



Effects of montelukast on subepithelial/peribronchial fibrosis in a murine model of ovalbumin induced chronic asthma

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ABSTRACT

Montelukast, a leukotriene receptor antagonist, is used commercially as a maintenance treatment for asthma and to relieve allergic symptoms. In this study, we evaluated the protective effects of montelukast against the airway inflammation and fibrosis using a murine model of ovalbumin (OVA) induced chronic asthma. The animals received OVA challenge three times a week for 4 weeks. Montelukast (30 mg/kg) was administered orally once a day for 4 weeks. The administration of montelukast caused a reduction in elevated interleukin (IL)-4, IL-13, eotaxin, immunoglobulin (Ig), inflammatory cell infiltration into the airways, and mucus production after repeated OVA challenges. To investigate the antifibrotic mechanism of montelukast, we examined the expression of profibrotic mediators, including vascular endothelial growth factor (VEGF), transforming growth factor (TGF)- β_1 , and Smad3 proteins in the lung tissue using western blotting and immunohistochemistry. The administration of montelukast reduced the overexpression of profibrotic proteins in the lung tissue, which was confirmed by immunohistochemistry. These results are consistent with a histopathological examination of lung tissue with Masson's trichrome stain. In conclusion, the administration of montelukast reduced airway inflammation and pulmonary fibrosis by reducing the release of Th2 cytokines and the expression of VEGF, TGF- β_1 /Smad3 in the lung tissue.

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1. Introduction

Chronic asthma is characterized by chronic inflammation, mucus hypersecretion, and subepithelial/peribronchial fibrosis. It develops when an individual is exposed to various risk factors, such as allergen, drugs, radiation, infection, and toxic particles, and causes pathophysiological changes. Increases in T helper (Th)2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13, are crucial pathophysiological changes in chronic asthma [1,2]. Th2 cytokines are produced by activated Th2 cells, which induce inflammatory cell infiltration into the airways [3]. Th2 cytokines are also associated with several growth factors, including vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)- β_1 [1,4], which are important and well-known mediators of airway remodeling processes, including chronic inflammation, mucus hypersecretion, and fibrosis. According to previous studies, TGF- β_1 induces the deposition of collagen, resulting in pulmonary fibrosis [5]. VEGF increases inflammatory cell infiltration into the airways by enhancing vascular permeability and Th2 cytokine release [6]. Recently, Vasquez-Pinto et al. [7] demonstrated that VEGF causes pulmonary fibrosis by increasing the expression of Th2 cytokines and TGF- β_1 , and these observations are consistent with the results of Lee et al. [5].

Montelukast, a leukotriene receptor antagonist, is used commercially used as a maintenance treatment for asthma and to relieve allergic

symptoms. Montelukast inhibits the action of leukotriene D4 on the cysteinyl leukotriene receptor (CysLTR)1 in the lung, which reduces bronchoconstriction and airway inflammation [8,9]. Previous studies have demonstrated that montelukast attenuates the airway remodeling induced by several allergens by modulating the expression of Th2 cytokines and growth factors [10–12]. In addition to these features, several researchers have demonstrated that montelukast exerts beneficial effects against experimentally induced toxicities. Mohamadin et al. [13] reported that montelukast reduced lipopolysaccharide-induced liver damage via its antioxidant properties. Montelukast also displayed protective effects against experimentally induced renal failure and gastrointestinal injury in many experiments *in vivo* and *in vitro* [14–17].

In this study, we investigated the antifibrotic effects of montelukast in chronic asthma induced by repeated ovalbumin (OVA) challenge. To understand the antifibrotic mechanism of montelukast, we also evaluated the expression of profibrotic mediators, including VEGF, TGF- β_1 and Smad3 using immunoblotting and immunohistochemistry.

2. Materials and methods

2.1. Animals

Specific-pathogen-free female BALB/c mice (7 weeks old) were purchased from the Orient Co. (Seoul, Korea) and used after 1 week of quarantine and acclimatization. The mice were allowed sterilized tap water and standard rodent chow *ad libitum*. All experimental

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procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Chungnam National University Animal Care and Use Committee. The animals were cared for in accordance with the dictates of the National Animal Welfare Law of Korea.

2.2. Experimental procedures

OVA sensitization and airway challenge were performed as described previously [18]. In brief, mice were sensitized on days 0 and 14 with an intraperitoneal injection of 20 µg of OVA emulsified in 2 mg of aluminum hydroxide in 200 µL phosphate-buffered saline (PBS; pH 7.4). One week after the final sensitization, the mice underwent airway challenge with OVA (1%, w/v, in PBS) for 1 h with an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) three times a week for 4 weeks. Montelukast was obtained from the Korea Research Institute of Bioscience and Biotechnology and was dissolved in PBS and was prepared fresh daily before each treatment. It was administered by gavage to the mice at doses of 30 mg/kg once daily for 4 weeks.

