

The effects of low dose leukotriene receptor antagonist therapy on airway remodeling and cysteinyl leukotriene expression in a mouse asthma model

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Abbreviations: EIA, enzyme immunoassay; LT, leukotriene; PBS, phosphate-buffered saline; OVA, ovalbumin

Abstract

Airway structural changes that occur in patients with asthma in response to persistent inflammation are termed airway remodeling. The cysteinyl leukotrienes (LTC₄, D₄ and E₄) are known to play important roles in the pathobiology of asthma. To evaluate the effect of low dose montelukast (MK) on the development of airway remodeling using a chronic murine model of allergic airway inflammation with subepithelial fibrosis, BALB/c mice, after intraperitoneal ovalbumin (OVA) sensitization on days 0 and 14, received intranasal OVA periodically on days 14-75. MK treated mice received montelukast sodium intraperitoneally on days 26-75. The OVA sensitized/challenged mice developed an extensive eosinophil cell inflammatory response, goblet cell hyperplasia, mucus occlusion, and smooth muscle hypertrophy of the airways. In addition, in OVA sensitized/challenged mice, dense collagen deposition/fibrosis was seen throughout the lung interstitium surrounding the airways, blood vessels, and alveolar septae. The cysteinyl leukotriene 1 (CysLT1) receptor antagonist, MK significantly reduced the airway eosinophil infiltration, goblet cell hyperplasia, mucus occlusion, and lung fibrosis except airway smooth muscle hypertrophy in the OVA sensitized/challenged mice. The OVA sensitized/challenged mice had significantly increased

epithelial desquamation compared with control mice. MK markedly reduced epithelial desquamation of airways in OVA/MK treated animals compared with OVA sensitized/challenged mice. MK treatment did not affect the levels of CysLT in lung tissue. Our results show that the important role of cysteinyl leukotrienes in the pathogenesis of asthma. Lower dose of CysLT1 receptor antagonism has a significant anti-inflammatory effect on allergen-induced lung inflammation and fibrosis but not airway smooth muscle hypertrophy in an animal model of asthma.

Keywords: anti-asthmatic agents; asthma; leukotriene antagonists; leukotrienes; montelukast; SRS-A

Introduction

The inflammatory process that characterizes the pathophysiology of asthma involves a number of cells and mediators. Airway structural changes that occur in patients with asthma in response to persistent inflammation are termed airway remodeling and include airway wall thickening, subepithelial fibrosis, and hyperplasia of mucus glands, myofibroblasts, smooth muscle, and vasculature (Elias *et al.*, 1999). The cysteinyl leukotrienes (CysLTs; LTC₄, D₄, E₄) are known to play an important role in the pathobiology of asthma. These compounds, derived from arachidonic acid *via* the 5-lipoxygenase pathway, are produced in cells of inflammation such as eosinophils, mast cells, monocytes, and basophiles (Lewis *et al.*, 1990). The leukotrienes have multiple effects that contribute to the airways obstruction and inflammation that characterize asthma. Inhalation of LTE₄ causes eosinophil influx into the airway wall (Gauvreau *et al.*, 2001). *In vitro* studies with the CysLTs increased venous permeability (Joris *et al.*, 1987), mucus secretion (Marom *et al.*, 1982), and decreased activity of the respiratory cilia were shown (Bisgaard and Pederson, 1987). It is now widely recognized that CysLTs play an important role in asthma, participating in both the bronchoconstriction and the chronic inflammatory component of the disease.

Clinical studies of antileukotriene agents have provided direct evidence of the role of the CysLTs in

clinical asthma. In a previous study with MK-0571, a leukotriene receptor antagonist, protection against both early- and late-phase bronchoconstriction due to inhaled allergens was demonstrated (Rasmussen *et al.*, 1992). Montelukast (MK-0476) is a potent and specific CysLT1 receptor antagonist (Jones *et al.*, 1995). In previous studies, it has been shown that Montelukast (MK) has a high dose (10.25 or 62.5 mg/kg) or dose-dependent (1-10-100 mg/kg) anti-inflammatory response on allergen induced lung inflammation in an animal model of persistent asthma (Blain and Sirois, 2000; Wu *et al.*, 2003).

The aim of this study was to determine the effects of lower dose MK on the development of airway remodeling using a chronic murine model of allergic airway inflammation with subepithelial fibrosis. In addition, we investigated its influence on the levels of CysLTs in a murine model of asthma.

Materials and Methods

Experimental design

The local ethics committee of Firat (Euphrates) University has approved the experimental protocol. BALB/c mice were received from Department of Virology, School of Veterinary Medicine, and Firat (Euphrates) University. Six- to 8-week-old BALB/c mice ($n=22$) were divided into three groups. Ovalbumin (OVA) ($n=6$) and MK treated (OVA/MK) ($n=10$) groups were immunized by intraperitoneal (I.P.) injections of 100 μg of OVA complexed with alum on days 0 and 14 (Henderson *et al.*, 1996). Mice received an intranasal dose of 500 μg OVA on days 14, 27, 28, 29, 47, 61, 73, 74, and 75. The control group (PBS) ($n=6$) received normal phosphate buffered saline (PBS) with alum i.p. on days 0 and 14 and PBS without alum intranasally on days 14, 27, 28, 29, 47, 61, 73, 74, and 75 (Henderson *et al.*, 2002). A group of MK treated mice was administered with montelukast sodium [Merck&Co., Inc. (Rahway, NJ)] that was dissolved in PBS. Then MK (1 mg/Kg, once a day) was injected intraperitoneally beginning on day 26 of the protocol and continuing until day 75. In accordance with previous studies 1 mg/Kg dose was accepted as low dose (Blain and Sirois, 2000; Eum *et al.*, 2003; Wu *et al.*, 2003).

