Effect of Selective Cysteinyl Leukotriene Receptor Antagonists on Airway Inflammation and Matrix Metalloproteinase Expression in a Mouse Asthma Model

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Key Words
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Background: Cysteinyl leukotrienes (CysLTs) play a major role in the pathogenic changes of airway inflammation in asthma treatment. The matrix metalloproteinase (MMP) family, especially MMP-9 and MMP-2 levels, can reflect the status of airway remodeling. This study was undertaken to determine the role of a specific CysLT receptor antagonist in inhibition of airway inflammation and reversal of airway remodeling.

Methods: Ovalbumin (OVA)-sensitized BALB/c mice were fed with a specific leukotriene receptor antagonist (MK-679), prednisolone or placebo from Days 15 to 27. Airway hyperreactivity, bronchoalveolar lavage fluid (BALF), and sera were analyzed. Pulmonary histology was obtained, and the levels of MMP-2 and MMP-9 in BALF were measured.

Results: The OVA-sensitized mice developed significant airway inflammatory responses, including extensive eosinophils trafficking into BALF and lung interstitium, goblet cell hyperplasia, mucus hypersecretion, elevated serum immunoglobulin (Ig) E, and decreased level of serum IgG2a. Administration of MK-679 could reduce airway inflammation but was not as effective as prednisolone. However, MK-679 was more effective than prednisolone for reversing subepithelial fibrosis.
subepithelial fibrotic and myofibrotic reactions of airway remodeling. The levels of MMP-2 and -9 in BALF were proportional to the extent of airway remodeling, which can reflect the effects of treatment. Both prednisolone and MK-679 reverse airway hyperresponsiveness induced by OVA-sensitized mice.

**Conclusion:** Cysteinyl leukotriene receptor plays a more important role than CysLT in the pathogenesis of allergic airway inflammation. MMP-2 and -9 may be more sensitive indicators of airway remodeling.

1. **Introduction**

Asthma has long been recognized as a complicated disease that affects millions of people. It is first characterized by airway hyperresponsiveness (AHR) to a variety of specific or non-specific stimuli, mucus hypersecretion, pulmonary eosinophilia, airway edema and elevated serum immunoglobulin (Ig) E. After a while, persistent allergen stimulation induces chronic inflammatory changes in airways. Airway remodeling is accompanied by subepithelial fibrosis, fragmentation of elastic fibers, airway smooth muscle thickening, goblet cell metaplasia, changes in airway epithelium, and vascular hyperplasia. The major cause that results in airway narrowing after remodeling is subepithelial fibrosis. This striking change comes from the deposition of extracellular matrix (ECM) proteins (e.g., collagen, fibronectin, laminin, tenascin) in the lamina reticularis, which makes chronic asthma difficult to control with inhaled β2 agonist only. To date, there is still no satisfactory therapy available to better control asthma. The main issues to tackle in devising asthma treatment is understanding how repeated airway inflammation during asthma attacks results in airway remodeling, and identifying the major cytokines involved in this process. Cysteinyl leukotrienes (CysLTS) are now known as a major cytokine that are involved in the pathogenic process. Cysteinyl leukotriene receptor plays a more important role than CysLT in the pathogenesis of allergic airway inflammation. MMP-2 and -9 may be more sensitive indicators of airway remodeling.

Maternal metalloproteinases (MMPs) are a family of extracellular proteases that are responsible for degradation of extracellular matrix during tissue remodeling. Among the family of MMPs, the gelatinases MMP-2 and MMP-9 are specific to denatured collagens and collagen-IV of the basement membrane. Deposition of collagen over the basement membrane is a predominant finding during peribronchial fibrosis of airway remodeling. In all studies to date, MMP-9 was thought to be the major MMP in the airways of asthmatics. This suggests an important role of MMP-9 during asthma treatment and monitoring of the process of airway remodeling. In this study, we investigated the expression of MMP-2 and MMP-9 in bronchoalveolar lavage fluid (BALF) after treatment.

