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ORIGINAL ARTICLE



Effect of nintedanib on airway inflammation and remodeling in a murine chronic asthma model

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ABSTRACT

Introduction: Nintedanib is a multi-tyrosine kinase receptor inhibitor recently approved for treatment of idiopathic pulmonary fibrosis. Although angiogenesis is a key process involved in airway structural changes in patients with bronchial asthma, the effect of nintedanib targeting the angiokine pathway on airway inflammation and remodeling has not been evaluated. **Methods:** We used a 3-month ovalbumin (OVA) challenge mouse model of airway remodeling. Nintedanib was orally administered during the challenge period, and the effects were examined based on the percentage of airway inflammatory cells, airway hyper-reactivity (AHR), peribronchial goblet cell hyperplasia, total lung collagen and smooth muscle area. The expression of growth factor receptors was analyzed in mice lung tissues. **Results:** The OVA challenged group showed a significant increase in airway eosinophilic inflammation, elevated Th2 cytokines, AHR, and airway remodeling compared to those in the control group. The airway remodeling process, as evaluated by goblet cell hyperplasia, total lung collagen level, and airway smooth muscle area, was suppressed by nintedanib compared to that by OVA. Nintedanib effectively suppressed the phosphorylation of vascular endothelial growth factor/platelet derived growth factor subunit2/fibroblast growth factor3 receptors in the mice lung. **Conclusions:** Nintedanib effectively ameliorated airway inflammation and remodeling in an OVA-induced chronic asthma model. These results suggest that nintedanib could be a new treatment agent targeting airway remodeling in patients with severe asthma.

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chronic asthma; fibroblast growth factor receptor (FGFR); nintedanib; platelet-derived growth factor receptor (PDGFR); vascular endothelial growth factor receptor (VEGFR)

Introduction

Bronchial asthma is a chronic disease characterized by airway inflammation and remodeling. Among patients with bronchial asthma, 5–10% of the total population have been estimated to have severe asthma and are refractory to high-dose inhaled corticosteroids plus a second controller and/or systemic corticosteroids.^[1] There is still an unmet need for asthma treatment, as well as strategies for ameliorating major morbidity, and the socioeconomic burden.

Airway remodeling involves structural changes consisting of subepithelial fibrosis, hyperplasia of myofibroblasts and myocytes, and an increase in the number of smooth muscle fibers.^[2] This process

establishes an irreversible airway obstruction that is unresponsive to conventional asthma treatment. Persistent efforts have been made to investigate the pathophysiology of airway remodeling, and several studies have suggested a role for the increased microvascular network of the asthmatic airway.^[3, 4] Vascular areas increase in patients with bronchial asthma and these areas promote an influx of inflammatory mediators and abnormal cell growth and proliferation.^[5, 6] Angiogenesis is a key component leading to airway wall thickening and narrowing of the bronchial lumen in patients with asthma.^[7]

Vascular endothelial growth factor (VEGF) plays a fundamental role in angiogenesis. In addition, a direct

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contribution to the allergic airway response in patients with asthma has been suggested. VEGF amplifies the Th2-mediated response and interleukin (IL)-13, a key Th2 cytokine, induces structural cells to produce VEGF, leading to airway inflammation and remodeling.^[7, 8] Moreover, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) induce angiogenesis and are involved in bronchial vascular remodeling in patients with asthma. FGF and PDGF are secreted from mast cells along with other proangiogenic factors.^[6, 7] Besides these effects, FGF and PDGF contribute to airway remodeling by inducing synthesis of the extracellular matrix (ECM) and promoting proliferation of airway smooth muscle (ASM).^[9–11]

Nintedanib is a multiple tyrosine kinase inhibitor, recently approved for treating idiopathic pulmonary fibrosis (IPF). Nintedanib simultaneously inhibits phosphorylation of VEGF, FGF, and PDGF.^[12] Originally investigated as an anti-cancer agent, nintedanib had shown potential efficacy and safety in multiple malignancies, such as advanced non-small cell lung cancer, ovarian cancer, and multiple myeloma.^[13] Among the chronic diseases, nintedanib reduced the annual rate of decline in forced vital capacity in the INPULSIS trial, which was a phase III evaluation of patients with IPF.^[14]

Administering the multi-kinase inhibitor sunitinib or the monoclonal antibody bevacizumab has been shown to have significant inhibitory effects on airway remodeling in a chronic experimental asthma model.^[15–17] Moreover, imatinib and nilotinib, which are PDGFR kinase inhibitors, also significantly inhibited airway remodeling in a chronic asthma model.^[11, 18] However, the effects of nintedanib have not been reported. This study investigated the effects of nintedanib on airway inflammation and remodeling in an ovalbumin (OVA)-challenged chronic bronchial asthma mouse model.

Materials and methods

Animals

Female BALB/c mice (KOATECH, Gyeonggi-do, Korea), 7 to 8 weeks of age, were used in the experiments and divided into 5 groups ($n = 6–10$ per group): control (CON), ovalbumin (OVA) challenge, OVA challenge plus ethanol (EtOH), OVA challenge

plus 50 mg/kg nintedanib and OVA challenge plus 100 mg/kg nintedanib treatment.

