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Effect of nintedanib on airway inflammation in a mouse model of acute asthma

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ABSTRACT

Objective: New treatments are needed for cases of asthma that are refractory to traditional therapies. In this study, we examined the effect of oral nintedanib, an intracellular inhibitor of tyrosine kinases, on airway hyper-responsiveness (AHR) and airway smooth muscle cells, using a mouse model of experimental asthma. **Methods:** Asthma was experimentally induced in mice via subcutaneous injection of ovalbumin (OVA). A group of saline-injected mice served as a control group. The OVA mice were then divided into four treatment groups according to the dose of nintedanib. AHR was examined via exposure to vaporized methacholine. Airway inflammation was assessed via bronchoalveolar lavage fluid (BALF) cell counts and Th2 cytokine concentrations. **Results:** Baseline levels of AHR and airway inflammation were higher in OVA mice than in the control group. Treatment with nintedanib lowered AHR, BALF cell counts and BALF cytokine levels in a dose-dependent fashion. The effect of nintedanib was comparable to that of dexamethasone. In particular, treatment with nintedanib lowered the expression of transforming growth factor- β 1 and inhibited the expression and phosphorylation of platelet-derived growth factor receptor- β , vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, fibroblast growth factor receptor 2 (FGFR2), FGFR3, and extracellular signal-regulated kinase. **Conclusions:** Nintedanib lowered AHR and the expression of factors associated with airway inflammation and remodeling in a mouse model of experimental asthma. Our results suggest that nintedanib may be useful in the treatment of asthma.

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Introduction

Asthma is characterized by airway inflammation, airway hyper-responsiveness (AHR), and variable expiratory airflow limitation [1]. Several pharmacologic therapies have been developed to prevent and control asthma symptoms, reduce the frequency and severity of asthma exacerbations and reverse airflow obstruction [2]. Glucocorticoids are known as the most effective anti-inflammatory medications for effective long-term management of asthma [3]. In particular, inhaled corticosteroids (ICS) are the cornerstone of maintenance therapy for asthma. However, when the amount of drug deposited in the respiratory tract increases due to an increase in disease severity, the

risk of adverse drug reactions also increases. High dose ICS use may be associated with systemic side effects including osteoporosis, skin thinning, cataracts, glaucoma and local side effects such as oropharyngeal candidiasis and dysphonia [4–6]. Poor adherence to medication is another problem of ICS. There is a high percentage of patients not taking their ICS as prescribed, and this poor adherence might be one of the reasons for poorly controlled asthma [7]. Therefore, another treatment that could be an alternative choice to use of glucocorticoids would be desirable.

The pathophysiology of AHR is complex, and many factors contribute to its development. Airway mucosal inflammation is characterized by an influx of

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activated eosinophils and T lymphocytes [8], and it is well established that Th2 cytokines play critical roles in the allergic inflammatory response leading to AHR [9,10]. Allergen exposure leads to upregulation of platelet-derived growth factor (PDGF) in animals with experimental asthma. Signaling through the PDGF pathway leads to airway smooth muscle (ASM) proliferation and enhanced AHR [11,12]. Additionally, fibroblast growth factor (FGF) levels are higher in bronchoalveolar lavage fluid (BALF) from asthmatic patients with mild and moderately atopic disease than in BALF from nonasthmatic controls [13]. Vascular endothelial growth factor (VEGF) also plays an important role in Th2 inflammation [14]. Furthermore, formoterol/budesonide treatment decreases airway remodeling, bronchoconstriction, VEGF expression and VEGF receptor (VEGFR) 1 expression in asthmatic patients with moderate disease [15].

Nintedanib was recently approved for the treatment of patients with idiopathic pulmonary fibrosis (IPF) [16]. Nintedanib is an intracellular inhibitor of tyrosine kinases, including VEGFR-1, -2, and -3, PDGFR- α and - β , and FGF receptor (FGFR)-1, -2, and -3. A previous study revealed that nintedanib targets transforming growth factor β (TGF- β), a profibrotic cytokine that is involved in fibrotic tissue remodeling in asthma and other fibrotic diseases [17]. Our current study was undertaken to examine the effect of nintedanib and compare the effect of nintedanib with steroid on mice with experimentally induced acute asthma. The effects of nintedanib on AHR and ASM growth factors (including VEGFR, PDGFR, FGFR and TGF- β) were also investigated.

