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ORIGINAL ARTICLE

Effects of Tranilast and Pentoxifylline in a Mouse Model of Chronic Asthma Using House Dust Mite Antigen

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Tranilast has been used in allergic diseases because of its inhibitory effect on mast cells; it also has an anti-fibrotic effect in several diseases. Pentoxifylline (PTX), a methylxanthine derivative, is a potent anti-inflammatory drug that is known to manifest its effect through the inhibition of Th1 cytokine, but with an uncertain effect on Th2 cytokine. Seven-week-old female BALB/c mice were studied as a chronic asthma model. The mice were challenged with house dust mite (HDM) antigen for 7 weeks. Each group of mice was given an intraperitoneal injection of tranilast, PTX, or tranilast plus PTX before antigen administration. In this mouse model of chronic asthma, tranilast, and PTX each had an inhibitory effect on airway remodeling as well as on airway hyperresponsiveness (AHR) and airway inflammation. The improved events of these drugs were related with the inhibition of the Th2 cytokine IL-13 and TGF- β 1. Immunohistochemical analysis showed that decreases in the peribronchial trichrome stained area in each treatment group were associated with improvements in the peribronchial smooth muscle hyperplasia, collagen type I, and collagen type III deposition. These drugs could have potential beneficial effects on chronic asthma, especially with respect to airway remodeling.

Keywords asthma; house dust mite; mouse; pentoxifylline; tranilast

INTRODUCTION

Asthma is a chronic inflammatory disease characterized by reversible airflow obstruction and airway hyperresponsiveness (AHR). In the pathogenesis of asthma, a relative excess of Th2 cytokines (IL-4, IL-5, IL-13) and depletion of Th1 cytokines (IFN- γ , IL-12) have been proposed to mediate the disease, together with a variety of structural changes such as subepithelial fibrosis, mucous cell hyperplasia/metaplasia, and increased smooth muscle mass. These structural changes are associated with airway remodeling, and remodeling changes could be associated with the development of both AHR and persistent airflow limitation in chronic asthmatics (1).

Dust mites are ubiquitous and major allergens in humid areas of the world, and a number of people in the worldwide have positive skin test reactions to at least one species of dust mite. Sensitization to allergens derived from dust mites is strongly associated with asthma. In particular, the European house dust mite, *Dermatophagoides pteronyssinus* (Der P), is one of the most frequently associated mite-related respiratory allergens (2, 3).

The pharmacological characteristics of tranilast, N-(3',4'-dimethoxycinnamoyl)-anthranilic acid, were first described as early as the 1970s (4). Because of its ability to suppress

mast cell degranulation and histamine release (5–7), tranilast is used to treat allergic diseases such as allergic rhinitis, atopic dermatitis, and bronchial asthma. Although the precise mechanisms underlying the anti-fibrotic effects of tranilast are incompletely understood, known actions of tranilast include inhibiting the extracellular matrix synthesis induced by transforming growth factor- β 1 (TGF- β 1) (4, 8, 9). Until now, the effect of tranilast on the pathogenesis of airway remodeling in asthma has not been examined.

Pentoxifylline (PTX), a methylxanthine derivative, is a nonselective phosphodiesterase inhibitor. It has a strong anti-inflammatory effect and inhibits the development of Th1-mediated disorders such as autoimmune diabetes mellitus and experimental allergic encephalomyelitis (10). However, there are a number of studies illustrating that PTX intervention has not always the same effect toward Th2 cytokine production (11–15). Also there has been little report of PTX on the effect of airway remodeling related with the AHR and airway inflammation in the pathogenesis of chronic asthma.

We investigated the effects of tranilast and PTX whether these drugs have beneficial effects on the airway remodeling as well as AHR, airway inflammation, and the related pathogenesis in this mouse model of chronic asthma.

MATERIALS AND METHODS

Animal Model

Specific pathogen-free female BALB/c mice were used when they reached 7 weeks of age. Mice were housed in environmentally controlled, specific pathogen-free conditions for 1 week before the study and for the duration of the experiments. All animal experimental protocols were

BALB/c is an albino, laboratory-bred strain of House Mouse from which a number of common substrains are derived.

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TABLE 1.—Serum immunoglobulin E and cytokine production, and BALF cytokine levels. Data are the mean $\pm$ standard error for each group of mouse asthma model.

	Control	HDM	HDM+TRA	HDM+PTX	HDM+TRA+PTX
Serum					
IgE (A.U.) [†]	0.1 $\pm$ 0.0	9.6 $\pm$ 7.9	5.1 $\pm$ 2.1	1.9 $\pm$ 1.3	0.9 $\pm$ 0.6
IL-13 (pg/mL)	54.4 $\pm$ 7.4	223.2 $\pm$ 49.8	74.7 $\pm$ 16.3*	134.7 $\pm$ 41.0	61.4 $\pm$ 9.6*
IFN- γ (pg/mL)	11.9 $\pm$ 1.7	17.6 $\pm$ 4.6	7.9 $\pm$ 2.3	5.9 $\pm$ 0.9*	5.7 $\pm$ 0.9*
BALF					
IL-13 (pg/mL)	7.2 $\pm$ 0.9	33.1 $\pm$ 10.7**	8.4 $\pm$ 1.1	8.9 $\pm$ 1.3	8.8 $\pm$ 0.7
IFN- γ (pg/mL)	3.2 $\pm$ 0.1	3.3 $\pm$ 0.2	2.8 $\pm$ 0.1	3.3 $\pm$ 0.1	3.5 $\pm$ 0.2
TGF- β 1 (pg/mL)	120.4 $\pm$ 24.4	252.3 $\pm$ 40.9**	159.9 $\pm$ 20.2	127.7 $\pm$ 17.4	130.9 $\pm$ 16.8

HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

[†]IgE indicates HDM-specific IgE and expressed as arbitrary units (A.U.) relative to standard mouse serum.

* $p < 0.05$ vs. HDM.

** $p < 0.05$ vs. control, HDM+TRA, HDM+PTX, and HDM+TRA+PTX.

approved by the Animal Subjects Committee, Catholic University of Korea.