Sensitization and antigen challenge protocol

Mice were immunized by subcutaneous injection on once a week for 1 month with 25 µg of OVA (grade V; Sigma-Aldrich, St. Louis, MO., USA) adsorbed to 1 mg of aluminum hydroxide (Aldrich, Milwaukee, Wisc., USA) in 200 µL of phosphate-buffered saline (PBS). And then intranasal OVA challenges (20 µg/50 µL in PBS) were performed under isoflurane (Vedco, St. Joseph, MO., USA) anesthesia starting on day 31 and were repeated twice a week for 3 months. Control mice were treated in the same way with PBS but without OVA. Mice were killed 24 hrs after the final OVA challenge. All animal experimental protocols were approved by the Animal Subjects Committees of the Catholic University of Korea.

Administration of nintedanib

Nintedanib was obtained from Biovision Inc. (Milpitas, CA, USA). Dry nintedanib powder was dissolved in 10% EtOH. Allergen-sensitized mice were treated by oral gavage with 0.2 mL of PBS containing nintedanib (50 or 100 mg/kg). Nintedanib was given once a day starting on day 35, and then during the OVA challenges for 3 months. CON and OVA mice were treated in the same way with PBS.

Measurement of airway hyperresponsiveness (AHR)

AHR was assessed by measuring changes in the airway resistance (cmH₂O/mL/s) after mice were subsequently exposed to increasing concentrations of methacholine (Mch; 6.25–50 mg/mL; Sigma-Aldrich) in PBS using the Flexivent system (SCIREQ, Montreal, Quebec, Canada). After the mice were anesthetized with a mixture of tiletamine/zolazepam (30 mg/kg, Virbac Laboratories, Carros, France) and xylazine (10 mg/kg, Bayer, Leverkusen, Germany), a tracheostomy was performed. Tracheostomized (18 G cannula) mice were mechanically ventilated (160 breaths/min, tidal volume of 10 mL/kg) under appropriate anesthesia. Methacholine aerosol was administered for 10 sec, after which airway resistance was continuously monitored and recorded.

Bronchoalveolar lavage (BAL) fluid

Immediately after blood collection, the trachea was cannulated with silicone tubing attached to a 23G needle on a tuberculin syringe. BAL fluid was collected instillation of 1 mL of PBS through the trachea into the lung. BAL fluid was centrifuged at 2000 rpm for 5 min at 4°C. The pellets were resuspended in 300 μ L PBS, and total cell numbers were counted by trypan blue exclusion using a hemacytometer. Differential leukocytes were obtained from a cytospin (5 min at 750 $\times$ g) slide preparation stained with Diff-Quick (Sysmax, Tokyo, Japan). The percentages of differential inflammatory cell were obtained by counting at least 200 macrophages, neutrophils, eosinophils or lymphocytes on randomly selected areas of the slide using light microscopy. The supernatant of BAL fluid was aliquoted and frozen at -80°C until further analysis.

Lung tissue histology

Lungs tissue sections embedded in paraffin (3 μm) were stained with hematoxylin and eosin (H&E) to observe the general morphology and periodic acid Schiff (PAS) stain to identify goblet cell hyperplasia and submucosal gland hypertrophy. To quantify goblet cell hyperplasia, the pathological changes were evaluated according to the method described by Padrid et al.^[19] The percentage of goblet cells in the epithelium scored as follows by the two researchers separately and took the average values: no goblet cells = 0, <25% = 1, 25% to 50% = 2, 51% to 75% = 3, >75% = 4.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of interleukin (IL)-4, -5, and -13 were measured in the BAL fluid with an ELISA kit (R&D Systems, Minneapolis, Minn., USA). The protocol followed was according to the manufacturer's instructions. The sensitivities of the assay were 2 pg/mL, 7 pg/mL and 1.5 pg/mL, respectively. The anti-OVA immunoglobulin (Ig) E EIA kit was purchased from BioLegend (San Diego, California, USA). Reactions were read using an ELISA plate reader at 450 nm.

Western blot

Total protein was isolated from lung by homogenization in radio immunoprecipitation assay (RIPA) cell

lysis buffer containing a mixture of and phosphatase inhibitor (GenDEPOT, CA, USA) and then centrifuged at 13,000 rpm for 30 min at 4°C. Protein concentration was quantified using the BCA assay. An equal amount of sample protein (40 $\mu\text{g/mL}$) was then separated by % sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Little Chalfont, UK). The membranes were blocked with 5% skim milk (Difco/Becton Dickinson, Atlanta, GA, USA) for 1 hrs at room temperature, followed by incubation with a 1:200 dilution of anti-FGFR3, anti-p-FGFR3 (Santa Cruz Biotechnology, Inc., CA, USA) or a 1:1,000 dilution anti-PDGFR β , anti-p-PDGFR β antibody, anti-VEGFR2, anti-p-VEGFR2 antibody (Cell Signaling Technology; CST, Inc., MA, USA) in 5% BSA in Tris buffered saline containing 0.1% Tween 20 (TBS-T) overnight at 4°C. After incubation, the blot was washed and incubated with a 1:2,000 dilution of anti-rabbit-IgG, horseradish peroxidase (HRP)-linked secondary antibody (CST) for 2 hrs at room temperature. After washing, the protein bands were visualized by enhanced chemiluminescence using a RAS 3000 Image Analysis System (Fuji Film, Japan).