On day 77, 48 h following the final intranasal or PBS treatment of mice from each experimental group, the mice were exsanguinated by cervical dislocation and the lungs were removed.

Lung histology

The lungs of the mice were obtained and fixed in 10% formalin solution. After embedding in paraffin,

the tissues were cut into 5- μm sections and stained with hematoxylin-and-eosin (H&E) to assess the inflammatory cell infiltrate (0-4+ scale; 0, no cell infiltration; +, ~0-25% cell infiltration; ++, ~25-50% cell infiltration; + + +, ~50-75% cell infiltration; + + + +, ~75-100% cell infiltration). Luna's method was performed to identify eosinophil infiltration (as a percent of total inflammatory cells) into the airway area (0-4+ scale, as described above) (Luna, 1979). Masson's trichrome staining was performed to determine collagen deposition in the lungs (0 to 4+, as described above) (Sheehan and Hrapchac, 1980), Alcian Blue, pH 2.5, with nuclear fast red counterstaining to identify airway goblet cells (as percent of total airway cells). The degree of mucus plugging of the airways was classified on a semiquantitative scale from 0 to 4+ as follows; 0, no mucus; +, ~0-25% occlusion; ++, ~25-50% occlusion; + + +, ~50-75% occlusion; + + + +, ~75-100% occlusion. Airway smooth muscle thickness was evaluated in lung sections stained by H&E by measuring the thickness of the smooth muscle cell layer beneath the airway epithelial cell basement membrane at the three sites tangential to each airway cross section examined by ocular micrometer (Henderson *et al.*, 2002). The ratio of airways that occurred epithelial desquamation to total airways was evaluated on a scale as described above. Two observers blinded to the protocol design performed the histological and morphometric analysis by light microscopy. A minimum of 10 fields throughout the upper and lower left lung tissue were randomly examined for the morphometric analysis.

Quantitation of LTC₄, LTD₄, LTE₄ in lung tissues

Levels of CysLTs in lung tissues of three groups of mice were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cysteinyl Leukotriene EIA kit, Assay Designs Inc. MI) according to the manufacturer's instruction. Briefly, one lung from each mouse was ground, using a mortar and pestle and ground glass [1 g lung tissue was broken with 1 ml Assay Buffer (Tris-buffered saline)]. The solution of ground lung and ground glass was then centrifuged at 3,000 rpm for 10 min. The prostaglandin synthetase inhibitors (10 $\mu\text{g}/\text{ml}$) (meclofenamic acid, SIGMA Chemical Co) were added to supernatants. The supernatant was then frozen at -80°C for later use. The supernatant was added to 2 plates for each sample on EIA LT standards (2,500 pg/ml, 1,250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml and 78 pg/ml) and superior aliquot of tissue homogenates. After incubating with chromogen, the reaction was stopped by adding stop solution and color reactions were read on 450 nm optic density.

Statistical analysis

All statistical analyses were performed using SPSS v10.0 software. Results were expressed as mean \pm standard deviation (SD). Kruskal Wallis and Mann-Whitney U test were used to compare the variables. A *P*-value of less than 0.05 was considered statistically significant.

Results

In our study, 48 h after the final OVA or PBS treatment in mice from each experimental group, lung tissue was obtained to assess inflammatory cell infiltration, mucus release, and collagen deposition. The effect of MK on airway inflammation and remodeling was determined.

Cellular infiltration of lung interstitium

The mean total inflammatory cell infiltration score in OVA-sensitized/challenged animals was significantly

higher than that in control animals (2.33 ± 0.51 , 1.0 ± 0.89 , $P = 0.006$, respectively). In the OVA-sensitized/challenged animals, a mean eosinophil number score of 2.66 ± 0.81 was seen representing 25-50% eosinophil cell infiltration of the lung interstitium. It was significantly higher than that of control animals (2.66 ± 0.81 , 0.5 ± 0.54 , $P = 0.003$, respectively) (Figures 1A and B). CysLT1 receptor antagonism by MK inhibited the eosinophil infiltration but not total inflammatory cells infiltration of the lung tissue in OVA-sensitized/challenged mice ($P = 0.002$, OVA/MK versus OVA for eosinophils) (Figures 1C, 2A and 2B). The mean eosinophil number score in OVA/MK treated group was 0.90 ± 0.73 .