Methods

Animals

Female BALB/c mice (KOATECH, Gyeonggi-do, Korea), 7–8 weeks of age, were used in all experiments. A total of 37 mice were divided into the following five groups: control ($n=6$ mice), ovalbumin challenge plus ethanol (OVA + EtOH, $n=8$ mice), OVA mice treated with 50 mg nintedanib/kg/day (OVA + BIBF50, $n=8$ mice), OVA mice treated with 100 mg nintedanib/kg/day (OVA + BIBF100, $n=7$ mice), and OVA mice treated with 4 mg dexamethasone/kg body weight (OVA + DEX, $n=8$ mice). All experimental animal protocols were approved by the Animal Subjects Committee at the Catholic University of Korea, in accordance with Article 14 of the Korean Animal Protection Law. The use of animals in the study complied with Article 13 of the Korean Animal Protection Law and relevant institutional policies.

Mouse sensitization

Acute asthma was induced in mice using a previously established protocol [18]. Briefly, mice were immunized on Days 0 and 7 with a subcutaneous injection of 25 μ g OVA (grade V, Sigma-Aldrich, St. Louis, MO, USA), adsorbed to 1 mg aluminum hydroxide (Aldrich, Milwaukee, WI, USA), in 200 μ L normal saline. Intranasal OVA challenges (20 ng OVA in 50 μ L phosphate-buffered saline [PBS]) were also administered on Days 21, 23, 25, and 28 while animals were anesthetized under isoflurane (Vedco, St. Joseph, MO, USA). Control mice were treated in the same manner, but were challenged with 50 μ L PBS in place of OVA. Mice were sacrificed 24 h after the final OVA challenge. Lung tissue and BALF were then collected for analyses.

Treatment with nintedanib

Nintedanib treatment was performed using our previously described protocol [19]. Dry nintedanib powder (Biovision, Inc., Milpitas, CA, USA) was dissolved in 10% ethanol, yielding a solution of 0.01 g nintedanib/mL. The final concentration of the ethanol used in the preparation of nintedanib was 5% EtOH for 50 mg/kg and 10% EtOH for 100 mg/kg. The nintedanib solution was further diluted using PBS. Allergen-sensitized mice were administered 50 or 100 mg nintedanib/kg in 0.2 mL of solution, via oral gavage. Nintedanib was administered once per day via oral gavage on Days 21 through 25. An additional, final nintedanib dose was administered on Day 28. Control mice underwent nearly identical procedures; however, 0.2 mL PBS was used in place of nintedanib solution during oral gavage.

Measurement of AHR

AHR was assessed by measurement of airway resistance (cmH₂O/mL/s) before and after exposure to increasing concentrations of methacholine (Mch, 6.25–50.00 mg/mL, Sigma-Aldrich) dissolved in PBS. Mice were anesthetized with a mixture of tiletamine/zolazepam (30 mg/kg, Virbac Laboratories, Carros, France) and xylazine (10 mg/kg, Bayer, Leverkusen, Germany). Then, a tracheotomy was performed and an 18 G tracheal cannula was inserted. Once the appropriate level of anesthesia was reached (confirmed via pain response to a toe pinch), mice were mechanically ventilated (160 breaths/min, 10 mL/kg tidal volume). Mice were exposed to aerosolized Mch for periods of 10 s, using the Flexivent system (SCIREQ,

Montreal, Quebec, Canada). Airway resistance was then continuously monitored and recorded.

Lung tissue histology

Lung tissue sections embedded in paraffin (3 μ m) were stained with hematoxylin and eosin (H&E) to observe the effect of nintedanib on lung tissue histopathology. The level of perivascular or peribronchial inflammation was also assessed. Briefly, a grade of 0 was assigned when no inflammation was detectable; a grade 1 for occasional cuffing with inflammatory cells; and grades 2, 3 and 4 when most bronchi or vessels were surrounded by a thin layer (1–3 cells), a moderate layer (3–5 cells), and thick layer (more than 5 cells deep) of inflammatory cells, respectively [20]. An increment of 0.5 was given if the inflammation fell between two grades. Total inflammation score was calculated by the addition of both peribronchial and perivascular inflammation scores [20].

Bronchoalveolar lavage

A blood sample was collected and the trachea was cannulated with silicone tubing attached to a 23 G needle on an 800- μ L tuberculin syringe. The left main bronchus was tied and, following instillation of 1.2 mL sterile PBS into the right lung, a BALF sample was withdrawn. Recovered BALF was subjected to centrifugation at $750 \times g$ at 4 °C for 5 min in a Cytospin 3 Cytocentrifuge (Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was collected and stored at –80 °C. The total number of cells in the BALF was counted using a hemocytometer and adjusted to 1×10^6 cells/mL. Then, 50 μ L aliquots of BALF were centrifuged onto microscope slides for 5 min at $43 \times g$ and stained with Diff-Quick (Sysmax, Kobe, Japan). The percentages of macrophages, eosinophils, lymphocytes and neutrophils in the BALF were obtained by counting 500 leukocytes in randomly selected light microscope fields.