Hydroxyproline analysis

The concentrations of total collagen content were measured in the peripheral portions of the lung with a hydroxyproline assay kit (Biovision Inc., Milpitas, CA, USA). The protocol followed was according to the manufacturer's instructions. Briefly, the lung tissue was homogenized in dH_2O , using 300 μL H_2O for 30 mg of tissue. The samples were subsequently added to 300 μL of 12 N HCl for at 120°C , overnight. After centrifugation, 100 μL of the chloramine T reagent was added to 10 μL of each hydrolyzed sample and standard and incubated at room temperature for 5 min, and then 100 μL of the DMAB reagent was added to each well. These samples were incubated for 90 min at 60°C , and were read at 560 nm in a spectrophotometer.

Immunohistochemistry for α -smooth muscle actin

Fixed tissues with 4% paraformaldehyde were embedded in paraffin and sectioned at 3 μm thickness. The slides were stained for α -smooth muscle actin (α -SMA) to assess the increase in ASM area. The lung sections were incubated overnight at 4°C either with a

primary mouse monoclonal antibody directed against α -SMA (Immunohistology kit, IMM-2; Sigma-Aldrich). Immunoreactivity was detected by peroxidase reagent and AEC (3-amino-9-ethylcarbazole in N,N-dimethylformamide) chromogen to produce a red-brown precipitate in the cytoplasm of α -SMA-positive cells. The immunostained area of α -SMA was estimated from the α -SMA positive area using the image processing program (BX50, Olympus, Tokyo, Japan). Results are expressed as the area immunostained per micrometer length of basement membrane of bronchioles (internal diameter 150–200 μ m).

Statistical analysis

The data were subjected to one-way ANOVA followed by Dunnett's multiple range test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). All data are expressed as means $\pm$ S.E.M. and in all cases, $p < 0.05$ was considered statistically significant.

Results

Preventive effects of nintedanib on airway inflammation and AHR

Total cell counts from bronchoalveolar lavage (BAL) fluid increased after a 3-month OVA challenge, as compared to those in the control group, but decreased in response to nintedanib treatment ($p < 0.05$, Figure 1).

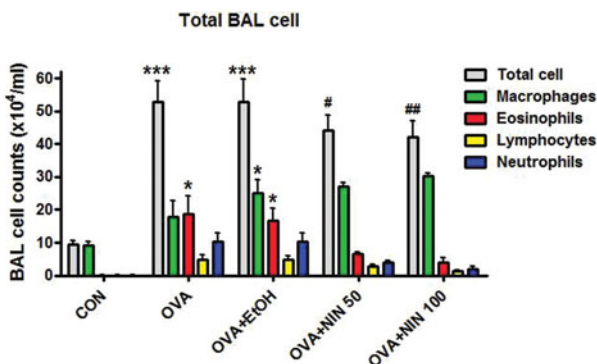


Figure 1. Effect of nintedanib on total and differential cell counts in bronchoalveolar lavage (BAL) fluid. Mice were sacrificed 24 h after the final OVA challenge, and BAL cells were isolated. Values are expressed as mean $\pm$ SEM ($n = 6$ –10/group). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. ## $p < 0.01$ and ### $p < 0.001$ compared with the OVA + EtOH group.

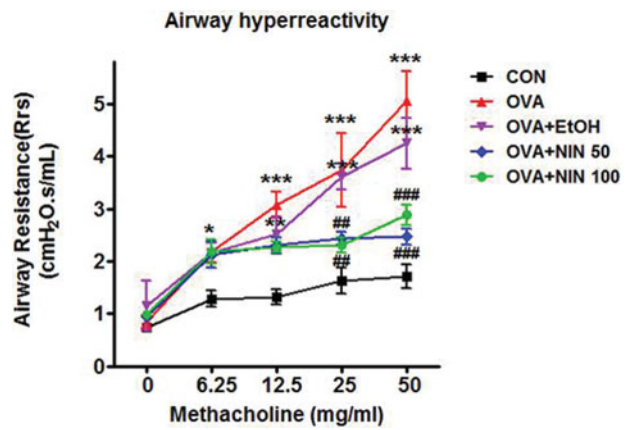


Figure 2. Effect of nintedanib on airway hyperresponsiveness (AHR) to methacholine. AHR was measured 24 h after the final OVA challenge using a Flexivent system in which mice were exposed to increasing concentrations of methacholine (6.25–50 mg/mL). CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, Ethanol administered to sensitized/challenged mice; OVA + NIN 50, 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN 100, 100 mg/kg nintedanib/kg administered to sensitized/challenged mice. Values are expressed as mean $\pm$ SEM ($n = 6$ –10/group). * $p < 0.05$ and *** $p < 0.001$ in comparison with control group. # $p < 0.05$ and ## $p < 0.01$ compared with the OVA + EtOH group.