Following the OVA challenge, samples of bronchoalveolar lavage fluid (BALF) were collected from the mice and processed, and the inflammatory cells were counted as described previously [2]. In brief, the mice were killed with an intraperitoneal injection of pentobarbital (50 mg/kg; Hanlim Pharmaceutical Co., Seoul, Korea) 48 h after the last challenge, and a tracheostomy was performed. To obtain the BALF, ice-cold PBS (0.5 mL) was infused three times into the lungs and withdrawn via tracheal cannulation (total volume of 1.5 mL). The total numbers of inflammatory cells were assessed by counting the cells in at least five squares of a hemocytometer after the dead cells were excluded by Trypan blue staining. To prepare the cells for counting, 100 µL of BALF was centrifuged onto slides at 200 ×g and 4 °C for 10 min using a Cytospin (Hanil Science Industrial, Seoul, Korea). The slides were dried, and the cells were fixed and stained using Diff-Quik® staining reagent (B4132-1A; IMEB Inc., Deerfield, IL), according to the manufacturer's instructions. The supernatant obtained from the BALF was stored at –70 °C for biochemical analysis.

2.3. Measurement of cytokines, chemokine, and IgE levels in BALF or plasma

The levels of IL-4, IL-13, and eotaxin in the BALF were measured using enzyme-linked immunosorbent assay (ELISA) kits (BioSource International, Camarillo, CA) according to the manufacturer's protocols. The levels of total IgE and OVA-specific IgE in BALF and plasma were measured using an ELISA. Microtiter plates were coated with anti-IgE antibody (anti-mouse IgE; 10 g/mL; Serotec, Oxford, UK) in PBS-Tween 20, and incubated with a BALF or plasma sample. The plate was then washed four times, and 200 µL of o-phenylenediamine dihydrochloride (Sigma-Aldrich, St. Louis, MO) was added to each well. The plates were incubated for 10 min in the dark and the absorbance was then measured at 450 nm. This assay was performed in duplicate.

2.4. Measurement of TGF-β₁ and VEGF levels in lung tissues

The levels of TGF-β₁ and VEGF in the BALF were measured with ELISA kits (R&D Systems, Inc., Minneapolis, MN and Immuno Biological Laboratories Co., Ltd., Minneapolis, MN, respectively), according to the manufacturers' protocols. Total proteins were determined using a protein assay reagent (Bio-Rad, Hercules, CA). The results were expressed as pg/mg protein. This assay was performed in duplicate.

2.5. Immunoblotting

Equal amounts of total lung proteins (30 µg) were heated at 100 °C for 5 min, loaded onto 8% SDS-PAGE gels, and separated electrophoresed. The proteins were then transferred to a nitrocellulose membrane (at 100V for 2 h), and the membrane was blocked for 1 h with Tris-buffered saline

containing 0.05% Tween-20 (TBST) plus 5% skim milk. The membrane was then incubated overnight at 4 °C with anti-VEGF antibody (1:1000 dilution; Abcam, Cambridge, MA), anti-TGF-β₁ antibody (1:1000 dilution; Abcam), anti-pSmad3 and -smad3 antibody (1:1000 dilution; Abcam), and anti-β-actin antibody (1:1000 dilution; Cell Signaling Technology, Danvers, MA). The membrane was washed three times with TBST and then incubated with a horseradish peroxidase-conjugated secondary antibody (1:3000 dilution; Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. The membrane was washed three times again with TBST and developed using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Uppsala, Sweden). For the quantitative analysis, densitometric value of each band was determined using Chemi-Doc (Bio-Rad). This assay was performed in 7 mice per groups.

2.6. Histology

After the BALF samples were collected, the lung tissue was fixed in 10% (v/v) neutral-buffered formalin. The tissues were embedded in paraffin, sectioned to 4 µm thickness, and stained with hematoxylin and eosin (H&E) solution (hematoxylin, Sigma MHS-16; eosin, Sigma HT110-1-32) and periodic acid-Schiff (PAS; IMEB Inc., San Marcos, CA) to estimate inflammation and mucus production, respectively. To evaluate peribronchial fibrosis, paraffin-embedded tissue was stained with Masson's trichrome. Quantitative analysis of inflammation, mucus production, and collagen deposition was determined using an image analysis (Molecular Devices, Inc., CA, USA). Slides were prepared for two per animal (n = 7/group). In quantitative analysis, we randomly chosen five histological fields per slide focused on bronchial (×200 magnification).

For the immunohistochemical analysis, paraffin sections were deparaffinized, dehydrated, washed in PBS containing 0.3% Triton X-100, and preincubated for 10 min at room temperature with 10% goat serum to block nonspecific staining. The slides were then incubated overnight at 4 °C with primary mouse anti-rabbit VEGF antibody (1:200 dilution; Abcam) and TGF-β₁ (1:1000 dilution; Abcam). After the primary antibodies were removed, the sections were washed and incubated at 37 °C for 1 h with biotinylated secondary antibody, and then incubated with an avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Excess complex was removed, and the sections washed with PBS and incubated with 0.05% diaminobenzidine (1:200; Millipore, Billerica, MA) for a further 10 min. The sections were counterstained, rinsed in PBS to terminate the reaction, and protected with coverslips for microscopic examination. This assay was performed in all mice of each group.