Groups of mice (eight or nine mice/group) were exposed to intranasal house dust mite (HDM) purified extract (Der P antigen, 25 μ g of protein, LPS concentration of 0.024 EU/ μ g; Yonsei University, Seoul, Korea) in 20 μ L of phosphate buffered saline (PBS) 5 days per week for 7 consecutive weeks. The mice were given intraperitoneal (i.p.) injections of tranilast (50 mg/kg/day), PTX (50 mg/kg/day i.p. injection), or both tranilast and PTX before each allergen challenge for 7 consecutive weeks. HDM-challenged mice without treatment received the same volume of saline injection instead of tranilast or PTX. Control mice were exposed to PBS without HDM extract and received saline injection instead of tranilast or PTX. All groups of mice were killed after 7 weeks of this experiment.

Measurement of Airway Function

Before the mice were killed, airway responsiveness to methacholine was estimated indirectly using a non-invasive whole-body mouse plethysmograph (Allmedicus, An Yang, Korea) to measure a derived index known as enhanced pause

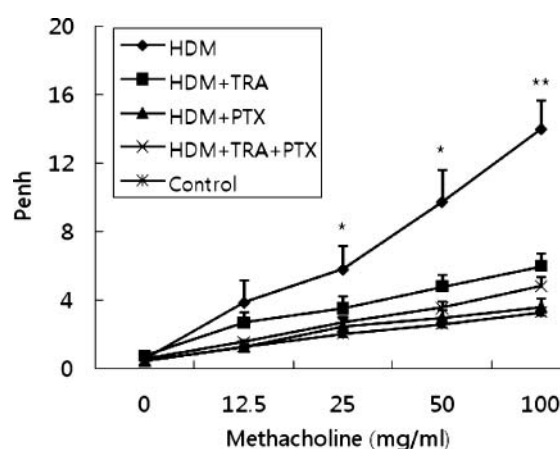


FIGURE 1.—Airway hyperresponsiveness to methacholine of each group in a mouse model of chronic asthma. Compared with HDM, other groups showed significantly decreased airway hyperresponsiveness. * $p < 0.05$ vs. Control, HDM+TRA, HDM+PTX, and HDM+TRA+PTX; ** $p < 0.01$ vs. Control, HDM+TRA, HDM+PTX, and HDM+TRA+PTX. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline. Bars were representative of standard errors.

(Penh). Penh could be a reasonable analogue of airway responsiveness to a nonspecific inhaled stimulus such as methacholine.

Measurement of HDM-specific Immunoglobulin E

Serum was obtained by the centrifugation of blood collected from retroorbital sinus of each mouse and stored at -70°C . HDM-specific immunoglobulin E (IgE) was determined using enzyme-linked immunosorbent assay (ELISA) as follows. A 100- μ L aliquot of HDM extract (5 μ g/mL in PBS) was dispensed into each well of a 96-well flat microtiter plate and incubated overnight at 4°C . The antigen-coated plates were washed three times with 0.05% PBS-Tween 20 buffer (washing buffer) and incubated with mouse sera overnight at 4°C . Plates were washed three times with washing buffer and incubated with antimouse IgE (Pharmingen, San Diego, CA) antibody at room temperature for 1 hour. They were then washed an additional six times before adding citric acid-phosphate buffer containing 0.15 mg/mL o-phenylenediamine (Sigma, St. Louis, MO). The color was developed at room temperature, the reaction was stopped with 2.5 M sulfuric acid, and the color was then measured at 492 nm. The HDM-specific IgE level was expressed as arbitrary

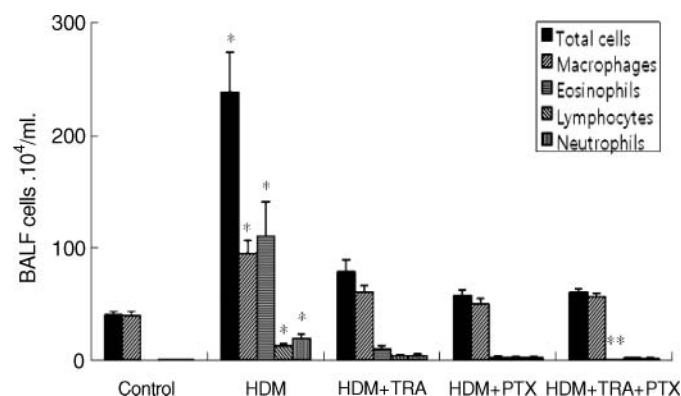


FIGURE 2.—Bronchoalveolar lavage fluid inflammatory cells of each group in a mouse model of chronic asthma. Compared with HDM, other groups showed significantly decreased inflammatory cells, including total cells, macrophages, eosinophils, lymphocytes, and neutrophils (* $p < 0.01$). In HDM+TRA+PTX, eosinophils were significantly decreased compared with HDM+TRA (** $p < 0.05$). HDM=house dust mite; TRA = tranilast; PTX = pentoxifylline. Bars were representative of standard errors.