The changes of eosinophil and neutrophil counts also showed similar pattern, increased after OVA challenge and decreased with nintedanib treatment. AHR to methacholine increased, dose dependently, in OVA-challenged mice, but decreased significantly after nintedanib treatment ($p < 0.001$, Figure 2). Levels of the Th2 cytokines IL-4, IL-5, and IL-13 from BAL fluid decreased after nintedanib treatment compared to those in the OVA-challenged mice group (Figure 3A). In addition, serum OVA-specific IgE level was significantly increased in OVA-challenged mice compared to control and decreased with nintedanib treatment ($p < 0.05$) (Figure 3B).

Effect of nintedanib on angiogenesis in lung tissues

We evaluated the effect of nintedanib on angiogenesis by perivascular cell infiltration, blood vessel count and perivascular α -smooth muscle actin (SMA) area. We analyzed the changes of infiltrating inflammatory cells by gross examination in lung tissues around the blood vessels. Figure 4A showed the decreased perivascular inflammatory cell infiltration (triangle) after nintedanib treatment. The number of blood vessels and α -SMA stained area had significantly decreased with

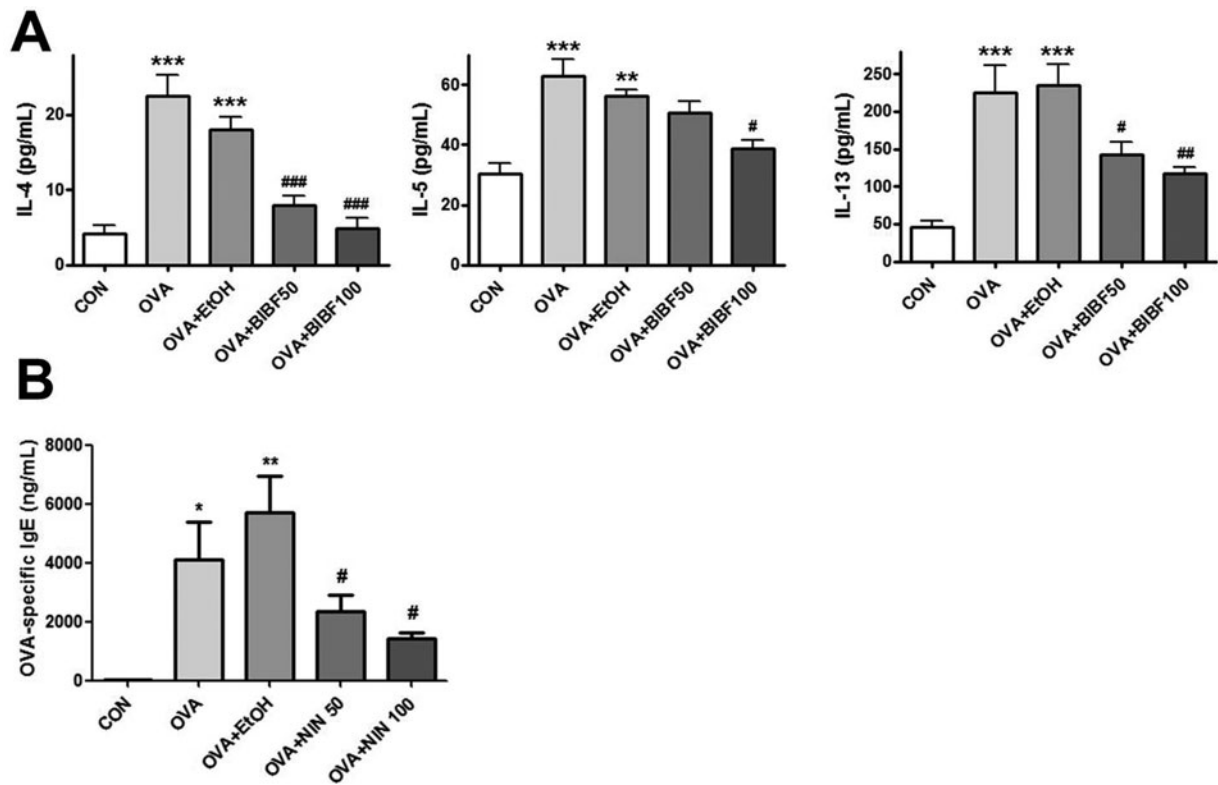


Figure 3. Effect of nintedanib on cytokine levels in BAL fluid and serum OVA specific IgE. (A) The concentration of interleukin (IL)-4, -5 and -13 were measured in the BAL fluid with an ELISA. (B) Serum levels of OVA specific immunoglobulin E were assessed by ELISA. CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, Ethanol administered to sensitized/challenged mice; OVA + NIN 50, 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN 100, 100 mg/kg nintedanib/kg administered to sensitized/challenged mice. Values are expressed as mean $\pm$ SEM ($n = 6-10$ /group). ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ compared with the OVA + EtOH group.

nintedanib treatment compared to OVA + EtOH control group (Figure 4B and C).

