Therapeutic potential of an orally effective small molecule inhibitor of plasminogen activator inhibitor for asthma

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Asthma is one of the most common respiratory diseases affecting ~5% of the population in the United States. The pathological features of chronic asthmatic airways include infiltration of eosinophils, hyperresponsiveness, mucus hypersecretion, and subepithelial fibrosis. Despite intensive studies, the precise mechanisms underlying the pathogenesis of chronic asthma remain unclear. Most importantly, there are no effective treatments to cure chronic asthma, although anti-inflammatory steroids plus bronchodilators provide temporal relief of the symptoms during asthma exacerbation. Understanding the precise mechanisms underlying the pathogenesis of chronic asthma have been and will continue to be a major challenge for clinicians and basic science researchers.

Plasminogen activator inhibitor 1 (PAI-1) is a primary inhibitor of tissue-type and urokinase-type plasminogen activators (tPA and uPA, respectively). Besides suppressing fibrinolysis through inhibition of tPA and uPA and thereby plasminogen activation, PAI-1 has pleiotropic functions, including inhibition of extracellular protein degradation, modulation of cell attachment and migration, and regulation of cell senescence and apoptosis processes (25). Importantly, it has been reported that PAI-1 protein is elevated in sputum of allergic asthmatic patients (1–3, 21, 22) and in experimental asthma models (23, 31). Individuals carrying polymorphic allele of the PAI-1 gene (4G instead of 5G), which leads to increased plasma PAI-1 level, have higher risk to develop asthma and show more severe asthmatic symptoms than 5G/5G carriers (28, 30, 32). Most importantly, it has been reported that intratracheal administration of small-interfering RNA targeting PAI-1 (29) or the PAI-1 inhibitor tiplaxtinin (24) attenuated allergen-induced pathological changes in experimental asthma models. Together, the data suggest that PAI-1 plays a critical role in the pathogenesis of asthma (3, 4, 28, 37) and therefore may serve as an ideal therapeutic target for the treatment of asthma. Nonetheless, despite strong evidence supporting the role of PAI-1 in asthma pathogenesis, no therapeutic drugs that target PAI-1 for the treatment of chronic asthma have yet been developed.

5-Chloro-2-[(2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethoxy][28]acetyl]aminobenzoate (TM5275) is an orally effective small-molecule PAI-1 inhibitor. It has potent antithrombotic activity in both rodents and nonhuman primates (cynomolgus monkey) (18). Importantly, TM5275 does not interfere with other serpin/serine protease systems such as α1-antitrypsin/trypsin and α2-antiplasmin/plasmin and causes no obvious toxicity to the liver, kidney, hematopoietic system, central nervous system, or cardiovascular system of rats and monkeys when given in doses up to 2,000 mg·kg−1·day−1 for 2 wk (18). It has no significant effect on activated partial thromboplastin time, prothrombin time, or bleeding time, the most common side effects of anticoagulation agents (18). These data suggest that TM5275 is relatively specific for PAI-1 with low toxicity. Therefore, we explored, in this study, the therapeutic potential of TM5275 for asthma in an ovalbumin (OVA)-induced chronic asthma model. Our results show that oral administration of TM5275, 25 days after OVA sensitization and challenge, significantly ameliorated airway hyperresponsiveness (AHR), eosinophil and neutrophil infiltration, mucin production, and subepithelial fibrosis. The results suggest that TM5275 may have therapeutic potential for asthma.
MATERIALS AND METHODS

Mouse model of chronic allergic asthma. A well-established chronic asthma model induced by ovalbumin (OVA) was used in this project to test the therapeutic value of TM5275 for chronic asthma (10, 11). Specifically, 6- to 8-wk-old female BALB/cJ mice were divided into three groups, 16 mice/group. Group 1 (nonasthmatic) mice were sensitized and challenged with saline. Group 2 (asthmatic) mice were sensitized with 50 µg of alum-precipitated chicken egg OVA by intraperitoneal injection two times (day 0 and day 14) and then challenged with OVA by aerosol inhalation, 5 mg/ml, 30 min/day, daily from day 21 to day 25, and 10 min/day every other day from day 26 to day 35. This group of mice was also treated with vehicle (20% DMSO in saline) by oral gavage daily from day 26 to day 35. Group 3 (asthmatic + TM5275 treatment) mice were sensitized and challenged with OVA as the group 2 mice and treated with 40 mg/kg TM5275 (dissolved in 20% DMSO) by oral gavage daily from day 26 to day 35. The dose of 40 mg/kg TM5275 was chosen based on our previous study in which we showed that oral administration of 40 mg/kg TM5275 for 10 consecutive days completely blocked TGF-β1-induced lung fibrosis with no obvious toxicity (no body weight loss) (13). A schematic diagram with the detailed information about sensitization, challenge, and treatment is presented in Fig. 1. The whole experiment has been repeated one time. Twenty-four hours after the final challenge and TM5275 treatment, eight mice from each group were used for the lung function test using the SCIREQ FlexiVent system, whereas another eight mice from each group were killed for histology and biochemistry analyses. All procedures involving animals were approved by the Institutional Animal Care and Use Committees at the University of Alabama at Birmingham (UAB) and conducted at the UAB animal facilities under specific pathogen-free conditions.