Airway goblet cell hyperplasia and mucus occlusion

Goblet cell hyperplasia was observed in the OVA-sensitized/challenged animals but not in PBS control animals (Figures 1D and 1E). In the OVA-sensitized/challenged animals, goblet cells increased to $25.83 \pm 15.62\%$ of airway cells compared to $1.5 \pm 1.37\%$

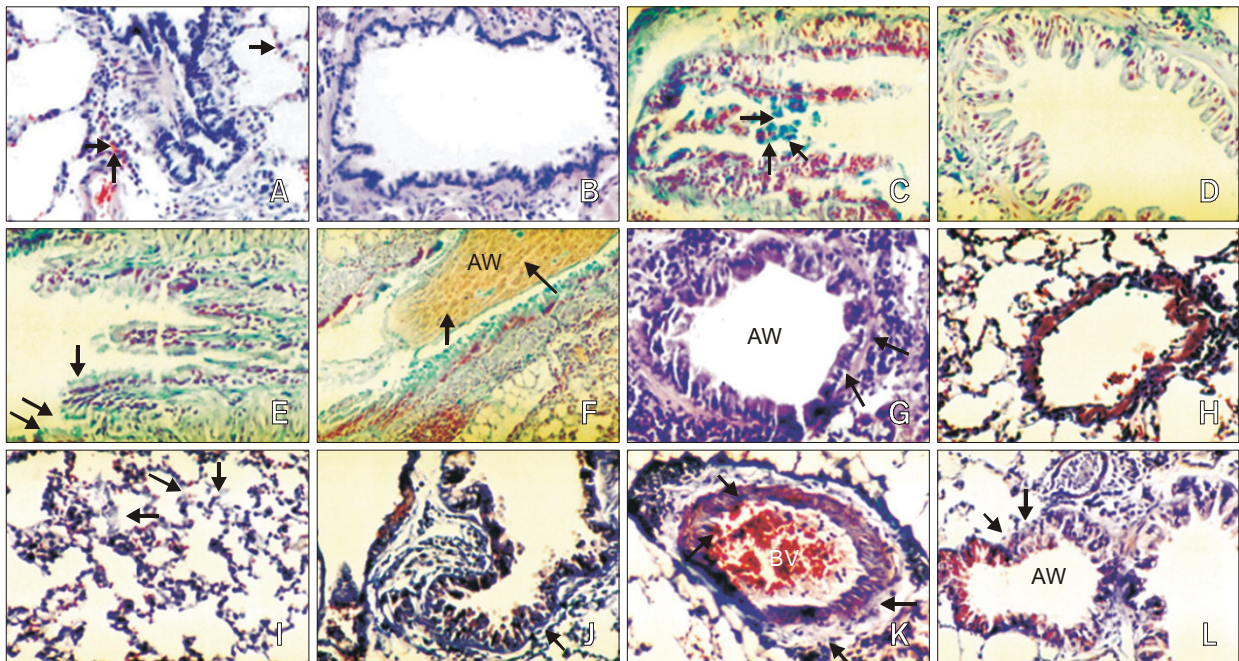


Figure 1. In the OVA sensitized/challenged mice, eosinophil infiltrate (arrows) around the airways is observed. Luna method, $100 \times$ (A). Montelukast treatment decreased the eosinophil infiltration (arrows) of the the lung interstitium around the airways. Luna method, $100 \times$ (B). Airway goblet cell hyperplasia (arrows) was observed in the OVA sensitized/challenged mice. Alcian blue, pH 2.5, $100 \times$ (C). There were no goblet cell hyperplasia and mucus occlusion in the PBS control mice. Alcian blue, pH 2.5, $100 \times$ (D). MK treatment decreased goblet cell hyperplasia (arrows) and mucus occlusion in the lung interstitium. Alcian blue, pH 2.5, $100 \times$ (E). Mucus occlusion (arrows) of the airway (AW) lumen is seen in the OVA sensitized/challenged mice. Alcian blue, pH 2.5, $100 \times$ (F). Airway smooth muscle hypertrophy (arrows) was observed surrounding the airways in the OVA sensitized/challenged mice. Hematoxylin-and-eosin, $100 \times$ (G). There was no collagen deposition/fibrosis in the lung interstitium and blood vessels in PBS control mice. Masson's trichrome, $100 \times$ (H). Extensive collagen deposition (arrows) was seen in the lung interstitium in the OVA sensitized/challenged mice. Masson's trichrome, $100 \times$ (I). In the OVA sensitized/challenged mice, subepithelial fibrosis (arrows). Masson's trichrome, $100 \times$ (J) and blood vessel fibrosis (arrows). Masson's trichrome, $100 \times$ (K) were observed. MK treatment decreased collagen deposition/fibrosis (arrows) in the lung interstitium around the airways. Masson's trichrome, $100 \times$ (L).

