ABSTRACT

Allergic diseases such as rhinitis, eczema, conjunctivitis, food allergy, urticaria etc. are major public health concerns. Approximately, 700 million people all over the world are affected with at least one form of an allergic disorder. Prevalence of allergic diseases is increasing rapidly. Asthma is one of the manifestations of an allergic disorder. Allergen exposure in asthmatics leases to several symptoms like lung inflammation, eosinophilic/neutrophilic infiltration, bronchial hyper-responsiveness and variable airflow obstruction, whereas allergen exposure in an atopic activates the immune cells like mast cells, eosinophils and neutrophils, which release inflammatory mediators including serine proteases such as tryptase, chymase and elastase. Protease aggravates allergic reaction by enhancing histamine mediated response and mast cell mediated responses. Additionally proteases facilitate inflammation along with the induction of inflammatory mediators while serine proteases play an important role in development of an allergic disease, serine protease inhibitors possess therapeutic potential for in allergic airway disease treatment.

Currently available pharmacotherapies like antihistamines, anticholinergic agents, intranasal corticosteroids, mast cell stabilizers, bronchodilators like β-agonists, leukotriene inhibitors etc. provide only symptomatic relief and require regular uses. On stopping or withdrawal of such a therapy may lead to symptom reoccurrence. However, long term use of such drugs is associated with adverse effects; hence there is a need of an alternative therapy. Previous studies have shown that serine proteases have role in allergic airway diseases. Therefore, serine protease inhibitor may be an option for therapy in allergic diseases; some inhibitors have undergone clinical trials. AEBSF is an irreversible serine protease inhibitor with broad specificity and high affinity. It has low toxicity (LD50 of 76mg/kg), excellent stability and high solubility in water (200mg/ml) Owing to these properties; AEBSF may be a suitable molecule to be studied for its therapeutic potential in allergic diseases. The present study is therefore aimed to explore the therapeutic effect of AEBSF in allergic airway disease.

Prophylactic and therapeutic effect of a serine protease inhibitor was evaluated in ovalbumin induced mice model of allergic airway disease. Mice were sensitized
and challenged with ovalbumin. Mice were administered with AEBSF one hour before or after ovalbumin challenge. AEBSF was administered to mice through intranasal route to maximize the local effect of inhibitors in airway of allergic mice. After euthanization, immunological and inflammatory parameters were analyzed in serum BALF and lung sample of mice.

Ovalbumin immunization to mice induced cellular infiltration in lungs with increase in serum IgE and IgG1 as well as Th2 cytokines in BALF. AEBSF treatment to ovalbumin immunized mice significantly reduced cellular infiltration including eosinophils and neutrophils. AEBSF given to mice either before or after allergen challenge reduced specific serum IgE and IgG1. Additionally, on AEBSF treatment Th2 cytokines levels were decreased along with increment in IL-10 levels, indicating shift of Th2 immune response to regulatory T cell response. AEBSF could reduce the ovalbumin induced oxidative stress in lungs. Lung histology showed AEBSF administration to mice suppressed ovalbumin induced lung inflammation and mucus secretion. In conclusion, AEBSF significantly reduces allergic airway inflammation and has potential for adjunct therapy in allergic diseases including asthma.

Ovalbumin is a purified allergen; however, in nature allergens are existing in the form of complex mixture. To be used in therapy, therapeutic effects of AEBSF need to be determined against different allergen/allergen extract. The effect of AEBSF was evaluated in CE (extract possess multiple allergen including protease) and Per a 10 (purified protease allergen) induced mice model of airway inflammatory disease.

CE or Per a 10 immunization increased the airway resistance in mice. AEBSF given to mice before allergen challenge could reduce the airway resistance more effectively than treatment given after challenge possibly because of mast cell stabilizing property of protease inhibitor. CE or Per a10 immunization to mice induced cellular infiltration, Th2 cytokines, airway inflammation and mucus secretion in lungs. The present study showed that AEBSF treatment effectively reduced cellular infiltration in CE and Per a 10 immunized mice. AEBSF administration could also lower the specific IgE and IgG1 in both CE and Per a 10 immunized mice. Allergen immunization increased Th2 cytokines in BALF which on AEBSF treatment reduced significantly. AEBSF administration also reduced airway inflammation, mucus plugging and mast cell accumulation. AEBSF treatment to mice before challenge (10
μg) and after challenge (2 μg and 10 μg) showed significant decrease in 8-isoprostane level in BALF. Same treatment also lowered the level of ROS in BAL cell pellet indicated reduction in oxidative stress in lung. Significant reductions in NF-κB activation in lungs indicated the therapeutic potential of AEBSF in allergic airway diseases.

The study demonstrated that AEBSF will be useful as an add-on therapy for allergic airway diseases. Moreover, the therapeutic effect of AEBSF treatment in mice indicated that the effect of treatment is independent of protease activity of allergen. The therapeutic effect of serine protease inhibitor targets on endogenous proteases.

Activation of kinases PI3K, ERK1/2 and p38 are important for the expression of inflammatory mediators in allergic diseases. These kinases can be targeted for the management of allergic disorders. Moreover, protease inhibitors are also considered as potential therapy which blocks the protease to lower the release of inflammatory mediators. Both serine protease inhibitors and kinase inhibitors have therapeutic potential in allergic diseases, but combination may have better therapeutic potential. The therapeutic effect of serine protease inhibitor in combination with PI3K, ERK1/2 and the p38 kinase inhibitor were evaluated in CE induced mice model of allergic disease.

CE immunization to mice activated PI3K, ERK1/2 and p38 kinase in lung tissue and induced inflammatory mediators. Western blot confirmed that kinase inhibitors alone or in combination with AEBSF lowered the activation of corresponding kinase. Enhanced effect of reduction in cellular infiltration including eosinophils was observed on treatment of AEBSF in combination with iPI3K and the iERK1/2 compared to monotherapy. Combination of AEBSF with iPI3K and the iERK1/2 augment the reduction of Th2 cytokine in comparison to monotherapy in mice. Combination of AEBSF with iERK1/2 and the iPI3K showed enhanced level of IL-12 compared to monotherapy. The augmented effect of AEBSF to attenuate the Th2 cytokine in combination with iERK1/2 and the iPI3K was probably attributed to increased level of IL-12.

In conclusion, PI3K inhibitor had the best therapeutic effect with serine protease inhibitor to attenuate allergic diseases and has a potential to be used as therapy in allergic disease.