Molecular assessments

Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISAs) were used to measure cytokine concentrations in the BALF supernatant; specifically, interleukin (IL)-4, IL-5, and IL-13 (R&D Systems, Minneapolis, MN, USA) and TGF- β 1 (BioSource International, Camarillo, CA, USA) were measured. All assays were performed in accordance with manufacturer recommendations.

Assay sensitivities were 2, 7, 1.5 and 2 pg/mL, for IL-4, IL-5, IL-13 and TGF- β 1, respectively. An anti-OVA immunoglobulin (Ig) E enzyme immunoassay (EIA) kit (BioLegend, San Diego, CA, USA) was also used and reactions were measured using an ELISA plate reader at 450 nm.

Real-time polymerase chain reaction

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) analysis was performed to determine PDGFR β , VEGFR1, VEGFR2, FGFR2, FGFR3 and extracellular signal-regulated kinase (ERK) levels. Total RNA was isolated from lung homogenates using TRIzol (Gibco-BRL, Gaithersburg, MD), then reverse transcribed. Each reaction was performed in a final volume of 25 μ L, containing 2 μ L cDNA, 0.2 μ mol/L forward and reverse primers, 1.5 mol/L $MgCl_2$, 0.12 μ mol/L TaqMan probe, and 180 μ mol/L dNTPs in $1 \times$ PCR Gold buffer. Fluorescence emitted by the reporter dye was detected in real time, with the threshold cycle (Ct) of each sample representing the quantity of PCR product in the sample [21]. The Ct is the cycle at which the fluorescence generated by the reporter dye exceeds a set level above baseline. When indicated, the target signal was normalized against a relative quantity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and expressed as $\Delta Ct = Ct_{\text{Target}} - Ct_{\text{GAPDH}}$. The change in the target signal relative to the total amount of genomic DNA was expressed as $\Delta\Delta Ct = \Delta Ct_{\text{treatment}} - \Delta Ct_{\text{control}}$. Relative changes were then calculated as $2^{-\Delta\Delta Ct}$.

Western blot analyses

Proteins were isolated from the lung by homogenization in radio immunoprecipitation assay (RIPA) cell lysis buffer that contained a mixture of phosphatase inhibitors (GenDEPOT, CA, USA). Samples were centrifuged at $15,928 \times g$ for 30 min at 4 °C. Protein concentration was quantified using the bicinchoninic acid (BCA) assay. Equal amounts of sample protein (40 μ g/mL) were then separated via 10%–12% 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 h at room temperature. They were then incubated overnight at 4 °C with a 1:200 dilution of either anti-FGFR3 or anti-p-FGFR3 (Santa Cruz Biotechnology, Inc., CA, USA), or with a 1:1000 dilution of either anti-PDGFR β , anti-p-PDGFR β antibody, anti-VEGFR2, or anti-p-VEGFR2 antibody (Cell Signaling

Technology; CST, Inc., Danvers, MA, USA) in 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween-20. After incubation, the blot was washed and incubated for 2 h at room temperature with a 1:2000 dilution of anti-rabbit-IgG and horseradish peroxidase (HRP)-linked secondary antibody (CST). After washing, protein bands were visualized by enhanced chemiluminescence, using a RAS 3000 Image Analysis System (Fuji Film, Tokyo, Japan).

Statistical analyses

All results are presented as mean $\pm$ standard error of the mean. Data were compared using a one-way pairwise analysis of variance, followed by Dunnett's multiple range test. Statistical analyses were performed using Graph-Pad Prism (version 5.00 for Windows, GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $p < 0.05$.

Results

Effects of nintedanib on airway inflammation and AHR

Cell counts and Th2 cytokine concentrations in the BALF were used as measures of airway inflammation. In the acute asthma model, the numbers of total cells, macrophages, neutrophils and eosinophils were significantly higher in BALF from the OVA challenge group than in BALF from the control group (Figure 1, $p < 0.05$). The number of eosinophils in both nintedanib treatment groups was significantly lower than in the OVA + EtOH group (Figure 1, $p < 0.001$). Treatment with dexamethasone also significantly reduced the number of eosinophils (Figure 1, $p < 0.001$), and the effect of dexamethasone was not significantly different from that of nintedanib.