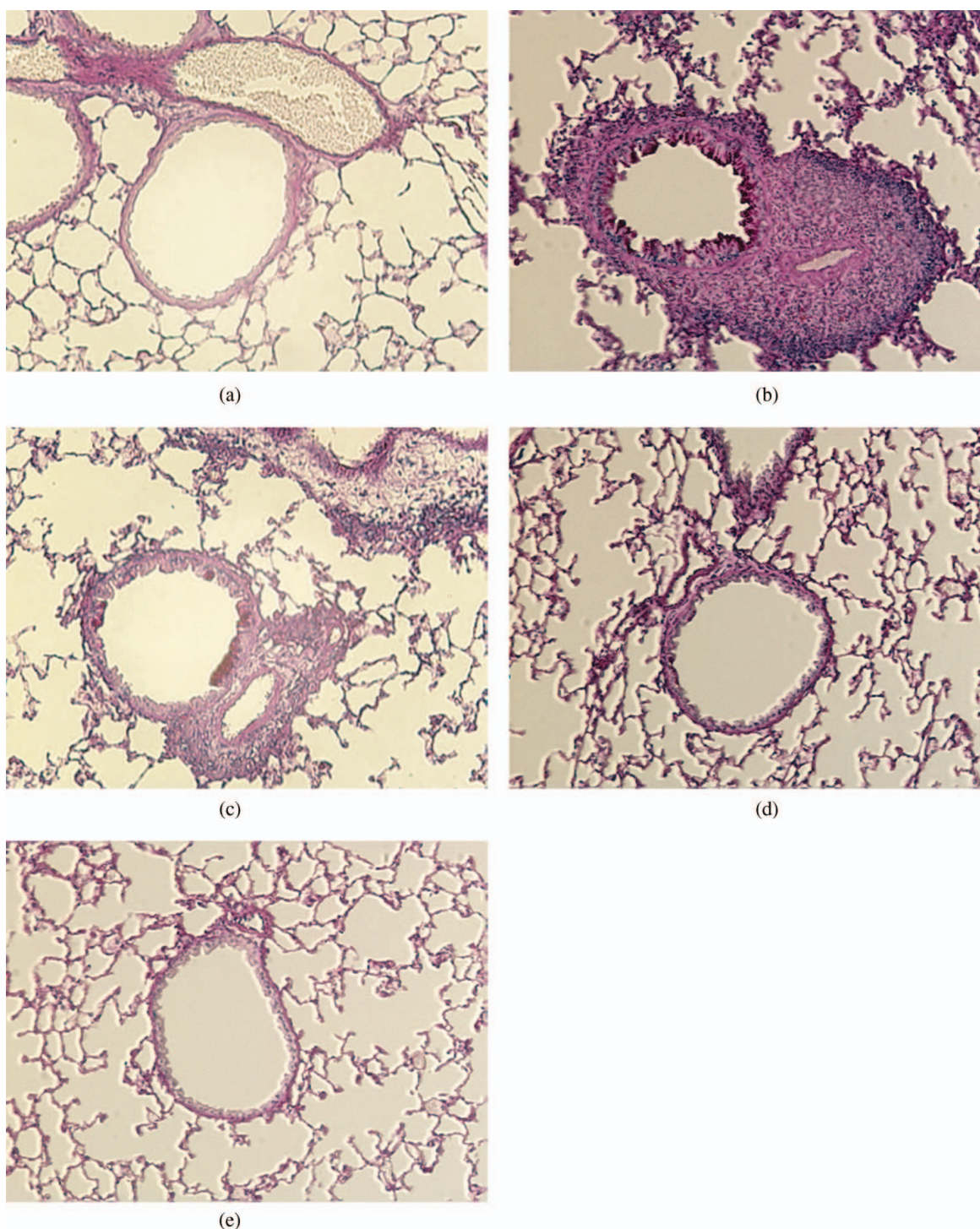


FIGURE 3.—Photomicrographs of PAS staining of each group in a mouse model of chronic asthma ($\times 200$). (a) Control, (b) HDM, (c) HDM+TRA, (d) HDM+PTX, (e) HDM+TRA+PTX. Compared with HDM, other groups showed decreased goblet cell hyperplasia in the bronchial epithelium. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

units (A.U.), which is a relative value based on the standard mouse serum that was exposed to HDM in the same manner.

Bronchoalveolar Lavage Fluid (BALF) Analysis

After the determination of airway responsiveness to methacholine, the animals were killed with intraperitoneal injection

of tiletamine/zolazepam (30 mg/kg) and xylazine (10 mg/kg). The right lung of each mouse was lavaged with 0.4 mL of calcium- and magnesium-free PBS after the trachea was cannulated and the left bronchus was tied. This procedure was repeated three times (total volume; approximately 1.0 mL, recovery $> 80\%$). Bronchoalveolar lavage fluid (BALF) from

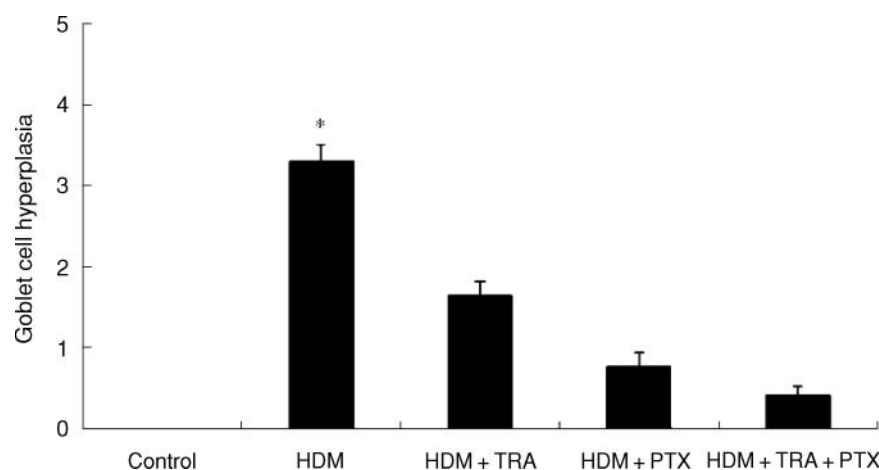


FIGURE 4.—Goblet cell hyperplasia of each group in a mouse model of chronic asthma. The degree of goblet cell hyperplasia in the epithelial lining was expressed as a score according to the percentage of goblet cells in the epithelial cells: grade 0, no goblet cells; grade 1, < 20%; grade 2, 20–39%; grade 3, 40–59%; grade 4, 60–79; grade 5, $\geq 80\%$ or total bronchial obstruction due to mucus secretion. Compared with HDM, other groups showed significantly decreased goblet cell hyperplasia in the bronchial epithelium (* $p < 0.01$). HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline. Bars were representative of standard errors.

each animal was collected in a plastic tube, cooled on ice, and centrifuged at 930 g at 4°C for 7 minutes. The BALF supernatants of each experimental mouse were stored at –70°C. Cell pellets were resuspended in PBS and stained with trypan blue, and the number of nucleated cells was counted using a hemacytometer. The cells were prepared on a smear using a cytocentrifuge, and a differential cell count of at least 500 cells was made after staining with Diff-Quik (Sysmex Corporation, Kobe, Japan).

Measurement of Cytokines in BALF and Serum

The amount of cytokine in the supernatant of BALF and serum was measured using enzyme immunoassay (R&D Systems, Minneapolis, MN for IL-13 and IFN- γ , and BioSource International, Camarillo, CA for TGF- β 1). Each sample was measured according to the manufacturer's recommendations. The detection limit of each kit is 1.5 pg/mL for IL-13, 2 pg/mL for IFN- γ , and 15.6 pg/mL for TGF- β 1.

