animal received triamcinolone treatment.

obtained from actual measurements were standardized by dividing each area by luminal area at fully dilated status and expressed as indice of dimensions without any unit. In the remodeling group, both smooth muscle and mucosal layers were significantly greater than those of
the control group. In the animals received triamcinolone treatment, the increases in the areas of smooth muscle layer and mucosal layer were significantly inhibited. As a result, total wall area was significantly greater in the remodeling group than in the control group. Similarly, in the triamcinolone treated group, the increase in total wall area was significantly inhibited.

The thickness of the basement membrane of membranous bronchi was shown in Fig. 5. The basement membrane thickness in the remodeling group was approximately 5 times greater than that in the control group. The increase was significantly less in the triamcinolone-treated group, but the degree of inhibition was less prominent as compared with the triamcinolone inhibition of thickening of smooth muscle and mucosal layers.

Airway responsiveness to aerosolized histamine (Fig. 6)  
Histamine concentrations needed to increase Rrs to 0.5 cmH₂O/mL/sec or more were 2083.3 ± 320.3 µg/ml for the control group and 636.2 ± 81.0 µg/ml for the remodeling group. The difference is significant (p < 0.0001), suggesting that airway remodeling accompany an increase in airway responsiveness. The treatment with triamcinolone partially inhibited the increase in airway responsiveness to histamine. The threshold values were 1198.0 ± 206.5 µg/ml for the 40 mg/ml triamcinolone-treated group and 906.3 ± 118.3 µg/ml for the 20 mg/ml triamcinolone-treated group.

Tracheal and bronchial smooth muscle contraction and relaxation  
As shown in Table 1, contractile responses to carbachol of tracheal and bronchial smooth muscle were significantly greater in the remodeling group than in the control group. The treatment with 40 mg/kg triamcinolone significantly inhibited the increase in contractile response to carbachol both in the tracheal and bronchial smooth muscle. However, in the case of 20 mg/kg triamcinolone, the increase only in the bronchial smooth muscle was significantly blocked.

In the contrast, relaxing responses to isoproterenol of tracheal and bronchial smooth muscle were significantly lower in the remodeling group than in the control group. The reduced response to isoproterenol that occurred associated with airway wall remodeling was significantly inhibited by the treatment with 20 or 40 mg/kg triamcinolone.
DISCUSSION

Besides subepithelial thickening of the basement membrane, the total wall is thickened in asthmatic patients. Mathematical modelings that incorporate morphologic and physiologic measurements made on asthmatic and non-asthmatic tissues have demonstrated that the airway wall thickening can enhance the degree of luminal ef-face-
Table 1  Contractile and relaxing responsiveness to carbachol and isoproterenol of tracheal and bronchial smooth muscles isolated from control, remodeling and triamcinolone-treated guinea pigs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>pEC_{50} for carbachol</th>
<th>pEC_{50} for isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trachea</td>
<td>bronchus</td>
</tr>
<tr>
<td>Control</td>
<td>6.27 ± 0.05</td>
<td>6.29 ± 0.08</td>
</tr>
<tr>
<td>Remodeling model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remodeling model</td>
<td>6.50 ± 0.06 **</td>
<td>0.54 ± 0.04 *</td>
</tr>
<tr>
<td></td>
<td>7.33 ± 0.09 *</td>
<td>7.71 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>7.27 ± 0.09 *</td>
<td></td>
</tr>
<tr>
<td>Triamcinolone-treated model</td>
<td>6.44 ± 0.05 *</td>
<td>6.36 ± 0.11</td>
</tr>
<tr>
<td>(20 mg/kg)</td>
<td>7.55 ± 0.15</td>
<td>7.60 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>6.35 ± 0.05</td>
<td>6.34 ± 0.05</td>
</tr>
<tr>
<td>Triamcinolone-treated model</td>
<td>7.70 ± 0.10</td>
<td>7.70 ± 0.10</td>
</tr>
<tr>
<td>(40 mg/kg)</td>
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Pharmacological experiments were done as described in the method section. Each value represents mean ± S. E. M. * p < 0.05, ** p < 0.005. These were compared with the values obtained in control group.

ment caused by a given degree of muscle contraction. Thus, airway wall thickening has been theoretically expected to increase airway hyperresponsiveness. However, there has been no direct in vivo evidence for this relationship. We therefore made a guinea pig model of chronic asthma with striking airway remodeling by repeating weekly antigen challenge in sensitized animals over a period of 24 weeks. These animals displayed significant thickening of the basement membrane as well as significantly increased areas of the smooth muscle, mucosal, and entire layers of the airways. Our study showed that airway responsiveness to inhaled histamine was significantly greater in these animals than those without airway remodeling, supporting the view proposed by James and his colleagues\(^1\). This finding also suggests that in airways with a lumen narrowed by wall thickening even mild smooth muscle contraction can trigger airway obstruction.