Nintedanib treatment significantly decreased AHR (Figure 2); notably, AHR increased with Mch exposure in a dose-dependent manner in OVA-challenged mice, and this effect was alleviated by nintedanib administration. Nintedanib also decreased the number of infiltrating inflammatory cells, as determined by gross examination of perivascular lung tissue (Figure 3) and inflammation score (Figure 4).

Dexamethasone also significantly decreased AHR (Figure 2), the number of infiltrating inflammatory cells (Figure 3), and inflammation score (Figure 4); the effect of dexamethasone was not significantly different from that of nintedanib.

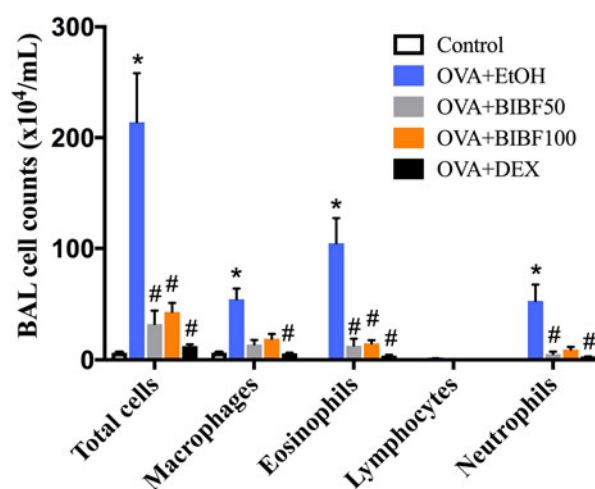


Figure 1. Effects of nintedanib on total and differential cell counts in bronchoalveolar lavage fluid (BALF). Values are expressed as mean $\pm$ SEM ($n = 6-8$ /group). * $p < 0.05$ versus control group and # $p < 0.05$ versus OVA group. OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

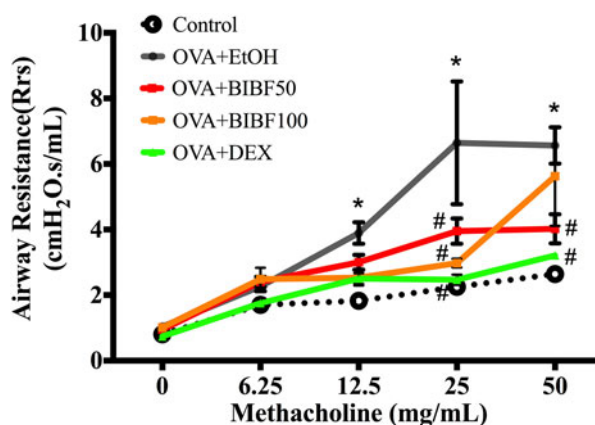


Figure 2. Effect of nintedanib on AHR to methacholine. The AHR was measured 24 h after the final OVA challenge, using a Flexivent system in which mice were exposed to increasing concentrations of methacholine (6.25–50 mg/mL). * $p < 0.05$ versus control group and # $p < 0.05$ vs. OVA group. OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

Molecular effects of nintedanib

Interleukin levels

Repeated OVA challenges induced significant increases in the levels of IL-4, IL-5 and IL-13 in BALF. In the OVA + EtOH group, IL-4, IL-5 and IL-13 levels were 218 ± 84 , 184 ± 60 and 260 ± 113 pg/mL, respectively. Treatment with nintedanib significantly reduced IL-4 (OVA + BIBF50: 139 ± 114 pg/mL; OVA + BIBF100: 71 ± 38 pg/mL, $p = 0.018$), IL-5