Histological Study

The left lung of each mouse was removed and fixed in 4% paraformaldehyde and then embedded in paraffin. At that time, the left lung was equally sectioned into three pieces from the apex of the lung, and the following stains were applied: hematoxylin and eosin for general morphology, periodic acid–Schiff (PAS) stain for the identification of goblet cells in the epithelium, and Masson's trichrome stain for airway fibrosis, respectively.

To quantify goblet cell hyperplasia, we used the method described by Padrid et al. (16), with some modifications. Briefly, the pathological changes were evaluated according to the modified six-point scoring system (grades 0–5) and were expressed by scores according to the percentage of goblet cells among the epithelial cells: grade 0, no goblet cells; grade 1, <20%; grade 2, 20–39%; grade 3, 40–59%; grade 4, 60–79%; grade 5, $\geq 80\%$ or total bronchial obstruction due to mucus secretion. We then calculated the mean scores of goblet cell hyperplasia in each mouse.

The areas of peribronchial fibrosis beneath the epithelial basement membrane in the Masson's trichrome-stained tissue sections were outlined and quantified using a light microscope attached to Polaroid DMC1e digital microscope color camera (Polaroid Corporation, Cambridge, MA) connected to the computerized image analyzer program, Optimas (Media Cybernetics, Silver Spring, MD). Results are expressed as the peribronchial trichrome stained area per micrometer length of basement membrane with round shaped bronchioles 150 to 200 μ m of internal diameter.

At least five bronchioles were counted in each slide to quantify goblet cell hyperplasia and the peribronchial trichrome stained area.

Immunohistochemistry

From the paraffin embedded tissue sections of the left lung, immunohistochemical stain was performed for analysis of the degree of peribronchial smooth muscle hyperplasia, collagen type I and collagen type III deposition. Endogenous peroxidase activity was quenched by incubating lung sections with 3% hydrogen peroxide in anhydrous methanol for 10 minutes. For smooth muscle immunostaining, a monoclonal anti-mouse α -smooth muscle actin (α -SMA) antibody (Sigma, St. Louis, MO) was added into the lung sections and incubated overnight in a moist chamber at 4°C. Immunoreactivity was detected by sequential incubations of the lung sections with a kit of biotinylated secondary antibody, followed by peroxidase reagent and AEC chromogen (3-amino-9-ethylcarbazole) (Sigma, St. Louis, MO). Similar methods were used for the detection of collagen type I and collagen type III using anti-collagen type I and type III (Santa Cruz Biotechnology, Santa Cruz, CA) for primary antibody. Antigen retrieval was performed in trypsin digest (Zymed Laboratories, CA). Mouse collagen type I and type III was detected using a biotinylated secondary antibody, followed by peroxidase reagent and DAB (3,3'-diaminobenzidine) chromogen (Vector Laboratories, CA).

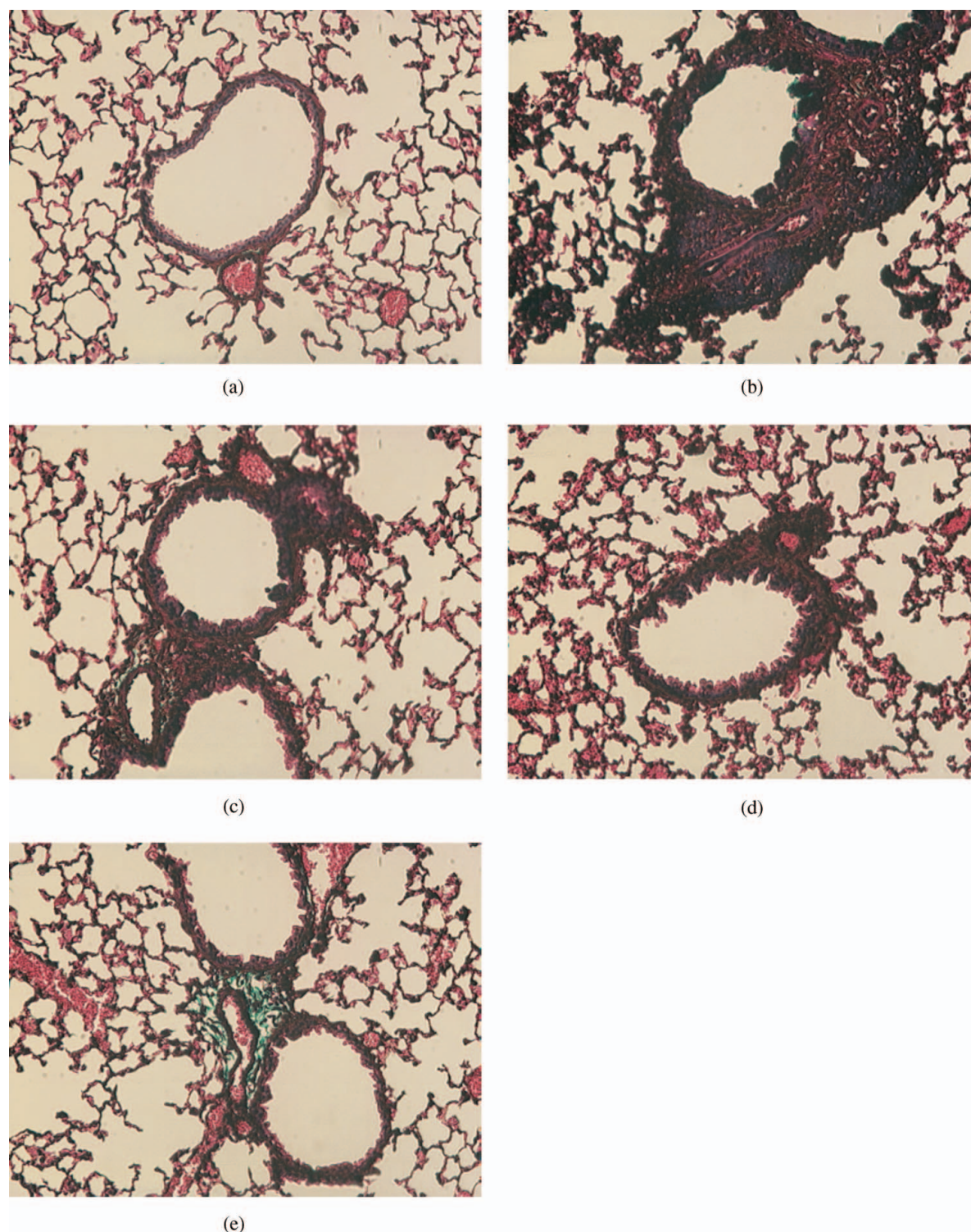


FIGURE 5.—Photomicrographs of Masson's trichrome staining of each group in a mouse model of chronic asthma ($\times 200$). (a) Control, (b) HDM, (c) HDM+TRA, (d) HDM+PTX, (e) HDM+TRA+PTX. Compared with HDM, other groups showed decreased peribronchovascular trichrome stained area in the bronchial wall. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

Statistical Analysis

A statistical software, The SAS System for Windows V 8.02 was used for the analysis. Results in the different groups of mice were compared by one-way analysis of variance

(ANOVA) and repeated measures ANOVA followed by post-testing using Scheffe's multiple comparison of means. A p value of less than 0.05 was considered to be statistically significant.