Effect of nintedanib on airway remodeling

We examined the inhibitory effect of nintedanib on the airway remodeling process using a histopathological examination and quantified goblet cells, total lung collagen level, and airway smooth muscle area. Hematoxylin and eosin-stained lung tissues revealed decreased infiltration of peribronchial inflammatory cells in lung tissues after nintedanib treatment (Figure 5A). Nintedanib decreased the number of goblet cells with a significantly lower PAS point score compared to that of the OVA-challenged mice (Figure 5B and C). The hydroxyproline assay and α -SMA staining revealed that total lung collagen level ($p < 0.01$, Figure 6) and airway smooth muscle area ($p < 0.01$, Figure 7) also decreased after nintedanib treatment, which were increased in response to the 3-month OVA challenge.

Effect of nintedanib on activation of PDGFR β , VEGFR2, and FGFR3

We performed a Western blot analysis with antibodies against PDGFR β , VEGFR2, and FGFR3 in mice lungs to evaluate the nintedanib mechanism. Figure 8 demonstrates the inhibitory effects of nintedanib on phosphorylation of these receptors. The OVA-challenged chronic asthma model demonstrated increased expression of PDGFR β , VEGFR2, and FGFR3 compared to that in control mice. Orally administered nintedanib exerted successive inhibitory effects on activation of these receptors in mice lungs.

Discussion

This study demonstrated that oral administration of nintedanib effectively inhibited airway inflammation, AHR, increased levels of Th2 cytokines, and airway remodeling in an OVA-induced murine chronic asthma model. Our mouse model showed significant

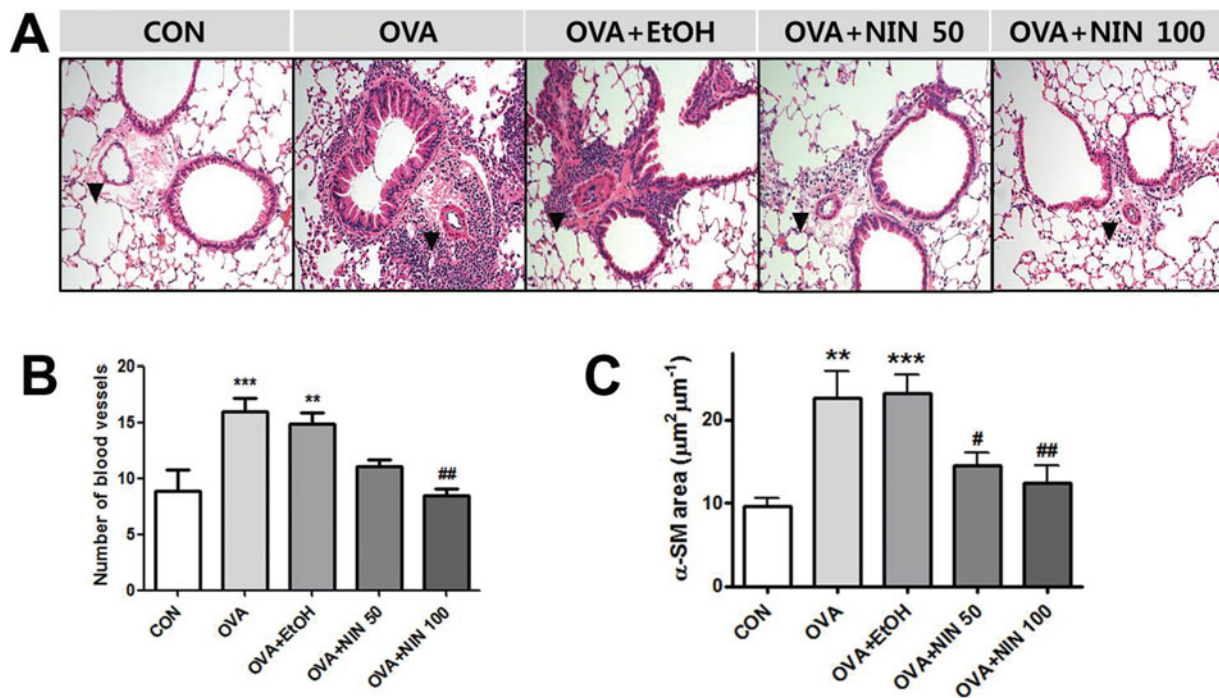


Figure 4. Effect of nintedanib on angiogenesis in lung tissues. (A) H&E stain of lung tissues ($\times 200$ magnification) presenting the changes of inflammatory cells around the peribronchial blood vessels. (B) Number of blood vessels in lung sections from mice. (C) Smooth muscle stained perivascular areas per micrometer length of endothelial membrane of blood vessels were calculated using an image analyzer. CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, Ethanol administered to sensitized/challenged mice; OVA + NIN 50, 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN 100, 100 mg/kg nintedanib/kg administered to sensitized/challenged mice, ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. # $p < 0.05$ and ## $p < 0.01$ compared with the OVA + EtOH group.