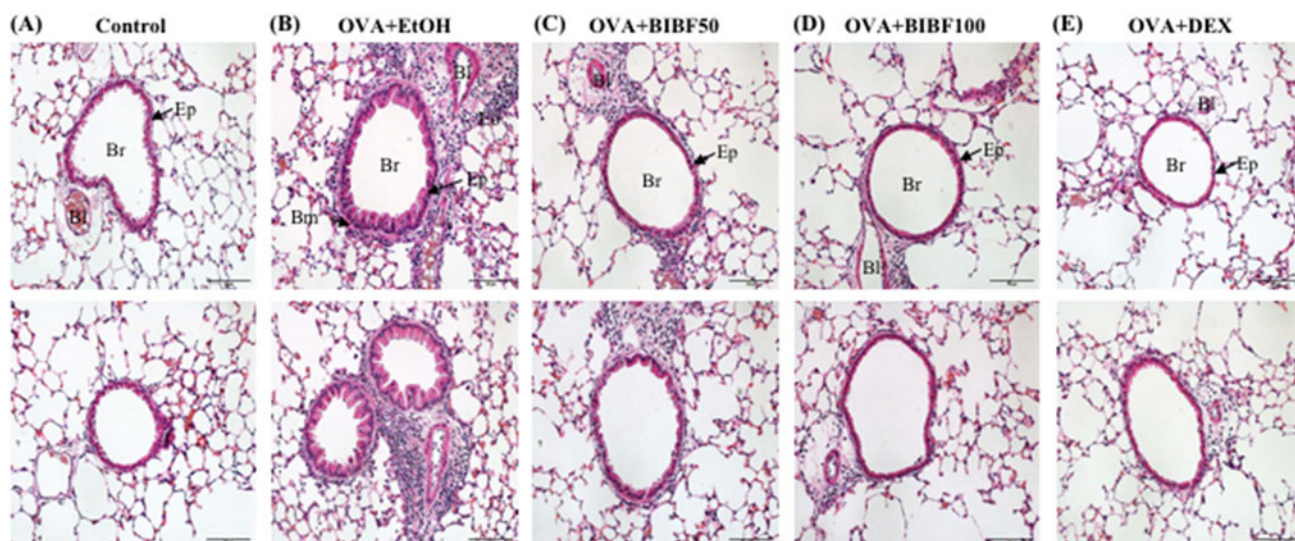


Figure 3. Effect of nintedanib on lung tissue histopathology. Sections were stained with hematoxylin and eosin. Magnification of 200 $\times$. (A) Control group; normal lung structure. (B) Ovalbumin-sensitized mice treated with ethanol (OVA + EtOH) group. (C) OVA mice treated with 50 mg nintedanib/kg/day (OVA + BIBF50). (D) OVA mice treated with 100 mg nintedanib/kg/day (OVA + BIBF100). (E) OVA mice treated with 4 mg dexamethasone/kg/day.

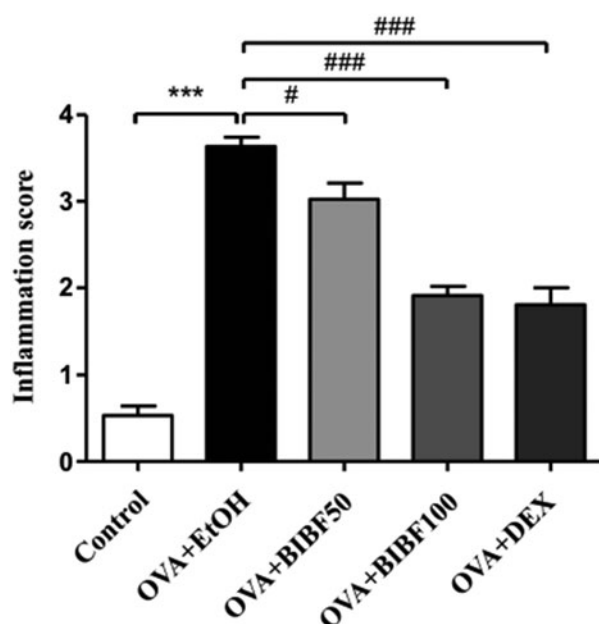


Figure 4. Effect of nintedanib on total lung inflammation scores. Total inflammation score was calculated by the addition of the peribronchial and perivascular inflammation scores. *** $p < 0.001$ versus control group. # $p < 0.05$ versus OVA group and ### $p < 0.001$ versus OVA group. OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

(OVA + BIBF50: 91 ± 60 pg/mL, $p = 0.003$; OVA + BIBF100: 48 ± 12 pg/mL, $p < 0.001$) and IL-13 (OVA + BIBF50: 178 ± 50 pg/mL; OVA + BIBF100: 31 ± 30 pg/mL, $p < 0.001$) levels in a dose-dependent manner (Figure 5). Treatment with dexamethasone

also significantly reduced IL-4 (81 ± 72 , $p = 0.026$), IL-5 (100 ± 43 , $p = 0.01$) and IL-13 (135 ± 75 , $p = 0.029$). There was no significant difference between the effect of nintedanib and dexamethasone on reducing up-regulated interleukins.

Growth factor receptor expression and activation

Treatment with nintedanib inhibited expression (measured by RT-PCR mRNA levels) and phosphorylation (evaluated by Western blot analyses) of PDGFR β , VEGFR1, VEGFR2, FGFR2 and FGFR3 (Figure 6). Importantly, mice displaying OVA-challenged acute asthma demonstrated greater expression of PDGFR β , VEGFR2 and FGFR3, compared with control mice.