2.7. Image capture and photomicrography

Photomicrographs were taken with a Photometric Quantix digital camera running a Windows program, and montages were assembled in Adobe Photoshop 7.0. The images were cropped and corrected for brightness and contrast, but were not otherwise manipulated.

2.8. Statistical analysis

Data are expressed as means ± standard errors of the mean (SEM). Statistical significance was determined using analysis of variance (ANOVA) followed by a multiple comparison test with Bonferroni adjustment. *P* values <0.05 or <0.01 were considered significant.

3. Results

3.1. Effect of montelukast on the number of inflammatory cells in BALF

The numbers of inflammatory cells in the BALF were significantly increased in the OVA-challenged mice compared with those in the

negative control mice (Fig. 1). In contrast, the montelukast-treated mice had significantly fewer inflammatory cells than the OVA-challenged mice.

3.2. Effect of montelukast on IL-4, IL-13, and eotaxin levels in BALF

The levels of IL-4 and IL-13 in the BALF were significantly higher in the OVA-challenged mice than in the negative controls. However, the montelukast-treated mice had significantly lower levels of IL-4 and IL-13 than the OVA-challenged mice. Similar to results for Th2 cytokines, the levels of eotaxin were higher in the OVA-challenged mice than in the negative controls, and the montelukast-treated mice had significantly lower levels of eotaxin than the OVA-challenged mice (Fig. 2).

3.3. Effects of montelukast on total IgE and OVA-specific IgE levels in BALF and plasma

The levels of total IgE and OVA-specific IgE in the BALF were much higher in the OVA-challenged mice than in the negative controls (Table 1). In contrast, the montelukast-treated mice had significantly lower levels of total IgE and OVA-specific IgE in the BALF than the OVA-challenged mice. These results are consistent with those for plasma. Montelukast-treated mice had significantly lower levels of total IgE and OVA-specific IgE in their plasma than the OVA-challenged mice.

3.4. Effects of montelukast on airway inflammation, mucus production, and fibrosis in lung tissue

The OVA-challenged mice showed marked infiltration of inflammatory cells into the peribronchiolar and perivascular lesions in the lung tissue sections compared with that in the negative control mice (Fig. 3A and B). In contrast, the montelukast-treated mice showed a reduction in the infiltration of inflammatory cells into the peribronchiolar

and perivascular lesions compared with that in the OVA-challenged mice. PAS-stained lung sections from the OVA-challenged mice exhibited mucus overproduction and goblet cell hyperplasia. Sections from the montelukast-treated mice showed less mucus production and goblet cell hyperplasia in the bronchial airways than the OVA-challenged mice. The OVA-challenged mice also displayed a marked increase in subepithelial and interstitial fibrosis compared with the negative controls, whereas the montelukast-treated mice displayed a significant reduction in subepithelial and interstitial fibrosis compared with the OVA-challenged mice.

3.5. Effects of montelukast on VEGF and TGF- β_1 levels in lung tissue

The levels of VEGF in the lung tissues were significantly higher in the OVA-challenged mice than in the negative controls (Fig. 4A). However, the montelukast-treated mice showed significantly lower VEGF than the OVA-challenged mice. Consistent with the result for VEGF, the levels of TGF- β_1 were higher in the OVA-challenged mice than in the negative controls and the montelukast-treated mice had significantly lower levels of TGF- β_1 than the OVA-challenged mice.

3.6. Effects of montelukast on VEGF, TGF- β_1 , and pSmad3 in lung tissue

The levels of VEGF in the lung tissues were significantly higher in the OVA-challenged mice than in the negative controls (Fig. 4). However, the montelukast-treated mice showed significantly lower VEGF than the OVA-challenged mice. Consistent with the result for VEGF, the levels of TGF- β_1 were higher in the OVA-challenged mice than in the negative controls and the montelukast-treated mice had significantly lower levels of TGF- β_1 than the OVA-challenged mice.

As shown in Figs. 5A and B, the OVA-challenged mice showed significantly greater VEGF, TGF- β_1 , and pSmad3 expression in the lung tissue than the negative controls. The montelukast-treated mice had significantly lower VEGF, TGF- β_1 , and pSmad3 expression