Thickening of airway smooth muscle layer is thought to occur as a result of smooth muscle cell hypertrophy and hyperplasia. Such remodeling process may affect contractility of airway smooth muscle. This is supported by studies showing that airway smooth muscle obtained from patients with asthma is hyperresponsive\(^{20,21}\). Other studies have demonstrated increased levels of contractile proteins in patients who have asthma associated with smooth muscle proliferation\(^2\). In our pharmacological study, we determined pEC_{50} (ED_{50}) for contractile response to carbamylcholine from logarithmic concentration - response curves based on the premise that the results are unaffected by tracheal smooth muscle area to specifically examine the drug responsiveness of airway smooth muscle. Both tracheal and bronchial smooth muscle isolated from animals of the remodeling group showed significantly elevated contractility to carbamylcholine. These results suggest that the increased airway responsiveness observed in vivo in the remodeling group resulted not only from airway wall thickening, but also from increased smooth muscle contractility. We similarly examined relaxing response to isoproterenol of tracheal and bronchial smooth muscle. Maximal contraction was induced by pretreatment with histamine, and isoproterenol-induced relaxation was assessed. Significantly higher isoproterenol concentrations were required to obtain same degree of relaxation in smooth muscle from remodeled animals than in that from control animals. These findings may explain the poor response to β-agonists of patients with chronic asthma in whom airway remodeling is thought to occur. Further studies are needed to elucidate the mechanism by which alterations in pharmacological properties occur in the remodeled airway smooth muscle.

Corticosteroids have long been used for asthma treatment and have markedly decreased asthma death\(^22\). Clinically, corticosteroids reduce sputum eosinophilia, suppress airway inflammation, and improve asthmatic symptoms\(^20\). At the cellular level, they bind to corticosteroid receptors, are activated after protein dissociation, enter the nucleus, and inhibit the transcription of various cytokines genes\(^{21,26}\). Corticosteroids also inhibit mast cell and eosinophil migration, suppress mast cell destruction, decrease eosinophil count, inhibit nitric oxide synthase and decrease its production, and suppress the syn-
thesis of adhesion factors\(^{27}\). They are used not only for the treatment of asthma exacerbations; recent asthma guidelines have recommended inhaled corticosteroids for both early and long-term management\(^{28-30}\). Inhaled corticosteroids have been reported to significantly improve peak expiratory flow (PEF). Studies examining the relation between PEF and the interval from disease onset until the start of inhaled corticosteroid treatment have shown adequate improvement in PEF in patients who started receiving inhaled corticosteroids soon after onset of asthma. In contrast, delayed initiation of inhaled corticosteroid treatment is associated with decreased improvement in PEF\(^{31-33}\). These findings suggest that airway wall remodeling, once established, is irreversible. Therefore, we investigated using our animal models whether corticosteroid treatment could prevent the development of airway wall remodeling. Corticosteroid treatment dose-dependently inhibited basement membrane thickening and other organic changes of the airway wall (increased areas of the smooth muscle layer, mucosa, and all layers). These results suggest that early treatment with corticosteroids may inhibit airway wall remodeling. In addition to directly decreasing the number of eosinophils, corticosteroids inhibit the migration and activation of a broad range of inflammatory cells and block the production of factors considered to have a role in airway wall remodeling. Our study also showed that corticosteroid treatment inhibited an increase in airway responsiveness. Increased contractility and reduced relaxing activity of airway smooth muscle that occurred associated with airway remodeling were also significantly prevented by the weekly administration of corticosteroids. Thus, the present experiments demonstrated that corticosteroid therapy can prevent not only structural change of airway walls but also alterations in physiological and pharmacological properties of airway smooth muscle. It should be examined by clinical trials whether early intervention therapy with inhaled corticosteroids would inhibit worsening of asthma by preventing airway remodeling.

In summary, our present studies demonstrated that airway remodeling induced by repetition of antigen-induced airway inflammation accompany the increase in airway responsiveness to stimuli, and that during the remodeling process alterations in pharmacological property of airway smooth muscle such as contractility and relaxing activity occur. It was also shown that corticosteroids have the ability to prevent not only the development of airway remodeling but also an increase in airway responsiveness and change in pharmacological property of airway smooth muscle. We believe that these findings will be the theoretical basis for early use of inhaled corticosteroids for newly diagnosed asthma.

**REFERENCE**

tion computed tomography findings are correlated with disease severity in asthma. Respiration, 69: 420-426, 2002.