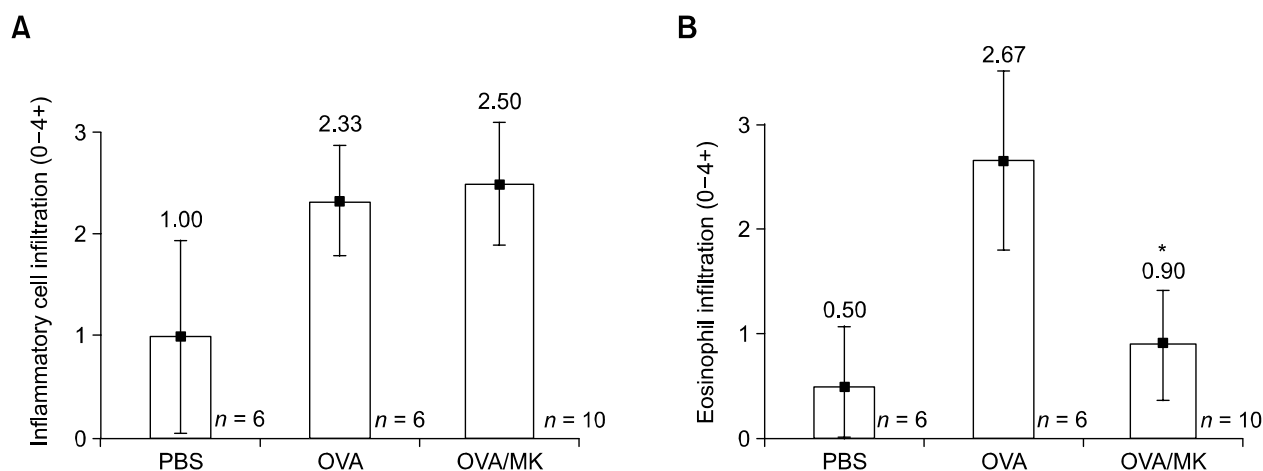


Figure 2. CysLT1 receptor blockade reduces the intensity of eosinophils in the lung interstitium after OVA challenge. The intensity of total inflammatory cells (0-4+ scale) (A) and eosinophils (0-4+ scale) (B) present in lung tissue from each group as the mean \pm SD. * $P = 0.002$ versus OVA by Mann-Whitney U test.

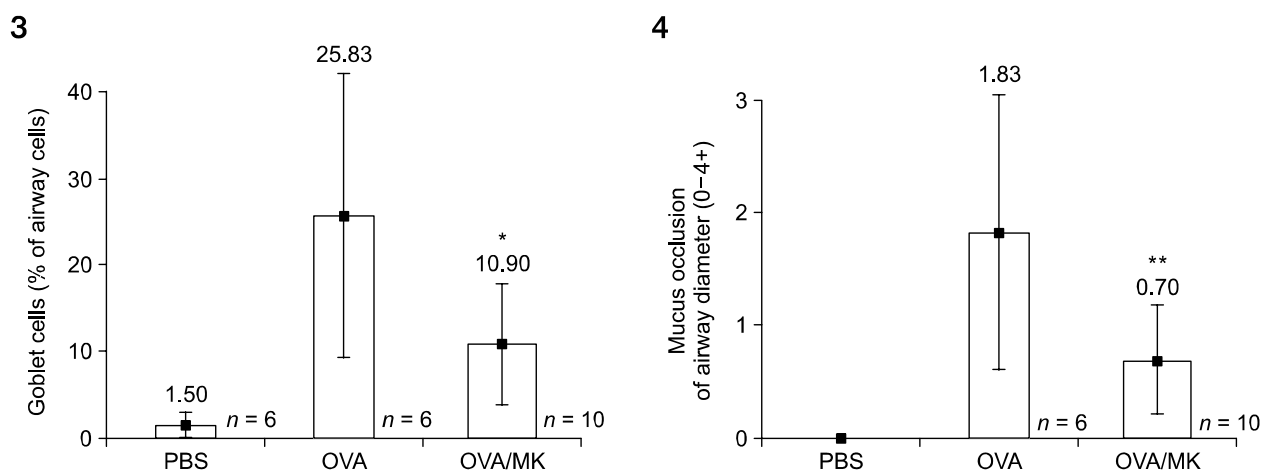


Figure 3 and 4. MK markedly reduced airway goblet cell hyperplasia and the airway mucus occlusion (respectively, * $P = 0.018$, ** $P = 0.016$, OVA/MK versus OVA-sensitized/challenged) in the OVA-sensitized/challenged animals. The percentage of goblet cells hyperplasia (3) and occlusion of airway diameter by mucus (0-4+ scale) (4) for each group.

of airway cells in control mice ($P = 0.004$, OVA versus PBS). MK markedly reduced airway goblet cell hyperplasia ($P = 0.018$, OVA/MK versus OVA) (Figures 1F and 3). In the OVA/MK animals, the percentage of goblet cell was 10.90 ± 9.66 . There was a significant increase in airway mucus secretion in the OVA-sensitized/challenged animals compared with PBS control animals ($P = 0.002$) (Figures 1E and 1G). The mean occlusion score was 1.83 ± 1.16 in OVA-sensitized/challenged animals and 0.0 ± 0.00 in PBS control mice. The OVA-sensitized/challenged animals had 0-25% occlusion of the airway lumen by mucus. MK blocked the airway mucus occlusion in the OVA-sensitized/challenged

animals. In the OVA/MK animals, the mean occlusion score was 0.81 ± 1.00 (Figure 1F, 4, $P = 0.016$, OVA/MK versus OVA-sensitized/challenged).

Airway smooth muscle hypertrophy

OVA-sensitized/challenged animals had a significantly increased airway smooth muscle thickness compared with PBS control animals ($8.33 \pm 1.25 \mu\text{m}$, $6.50 \pm 1.18 \mu\text{m}$, $P = 0.035$, respectively). MK treatment reduced the airway smooth muscle hypertrophy but not statistically significant ($8.33 \pm 1.25 \mu\text{m}$, $6.95 \pm 1.58 \mu\text{m}$, $P = 0.102$ OVA versus OVA/MK) (Figures 1B and 1H). In addition, there was no

significant difference in airway smooth muscle thickness between PBS control mice and OVA/MK treatment animals ($P = 0.742$) (Figure 5).