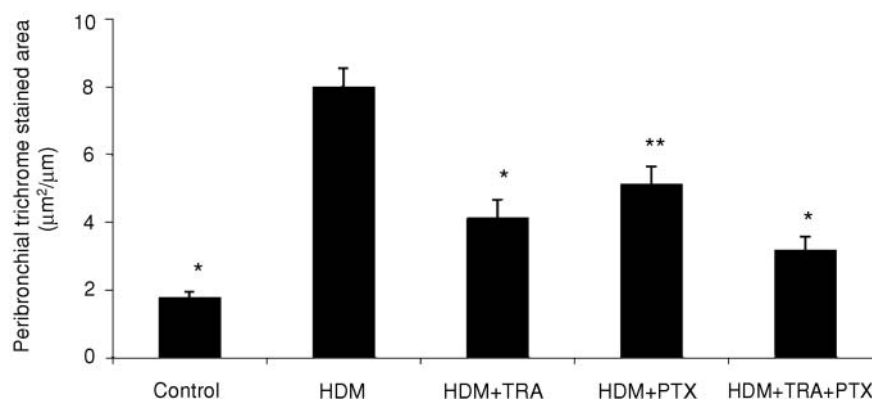


FIGURE 6.—Peribronchial trichrome stained area of each group in a mouse model of chronic asthma. Compared with HDM, other groups showed significantly decreased peribronchial trichrome stained area in the bronchial wall (* $P < 0.01$, ** $P < 0.05$). HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline. Bars were representative of standard errors.

RESULTS

Airway Hyperresponsiveness (AHR)

HDM-challenged mice without treatment had significantly greater AHR than did control mice. The increased AHR of the HDM-challenged mice was markedly reduced in groups treated with PTX, tranilast, or both PTX and tranilast. There was no statistically significant difference in AHR between the control and each treatment group (Figure 1).

Serum HDM-specific Immunoglobulin E, and Cytokines in BALF and Serum

HDM-challenged mice treated with tranilast, PTX, or both tranilast and PTX showed a tendency toward decreased serum HDM-specific IgE compared with HDM-challenged mice without treatment (Table 1). In BALF, HDM-challenged mice without treatment showed statistically significant increases in IL-13 (33.1 ± 10.7 pg/mL) and TGF- β 1 (252.3 ± 40.9 pg/mL) compared with control subjects (7.2 ± 0.9 pg/mL, 120.4 ± 24.4 pg/mL, respectively) ($p < 0.05$). Treatment with tranilast, PTX, or both tranilast and PTX showed statistically significant reductions in IL-13 (8.4 ± 1.1 pg/mL, 8.9 ± 1.3 pg/mL, 8.8 ± 0.7 pg/mL, respectively) ($p < 0.05$) and in TGF- β 1 (159.9 ± 20.2 pg/mL, 127.7 ± 17.4 pg/mL, 130.9 ± 16.8 pg/mL, respectively) ($p < 0.05$). BALF IFN- γ showed no statistically significant differences among the groups.

In serum, treatment with tranilast and both tranilast and PTX resulted in reduced levels of IL-13 (74.7 ± 16.3 pg/mL, 61.4 ± 9.6 pg/mL, respectively) compared with HDM-challenged mice without treatment (223.2 ± 49.8 pg/mL) ($p < 0.05$). Serum IFN- γ was reduced in the treatment with PTX and both tranilast and PTX (5.9 ± 0.9 pg/mL, 5.7 ± 0.9 pg/mL, respectively) compared with HDM-challenged mice without treatment (17.6 ± 4.6 pg/mL) ($p < 0.05$).

Cellular Response in BALF

HDM-challenged mice without treatment had significantly higher numbers of total BALF cells, including macrophages, eosinophils, lymphocytes, and neutrophils, than did control subjects (Figure 2). The increases in BALF cellular components were significantly reduced in groups treated with

tranilast, PTX, or both tranilast and PTX. The eosinophil counts in mice treated with both tranilast and PTX were significantly lower than in the group treated with tranilast alone.

Goblet Cell Hyperplasia

HDM-challenged mice without treatment had a greater degree of goblet cell hyperplasia than did control mice and this was statistically significant (grade 3.30 ± 0.21 vs. grade 0.00 ± 0.00 , $p < 0.01$). Treatment with tranilast, PTX, or both tranilast and PTX showed a significantly reduced level of goblet cell hyperplasia (grade 1.65 ± 0.17 , grade 0.77 ± 0.17 , grade 0.42 ± 0.10 , respectively) ($p < 0.01$) (Figures 3 and 4).

Peribronchial Trichrome Stained Area

HDM-challenged mice without treatment had a greater peribronchial trichrome stained area than did control mice (1.77 ± 0.16 vs. 7.98 ± 0.53 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole; $p < 0.01$). Treatment with tranilast (4.13 ± 0.53 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole; $p < 0.01$), PTX (5.13 ± 0.49 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole; $p < 0.05$), or both tranilast and PTX (3.20 ± 0.36 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole; $p < 0.01$) resulted in a statistically significant reduction in peribronchial trichrome stained area (Figures 5 and 6).

Immunohistochemistry for Smooth Muscle Hyperplasia and Collagen Deposition

The peribronchial components of airway remodeling were associated with smooth muscle and collagen type I and collagen type III. HDM-challenged mice without treatment had an increased smooth muscle hyperplasia, and collagen type I and type III deposition compared with control mice. Treatment with tranilast, PTX, or both tranilast and PTX resulted in a reduction in smooth muscle hyperplasia, and collagen type I and type III deposition (Figures 7 to 9).