Extracellular signal-regulated kinase

Real-time RT-PCR was used to analyze the effects of nintedanib on the mRNA expression of ERK in lung tissues after repeated OVA challenges. Expression of ERK was increased by OVA challenges and was significantly reduced by nintedanib treatment (Figure 7). Dexamethasone also significantly decreased the expression of ERK, and the effect of dexamethasone was not significantly different from that of nintedanib.

Transforming growth factor β

Repeated OVA challenges induced a significant increase in TGF- β 1 levels (control: 18 ± 6 pg/mL, OVA + EtOH: 938 ± 271 pg/mL; $p < 0.001$). However, nintedanib treatment significantly reduced TGF- β 1 levels in mice with OVA-induced acute asthma

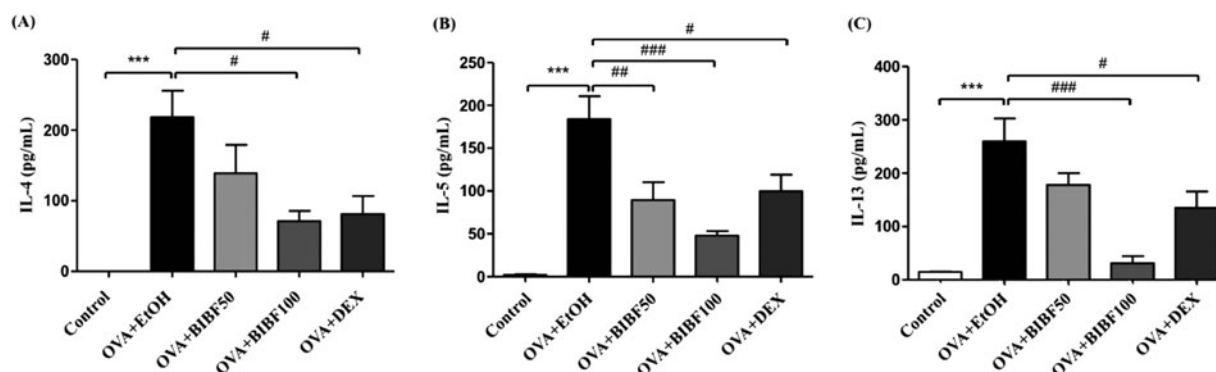


Figure 5. Effect of nintedanib on cytokine levels in BALF. Enzyme-linked immunosorbent assays were used to measure the concentration of (A) interleukin (IL)-4, (B) IL-5, and (C) IL-13. *** $p < 0.001$ versus control group. # $p < 0.05$ versus OVA group. ## $p < 0.01$ versus OVA group and ### $p < 0.001$ versus OVA group. OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

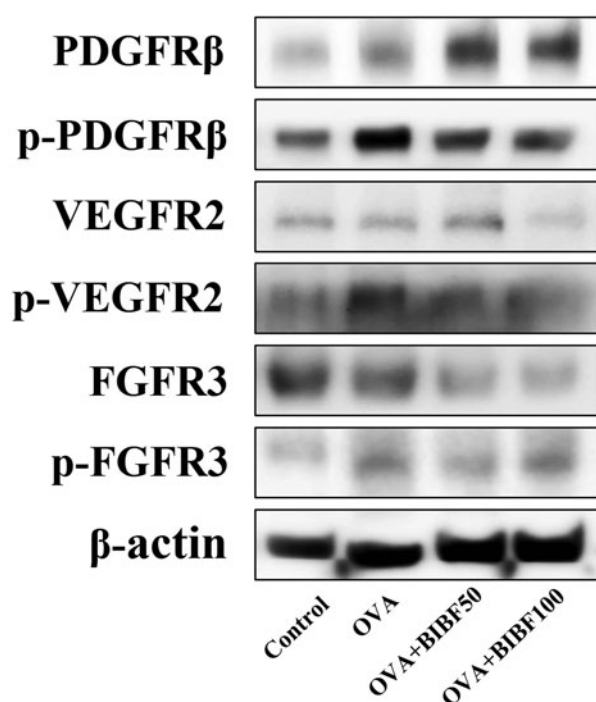


Figure 6. Effects of nintedanib on growth factor receptor expression and phosphorylation. Western blot showing the expression of total and phosphorylated platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR). β -actin was used as a control. OVA + EtOH, ovalbumin-sensitized mice treated with ethanol; OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day.