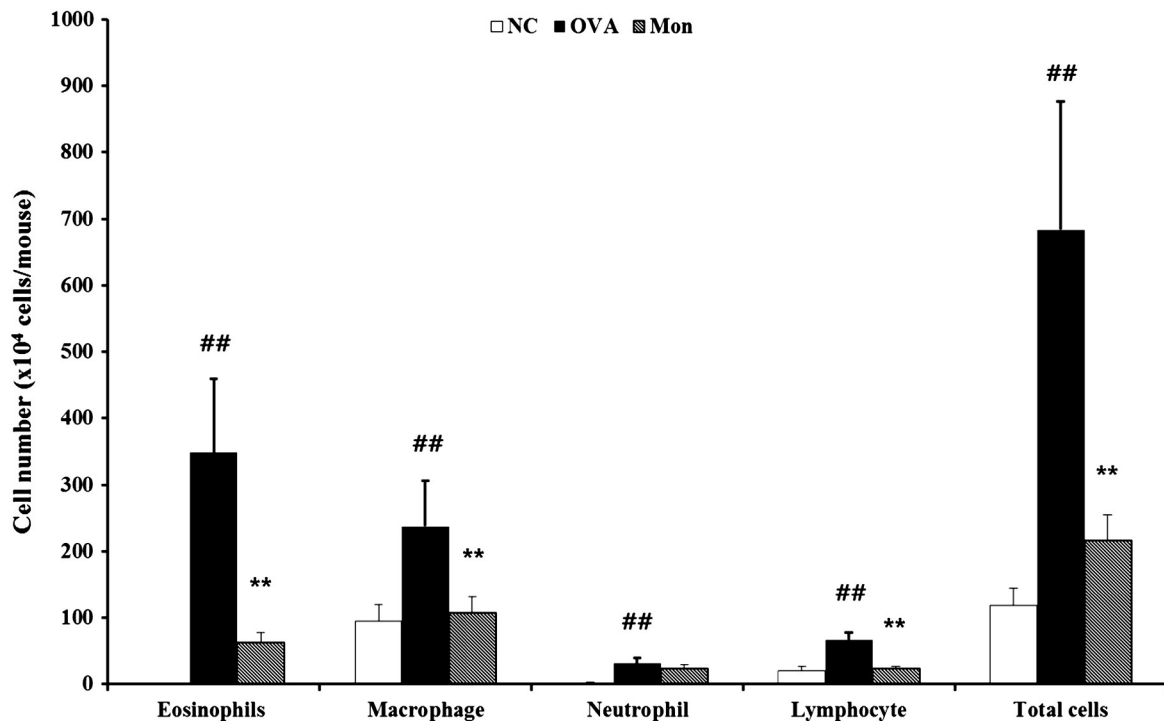


Fig. 1. Montelukast inhibited the recruitment of inflammatory cells to the bronchoalveolar lavage fluid (BALF) of mice 48 h after the final OVA challenge. Cells were isolated by centrifugation and stained with Diff-Quik® stain reagent. Cell numbers were counted under a light microscope in at least five squares of a hemocytometer after the dead cells were excluded with Trypan Blue. NC, normal control mice treated with PBS only; OVA, OVA-sensitized/challenged mice; Mon, montelukast (30 mg/kg) + OVA-sensitized/challenged mice. Values are expressed as means $\pm$ SEM (n = 7/group). ^{##}Significantly different from NC, $P < 0.01$; ^{**}significantly different from OVA, $P < 0.01$.