DISCUSSION

In the present study, both tranilast and PTX could attenuate airway remodeling as well as allergen-induced AHR and airway inflammation, which were mediated at least in part

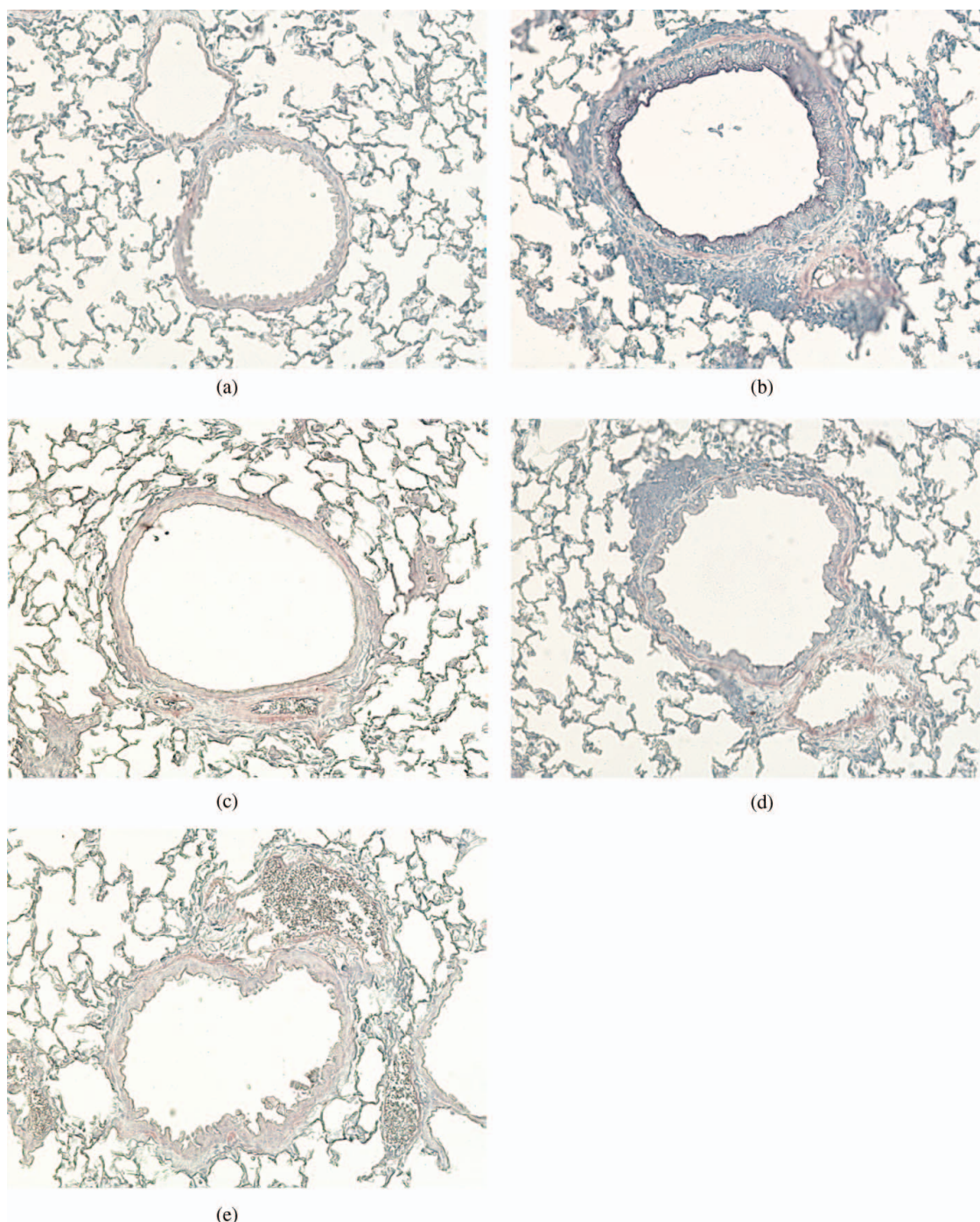


FIGURE 7.—Photomicrographs of α -smooth muscle actin immunohistochemical staining of each group in a mouse model of chronic asthma ($\times 200$). (a) Control, (b) HDM, (c) HDM+TRA, (d) HDM+PTX, (e) HDM+TRA+PTX. Compared with HDM, other groups showed decreased peribronchial smooth muscle in the bronchial wall. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

by the inhibition of the Th2 cytokine IL-13 and TGF- β 1 in BALF. Airway remodeling such as goblet cell hyperplasia and increased peribronchial thickness including smooth muscle hyperplasia, collagen type I, and collagen type III deposition could be improved by tranilast or PTX.

We used HDM antigen in this mouse model of chronic asthma because it is the antigen most frequently associated with human allergic disease. AHR, airway inflammation, and

airway remodeling such as goblet cell hyperplasia, smooth muscle hyperplasia, and collagen type I and collagen type III deposition developed in a manner similar to human chronic asthma.

For the measuring of Der P specific IgE, we chose the standard mouse serum that was exposed with the same Der P antigen and high IgE value. After serial dilution of the standard mouse serum, we can get relative ratio (OD) of each