(OVA + BIBF50: 819 ± 368 pg/mL; OVA + BIBF100: 379 ± 189 pg/mL, $p < 0.001$; Figure 8). Treatment with dexamethasone also significantly reduced TGF- β 1 levels (502 ± 227 , $p = 0.013$). There was no significant difference between the effect of nintedanib and dexamethasone on reducing upregulated TGF- β 1 levels.

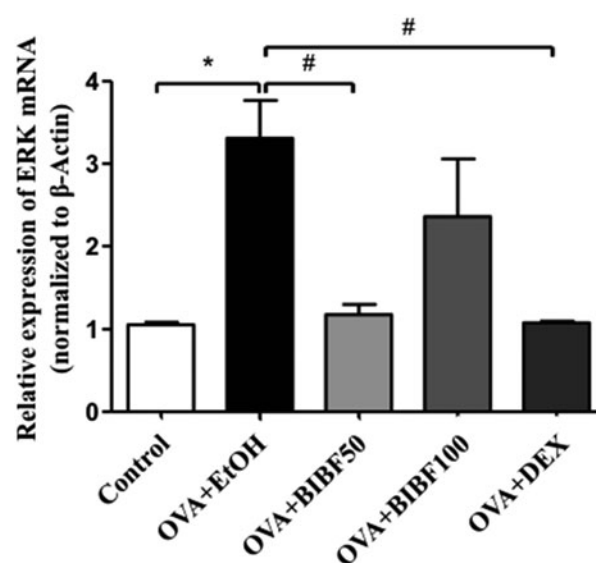


Figure 7. Effects of nintedanib on mRNA expression of ERK in lung tissues. Real-time RT-PCR was used to analyze the effects of nintedanib on the mRNA expression of ERK in lung tissues after repeated OVA challenges. * $p < 0.05$ versus control group and # $p < 0.05$ versus OVA group. OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

Discussion

The present study investigated the role of nintedanib in an OVA-challenged mouse model of acute asthma. In this model, nintedanib treatment significantly inhibited airway inflammation, AHR and upregulation of Th2 cytokines. Oral treatment with nintedanib significantly decreased phosphorylation of PDGF, VEGF and FGF receptors and TGF- β 1 levels, confirming the anti-inflammatory properties of the compound. Our results suggest that nintedanib could serve as a novel agent for inhibiting the airway inflammation in patients with asthma.

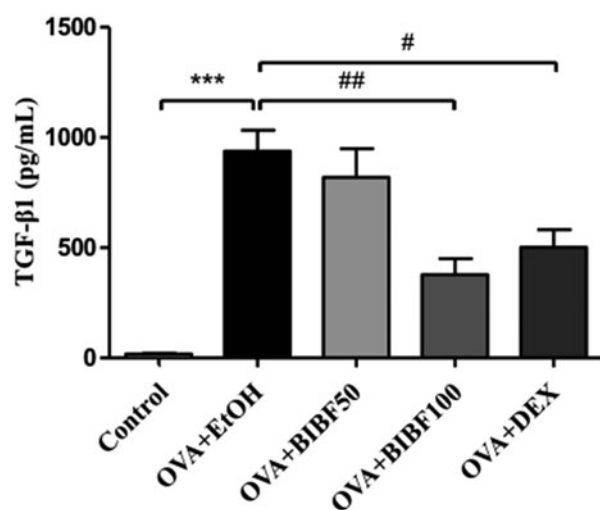


Figure 8. Effects of nintedanib on transforming growth factor beta 1 (TGF- β 1). Enzyme-linked immunosorbent assays were used to measure the concentration of TGF- β 1 in BALF. *** $p < 0.001$ versus control group. # $p < 0.05$ versus OVA group, ## $p < 0.01$ versus OVA group. OVA + EtOH, ovalbumin-sensitized mice treated with ethanol; OVA + BIBF50, OVA mice treated with 50 mg nintedanib/kg/day; OVA + BIBF100, OVA mice treated with 100 mg nintedanib/kg/day; OVA + DEX, OVA mice treated with 4 mg dexamethasone/kg/day.

Glucocorticoids are known as the most effective anti-inflammatory medications for effective long-term management of asthma [3]. In particular, ICS are the cornerstone of maintenance therapy for asthma. However, there are some concerns regarding potential systemic side effects of glucocorticoids. Using systemic or inhaled glucocorticoids may be associated with systemic side effects including osteoporosis, skin thinning, cataracts, glaucoma, and local side effects such as oropharyngeal candidiasis and dysphonia [4–6]. Furthermore, poor adherence to inhaler use is another problem of ICS treatment in patients with asthma. Suboptimal adherence to controller medication can lead to decline in lung function, poor symptom control, and increased risk of asthma-related hospitalizations [22–24]. Therefore, another treatment that could be an alternative choice to use of glucocorticoids would be desirable.