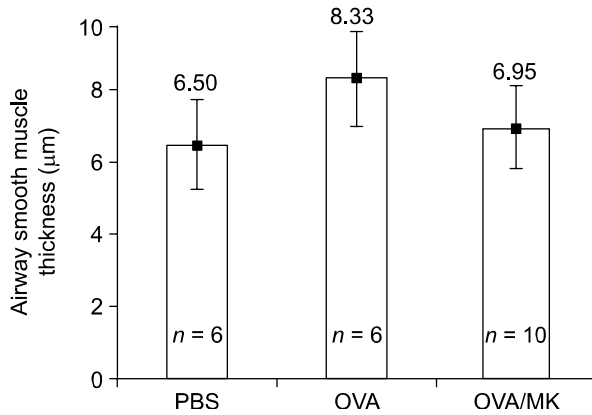


Figure 5. Airway smooth muscle thickness in the OVA-sensitized/challenged mice was reduced by MK treatment but not statistically significant. Airway smooth muscle thickness (µm) for each group.

Airway collagen deposition/fibrosis

In OVA sensitized/challenged mice, dense collagen deposition/fibrosis was seen throughout the lung interstitium surrounding the airways, blood vessels, and alveolar septae (Figure 11-L). The mean airway and alveolar septae fibrosis score was significantly higher in OVA sensitized/challenged animals than in PBS control mice (2.50 ± 1.04 , 1.66 ± 0.81 in OVA sensitized/challenged mice, 0.83 ± 0.40 , 0.00 ± 0.00 in PBS control animals, $P = 0.008$, $P = 0.02$, respectively). However, there were no significant differences in blood vessels fibrosis score between OVA sensitized/challenged mice and PBS control mice (2.66 ± 1.50 , 1.16 ± 0.75 , respectively, $P = 0.06$). MK therapy significantly reduced lung fibrosis. The mean airway, blood vessels, and alveolar septae fibrosis scores were significantly lower in OVA/MK animals compared with those of OVA sensitized/ challenged mice (0.90 ± 0.87 , 1.10 ± 1.10 , 0.60 ± 0.69 in OVA/MK mice, $P = 0.012$, $P = 0.039$, $P = 0.021$ OVA versus OVA/MK, respectively) (Figure 6).

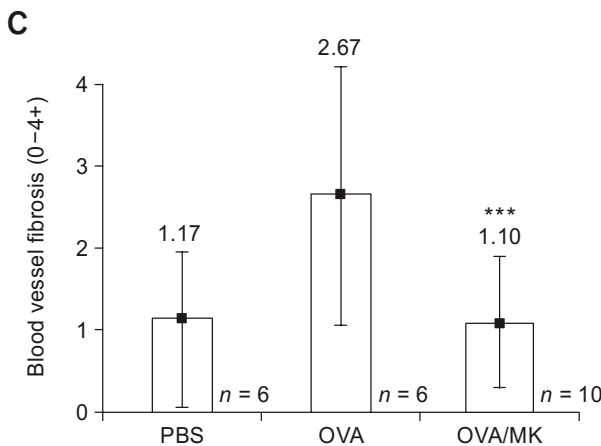
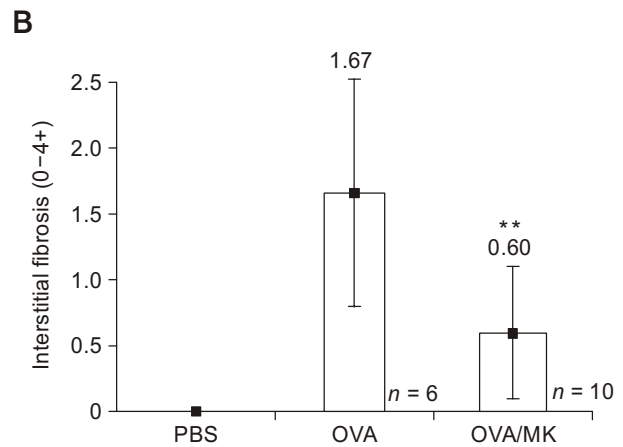
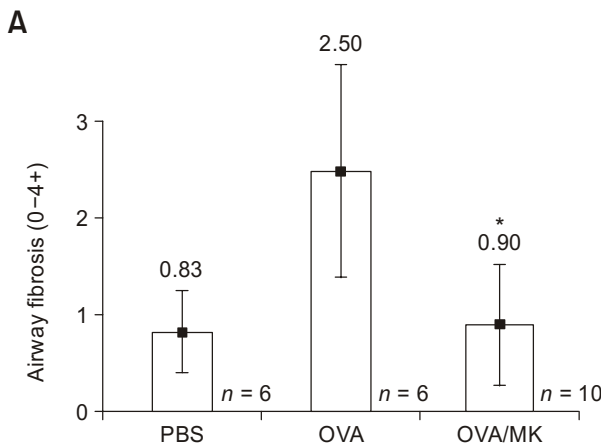


Figure 6. The intensity of airway (A), interstitial (B) and blood vessels (C) fibrosis for each group. MK therapy reduced lung fibrosis (respectively, * $P = 0.012$, ** $P = 0.021$, *** $P = 0.039$ OVA/MK versus OVA-sensitized/challenged).

