Effects of Tyrosine Kinase Inhibitors in a Mouse Model of Asthma

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ABSTRACT

Immune mechanisms in asthma display the importance of tyrosine kinases in the production, activation, proliferation, differentiation, release and survival of several inflammatory players involved. Blocking the activity of these enzymes and the signaling pathways they activate may serve as a strategy for drug development. This study investigated the effect of tyrosine kinase inhibition in asthma, particularly on cell infiltration and eosinophilia. Differential cell counting of mice BAL fluid was performed after OVA challenge and drug treatment. There was no significant decrease in total cell numbers and eosinophil counts observed in the genistein-treated mice. This may be due to the models being too inflamed, thus becoming unresponsive to the treatment or to the unknown potency and non-specific in vivo effects of the drug. Increasing the dose of genistein or reducing time exposure to antigen challenge is proposed to assess the feasibility of tyrosine kinase inhibition in a mouse model of asthma.

INTRODUCTION

Research efforts in discovering new therapies for asthma have been given much attention. To deal with this, it is important to identify the cells and signaling molecules underlying the disease process and how these players cause asthma symptoms. Asthma is characterized by hyperresponsiveness of lung airways, peripheral blood eosinophilia, presence of inflammatory cells predominantly by eosinophils, pathological changes in the lung and by spontaneous increases in airway resistance (Smith, 1989). Murine models have extensively been used to further improve our understanding of asthma. Findings from these models may bring a good outlook in the pharmacological treatment of asthma.

There currently is an increasing attention to examine upstream events occurring in the inflammatory or immune cascade. Signaling through immune receptors and intracellular proteins depend on the catalytic function of protein tyrosine kinases. These enzymes catalyze the intracellular transfer of information and are important for basic cellular processes. The inflammatory response associated with asthma is characterized by the recruitment of eosinophils from the bronchial microcirculation in response to signaling molecules released from other cells or from eosinophils themselves. The effect of tyrosine kinase inhibition on cell infiltration and eosinophilia in asthmatic mouse models was studied. Because the role of tyrosine kinases in the signaling cascade of allergic inflammation has been well documented, these enzymes serve as promising targets to prevent manifestation of asthma events.

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MATERIALS AND METHODS

Sensitization Protocol, Airway Antigen Challenge and Drug Treatment

Male BALB/c mice were immunized intraperitoneally with 20µg of ovalbumin (OVA) and 4mg/mL aluminum hydroxide (alum) gel suspended in 100µL saline. A booster injection of was given on day 14. Immunized mice were challenged with 1% aerosolized OVA in PBS for 20 mins. on days 18, 19 and 20. Control mice were challenged with aerosolized saline. Intraperitoneal injection of genistein (15 mg/kg, 30 mg/kg, 60 mg/kg), 8% dexamethasone (10 mg/kg) and daidzein (30 mg/kg) dissolved in DMSO was given before and after OVA challenge.

Bronchoalveolar Lavage (BAL) Fluid Collection, Differential Cell Counting and Data Analysis

BAL was performed 24 hrs. after the last antigen challenge. A tracheotomy was performed. The trachea was cannulated and PBS was massaged into the lungs. BAL fluid was collected. A total cell count was performed with a hemocytometer. For cytological examination, the isolated BAL cells were counted and cell smears were prepared through cytospin. Cell differentials were examined by counting cytospins of the BAL cells following staining with Liu’s stain. Eosinophil, macrophage, neutrophil and lymphocyte counting was performed on a minimum of 500 cells. One-way ANOVA test, Tukey and Duncan Post-hoc tests were performed for the statistical analysis of the data wherein p ≤ 0.05 was considered significant.

RESULTS

Microscopic examination of the BAL cell numbers and differentials displayed a marked increase of eosinophils and total cell numbers in the airway of OVA-challenged mice and not in saline-challenged and naïve mice (Fig. 1).