Nintedanib is a triple kinase inhibitor of VEGF, FGF and PDGF receptors, which was recently approved for the treatment of patients with IPF [16]. Previous studies have shown that nintedanib has not only antifibrotic activity but also anti-inflammatory activity in an experimental model of lung fibrosis [25,26]. Furthermore, our previous study has also shown the anti-inflammatory effect of nintedanib in a chronic asthma model [19]. In the present study, treatment with nintedanib reduces airway inflammation, AHR, and upregulated interleukins in a mouse

model of acute asthma, and the effect of nintedanib was not significantly different from that of dexamethasone. This finding may suggest that nintedanib could be an alternative choice to glucocorticoids.

Prior studies have shown that growth factors play an important role in airway inflammation and remodeling in asthma patients; notably, PDGF plays a prominent role in altering fibroblast activities linked to airway remodeling. PDGF is known to be a powerful stimulator of fibroblast chemotaxis, proliferation and collagen production [27–30]. Thus, targeting PDGF and/or its receptor could be effective in reducing collagen deposition and scar formation in the airway of asthma patients. The PDGFR tyrosine-kinase inhibitors (TKIs), tyrphostin AG1296 and PDGFR inhibitor III, suppress FGF-2/TGF- β 1 synergism *in vitro* and may be responsible for ASM cell hyperplasia that is associated with asthmatic airway remodeling [31]. Furthermore, our previous study showed that imatinib and nilotinib, both PDGFR TKIs, significantly attenuate airway inflammation, hyper-responsiveness and remodeling in a mouse model of chronic asthma [32–34]. Results from a phase II clinical trial with imatinib were also encouraging, as the compound effectively decreases bronchial hyper-responsiveness in patients with severe asthma [35]. Together, this evidence suggests that PDGFR inhibition may be useful for abating airway inflammation and ASM hyperplasia in asthma patients.

VEGF is well-known as a key regulator of blood vessel growth in the airways of asthma patients, acting via promotion of endothelial cell proliferation and differentiation, vascular leakage and vessel permeability [36]. Additionally, increased serum VEGF levels have been observed in pediatric patients with more severe asthmatic phenotypes [37]. A previous study showed that treatment with bevacizumab, an anti-VEGF monoclonal antibody, decreases epithelial thickness and goblet cell hyperplasia in a mouse model of chronic asthma [38]. In the current study, nintedanib had an inhibitory effect on VEGFR activation in mouse lung tissue. These results are consistent with a previous study that showed nintedanib inhibits VEGF, FGF and PDGF [26].

Prior studies revealed an over-expression of TGF- β 1 mRNA and TGF- β proteins in bronchial biopsies from subjects with severe and moderate asthma, compared with controls without asthma [39,40]. TGF- β is known as a major mediator that affects many different cell processes in the airways of asthmatic patients [41]. Recent studies using a mouse model of acute allergic inflammation have demonstrated *in situ*

activation of TGF- β signaling pathways in the airways [42,43]. In this study, nintedanib treatment significantly reduced elevated TGF- β 1 levels; this result is consistent with a previous study that showed nintedanib inhibits TGF- β signaling [17,44]. Further studies of the inhibitory effect of TGF- β in asthma are needed.

It is well-known that ERK is involved in downstream signaling of the PDGF and FGF signaling pathways. Previous studies have shown that nintedanib inhibits PDGFR phosphorylation and subsequent protein kinase B and ERK 1/2 phosphorylation in lung tissue from mice [44,45]. In this study, expression of ERK was increased by OVA challenges and was significantly reduced by nintedanib treatment. This could be additional evidence that nintedanib inhibits PDGF and FGF.

This study had several limitations. We only examined an experimental model of acute asthma; therefore, it was difficult to directly show the effects of nintedanib on airway remodeling. However, our previous study showed the effects of nintedanib on airway remodeling in a chronic asthma model [19], and the results of our present study and the previous one are consistent. Additionally, the effects of nintedanib on VEGF, PDGF, and FGF receptors should be further examined in acute asthma models. In particular, the downstream effects of this inhibitory interaction on asthma and airway remodeling should be explored.

Conclusion

In conclusion, orally administered nintedanib reduces airway inflammation, AHR, Th2 cytokine expression, and phosphorylation of receptors for PDGF, VEGF, FGF and TGF- β 1 in a mouse model of acute asthma. Our findings suggest that nintedanib may provide a novel therapy for patients with asthma.

Disclosure statement

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

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