2. **Methods**

MK-679(R(−)-3-[[3-((2-(7-chloro-2-quinolinyl)ethenyl)phenyl)(3-(dimethylamino)-3-oxopropyl)thiophenyl]thio]propanoic acid) is a potent and specific LTD4-receptor antagonist, selective inhibitor of [3H]leukotriene D4 binding. The binding site is different from that of popular prescription of leukotriene receptor antagonist, Montelukast. Otherwise, MK-679 had intravenous and inhaled forms in the clinical trial. It is more convenient than other medications for asthma control.

The key reagent MK-679 was provided by Merck Sharp & Dohme Corp. (One Merck Drive, Whitehouse Station, NJ, USA).

2.1. Sensitization and airway challenge for induction of airway remodeling

Female BALB/c mice, 6–8 weeks of age, were obtained from the National Laboratory Animal Center (seven mice/group). The mice were maintained on ovalbumin (OVA)-free diets. The mice were immunized with intraperitoneal (IP) injections of 100 μg OVA (0.2 ml of 0.5 mg/ml; grade V; Sigma Chem. Co., St. Louis, USA) complexed with alum (Sigma Chem. Co., St. Louis, USA) on Days 0 and 14. For the age- and sex-matched control groups, the unsensitized mice received an IP injection of 0.2 ml saline complexed with alum. Intranasal (IN) OVA challenge was first administered with a dosage of 100 μg (0.05 ml of 2 mg/ml) on Day...
14. IN OVA challenges were then repeated on Days 25, 26, and 27 with doses of 50 μg (0.05 ml of 1 mg/ml). Before the intranasal challenge, the mice were first anesthetized with IP 40 mg/kg sodium pentobarbital. For the control group, the unsensitized mice received an IN normal saline of 50 μl on Day 14 and Days 25, 26, and 27. In this mouse asthma model, normal saline was used as a negative control.

2.2. Therapeutic intervention with leukotriene receptor antagonist and corticosteroid

During Days 15 to 27, randomized treatment groups of mice were respectively fed with different agents by gavage. A group of OVA-treated mice was fed with selective leukotriene D4 receptor antagonist MK-679 (Verlukast, 60 mg/kg/day, dissolved in apple juice; Merck Sharp and Dome Co., Inc.).21,22 The positive control group of mice was fed with prednisolone sodium phosphate solution (3 mg/kg/day; Center Laboratories, Inc., Taipei, Taiwan).12 A group of unsensitized mice and a set of OVA-treated mice were given apple juice as placebo.

2.3. Determination of airway responsiveness by noninvasive pulmonary function test

On Day 28, 24 hours after the last intranasal challenge with either normal saline or OVA, noninvasive pulmonary mechanics were determined by whole-body plethysmography (Model PLY 3211; Buxco Electronic Inc., Sharon, CT, USA).23 Airway hyperreactivity to aerosolized methacholine was performed in conscious, freely moving, spontaneously breathing mice. Aerosol was first generated by placing 0.5 ml of saline or methacholine solution in the cup of an ultrasonic nebulizer. Different concentrations of aerosolized methacholine (0, 5, 10, and 20 mg/ml) were inhaled in turn. The degree of bronchoconstriction was expressed as enhanced pause (Penh), a calculated dimensionless value that correlates with measurements of airway resistance, impedance, and intrapleural pressure.