changes in the airway wall due to goblet cell hyperplasia, total lung collagen level, and airway smooth muscle area in lung tissues. The effects of decreased infiltration of airway inflammatory cells and remodeling by nintedanib were associated with significant amelioration of AHR, as compared to those in the OVA group. Moreover, oral treatment of nintedanib during the OVA challenge significantly decreased phosphorylation of VEGF, FGF, and PDGF receptors, simultaneously, in mouse lung tissues. In addition, perivascular inflammatory cell infiltration, α -SMA area and the counts of vessels were significantly decreased in nintedanib treatment group compared to OVA group. These results suggest that nintedanib could be a novel agent for targeting the airway remodeling process in patients with severe asthma.

Conventional therapies to treat severe asthma include long acting β -agonists or muscarinic receptor antagonists and/or systemic corticosteroids to relieve symptoms and control the disease. However, patients who are resistant to these standard treatments need a new therapeutic approach to control airway inflammation and remodeling. The 2014 European Respiratory

Society/American Thoracic Society guidelines recommend personalized treatment depending on phenotype.^[1] While there are no definite consensus on the phenotypes of severe asthma, biological agents such as monoclonal antibodies targeting IL-4, IL-5, IL-13, immunoglobulin E, and C-X-C motif chemokine receptor2 or tyrosine kinase inhibitors are suggested depending on the patient's clinical characteristics and biomarkers.^[1, 20]

Because the structural abnormalities affect airway mechanics, alterations in airway cells, such as epithelial, fibroblast, and smooth muscle cells, have been a focus in developing treatment agents targeting the airflow obstruction for patients with severe asthma. The bronchial epithelium is thicker in patients with severe asthma than in patients with mild to moderate asthma through altered proliferation, apoptosis, and release of pro-inflammatory factors.^[21] Fibroblasts interact with mast cells and eosinophils and proliferate and change into myofibroblasts, which have contractile properties and secrete ECM in the airway wall.^[22] ASM cells contract, proliferate, produce ECM and pro- or anti-inflammatory