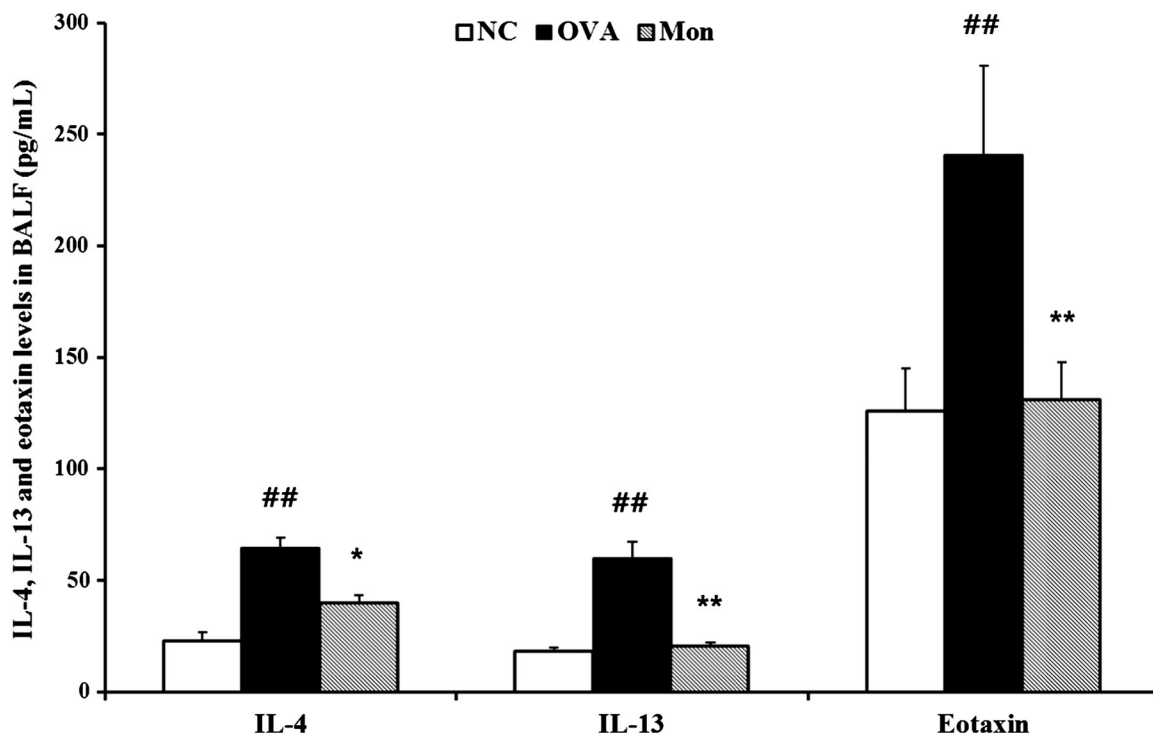


Fig. 2. Montelukast reduced the levels of IL-4, IL-13 and eotaxin in the bronchoalveolar lavage fluid (BALF) of mice 48 h after the final OVA challenge. The OVA-challenged mice had significantly higher levels of IL-4, IL-13, and eotaxin in their BALF. However, the montelukast-treated mice had significantly lower levels of IL-4, IL-13, and eotaxin than those of the OVA-challenged mice. Values are expressed as means $\pm$ SEM ($n = 7$ /group). ##Significantly different from NC, $P < 0.01$; *, **significantly different from OVA, $P < 0.05$ and < 0.01 , respectively.

than the OVA-challenged mice. Consistent with the results of immunoblotting, immunohistochemical analysis of the OVA-challenged mice showed that VEGF and TGF- β_1 were more strongly expressed by epithelial cells and inflammatory cells in airways of the lung tissue than in the normal controls (Fig. 5C). In contrast, the montelukast-treated mice showed reduced VEGF and TGF- β_1 expression compared with OVA-challenged mice.

4. Discussion

In this study, we investigated the beneficial effects of montelukast on airway inflammation and pulmonary fibrosis induced by repeated OVA-challenge. The OVA-challenged mice showed increases in the numbers of inflammatory cells, Th2 cytokines, eotaxin, mucus secretion, and subepithelial/interstitial fibrosis and the overexpression of VEGF, TGF- β_1 , and pSamd3 proteins in their lung tissue. In contrast, the administration of montelukast reduced inflammatory cell infiltration into the airways with decreases in Th2 cytokine release, pulmonary fibrosis, and the expression of profibrotic mediators.

Airway inflammation is an important feature of the pathogenesis of chronic asthma. The pathophysiology of asthma is extremely complex and involves a network of Th2 cells and effector cells. Exposure to several allergens leads to an increase in Th2 cytokines, such as IL-4, IL-5,

and IL-13 [2], and these cytokines promote the infiltration of inflammatory cells into the airways, IgE production, and mucus hypersecretion by epithelial goblet cells [19]. These increased Th2 cytokines also induce the expression of various growth factors, which further aggravate airway inflammation [20]. These pathophysiological changes were confirmed in the OVA-challenged mice in our study. Montelukast is a known as a CysLTR1 antagonist that blocks the action of leukotriene D4. This response reduces the bronchoconstriction induced by leukotriene and the inflammatory response in lung tissue [12]. Moreover, Wu et al. [21] also demonstrated the anti-inflammatory effects of montelukast via modulation of Th2 cytokines. Montelukast reduced not only the levels of IL-4 and IL-13 in the BALF and lung tissue but also the IgE levels in plasma. Consistent with a previous study, our results show that montelukast attenuates airway inflammation by reducing the levels of Th2 cytokines and IgE in the BALF and plasma. These results were confirmed in a histopathological analysis. Administration of montelukast reduced inflammatory cell infiltration into the peribronchial and perivascular lesions and mucus hypersecretion of epithelial goblet cells. Taken together, these data indicate that montelukast effectively attenuates airway inflammation via the suppression of Th2 cytokines.

Fibrosis is an irreversible change in a tissue and is a crucial feature of many chronic diseases. In particular, pulmonary fibrosis is an important cause of morbidity and mortality in patients with chronic pulmonary

Table 1
Levels of total IgE and OVA-specific IgE in BALF and plasma.

Groups	BALF (ng/mL)		Plasma (ng/mL)	
	Total IgE	OVA-specific IgE	Total IgE	OVA-specific IgE
NC	1.16 $\pm$ 0.28	–	420.2 $\pm$ 31.95	–
OVA	32.55 $\pm$ 6.78 ^{##}	1.44 $\pm$ 0.08 ^{##}	3973.3 $\pm$ 65.43 ^{##}	213.7 $\pm$ 12.30 ^{##}
Mon	14.42 $\pm$ 1.63 ^{**}	0.90 $\pm$ 0.08 ^{**}	2811.4 $\pm$ 230.36 ^{**}	101.3 $\pm$ 17.74 ^{**}

NC; normal control mice treated with PBS only; OVA; OVA-sensitized/challenged mice; Mon; montelukast (30 mg/kg) + OVA-sensitized/challenged mice.

^{##} Significantly different from NC, $P < 0.01$.