2.4. Immunoassay for serum IgE levels

After plethysmography, the mice were sacrificed; each mouse was then exsanguinated by cardiac puncture. The blood was transferred to a 2 ml microcentrifuge tube that contained gel and then centrifuged at 4°C, 6000 rpm for 5 minutes. Serum was removed and red blood cells were discarded. The serum was divided into three aliquots and stored at −70°C until antibody level analyses were performed. Indirect enzyme-linked immunosorbent assay (ELISA) was employed to determine IgE serum antibody titers. ELISA plates with 96 wells (Alpha Diagnostics Intl Inc., San Antonio, USA) were coated with anti-mouse IgE. All reagents in the procedure were allowed to reach room temperature. Twenty microliters of standards and serum were added separately with 80 μl of sample buffer in each well. After incubation for 60 minutes at room temperature, the plate was washed five times. One hundred microliters of horseradish peroxidase (HRP)-labeled anti-mouse IgE conjugate were added and incubated at room temperature for 30 minutes, followed by adding 100 μl of 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution and incubating at room temperature for 15 minutes. After 100 μl of stop solution was added, optical density (OD) readings of the samples were obtained from measuring the absorbance at 450 nm by an ELISA reader. The antibody titers of the samples were compared to pooled standards that were generated in the laboratory and expressed as nanogram per milliliter (ng/ml).

2.5. Acquisition of BALF and quantification of BALF cell level

BALF was done after sacrificed mice were exsanguinated. The right lung was separated by ligating at the right main-stem bronchus; BAL was performed on the left lung. The left lung was lavaged three times with 0.5 ml of normal saline. The first-collected BAL fluid was centrifuged at 4°C, 1000 g for 5 minutes. The supernatant (about 400 μl) was extracted and divided into three equal portions, and then frozen at −80°C for subsequent testing. The pellet was resuspended and mixed gently with second-collected and third-collected BAL fluid containing microcentrifuge tubes. Ten microliters of the resuspended BALF was stained with 0.06% methylene blue for total leukocyte count with a hemocytometer. Two hundred microliters of diluted BAL cell suspension was aliquoted into the single-chamber cytospin device in a cytocentrifuge (Rotofix 32; Andreas Hettich GmbH & Co. KG, Tutlingen, Germany), followed by centrifuging a slide at room temperature, 500 rpm for 4 minutes. The slide was stained with hematoxylin and eosin stain to assess eosinophil counts. Differential counts were performed in a blinded fashion by counting at least 300 cells under light microscopy.

2.6. Measurement of matrix metalloproteinases (MMP)-2 and MMP-9 activity

After bronchoalveolar lavage, the activity of MMP-2 and 9 was determined by gelatin zymography. Five microliters of samples (BALF) were added to 4 μl of loading buffer and separated by electrophoresis (140 V for 3 hours) in 8% Sodium dodecyl sulfate (SDS)-polyacrylamide gels that contained 0.1% gelatin. The SDS was removed by washing gels twice with 50 ml washing buffer (2.5% Triton X-100 in d-H2O, Sigma Chem. Co. St. Louis, USA), each wash lasting 30 minutes under room temperature. After incubation for 12 hours at 37°C in reaction buffer, the gels were stained with staining buffer (0.25% Coomassie Blue) for 30 minutes. The optical densities of the MMP-2, 9 bands were quantified by densitometer (AlphaImager 2000, Alphainotech Corporation, San Leandro, CA, USA). Results were expressed as zymography activity (relative density) on the basis of the computer analysis. An MMP-2 and MMP-9 standards were used as a positive control.

2.7. Lung histology

After BAL, trachea and right lung were obtained and fixed in formalin at room temperature for about 15 hours. The right lung tissue was then embedded in paraffin and cut into 5-μm sections. The lung sections were stained with hematoxylin and eosin to determine the airway inflammatory cell infiltration and smooth muscle thickness. Each slide was
randomly examined for five to eight fields to evaluate the morphometric variation. To analyze the anatomic distribution of collagen deposition/fibrosis in the airway, the lung sections were stained with Masson’s trichrome stain. The total area of Masson’s trichrome staining in each paraffin embedded lung was outlined and quantified by using a light microscope attached to an image analysis system (Image-Pro Plus; Media Cybernetics, Silver Springs, MD, USA). The unit area of captured images was calibrated and standardized with a slide micrometer. Results were expressed as the area of Masson’s trichrome staining per micrometer length of basement membrane of bronchioles (~150–200 μm of internal diameter, surrounded by smooth muscle cells). Between three and five bronchioles were counted in each slide. All slides were stained in the same set under identical staining conditions and analyzed under the same light microscope conditions. The lung tissue slides for morphologic survey were coded and analyzed by technicians who were blind to the protocol design. Each slide was also analyzed in the same predetermined sequence to minimize observer bias.