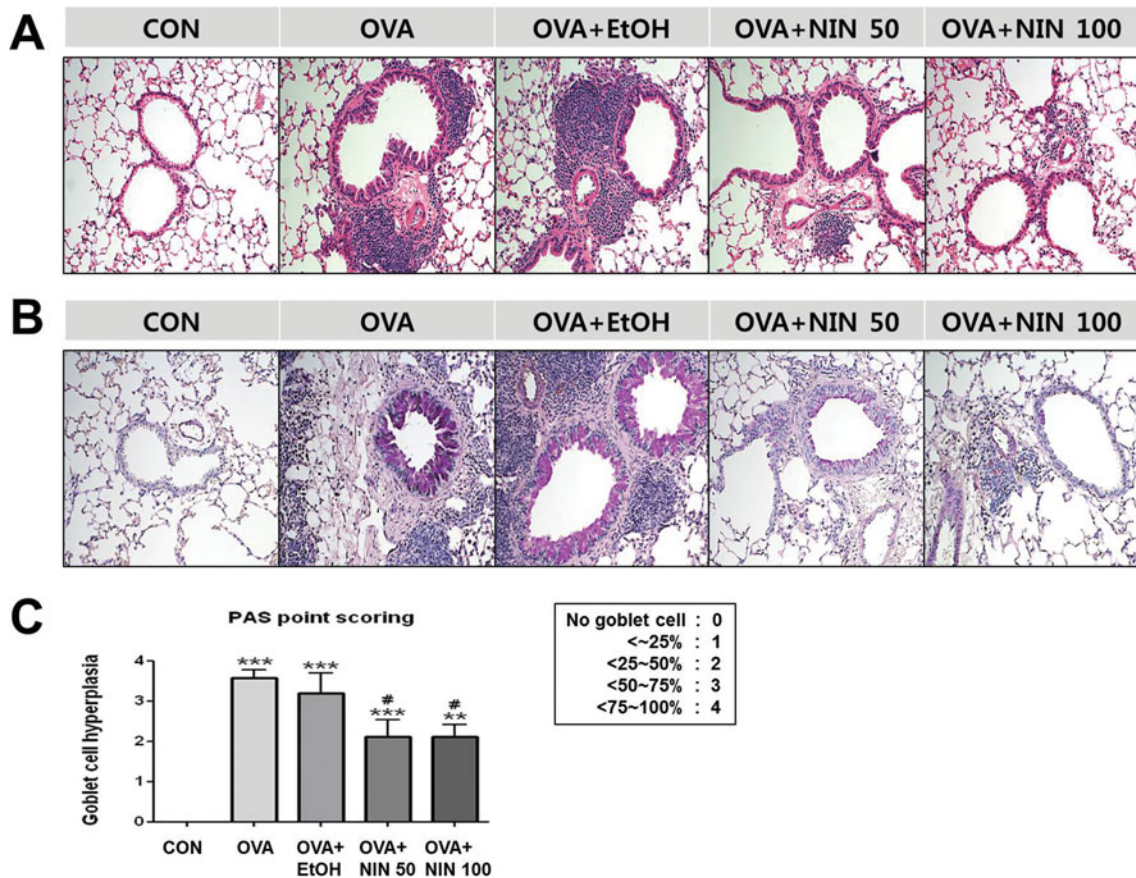


Figure 5. Effect of nintedanib on lung histopathology and goblet cell hyperplasia. Representative micrographs of H&E (A) and PAS stained (B) lung sections from mice of the treatment groups are shown. Quantification of the PAS-stained area by point scoring (C). CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, Ethanol administered to sensitized/challenged mice; OVA + NIN 50, 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN 100, 100 mg/kg nintedanib/kg administered to sensitized/challenged mice, ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. # $p < 0.05$ compared with the OVA group.

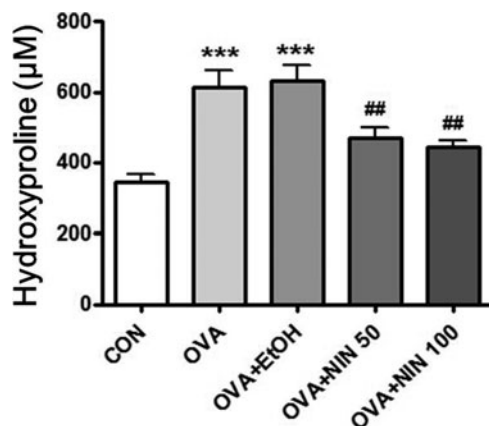


Figure 6. Effect of nintedanib on total lung collagen levels. Lung tissue was collected from each mouse for the hydroxyproline assay. CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, Ethanol administered to sensitized/challenged mice; OVA + NIN (50 mg/kg), 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN (100 mg/kg), 100 mg/kg nintedanib/kg administered to sensitized/challenged mice. Values are expressed as mean ± SEM ($n = 6-10$ /group). *** $p < 0.001$ compared with the control group. ## $p < 0.01$ compared with the OVA + EtOH group.

mediators resulting in AHR, airway thickening, and fibrosis.^[23]

Among the growth factors associated with activation of these airway cells, we focused on the roles of VEGF, FGF, and PDGF to reverse the airway remodeling process. VEGF plays diverse roles in airway structural changes, which are in addition to Lee's report that VEGF induces Th2-mediated sensitization and inflammation.^[8] For example, VEGF secreted from epithelial cells promotes subepithelial fibrosis^[24] and ASM-derived VEGF secretes ECM molecules.^[25] In addition, treatment with the anti-VEGF monoclonal antibody, bevacizumab, decreases epithelial thickness and goblet cell hyperplasia in a mouse model of chronic asthma.^[16] Moreover, PDGF mediates collagen production in airway fibroblasts and ASM proliferation, and FGF is involved in ASM hyperplasia and ECM synthesis.^[9, 10, 26, 27] In our experiment, the expression of phosphorylated forms of VEGF and PDGF receptors increased in the OVA group compared to that in the control, which is consistent with these previous reports.

