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ORIGINAL ARTICLE

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

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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

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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.[1,2] After awhile, 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.[3,4] 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,[3,5] which makes chronic asthma difficult to control with inhaled β_2 agonist only.[6–8] 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 changes of airway inflammation. They exert most of their bronchoconstrictive and proinflammatory effects through activation of a putative 7-transmembrane domain, G-protein–coupled $CysLT_1$ receptor.[9] CysLTs are also the most potent factors that aggravate airway remodeling through the $CysLT_1$ receptor.[9,10]

It has been shown that administration of corticosteroids during or after the allergen challenge period block the airway inflammatory responses.[5,11,12] However, the effect of corticosteroid on airway remodeling is still controversial. In some mouse asthma models, concomitant treatment of corticosteroids during the allergen challenge period prevented the development of structural changes, including subepithelial fibrosis.[13,14] However, others have reported only limited[14,15] or no effects[5] on reversing the established airway remodeling. In spite of the many challenges on airway remodeling that corticosteroid can exert, it still serves as the major agent in current asthma treatments. To resolve the above issues, we aimed to determine the effects of the selective CysLT receptor antagonist MK-679 (Verlukast), in comparison to those of the corticosteroid prednisolone on airway remodeling in a chronic mouse asthma model. In this study, applications of therapeutic agents were all by gavage, which is rarely performed by other studies[13] but may more closely reflect physiologic conditions in daily life.

Matrix metalloproteinases (MMPs) are a family of extracellular proteases that are responsible for degradation of extracellular matrix during tissue remodeling.[16] Among the family of MMPs, the gelatinases MMP-2 and MMP-9 are specific to denatured collagens and collagen-IV of the basement membrane.[17] Deposition of collagen over the basement membrane is a predominant finding during peribronchial fibrosis of airway remodeling.[16] 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)thio)methyl)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.[18,19] 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.[11,20] 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