2.8. Liver and kidney histology
Liver and kidney tissues of the MK-679-treated (60 mg/kg/day) group were embedded in paraffin and cut into 5-μm sections. The liver and kidney sections of the MK-679 treated group were stained with hematoxylin and eosin for toxic effect screening and evaluation. There was no pathogenic finding over the liver and kidney tissues of the MK-679-treated group. Comparing the tissues with the liver and kidney tissues from the saline-treated or OVA-treated group, no specific difference was found.

2.9. Statistical analyses
The data were reported as mean ± SEMs of the combined experiments. Results in the different groups were compared by analysis of variance (ANOVA) using the protected least significant difference method. Differences between each group were considered statistically significant when p values < 0.05.

3. Results

3.1. Effects of selective CysLT receptor antagonist and corticosteroid treatment on reversal of allergen-induced airway inflammation and remodeling serum IgE production
Serum IgE level was elevated significantly in the OVA-sensitized/challenged group compared with the saline-treated control group (1615.71 ± 403.59 ng/ml vs. 126.29 ± 13.39 ng/ml; p < 0.001). This is shown in Figure 1. Administration of both prednisolone (515.05 ± 117.84 ng/ml; p = 0.001 vs. sensitized group) and MK-679 (501.75 ± 96.42 ng/ml; p = 0.001 vs. sensitized group) to OVA-treated mice during repetitive ovalbumin challenges had significant effects on inhibiting the serum IgE release. Each respectively reduced the serum IgE level by 68.1% (steroid/OVA vs. OVA) and 68.9% (MK-679/OVA vs. OVA). The serum IgE levels of OVA-sensitized/challenged mice treated with prednisolone or MK-679 were not significantly different from those of the saline control group.

3.2. Airway inflammatory cell infiltration
Airway inflammatory cells markedly infiltrated into BALF of the OVA-sensitized/challenged group compared with the saline-treated control group (11.29 ± 1.94 × 10^5/ml vs. 7.79 ± 1.71 × 10^5/ml; p < 0.001). This is shown in Figure 2A. Administration of prednisolone to OVA-treated mice during repetitive ovalbumin challenges had significant effects on reducing infiltration of airway inflammatory cells into BALF (3.43 ± 0.65 × 10^5/ml; p = 0.001 vs. sensitized group). By contrast with the significant effects of prednisolone on inhibiting airway inflammatory cells infiltrating into BALF, the effects of MK-679 on reducing the levels of BALF inflammatory cells were not significantly different with the OVA-treated group (7.79 ± 2.26 × 10^5/ml vs. 11.29 ± 1.94 × 10^5/ml; p = 0.093). This is shown in Figure 2A. However, there appeared to be a trend that MK-679 inhibited the infiltration of airway inflammatory cells into BALF.

3.3. Eosinophilia in bronchoalveolar lavage fluid
The percentage of BALF eosinophils in OVA-treated mice was significantly greater than in control saline-treated mice (1.1 ± 0.29% vs 48.29 ± 4.48%; p < 0.001). This is shown in Figure 2B. Although both prednisolone and MK-679 had significant effects on reducing eosinophil influx into BALF in OVA-treated mice, administration of prednisolone...
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3.5. Airway collagen deposition and peribronchial fibrosis