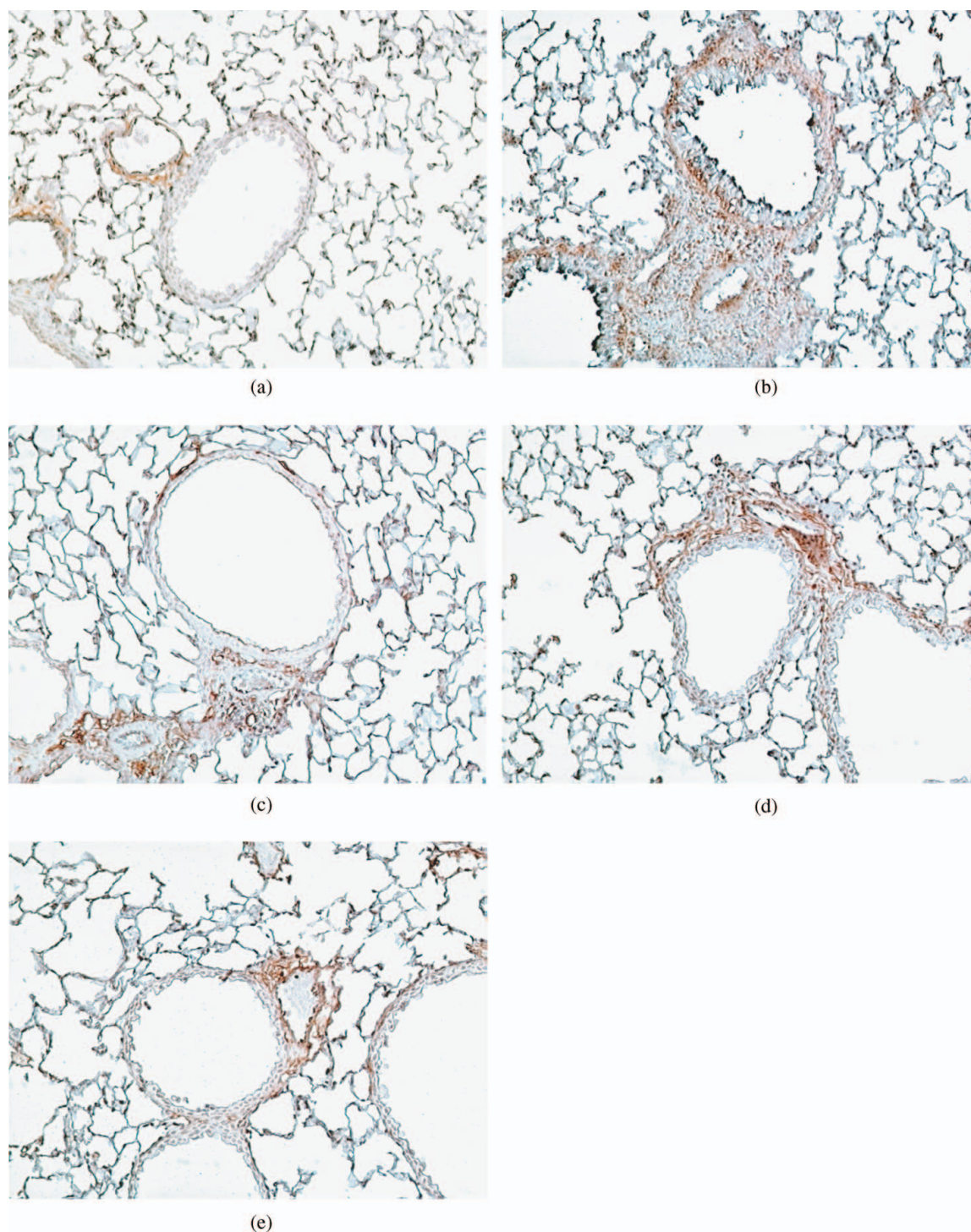


FIGURE 8.—Photomicrographs of collagen type I immunohistochemical staining of each group in a mouse model of chronic asthma ($\times 200$). (a) Control, (b) HDM, (c) HDM+TRA, (d) HDM+PTX, (e) HDM+TRA+PTX. Compared with HDM, other groups showed decreased peribronchial collagen type I deposition in the bronchial wall. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

experimental mice serum. Specific IgE level of each group yields positive results; however, owing to the large variation of standard errors, the results were statistically insignificant.

For the Th2 cytokine, we assessed IL-13 because it is one of the most important cytokines in allergic inflammation. For Th1, we measured IFN- γ because of its well-characterized

role as a Th1 cytokine. TGF- $\beta 1$ plays an important role in initiating the fibrotic process in the synthesis and accumulation of extracellular matrix.

Previous studies have shown that tranilast inhibits TGF- $\beta 1$ release (9, 17). This cytokine mediates collagen formation in a range of cells, including skin fibroblasts, cardiac