Measurement of AHR. AHR was measured using the FlexiVent system upon challenge with increasing concentrations of methacholine as we described previously (11). Briefly, 48 h after the last OVA challenge and TM5275 treatment, mice were anesthetized with ketamine (450 mg/kg), and a tracheotomy tube (18G) was inserted and connected to the inspiratory and expiratory ports of a ventilator (FlexiVent; SCIREQ, Montreal, PQ, Canada). Mice were mechanically ventilated at a rate of 160 breaths/min at a tidal volume of 0.2 ml with a positive end-expiratory pressure of 2–4 cmH2O. Methacholine (0, 5, 10, 15, and 20 mg/kg) was administered via aerosolization. From 20 s up to 3 min after each methacholine aerosol challenge, resistance, elastance, tissue damping, and total lung capacity were recorded continuously. The average value of each parameter was taken to express changes in murine airway function.

Zymography analysis of tPA and uPA activities. The activities of tPA and uPA in lung tissue were determined by zymographic analysis as we have described before (13, 39). Briefly, equal amounts of proteins were loaded on 12% polyacrylamide gel containing 2 mg/ml casein in the presence of 5 µg/ml plasminogen. After electrophoresis, the enzyme reaction was initiated by incubating the gel in 0.1 M glycine-NaOH (pH 8.3) at 37°C for 16 h, and the lytic areas were developed by staining the gel with a solution containing 30% methanol, 10% glacial acetic acid, and 0.5% Coomassie blue G250. The gels were destained in the same solution without dye and scanned using a Bio-Rad Fluor-s MultImaging system. To avoid the possible interference of matrix metalloproteinases, EDTA (2 mM) was included in the glycine-NaOH buffer during the incubation period. The tPA and uPA bands were identified based on the mobility of the molecular weight markers. The photonegative images of the gels are presented in Figs. 1–9 and assessed semiquantitatively using the Image J analyzing software (NIH website).

Bronchoalveolar lavage, bronchoalveolar lavage cell counting, and lung tissue processing. Bronchoalveolar lavage (BAL) was performed with 0.8 ml of phosphate-buffered saline (PBS). BAL fluid (BALF) was spun down at 400 g for 10 min; the supernatants were collected, and the cells were resuspended uniformly in saline. The BALF cells were centrifuged on a microscope slide using a CytoSpin and stained with a Diff-Quik stain set (B4132-1A; Siemens Health-care Diagnostics, Newark, DE). Total cell numbers were counted and calculated based on the volume of the BALF; differential cell counts were performed by counting 300–600 cells on each slide using an oil immersion (×100) lens of a Zeiss microscope, and the percentages of monocytes, neutrophils, lymphocytes, and eosinophils were calculated. After lavage, pulmonary artery vascular beds were perfused and then left lung fixed with 10% PBS-buffered formalin for histology and immunohistochemistry as we have described previously (27). The rest of the lung was frozen immediately in liquid nitrogen for biochemistry analyses.

Measurement of urea concentrations in the plasma and BALF. Concentrations of urea in BALF and in the plasma were measured using a commercially available kit (Teco Diagnostics, Anaheim, CA) following the protocol provided by the manufacturer. The ratio of urea concentrations in the plasma and in BALF (dilution factor) was calculated for each mouse.

Measurement of OVA-specific IgE in plasma and BALF. OVA-specific IgE in mouse plasma and BALF were measured by an anti-OVA mouse-IgE ELISA kit from BioVendor (Gunma, Japan) following the instruction provided by the manufacturer. The results were normalized with urea dilution factors, and the results are expressed as nanograms per milliliter of epithelial lining fluid (ELF).