The area of collagen deposition/fibrosis was assessed by Masson’s trichrome stain. Collagen deposition was markedly increased over perivascular and lung interstitium around airways in OVA-sensitized/challenged mice compared with the saline-treated control group (Figure 4A vs. 4B). This increase in airway collagen deposition could be reversed by prednisolone or MK-679 administered during OVA challenge (Figure 4C and D). The area of peribronchial trichrome staining in OVA-treated mice was significantly greater than that in control saline-treated mice (3.28 ± 0.28 vs. 0.19 ± 0.03 μm²/μm circumference of bronchiole; p < 0.001). This is shown in Figure 5. Administration of either prednisolone (0.98 ± 0.37 μm²/μm circumference of bronchiole; p < 0.001 vs. sensitized group) or MK-679 (0.38 ± 0.18 μm²/μm circumference of bronchiole; p < 0.001 vs. sensitized group) to sensitize mice significantly reduced the area of peribronchial trichrome staining. No statistical difference in peribronchial fibrosis was observed between the control group and the OVA-treated with MK-679 administered group. However, there was statistical difference between the control group and the OVA-treated with prednisolone administered group (0.79 ± 0.5 μm²/μm circumference of bronchiole; p = 0.02). The effect of peribronchial fibrosis reversal was greater after administration of MK-679 compared with prednisolone.

3.6. Detection of MMP-2 and MMP-9 activity in BALF

Quantitative analysis of MMP-2 and MMP-9 expression by zymography demonstrated minimal expression of either MMP-2 (168.63 ± 27.88 relative density) or MMP-9 (19.92 ± 2.94 relative density) in BALF (Figure 6) of saline-treated mice. In contrast, both levels of MMP-2 (284.27 ± 43.44 relative density; p = 0.006 vs. control group) and MMP-9 (333.29 ± 15.79 relative density; p < 0.001 vs. control group) in BALF (Figure 6) were significantly increased after repetitive ovalbumin challenge in the remodeled airway. Levels of MMP-2 and MMP-9 inhibition were induced by both prednisolone and MK-679. Administration of prednisolone to OVA-treated mice significantly
reduced the level of MMP-2 (113.08 ± 7.29 relative density; p < 0.001 vs. sensitized group) and MMP-9 (38.46 ± 5.57 relative density; p < 0.001 vs. sensitized group). This is shown in Figure 6B and C. Applying MK-679 to OVA-treated mice could also reduce the level of MMP-2 (76.74 ± 11.33 relative density; p < 0.001 vs. sensitized group) and MMP-9 (98.24 ± 8.84 relative density; p < 0.001 vs. sensitized group) significantly (Figure 6B and C). However, the levels of MMP-9 inhibition were, in general, greater after administration of prednisolone compared with administration of MK-679 (p = 0.003).

3.7. Effects of selective CysLT receptor antagonist and corticosteroid treatment on allergen-induced airway hyperreactivity to methacholine noninvasive in vivo plethysmography

Airway reactivity to aerosolized methacholine was evaluated on Day 28, 24 hours after the last intranasal challenge with OVA or saline, by noninvasive plethysmography (Figure 7). Penh (% of air) was significantly increased in the OVA-treated mice compared with saline controls after challenged with methacholine at 5 mg/ml (p = 0.008, OVA vs. saline; Figure 7A). However, at higher doses (10 and 20 mg/ml), no significant increases were seen in Penh in OVA-treated mice compared with the saline group (Figure 7B). Administration of prednisolone or MK-679 was similar in low-dose methacholine challenge conditions, in that Penh was significantly reduced in prednisolone/OVA (p = 0.031, OVA vs. prednisolone/OVA; Figure 7A) and MK-679/OVA (p = 0.05, OVA vs. MK-679/OVA; Figure 7A) group after methacholine challenge at 5 mg/ml. By contrast, after higher doses (10 and 20 mg/ml) of methacholine challenge, administration of prednisolone or MK-679 did not significantly change the airway responses to methacholine in OVA-sensitized/challenged mice (Figure 7B). The MK-679 even failed to reduce airway hyper-reactivity to 10 and 20 mg/ml of aerosolized methacholine in OVA sensitized/challenged mice.