^{**} Significantly different from OVA, $P < 0.01$.

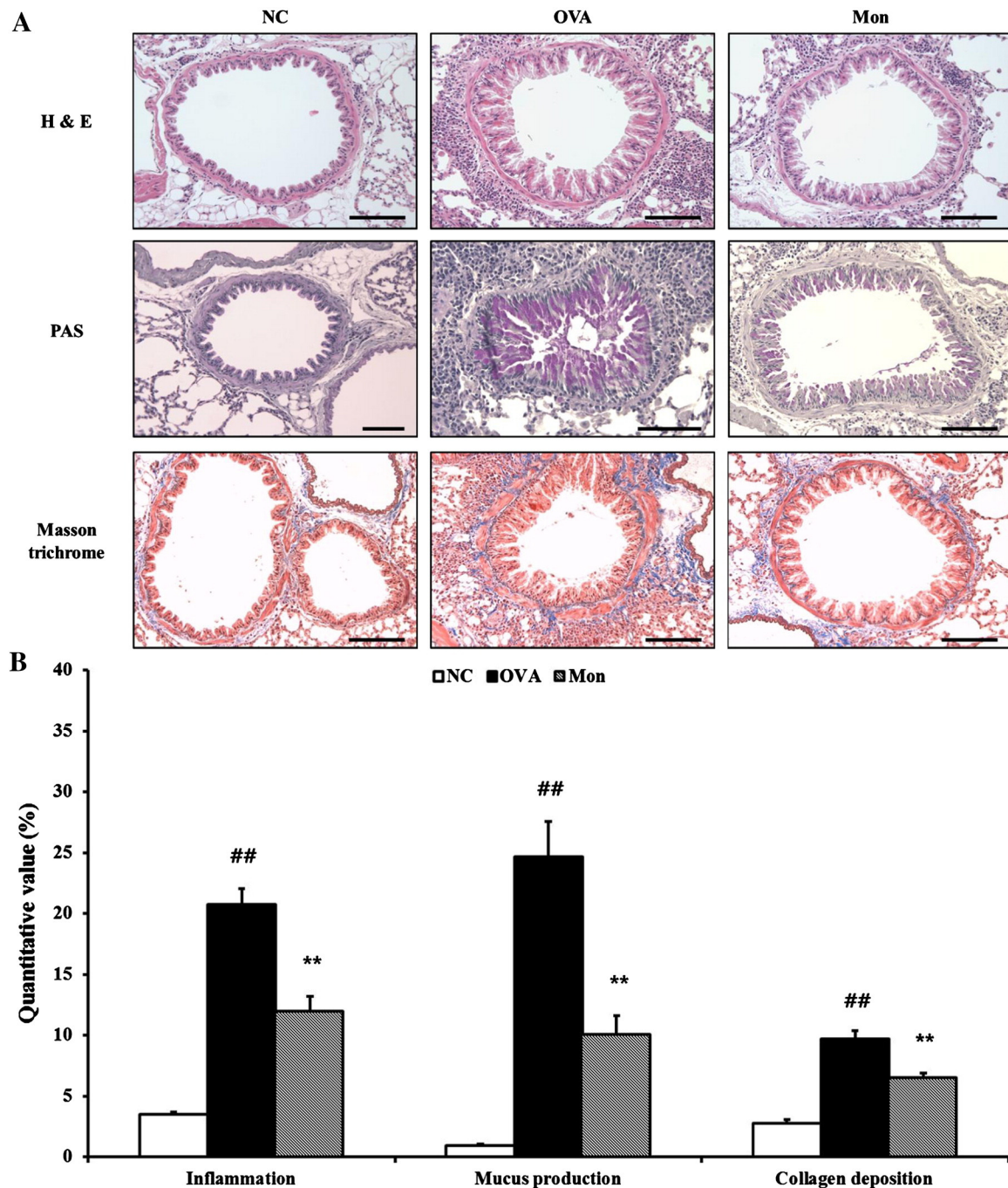


Fig. 3. Montelukast attenuated airway inflammation, mucus production, and fibrosis in lung tissue 48 h after the final OVA challenge. (A) Histological examination of the lung tissue with H&E staining, PAS staining, and Masson's trichrome staining (magnification $\times 200$). (B) Quantitative analysis for inflammatory response, mucus production, and collagen deposition. The administration of montelukast reduced the inflammatory response in the peribronchial and perivascular lesions, mucus production, and collagen deposition compared with those in the OVA-challenged mice. Bar indicates 100 μ m. Values are expressed as means $\pm$ SEM ($n = 7$ /group). ^{##}Significantly different from NC, $P < 0.01$; ^{*}, ^{**}significantly different from OVA, $P < 0.05$ and < 0.01 , respectively.