Measurement of cytokine/chemokines in BALF. The protein levels of cytokines and chemokines in mouse BALF were analyzed using a Bio-Plex multiplex suspension cytokine array (Bio-Rad Laboratories) according to the manufacturer’s instructions (26). The data were analyzed using Bio-Plex Manager software (Bio-Rad Laboratories). The results were normalized with urea dilution factors and expressed as nanograms per milliliter of ELF.

Measurement of goblet cell hyperplasia. Lung tissues fixed in 10% paraformaldehyde were stained with periodic acid-Schiff (PAS) to reveal mucin production in goblet cells as described previously (24). Goblet cell hyperplasia was quantified by determining the percentage of PAS-positive cells/length of bronchial basal membrane in 10 sites/mouse by quantitative morphometry techniques using Image J software as we have described previously (13).
Measurement of mucus hypersecretion. The amounts of mucin 5 subtype AC (MUC5AC) in BALF were measured using a mouse Mucin 5 AC ELISA kit (Biomitik, Ontario, Canada) following the instruction provided by the manufacturer. The results were normalized with urea dilution factors and expressed as nanograms per milliliter of ELF.

Collagen staining. Collagen deposition was revealed by Sirius red staining. High-quality images were taken at ×40 magnifications. The collagen deposition around large airways (including the bronchioles and tertiary bronchi, identified by their ciliated pseudostratified columnar epithelium and relative thick smooth muscle layer in the walls with no cartilage) and small airways (including the terminal bronchioles, identified by their low cuboidal epithium and intact walls, and the respiratory bronchioles, identified by their low cuboidal epithelium and occasional mural alveoli) (19) was quantified by quantitative morphometry techniques using Image J software as we have described previously (13).

Statistical analysis. AHR data (tissue damping, resistance, elastance, and total lung capacity) were analyzed by the two-way analysis of variance (ANOVA) trend test to compare the slopes of the methacholine dose-response curves from the three treatment groups (saline, OVA, and OVA + TM5275). Post hoc analyses were conducted with the Tukey’s test. The data presented in other figures were evaluated by one-way ANOVA, and statistical significance was determined post hoc by Fisher’s least-significant difference test wherein \( P < 0.05 \) was considered significant.

RESULTS

TM5275 attenuates OVA-induced AHR. Asthmatic airways are highly sensitive to allergens or irritants (AHR). To elucidate whether TM5275 has therapeutic potential for asthma, we first assessed the effect of TM5275 on OVA-induced AHR induced by methacholine using a FlexiVent system. Two-way ANOVA trend tests were conducted to compare the slopes of the methacholine dose-response curves from the three treatment groups (saline, OVA, and OVA + TM5275). The results show that OVA sensitization-challenge significantly increased total cell numbers as well as the numbers of neutrophils, lymphocytes, monocytes, and eosinophils in BALF after Diff-Quik staining. The results show that OVA sensitization-challenge significantly increased total cell numbers as well as the numbers of neutrophils, lymphocytes, monocytes, and eosinophils in BALF (Fig. 4). Of the total cells found in BALF of OVA-challenged mice, 62% are eosinophils, confirming an eosinophilic inflammatory response in our asthma model. Treatment with TM5275, 25 days after OVA sensitization-challenge, significantly mitigated OVA-induced inflammation of inflammatory cells (Fig. 4). Differential cell count results further show that TM5275 treatment significantly reduced OVA-induced infiltration of neutrophils (reduced by 80%), eosinophils (reduced by 57%), and monocytes (reduced by 43%), although it had no significant effect on OVA-induced lymphocyte infiltration. The results suggest that PAI-1 is involved in inflammatory cell infiltration and that TM5275 has potent anti-inflammatory function.

TM5275 suppressed OVA-induced infiltration of inflammatory cells in mouse lung. Inflammatory response in asthmatic airways is characterized by infiltration and activation of eosinophils, which in turn contribute importantly to the pathophysiology of asthma. The OVA-induced chronic asthma model is featured with eosinophilic inflammatory cell infiltration. To determine whether TM5275 can suppress the OVA-induced inflammatory response, we counted the total cell numbers as well as the numbers of neutrophils, lymphocytes, monocytes, and eosinophils in BALF after Diff-Quik staining. The results show that OVA sensitization-challenge significantly increased total cell numbers as well as the numbers of neutrophils, lymphocytes, monocytes, and eosinophils in BALF (Fig. 4). Of the total cells found in BALF of OVA-challenged mice, 62% are eosinophils, confirming an eosinophilic inflammatory response in our asthma model. Treatment with TM5275, 25 days after OVA sensitization-challenge, significantly mitigated OVA-induced infiltration of inflammatory cells (Fig. 4).