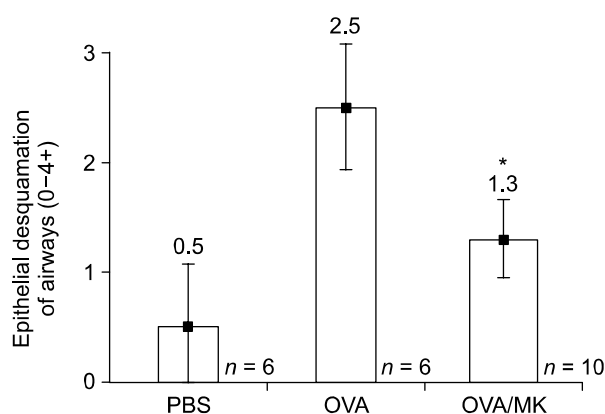


Figure 7. Effect of montelukast on OVA induced airway epithelial desquamation. (* $P = 0.003$ OVA/MK versus OVA-sensitized/challenged). The mean epithelial desquamation score of airway in all groups.

Airway epithelial desquamation

The mean epithelial desquamation of airway score was 0.5 ± 0.54 in PBS control mice, 2.5 ± 0.54 in OVA sensitized/challenged mice, and 1.30 ± 0.48 in OVA/MK mice. The OVA sensitized/challenged mice had a significantly increased epithelial desquamation score compared with PBS control animals ($P = 0.003$). The OVA/MK animals had a significantly lower epithelial desquamation score than OVA sensitized/challenged mice ($P = 0.003$) (Figure 7).

The levels of $\text{LTC}_4/\text{D}_4/\text{E}_4$ in lung tissue were elevated significantly in OVA sensitized/challenged mice (1193.33 ± 55.73 pg/ml, $P = 0.004$) and in OVA/MK mice (1164.00 ± 72.44 pg/ml, $P = 0.001$), compared with PBS control mice (61.83 ± 10.72). MK treatment did not affect the levels of CysLT in lung tissue. There was no significant difference in the levels of CysLT between OVA sensitized/challenged mice and OVA/MK treatment animals ($P = 0.293$) (Figure 8).

Discussion

In the current study, it was evaluated the effects of low dose leukotriene receptor antagonist, montelukast, on the airway remodeling for chronic asthma in a mouse model. Our findings suggest that pretreatment with low dose montelukast for chronic allergic asthma markedly decreased eosinophil infiltration into the lung interstitium, goblet cell hyperplasia with airway occlusion with mucus and increased collagen deposition around the airways and blood vessels without a significant decrease airway smooth muscle thickness. However, the levels of CysLTs were not affected by MK therapy.

It is now widely accepted that chronic airway

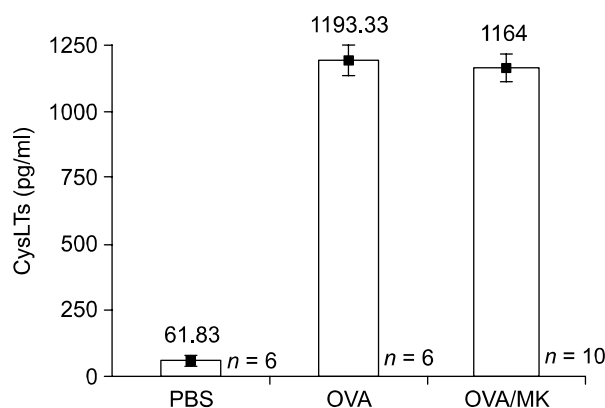


Figure 8. The CysLTs levels for each group. Montelukast did not reduce the levels of CysLTs in the OVA-sensitized/challenged mice.

inflammation plays a key role in asthma. The relationship between inflammation and structural changes is speculative. One generally accepted proposal is that remodeling is a direct result of a switch from acute to chronic inflammation followed by healing (Bousquet *et al.*, 2000). For this reason, anti-inflammatory agents will not necessarily prevent or attenuate the process of remodeling (Jeffery, 2001). All guidelines focus on the treatment of inflammation in asthma although there are differences between them and the process of airway wall remodeling, for which there is no defined treatment yet fully validated (Bousquet *et al.*, 2000).