diseases such as chronic asthma, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease [4]. The pathogenesis of pulmonary fibrosis is highly complex and is associated with various mediators. Growth factors such as VEGF, TGF- β_1 , basic fibroblast growth factor-2, insulin-like growth factor, and endothelin-1, are believed to be pivotal to the fibrotic process [22–26]. Of these, TGF- β_1 is well-known as a critical mediator of fibrogenesis, influencing the structural cells involved in the synthesis of extracellular matrix and affecting fibroblast proliferation and the differentiation of fibroblasts into myofibroblasts [4]. Moustakas and Heldin [27] demonstrated that when TGF- β_1 binds to its receptor, Smad complex is phosphorylated. This complex then

translocates to the nucleus, which mediates the fibrotic responses [28,29]. VEGF is also closely associated with the progress of pulmonary fibrosis. VEGF induces the expression of connective tissue growth factor and chemoattractant for mast cells, which increase the release of fibrogenic mediators and fibroblast proliferation [24,30]. Recently, a relationship between VEGF and TGF- β_1 in the progress of pulmonary fibrosis has been reported. Lee et al. [5] demonstrated that the inhibition of VEGF blocks TGF- β_1 production through a PI3K/Akt signaling pathway, resulting in the suppression of pulmonary fibrosis. However, few studies have examined the antifibrotic mechanism of montelukast in pulmonary fibrosis induced by repeated allergen challenge. According

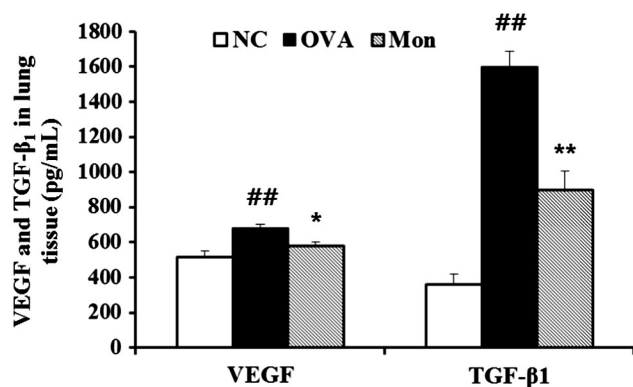


Fig. 4. Montelukast reduced the production of VEGF and TGF- β_1 in the lung tissues 48 h after the final OVA challenge. The OVA-challenged mice showed a significant increase in VEGF and TGF- β_1 compared with those in the normal controls. In contrast, the montelukast-treated mice showed significantly less VEGF and TGF- β_1 in the lung tissue than the OVA-challenged mice. The OVA-challenged mice showed increased VEGF, TGF- β_1 , and pSmad3 expression compared with that in the normal controls. In contrast, the montelukast-treated mice displayed significantly reduced expression of VEGF, TGF- β_1 , and Smad3 compared with that in the OVA-challenged mice. For calculating relative ratio, we measured the densities of each band. Relative ratio of VEGF and TGF- β_1 was divided by β -actin and that of pSmad3 divided by Smad3. Bar indicates 100 μ m. Values are expressed as means $\pm$ SEM ($n = 7$ /group). ##Significantly different from NC, $P < 0.01$; *, **significantly different from OVA, $P < 0.05$ and < 0.01 , respectively.

to a previous study, montelukast has a significant reduction in pulmonary fibrosis induced by allergen-induced asthma via its anti-inflammatory effects [31,32]. CysLTR antagonists also reduce airway fibrosis with reduction in the levels of Th2 cytokines and TGF- β_1 in BALF [3]. On the other hand, montelukast attenuated pulmonary fibrosis and airway inflammation with reduction in the Th2 cytokines and TGF- β_1 in the lung tissue of bleomycin-induced pulmonary fibrosis model [9]. Consistent with previous studies, OVA-challenged mice showed pulmonary fibrosis together with the overexpression of VEGF and TGF- β_1 /Smad3. However, administration of montelukast attenuated pulmonary fibrosis induced by repeated OVA-challenge by reducing the release of fibrotic mediators, which was confirmed by immunoblotting and immunohistochemistry. These results indicate that anti-fibrotic effects of montelukast correlate with the suppression of VEGF and TGF- β_1 /Smad3 signaling.

Montelukast, a cysteinyl leukotriene (CysLT)1 antagonist blocks the action of LTD4 on the CysLT1 in lung tissue by binding to it [33]. In asthmatic condition, CysLT1 receptor expressed in inflammatory cells and other structural cells such as bronchial smooth muscle cells and epithelial cells. LTD4 binds to CysLT1 receptor, which produces the pro-inflammatory cytokines, chemokines and growth factors [34]. These increases induce the inflammatory cell recruitment into airway, mucus hypersecretion, and smooth muscle hyperplasia, indicating asthmatic responses. Previous studies have demonstrated that elevated expression of cysLTs contributed to bronchial smooth muscle cell hyperplasia and clinical features of asthma via the increase in TGF- β_1 production by airway epithelial cells [35]. Therefore, the anti-fibrotic effects of