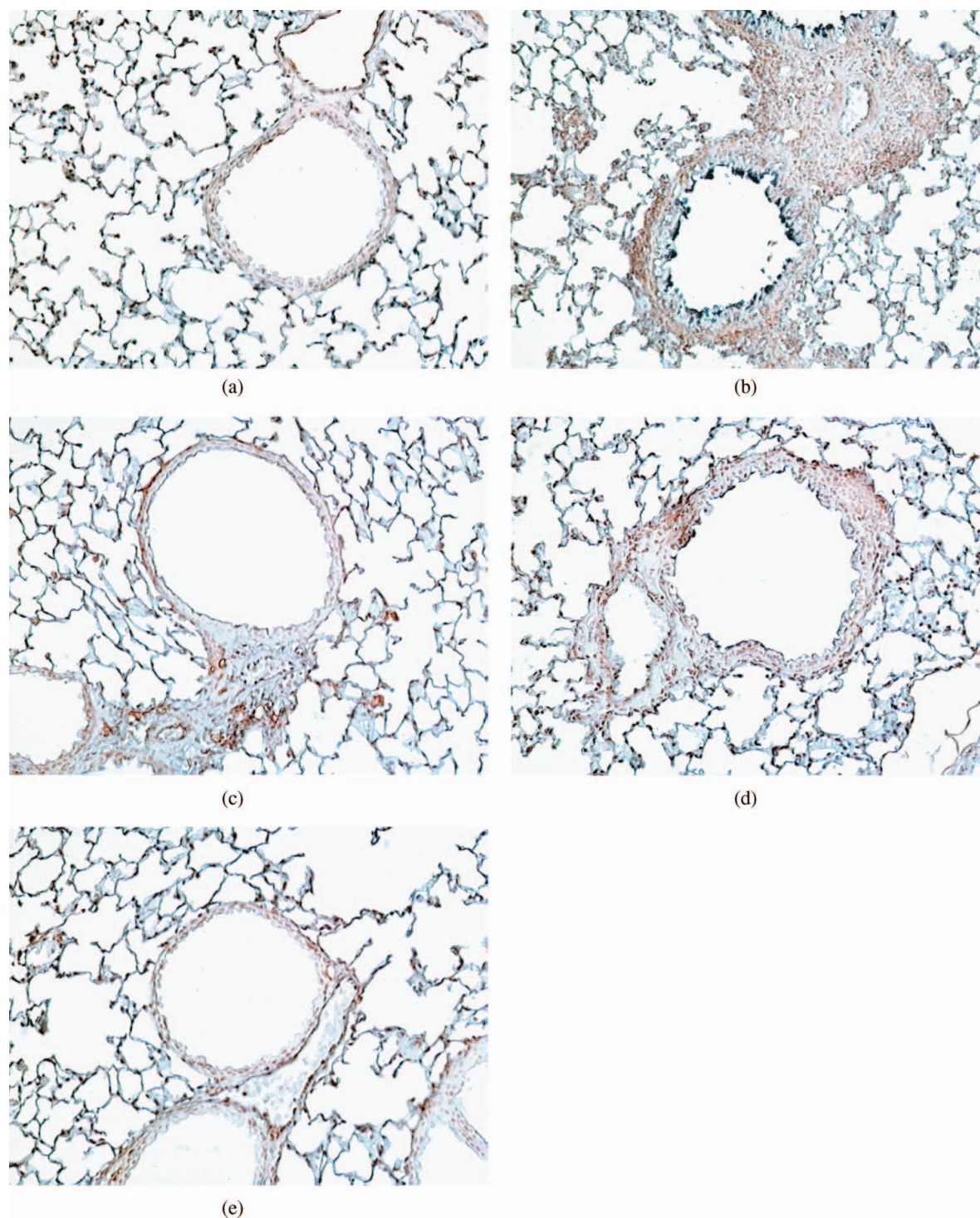


FIGURE 9.—Photomicrographs of collagen type III immunohistochemical staining of each group in a mouse model of chronic asthma ($\times 200$). (a) Control, (b) HDM, (c) HDM+TRA, (d) HDM+PTX, (e) HDM+TRA+PTX. Compared with HDM, other groups showed decreased peribronchial collagen type III deposition in the bronchial wall. HDM = house dust mite; TRA = tranilast; PTX = pentoxifylline.

fibroblasts, renal cortical fibroblasts, vascular smooth muscle cells, Tenon's capsule and corneal stromal fibroblasts, and hepatic stellate cells (9, 17–21). Therefore, because of its effective anti-fibrotic action, tranilast has been tried in patients for the prevention and treatment of several diseases

such as coronary restenosis and diabetic nephropathy (22, 23).

In the respiratory system, tranilast has a beneficial effect on allergic inflammation due to its well-known anti-allergic effect on mast cells. However, whether tranilast has a beneficial

effect on airway remodeling has not yet been investigated. We found that tranilast could attenuate goblet cell hyperplasia and increased peribronchial thickness, which are key features of airway remodeling, as well as AHR and airway inflammation.

At a cellular level, PTX exerts the nonspecific inhibition of phosphodiesterases, antagonism of the adenosine receptor, and inhibition of inflammatory mediators (10). However, compared with theophylline, it is tolerated relatively well over a wide dosage range and has a strong anti-inflammatory effect, mainly through its inhibitory influence on neutrophils, macrophages, and monocytes and the proinflammatory cytokines produced by these cells (24, 25). In human asthma, various inflammatory cells, including lymphocytes, neutrophils, alveolar macrophages, and eosinophils, play important roles in the pathogenesis of asthma (26–28). BALF inflammatory cells, including macrophages, eosinophils, lymphocytes, and neutrophils, were reduced after PTX treatment, which may be associated with improvements in AHR and airway remodeling.

In the animal model of pulmonary alveolitis, Entzian et al. (29) reported that PTX may more efficiently reduce inflammation than prednisolone or colchicine. They suggested that PTX could be an effective therapeutic alternative and should be investigated further regarding its anti-inflammatory and anti-fibrotic properties. We demonstrated that PTX treatment had beneficial effects on airway inflammation and increased peribronchial thickness.

Previous studies have shown that PTX inhibits Th1 cytokine production (IFN- γ , IL-2, TNF- α , IL-1, IL-2), with either no effect or a stimulatory effect on Th2 cytokines (IL-4, IL-10, IL-13) (11, 13–15). In this study, as we expected from previous studies, PTX had an inhibitory effect on the Th1 cytokine IFN- γ in serum, although it had no inhibitory effect in BALF. We also showed PTX had an additional inhibitory effect on the Th2 cytokine IL-13 and TGF- β 1 in BALF.

In an animal model of asthma, the administration of PTX led to the attenuation of AHR, which was associated with decreased BALF levels of the Th1 cytokine IFN- γ , without affecting the Th2 cytokine IL-13 and other hallmarks of pulmonary allergic inflammation (15). Therefore, the authors suggested that not only the Th2 immune response, but also the Th1 immune response, may be essential for the development of AHR. However, in the present study, PTX treatment led to the attenuation of AHR and the improvement of airway inflammation and airway remodeling through the suppression of the BALF Th2 cytokine IL-13 and TGF- β 1 without suppression of the Th1 cytokine IFN- γ in BALF.

For this reason, one could first consider the differences in the method of animal modeling, as well as the timing, concentration, and frequency of PTX given to the animal. Second, BALF IFN- γ levels were detected at very low concentrations in all groups. Thus, at least in this animal model, the IFN- γ in BALF could not attribute to the development of chronic asthma. In contrast to BALF, the reduction serum IFN- γ levels after PTX treatment was statistically significant, which could reflect only a systemic inhibitory effect of intraperitoneal PTX injection. Third, Benbernou et al. (12) examined the regulatory effect of PTX on Th1 and Th2 cell-derived

cytokines in human mononuclear cells. Cytokine production showed that PTX at the appropriate concentrations could induce selective suppression of the Th1 cytokine IL-2 and IFN- γ , whereas at high concentrations, it could act as a suppressive agent of both Th1- and Th2-derived cytokines. In this study, PTX reduced the Th1 cytokine IFN- γ in serum and the Th2 cytokine IL-13 in BALF.

In summary, using a mouse model of chronic asthma with continuous exposure to HDM antigen, we showed that tranilast and PTX each had an inhibitory effect on airway remodeling, AHR, and airway inflammation. The anti-inflammatory effect of tranilast was associated with the inhibition of the Th2 cytokine IL-13; PTX exerted its anti-inflammatory effects through not only the Th1 cytokine IFN- γ , but also the Th2 cytokine IL-13. Both tranilast and PTX had an inhibitory effect on a fibrotic cytokine TGF- β 1. These drugs could have inhibitory effects on goblet cell hyperplasia and peribronchial thickness including smooth muscle hyperplasia, collagen type I, and collagen type III deposition, key features of airway remodeling.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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