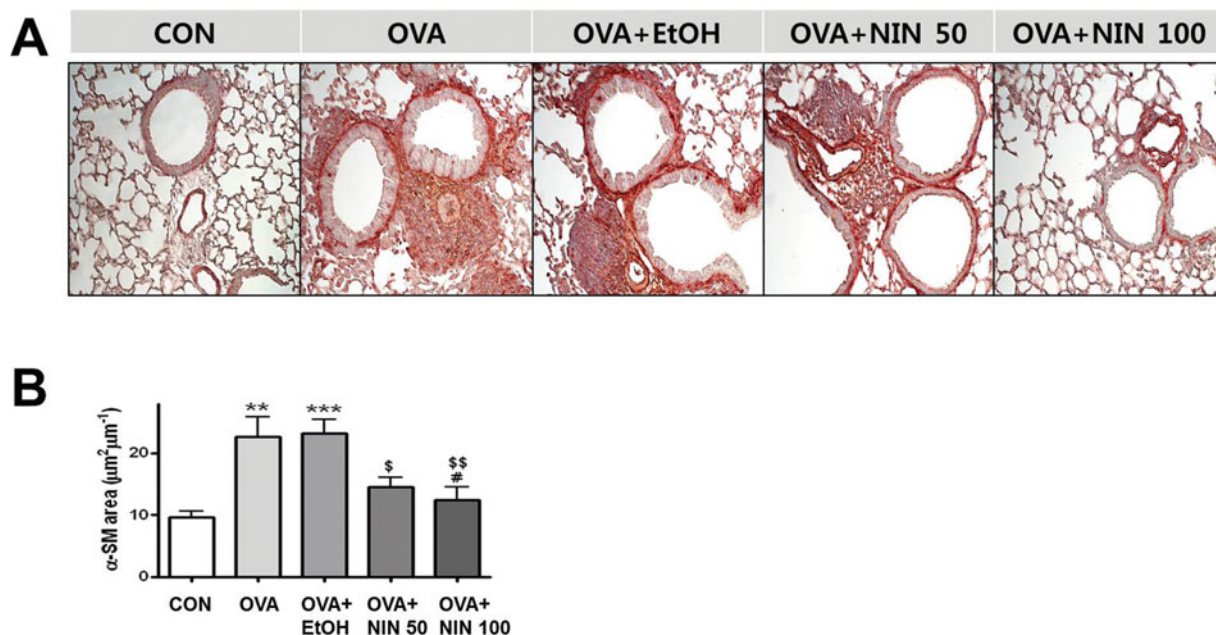


Figure 7. Effect of nintedanib on airway smooth muscle area. Representative micrographs of the area of smooth muscle-stained lung sections (A) (original magnification $\times 200$) and smooth muscle areas of immunostaining per micrometer length of basement membrane of the bronchioles, as assessed using an image analyzer (B). CON, control mice; OVA, OVA-sensitized/challenged mice; OVA + EtOH, ethanol administered to sensitized/challenged mice; OVA + NIN 50, 50 mg nintedanib/kg administered to sensitized/challenged mice; OVA + NIN 100, 100 mg/kg nintedanib/kg administered to sensitized/challenged mice. Values are expressed as mean $\pm$ SEM ($n = 6-10$ /group). ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. # $p < 0.05$ and ## $p < 0.01$ compared with the OVA + EtOH group.

The kinases targeted by nintedanib include VEGF 1, 2, and 3, PDGFR α and PDGFR β , FGFR1/2/3, FLT1, LCK, LYN, and SRC. Fibrogenic mediators, such as transforming growth factor (TGF)- β , IL-1, and tumor necrosis factor- α exhibit PDGF-dependent profibrotic activities. FGFs and their receptors control cellular proliferation, survival, and differentiation, which might be involved in the profibrotic process.^[28, 29] Nintedanib

shows sufficient anti-fibrotic and anti-inflammatory actions in an animal model of pulmonary fibrosis.^[30]

Based on these previous studies, combining the effects of anti-VEGF, FGF, and PDGF in nintedanib sufficiently suppressed Th2-mediated airway inflammation, goblet cell hyperplasia and collagen deposition, as in our study. The decreased ASM area could also be explained with reference to a pre-clinical nintedanib experiment for treating IPF. Lehtonen et al. reported that nintedanib reduces proliferation of fibroblasts from patients with IPF *in vitro* and decreases the amount of α -SMA and myofibroblasts.^[31] That is, the effects of nintedanib can be explained not only as those a multi-tyrosine kinase inhibitor but inhibiting fibroblast activation. Additionally, the level of TGF- β 1 in BAL fluid from OVA-induced acute asthma mice decreased compared to that in the OVA group (unpublished data) in our previous experiment. TGF- β 1 is a pivotal signaling molecule involved in airway remodeling and is implicated in subepithelial fibrosis, ASM remodeling, and microvascular changes.^[32] The effects of nintedanib on airway remodeling in a chronic asthma model via TGF- β 1 will be evaluated in further research.

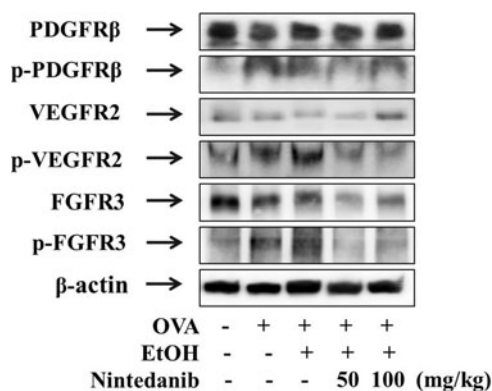


Figure 8. Effect of nintedanib on receptor expression and phosphorylation. Representative immuno-blots show the expression of total and phosphorylated PDGF-receptor (PDGFR), VEGF-receptor (VEGFR) and FGF-receptor (FGFR), in lung tissue. β -actin was used as a control.

The anti-VEGF monoclonal antibody bevacizumab shows anti-asthmatic effects comparable to

dexamethasone in an asthma animal model^[16], but an application to patients with asthma is problematic because of toxicity and the drug formula. Nintedanib is supplied as an oral agent and its safety and tolerability have been demonstrated in clinical trials and real world practice in patients with IPF.^[14, 33, 34] Considering that bronchial asthma is a chronic disease requiring long-term treatment, orally administrable drugs that are less toxic are preferred.

Some limitations in our study should be mentioned. As the most important concern when treating severe asthma is steroid resistance, the effect of nintedanib must be compared to that of a systemic steroid in our animal model. The effects of the VEGF, PDGF and FGF receptors on downstream signaling should also be evaluated in additional experiments.

In conclusion, orally administered nintedanib reduced eosinophilic airway inflammation and the remodeling process in a chronic mice asthma model. These effects were mainly mediated by inhibiting multi-kinase receptors. Nintedanib could be a potential treatment agent for patients with severe asthma by targeting the airway remodeling process.

Declaration of interest

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

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