Leukotrienes play important roles in asthma and are thought to have the ability to induce a variety of effects including bronchoconstriction and inflammatory cell recruitment. CysLTs have potent effect on leukocyte trafficking, airway mucus secretion, and collagen synthesis (Henderson *et al.*, 2002). CysLTs are released by most cells involved in the airways inflammation and particularly by eosinophils (Shaw *et al.*, 1984). Inhalation of LTE_4 (Laitinen *et al.*, 1993) or LTD_4 (Diamant *et al.*, 1997) induces the recruitment of eosinophils in the airways, possibly in part by inducing P-selectin expression on endothelial cells (Datta *et al.*, 1995). CysLT1 receptors are expressed in human eosinophils (Figuroa *et al.*, 2001), and their activation by CysLTs may lead to eosinophil recruitment to inflammatory sites. LTB_4 is a potent chemoattractant and activator of neutrophils (Claesson *et al.*, 1992). In our study, the increased eosinophil cell infiltrate was observed in the lung interstitium of OVA-sensitized/challenged mice. The migration of eosinophils into the airways involves several steps. Derived from myeloid progenitors in the bone marrow, mature eosinophils circulate briefly in the peripheral blood and home to the site of inflammation under the action of several factors

including cytokines and chemokines (Wardlaw *et al.*, 1995). The mechanisms by which CysLTs induce airway eosinophilia are unclear. It is plausible that CysLTs promote airway eosinophilia by modulating the expression of cytokines (Underwood *et al.*, 1996). In our study, we demonstrated that low dose of MK inhibited the eosinophil infiltration of the lung tissue in OVA-sensitized/challenged mice. Recent studies have shown a reduction in airway eosinophilia in asthmatic subjects treated with CysLT1 receptor antagonists (Nakamura *et al.*, 1998; Pizzichini *et al.*, 1999), suggesting chemotactic effects of the CysLTs *in vivo*. CysLTs have been shown to attract eosinophils, and LT antagonists could potentially block these effects. Chan *et al.* (1990) showed administration of LTD₄ induced a 2.5-fold increase in conjunctival radioactivity (a measure of eosinophil chemotaxis) *in vivo*. Utilizing aerosolized LTD₄, Underwood *et al.* (1996) demonstrated inhaled LTs elevated BAL eosinophils in guinea-pig airways; increased eosinophil numbers were confirmed histologically in the bronchial epithelium and subepithelium. In current study, marked eosinophilia developed following chronic allergen challenges, and eosinophilias were attenuated by MK therapy. These results are consistent with prior observations of CysLTs antagonists in allergic airway responses of humans (Taylor *et al.*, 1991), sheep (Abraham *et al.*, 1993), rats (Ihaku *et al.*, 1999) and mice (Blain and Sirois, 2000). Wu *et al.* (2003) demonstrated that the high dose CysLT1 receptor antagonist MK reduced eosinophil recruitment into airway by > 90% after allergen challenge in sensitized mice. This was associated with a dramatic reduction in the number of neutrophils, eosinophils and lymphocytes in the BAL, whereas in the study by Ihaku *et al.* (1999) only a reduction in mononuclear cells was found. In our study, total inflammatory cell infiltration was not changed by MK therapy. This situation can be explained by the lower dose of MK that was used in our study. In general, it is known that high dose leukotriene receptor antagonist therapy suppresses the inflammatory cell infiltration sufficiently, especially eosinophils in a mouse model asthma. Blain and Sirois (2000) showed that MK-571 produced a dose-dependent inhibition of the infiltration of the eosinophils in the bronchoalveolar lavage and a total inhibition of neutrophil infiltration. Wu and colleagues (Wu *et al.*, 2003) showed that a high dose MK reduced the number of neutrophils, eosinophils and lymphocytes in the BAL, whereas in the study by Ihaku *et al.* (1999) reduction in only mononuclear cells was found by lower dose MK therapy. On the other hand, Eum *et al.* (2003) demonstrated that the number of eosinophils and total cells were significantly reduced by both dose of MK (3 mg/kg and

10 mg/kg) in animal model of asthma. In this study, we tried to achieve the lower dose and long-term effect of MK on airway remodeling and the levels of CysLTs were measured. It was shown that MK reduced lung eosinophil but not total cell infiltration.

Epithelial goblet cells and mucus-secreting submucosal glands are the major sources of luminal mucus. Goblet cell hyperplasia is a feature of the airways in asthma (Jeffery, 2001). *In vivo* studies with the CysLTs have shown increased venous permeability (Joris *et al.*, 1987), mucus secretion (Marom *et al.*, 1982), and decreased activity of the respiratory cilia (Bisgaard and Pedersen, 1987). The CysLTs are also potent mucus secretagogues with greater activity in causing mucus secretion from isolated human bronchial tissue than LTB₄ or cyclooxygenase products of arachidonic acid metabolism (Larive'e *et al.*, 1994). CysLT1 receptor, whose expression has been demonstrated on bronchial smooth muscle cells and various inflammatory cell types in the lung, is responsible for the proinflammatory actions, including increased microvascular permeability with edema formation and increased mucus secretion, possessed by CysLTs (Samuelsson, 2000). Our results showed that CysLTs are also involved in airway goblet cell hyperplasia and mucus occlusion of the airway lumen as CysLT1 receptor blockade by this dose MK significantly reduced the allergen-induced goblet cell hyperplasia and mucus occlusion in the airways observed in OVA sensitized/challenged mice. Similarly, Henderson *et al.* (2002) demonstrated that the numbers of goblet cells in the airways in OVA sensitized/challenged mice were significantly decreased by the same dose of CysLT1 receptor blockade. They showed that inhibition of IL-4 and IL-13 production by LT receptor antagonism might be an important mechanism of reducing goblet cell hyperplasia and mucus hypersecretion in allergic airways.