Figure 1. Differential cell counting of naïve, challenged and drug-treated mice showing variance in macrophage (M), neutrophil (N), lymphocyte (L) and eosinophil (E) counts.
The majority of BAL cells in saline-challenged and naïve mice were macrophages. OVA challenge induced a substantial increase in macrophages, neutrophils, eosinophils, and lymphocytes in BAL (Fig. 2A).

Genistein had no effect in reducing eosinophilia and cell infiltration whereas dexamethasone (Dex) resulted in both a decrease in cell infiltration and eosinophilia (Fig. 1). DMSO and Daidzen were negative controls. Figure 2B shows the variations in differentials after drug treatment.

**DISCUSSION**

One of the hallmarks of asthma is inflammatory cell infiltration into the bronchoalveolar space (Stenton *et al*., 2002) and eosinophilic inflammation of the airway (Gleich, 2000). Eosinophils are a major source of inflammatory mediators and have been shown to play a pivotal role in allergic inflammation. The degree of their accumulation and activation in the airways correlates with the clinical severity of asthma, signifying a principal role for eosinophils in the pathogenesis of asthma (Walker *et al*., 1991). Mast cells, macrophages, neutrophils, eosinophils and lymphocytes interact and release several inflammatory mediators such as cytokines and chemokines that cause proliferation, differentiation, chemoattraction, adhesion, activation, enhanced survival and degranulation of target cells. Leukocyte activation through signal transduction by immune recognition receptors and cytokine receptors involves tyrosine kinases. Thus, the plethora of tyrosine kinase activities may form the basis for tyrosine kinase inhibition as a novel therapeutic target for allergic inflammation.

In this study, OVA-challenged mice have shown, indeed, an increase in total cell number due to cell infiltration. To test the hypothesis that tyrosine kinase inhibition can reduce eosinophilia, genistein was administered to asthmatic murine models. Genistein is a broad-spectrum tyrosine kinase inhibitor that competes with ATP, forming non-productive enzyme-substrate complexes (Akiyama *et al*., 1987). One assumption that was taken into consideration is that the drug was absorbed in the lungs. This is based on studies that have shown the effectiveness of genistein in attenuating bronchial contraction in antigen-challenged guinea pigs (Tsang and Wong, 2000) and in cancer prevention. The results of this study did not show significant reduction in
eosinophil counts. This could have arisen from the challenged mice being over inflamed and hence, becoming insensitive to the effects of genistein or to the unknown potency and non-specific effects of the drug. In contrast, dexamethasone-treated mice resulted in a substantial decrease in total cell number and reduction of eosinophilia, which may be attributed to the high potency of the drug. Although dexamethasone seems to be an effective treatment for asthma, adverse consequences that entail steroid use should be considered. Administering a larger dose of genistein or modifying the model by decreasing the degree of inflammation via reducing the time for allergen exposure could be done to improve the investigation.

To extend the study on the effect of tyrosine kinase inhibitors on eosinophilia, quantification of cytokine IL-5 and chemokines can be performed. IL-5 is an inflammatory signaling molecule that primarily stimulates proliferation, maturation and differentiation of eosinophils in the bone marrow and their release into the circulation. Recruitment and migration of eosinophils in the airway are induced by several chemokines such as eotaxin and RANTES. The release and effects of cytokines and chemokines are mediated by specific receptors that involve multiple tyrosine kinase signaling pathways. Therefore, inhibiting tyrosine phosphorylation serves as a broad-spectrum target to prevent allergic inflammation.

The initial events associated with generation of signals from the surface receptors in immune cells render tyrosine phosphorylation and the physical interactions of regulatory proteins at the core of the signal transduction cascade. Target inhibition of tyrosine kinase activity can thus intervene with the activation and growth of leukocytes and serve as a potential strategy to alleviate the pathophysiology of asthma. Improvement of the murine models used by reducing time exposure to allergen or increasing the dose of genistein is proposed.

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REFERENCES