### Table 1. Two-way ANOVA trend test of the slopes of the methacholine dose-response curves among three treatment groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( F(2,4) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue damping</td>
<td>23.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Resistance</td>
<td>23.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Elastance</td>
<td>30.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total lung capacity</td>
<td>32.83</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 2. Two-way ANOVA trend test of the slopes of the methacholine dose-response curves between two treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue Damping</th>
<th>Resistance</th>
<th>Elastance</th>
<th>Total Lung Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline vs. OVA</td>
<td>23.67</td>
<td>&lt;0.0001</td>
<td>22.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Saline vs. OVA + TM5275</td>
<td>11.69</td>
<td>&lt;0.0001</td>
<td>11.66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OVA vs. OVA + TM5275</td>
<td>20.18</td>
<td>&lt;0.0001</td>
<td>20.33</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

OVA, ovalbumin.
challenge significantly increased OVA-IgE in the plasma and in BALF. TM5275 treatment, on the other hand, partially blocked the OVA-stimulated increase in OVA-IgE in the BALF, although it had no significant effect on the level of plasma OVA-IgE (Fig. 5). The results further confirm that the PAI-1 inhibitor TM5275 suppressed the OVA-induced eosinophilic inflammatory response in mice.

**Effects of TM5275 on OVA-induced cytokine production.** Increased Th2 cytokine production is associated with asthma and contributes importantly to asthmatic airway pathology. To further elucidate the mechanism whereby TM5275 mitigates OVA-induced AHR, the amounts of Th1 and Th2 cytokines in BALF were measured by a Bio-Plex mouse cytokine ELISA kit. The results show that OVA sensitization-challenge significantly increased the amounts of IL-4 and IL-5 in BALF, although it had no significant effect on other cytokines, including IL-2, IL-10, IL-12, interferon-γ, tumor necrosis factor-α (TNF-α), and granulocyte macrophage colony-stimulating factor (Fig. 6). TM5275 treatment, on the other hand, reduced OVA-stimulated IL-4 and IL-5 production (Fig. 6).

**TM5275 reduced OVA-stimulated mucin production.** Mucin overproduction, one of the pathological features of asthmatic airways, was assessed by PAS staining and by measuring MUC5AC, a major form of mucin secreted by airway epithelial cells and associated with asthma, in BALF by ELISA. PAS staining results show that OVA sensitization-challenge significantly increased the amount of mucin in airway epithelial cells, whereas treatment with TM5275 partially blocked the OVA effect (Fig. 7, A and B). MUC5AC ELISA results further show that OVA sensitization-challenge significantly increased the amount of MUC5AC in BALF. TM5275 administration again reduced the amount of secreted MUC5AC stimulated by OVA (Fig. 7C). Together, the results suggest that TM5275 may have therapeutic potential for asthma through reducing allergy-induced airway mucin production.

**TM5275 reduced subepithelial collagen deposition in airways.** Collagens are the major type of extracellular matrix. Subepithelial collagen deposition (airway remodeling) leads to airway narrowing and airflow obstruction. Because PAI-1 plays a critical role in the regulation of collagen degradation, we further examined whether TM5275 treatment reduces collagen deposition in large and small airways by Sirius red

Fig. 3. Effect of TM5275 on the activities of urokinase-type and tissue-type plasminogen activators (uPA and tPA, respectively) in mouse lung. The activities of tPA and uPA in lung tissue were determined by zymographic analysis as described in MATERIALS AND METHODS. The photonegative images of the gels are presented, and the band intensities were semiquantified using the Image J analyzing software. “Significantly different from saline control mice; †significantly different from the OVA alone group (P < 0.05, n = 4–5).
staining. The results show that mice sensitized and challenged with OVA had a significantly increased amount of collagen deposition around large airways, although OVA sensitization-challenge had no significant effect on collagen deposition in small airways. Oral administration of TM5275, 25 days after OVA sensitization and challenge, significantly reduced the amount of collagen in the large airways (Fig. 8). The results suggest that TM5275 may have therapeutic potential for chronic asthma by reducing allergy-induced subepithelial collagen deposition.