4. Discussion

We employed an allergen-induced airway inflammatory model in mice to address the role of cysteinyl leukotrienes (CysLTs) in mediating pulmonary inflammation, which promotes airway remodeling and hyperreactivity to
methacholine. The levels of serum IgE and airway inflammatory cells, including eosinophils, can be suppressed by corticosteroid during allergen challenge. However, in our study, intervention with MK-679 did not as function well as corticosteroid on inhibiting serum IgE release and eosinophil infiltration. This may be due to MK-679 having limited effects in acute airway inflammation.

In vitro studies showed that CysLTs induced release of collagen and fibroblast growth factors from mouse alveolar macrophages. Alveolar macrophages can also express MMPs after repeated ovalbumin challenge. MMPs, especially MMP-9 and MMP-2, are known to regulate collagen synthesis and breakdown, which can then promote airway remodeling. Since alveolar macrophages express LTD4 high-affinity receptors, blocking the receptors by a potent and selective LTD4 receptor antagonist, Verlukast (MK-679), may inhibit the positive cycles of collagen release.

A defective repair response or recurrent injury may lead to a repeated cycle of chronic inflammation. This is modulated by various cytokines and growth factors, including transforming growth factor-β (TGF-β) and epidermal growth factor. These may illustrate why corticosteroid inhibits most inflammatory effects in airways but remodeling after recurrent injury still progresses. Since corticosteroid does not inhibit the major mediators or cytokines mentioned above, abnormal repair responses will proceed after repeated episodes of asthma attack. In our study, neither corticosteroid nor selective CysLT receptor antagonist MK 679 alone could completely reverse effects of airway remodeling. There have been reports of total reversal of airway remodeling by CysLT1 receptor antagonist; however, according to our results, selective LTD4 receptor antagonists such as MK-679 seem to be more effective in modulating peribronchial fibrosis than corticosteroid, and may prevent further airway remodeling. More recent studies have also demonstrated that CysLT receptor antagonists can reverse established airway remodeling in mice when the antagonist is first administered after the development of allergen-induced airway remodeling. The intervals of long-term follow-up, the time of treatment intervention and different pathways of the acting CysLT receptor antagonists may be the crucial factors that differentiate results of our study from others.

In all studies to date, MMP-9 was the predominant MMP in the airways of asthmatics. There are reports of increased levels of MMP-2 and MMP-3 in sputum or BALF; however, the

Figure 4  Effect of selective CysLT receptor blockade and corticosteroid treatment on allergen-induced goblet cell hyperplasia and collagen deposition/fibrosis. Lung tissues were obtained on Day 28 from saline controls (A) and OVA-treated mice in the absence (B) or presence of prednisolone (C) or MK-679 (D) and stained with Masson’s trichrome stain. (A) The airway is clear of mucus in lumen and fewer collagen deposits in saline-treated controls. (B) Accumulation of mucus (*) in the airway lumen is observed. Large amounts of collagen deposition (arrow) are identified around perivascular and peribronchial tissue of OVA-treated mice. Many goblet cells (arrowheads) are seen in the airway. (C) Extensive collagen (arrow) deposition and goblet cells (arrowheads) hyperplasia are still noted in prednisolone-treated mice. (D) The dense collagen deposits and abundant goblet cells were reduced after MK-679 treatment compared to the OVA-treated group. CysLT = cysteinyl leukotriene; OVA = ovalbumin.
levels were considerably less than those of MMP-9. In our study, both MMP-2 and MMP-9 were significantly increased in OVA-sensitized/challenged mice. The levels of MMP-2 and 9 in BALF were proportional to the severity of airway inflammation and the extent of airway remodeling.17 These results were confirmed by serum IgE levels and pulmonary histologic findings. Such change can be modulated after effective treatment; therefore, MMP-2 and -9 may act as more sensitive indices for monitoring the process of airway remodeling. Interestingly, a distinctly different expression of MMP-2 in BALF was noted between MK-679-treated and Montelukast-treated OVA-sensitized/challenged mice (data not shown). Inhibition of MMP-2 expression by MK-679 was more effective than by Montelukast. This suggests a more extensive action site (other than macrophages and eosinophils)9 of MK-679 than Montelukast, another CysLT receptor antagonist.