Smooth muscle mass is usually increased in large and/or small airways in both fatal and non-fatal cases of asthma (Carroll *et al.*, 1993). There is a 3-to-4-fold increase in muscle volume in asthmatic airways by comparison to normal subjects (Hogg, 1993). CysLTs may alter remodeling because they increase proliferation of airway smooth muscle (Cohen *et al.*, 1995; Panettieri *et al.*, 1998) and airway epithelial cells (Leikauf *et al.*, 1990) and LTC₄ was shown to up-regulate collagenase expression in human lung fibroblasts (Medina *et al.*, 1994). LTD₄ augments epidermal growth factor-induced human airway smooth muscle proliferation *in vitro* (Panettieri *et al.*, 1998). Previous studies of cysLT antagonists in allergic airway responses of Brown Norway rats (Wang *et al.*, 1993; Salmon *et al.*, 1999) and mice (Henderson *et al.*, 2002) demonstrated

that allergen-induced increases in airway smooth muscle are significantly reduced by treatment with a CysLT1 receptor antagonist. In contrast to these studies especially Henderson *et al.* (2002) where same dose MK was used, we did not observe significant differences in airway smooth muscle hypertrophy after MK therapy although there was a trend toward lower levels in OVA/MK treatment animals. The reason for this difference requires further investigation.

In asthma, remodeling is usually present in biopsies, as shown by collagen deposition on the reticular basement membrane (Bosquet *et al.*, 2000). Airway thickening beneath the basement membrane occurs with deposition of collagen and other extracellular matrix proteins, including fibronectin, tenascin, and laminin (Elias *et al.*, 1999). The thickened reticular layer is associated with deposition of immunoglobulin and/or collagen I and II and fibronectin (Roche *et al.*, 1989). The additional reticulin is likely produced after activation of myofibroblasts leading to a so-called "fibrosis" of the airways (Brewster *et al.*, 1990). Collagen deposition occurs in the connective tissue layer surrounding the blood vessels and alveolar interstitium. Our findings showed that airway collagen deposition/fibrosis was significantly reduced by MK therapy. These results indicate an important role of CysLTs in collagen deposition/lung fibrosis in this model of chronic asthma. Recent evidence also suggests that MK, a CysLT1 receptor antagonist, can significantly reduce airway remodeling in a mouse model of chronic asthma (Henderson *et al.*, 2002).

The CysLTs are released from human lung due to allergic subjects in response to challenge with allergen (Dahlen *et al.*, 1983). Hanazawa *et al.* (2000) showed significant increases in CysLTs in breath condensates of patients with moderate and severe asthma, compared with normal subjects. Early studies demonstrated that leukotrienes could be measured in biological fluids after an allergic or inflammatory insult (Jones *et al.*, 2001). A variety of inflammatory cells that are intimately associated with the asthmatic condition have the necessary biochemical machinery to produce leukotrienes. These cells are of myeloid origin and are either constitutive to the lung (e.g. mast cells, macrophage) or are recruited to the lung by specific chemoattractant stimuli (e.g. eosinophils, neutrophils, basophils, lymphocytes) (Williams *et al.*, 1984). Increased amounts of CysLTs are found in the BAL fluid of asthmatics compared with normal subjects (Wenzel *et al.*, 1990) and allergen challenge induces the release of LTs into the airways in several animal models of asthma (Powell *et al.*, 1995; Henderson *et al.*, 1996). Volovitz *et al.* (1999) have shown that increased

amounts LTC₄ were found in the respiratory mucosa of children with persistent asthma and that MK can suppress these elevated levels. Possible explanation of these situation in this study; CysLTs promote eosinophil recruitment in the airways and eosinophil, in turn, releases leukotrienes. MK, which is a CysLT1 receptor antagonist, indirectly leads to a decrease in leukotriene concentration in the airways by reducing the influx of eosinophils. In our study, in this chronic mouse model of asthma, we found that levels of CysLTs were significantly increased in lung tissue after airway allergen challenge. The levels of CysLTs in the lung tissue of OVA sensitized/challenged mice were not significantly different from those of the OVA/MK treated mice, although there was a significant decrease for eosinophil infiltration by MK therapy. These results suggest that decreased eosinophil cells may not be only one reason for affecting CysLTs levels. Most recently, it has been shown that primary airway epithelial cells contain a full set of CysLTs synthetic enzymes, and when activated, they release appreciable amounts of LTC₄. Epithelial cells do not express the CysLT1 receptor (James *et al.*, 1999). Thus, the production of CysLTs can occur in the absence of inflammation, and their actions on precursor mesenchymal cells may be important in augmenting airway remodeling in asthma (Holgate *et al.*, 2003). In addition impairment of PGE₂ synthesis from epithelial cells, as may exist in asthma, increases the extent to which epithelial cell-derived arachidonic acid is available for leukotriene synthesis by leukocytes (Holgate *et al.*, 2003).

In conclusion, our results suggest that CysLTs are important in the pathogenesis of chronic allergic airway injury and fibrosis. Evidence from a mouse model of asthma demonstrated that CysLT1 receptor antagonist inhibits the airway remodeling process, including eosinophil infiltration, goblet cell hyperplasia, mucus hypersecretion, collagen deposition and lung fibrosis except airway smooth muscle hypertrophy, but does not affect the levels of CysLTs in the lung tissue. These results suggest that low dose of Montelukast may modulate the parameters of inflammation and fibrosis.

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