DISCUSSION

PAI-1 expression is increased in the sputum of asthmatic patients and in experimental asthma models, and has been shown to play a critical role in the pathogenesis of asthma [1–3, 21–24, 28–32]. Although PAI-1 small-interfering RNA (siRNA) and the small-molecule PAI-1 inhibitor tiplaxtinin have been tested in animal models, no therapeutics that target PAI-1 for the treatment of asthma have been yet developed [24, 29]. TM5275 is an orally effective small-molecule PAI-1 inhibitor. It has potent antithrombotic activity in rat and monkey thrombosis models [18]. Most importantly, it does not interfere with other serpin/serine protease systems nor prolong bleeding time or cause obvious toxicity in rats or monkeys when administered with up to 2,000 mg/kg for 2 wk [18], suggesting that TM5275 is relatively specific for PAI-1 and safe to use (low toxicity). In this study, we show that oral administration of 40 mg/kg of TM5275, a dose that is much
lower than its no observed adverse effect level (NOAEL, 2,000 mg/kg), 25 days after OVA sensitization and challenge, ameliorated OVA-induced AHR and suppressed the OVA-induced eosinophilic inflammatory response, mucin production, and airway remodeling. The results suggest that TM5275 may have therapeutic potential for chronic asthma. It should be pointed out that, although tiplaxtinin, another small-molecule PAI-1 inhibitor, has been shown previously to ameliorate airway pathological changes when given simultaneously with OVA in a similar asthma model (24), TM5275 may be a better candidate drug, since its toxicity is lower (NOAEL of TM5275 in monkey is &gt;2,000 mg/kg, whereas NOAEL of tiplaxtinin in dogs is 100 mg/kg) and the bioavailability is higher (96% in monkeys for TM5275 vs. 36% in dogs for tiplaxtinin) compared with tiplaxtinin (9, 18).

One of the pathological features of asthmatic airways is inflammation. Eosinophils play an important role in allergic response and in asthma pathology. In this study, we show that challenge with OVA dramatically increased the number of eosinophils and the amount of OVA-IgE in BALF, confirming the nature of the allergic response in our OVA-induced asthma model. Treatment with TM5275 significantly reduced the number of eosinophils (by 57%) and the amount of OVA-IgE in BALF of OVA-challenged mice, indicating that PAI-1 is involved in the eosinophilic allergic response in asthma. Besides eosinophils, TM5275 administration also significantly reduced the numbers of neutrophils and monocytes in BALF of OVA-challenged mice. It should be pointed out that, although the percentages of cell types in BALF are similar between OVA and OVA plus TM5275 groups (4, 29.3, 62.4, and 4.3% in OVA-challenged mice vs. 6.4, 35.4, 56.4, and 1.8% in OVA + TM5275-treated mice for lymphocytes, monocytes, eosinophils, and neutrophils, respectively), TM5275 had a more dramatic effect on BALF cell counts of neutrophils (reduced by 80%), eosinophils (reduced by 57%), and monocytes (reduced by 43%) than on lymphocytes (no significant decrease). The mechanism and biological significance underlying the selective effect of TM5275 on BAL cells is unclear and warrants further investigation. Associated with suppression of inflammatory cell infiltration, TM5275 reduced the production of OVA-IgE as well as IL-4 and IL-5, two Th2 cytokines that promote eosinophil response and IgE production. The results suggest

Fig. 6. Effect of TM5275 on OVA-stimulated production of cytokine/chemokines in BALF. The protein levels of cytokines/chemokines in mouse BALF were analyzed using a Bio-Plex multiplex suspension cytokine array, and the data were analyzed using Bio-Plex Manager software as described in MATERIALS AND METHODS. IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; GM-CSF, granulocyte macrophage colony-stimulating factor. *Significantly different from saline control mice; †significantly different from OVA alone-treated mice (P < 0.05, n = 11–13).

Fig. 7. Effect of TM5275 on OVA-induced mucin production. Mucin production in airway epithelial cells was revealed by periodic acid-Schiff (PAS) staining and by measuring mucin 5 subtype AC (MUC5AC) in BALF using an ELISA kit as described in MATERIALS AND METHODS. Left, representative images of PAS staining; middle, semiquantified results of PAS staining; right, ELISA results of MUC5AC in BALF. *Significantly different from saline control mice; †significantly different from the OVA alone group (P < 0.05, n = 4–7).
that TM5275 has a potent anti-inflammatory effect. Similar effects on inflammatory cell infiltration have been reported by other groups with another PAI-1 inhibitor, tiplaxtinin (24), and PAI-1 siRNA (29). Together, the results suggest that PAI-1 inhibitors may have therapeutic potential for asthma through suppressing the inflammatory response, specifically the eosinophilic response (Fig. 9).