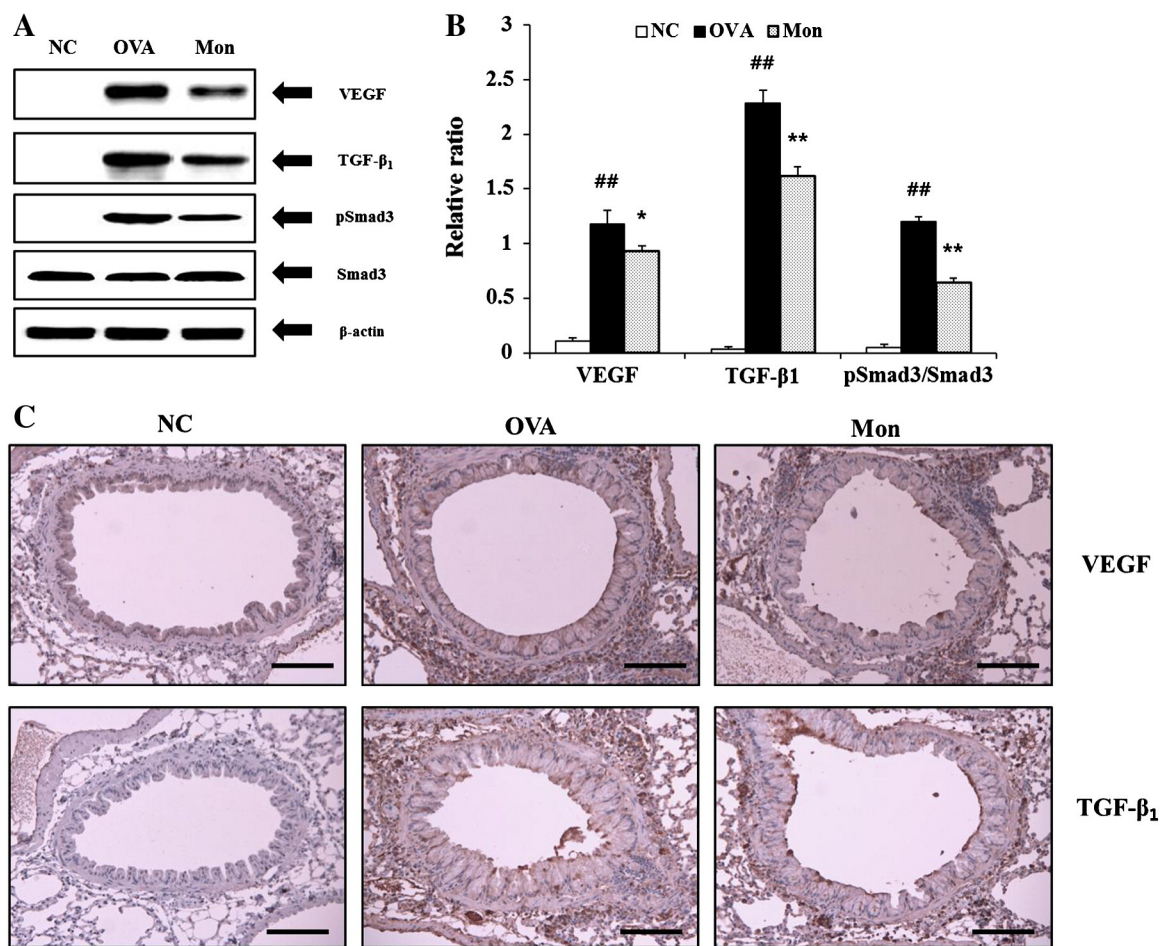


Fig. 5. Montelukast reduced the expression of VEGF, TGF- β_1 , and pSmad3 proteins in the lung tissues 48 h after the final OVA challenge. (A) VEGF, TGF- β_1 , and pSmad3 protein expression in the gel. (B) Relative units of VEGF, TGF- β_1 , and pSmad3 protein expression. (C) VEGF and TGF- β_1 expression in the lung tissue. The OVA-challenged mice showed increased VEGF, TGF- β_1 , and pSmad3 expression compared with that in the normal controls. In contrast, the montelukast-treated mice displayed significantly reduced expression of VEGF, TGF- β_1 , and Smad3 compared with that in the OVA-challenged mice. ##Significantly different from NC, $P < 0.01$; *, **significantly different from OVA, $P < 0.05$ and < 0.01 , respectively.

montelukast observed in this study were considered to be closely associated with its CysLT1 antagonistic effects.

In summary, montelukast attenuates the pathophysiological changes of asthma including airway inflammation, mucus hypersecretion and pulmonary fibrosis. Montelukast not only reduces the release of Th2 cytokines, eotaxin, and IgE, but also suppresses the overexpression of VEGF and TGF- β ₁/Smad3 in the lung tissue. This study suggests that montelukast exerts its beneficial effects on pulmonary fibrosis through the suppression of VEGF and TGF- β ₁/Smad3.

Author contributions

Study conception and design: SIS, SHK and LMY. Analysis and interpretation of data: SIS, JWY and LMY. Draft of this work: SIS and LMY. Final approval and overall responsibility for this work: LMY.

Conflict of interest

No competing financial interests exist.

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