Airway hyperresponsiveness to methacholine was observed in OVA-treated mice. Both prednisolone and MK-679 reversed airway hyperresponsiveness. However, such suppressive effects disappeared in the MK-679 group when challenged with higher doses of methacholine. It is possible that although leukotrienes mediate the mucus release and eosinophil infiltration of the airways, they do not control the airway hyperresponsiveness of the late-phase asthma response.20 The augmentation of growth factor-induced human airway smooth muscle cells proliferation by leukotriene D4 is mediated by a CysLT receptor distinct from that which mediates LTD4-induced airway contraction.29 Such a characteristic may account for our unsatisfactory results on inhibiting airway hyperresponsiveness by MK-679, in spite of its effective actions on airway remodeling. Increased airway thickness and airway contractile tissues were supposed to positively correlate with airway hyperresponsiveness in these studies.1,30 However, in other models, dissociation or even negative relationship between airway hyperresponsiveness and airway structure thickness was obtained.5,31 Therefore, presumably, airway hyperactivity is mediated not just by structural changes but also by multiple mediators and receptor distributions over

![](image1.png)

**Figure 5** Effects of selective CysLT receptor blockade and corticosteroid treatment on peribronchial collagen deposition. Area of peribronchial collagen deposition was assessed by Masson’s trichrome staining. Results are expressed as the area of trichrome staining per micrometer length of basement membrane. The results are expressed as means ± SEM. N = 7 in each group. *p < 0.05, compared with the all other groups. †p < 0.05, compared with the OVA-treated sensitized group by analysis of variance. CysLT = cysteinyl leukotriene; OVA = ovalbumin.

![](image2.png)

**Figure 6** Expression of MMP-9 and MMP-2 in bronchoalveolar lavage fluid: modulation by selective CysLT receptor blockade and corticosteroid. (A) Levels of MMP-9 and MMP-2 were assessed by zymography; (B) activity of MMP-9 was expressed as relative density in each group; (C) activity of MMP-2 was expressed as relative density in each group. The results are expressed as means ± SEM. N = 7 in each group. *p < 0.05, compared with the all other groups. †p < 0.05, compared with the OVA-treated sensitized group by analysis of variance. CysLT = cysteinyl leukotriene; MMP = matrix metalloproteinase; OVA = ovalbumin.
airway smooth muscles and nerve fibers. These may explain why corticosteroids seem more effective than MK-679 in reversing airway hyperresponsiveness.

Clinically, airflow obstruction in asthma is often not fully reversible, even with good management. The mechanisms and factors involved in airway inflammation and remodeling changes need to be determined in future studies, as well as the therapeutical potential of MK-679 for treating asthma.

In summary, our results indicated that both corticosteroid and the selective CysLT receptor antagonists are effective to some degree in preventing airway inflammation and remodeling when they are treated during sensitization. Corticosteroid was found to exert more significant effects than the selective CysLT receptor antagonists on inhibition of airway inflammation. In contrast, compared with corticosteroid, the selective CysLT receptor antagonists have valid outcomes on reversal of airway remodeling in an animal model of human asthma. These may not only suggest CysLTs have different immunopathogenic pathways of influencing on remodeling process that can not be modulated by corticosteroid, but also display time-dependent changes in the structures and functions of pulmonary physiology. Long-term study on airway remodeling and the influence of interventions during different pathogenic periods will continue in the future. MK-679 needs more studies to be used in clinical setting, too.

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