The mechanism whereby TM5275 reduces the numbers of neutrophils, eosinophils, and monocytes in BALF is unknown at the moment. PAI-1 has pleiotropic functions besides inhibiting the activities of uPA and tPA. It binds to the extracellular matrix protein vitronectin and thereby blocks the binding of vitronectin to integrins and uPA receptor (uPAR) on the cell surface, leading to cell detachment/migration (6, 36, 38). PAI-1 can also affect cell migration and function through binding to uPA-uPAR and then low-density lipoprotein receptor-related protein (LRP) on the cell surface (7, 16). Interestingly, it has been reported that macrophage migration depends on the interaction between PAI-1 and LRP, and that TM5275 inhibits macrophage migration through interfering in such interaction (16). Therefore, one of the potential mechanisms whereby TM5275 reduces the numbers of neutrophils, eosinophils, and monocytes in BALF is to block PAI-1-mediated cell migration (infiltration) through interfering with the interaction between PAI-1 and vitronectin and/or LRP. Emerging evidence also shows that PAI-1 modulates the survival/apoptosis process in different types of cells (12, 13, 34, 41). Previous study from this lab showed that TM5275 induces p53, a master apoptosis inducer, and apoptosis in primary human (13) and mouse (12) lung fibroblasts. A previous study from this group also showed that treatment with PAI-1 protein inhibited spontaneous and TNF-α-induced apoptosis of neutrophils, whereas knockout of the PAI-1 gene (PAI-1−/− mice) promoted neutrophil apoptosis induced by lipopolysaccharide (41). Therefore, another potential mechanism whereby TM5275 reduced the numbers of eosinophil, neutrophils, and/or monocytes in BALF of OVA-challenged mice is to induce apoptosis of these inflammatory cells. More studies are needed to decipher the mechanisms whereby TM5275 reduces inflammatory cell numbers in BALF.

Hypertrophy and hyperplasia of goblet cells, which leads to overproduction of mucin, is another important pathological feature of asthmatic airways. Using the PAS staining technique, Lee et al. showed that treatment with the PAI-1 inhibitor tiplaxtinin reduced OVA-induced mucin overproduction, although the mechanism by which tiplaxtinin reduces mucin overproduction was not explored (24). In this study, we show that OVA challenge dramatically increased, whereas TM5275 administration significantly reduced, OVA-stimulated mucin production in airways by PAS staining and by measuring the amount of MUC5AC in BALF. We further show that TM5275 administration attenuated the OVA-mediated increase in IL-4 and IL-5, two Th2 cytokines that have been shown to stimulate mucin production in goblet cells (17, 20, 35). Together, our results suggest that TM5275 administration ameliorated OVA-induced airway mucin production/accumulation probably through inhibition of the inflammatory response and Th2 cytokine production.

Airway remodeling or subepithelial fibrosis is another important pathological feature of chronic asthmatic airways. Collagens are the major types of extracellular matrix proteins and the major component of fibrotic tissue. Previous study from this lab and others has shown that PAI-1 inhibited collagen degradation in cultured fibroblasts (39) and plays an important role in the development of lung fibrosis (5, 8, 13, 33, 40). In this study, we show that OVA sensitization-challenge signifi-
cantly increased collagen deposition in large airways, whereas treatment with TM5275 partially blocked the OVA effect. These results are consistent with our previous findings, although in a different disease model (13). In that study, we showed that treatment of mice with TM5275 almost completely blocked collagen and hydroxyproline accumulation in the lung in a mouse lung fibrosis model induced by AdTGF-B1\textsuperscript{1223/225}, an adenovirus expressing constitutively active TGF-β, when given 4 days after AdTGF-B1\textsuperscript{1223/225} installation (13). It is unclear at this moment whether TM5275 reduces collagen deposition/accumulation by stimulating collagen degradation and/or by suppressing collagen synthesis. Because previous studies from this lab and others have shown that PAI-1 suppresses collagen degradation (14, 15, 39), it is speculated that TM5275 reduces collagen accumulation in large airways in part by increasing collagen degradation.

In summary, our data suggest that the orally effective small-molecule PAI-1 inhibitor TM5275 attenuates OVA-induced pathophysiological changes in mouse airways and may have therapeutic potential for chronic asthma.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


