The Effect of BTP on the Development of Allergic Asthma in Mice

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Abstract

Allergic Asthma is an inflammatory disease that causes an increase in airway hyper-responsiveness to a variety of stimuli. Of the many treatments available today, immunosuppressive drugs are a promising option. Unfortunately, many of these drugs have dangerous side effects. In a recent search for safer drugs, a series of 3,5-bistrifluoromethyl pyrazole (BTP) derivatives have been found to be effective immunosuppressants. In these experiments, we have determined whether BTP affects the development of symptoms of allergic asthma in a murine model of this disease.

Introduction

Allergic Asthma is an inflammatory disease that causes an increase in airway hyper-responsiveness to a variety of stimuli. This inflammation causes breathlessness, chest tightness, wheezing, and coughing, particularly at night or in the early morning (1). Thirty million Americans are living with asthma (2). 12% of all asthma sufferers are children (2). This disease causes much distress and anxiety to both adults and children living with the asthma. Because most episodes occur during the night, many asthma sufferers have trouble functioning properly during the day due to interrupted sleep.

Allergic Asthma is a T helper type-2 (Th2) mediated disease. It is thought to result from the expansion of $CD4^+$ T cells that produce cytokines Interleukin 4 (IL-4) and IL-5. IL-13 shares a receptor component with IL-4 and is necessary for the expression of allergic asthma (3). When a person is first exposed to an allergen, Th2 cells develop and then produce IL-4. IL-4 then causes the production of anti-allergen IgE antibodies. These antibodies then attach themselves to mast cells. Once enough antibodies are present on the mast cells, the mast cells activate. Their contents, mainly histamine, are released, bringing with it an allergic reaction (4).

The disease also has detrimental effects on the lungs as well. A great deal of inflammation is present in the lungs and a large degree of airway hyper-responsiveness. Hyper-responsiveness is an abnormal response of the lungs to minor stimulants (5, see Figure 1). There is a substantial amount of mucous produced as a result.

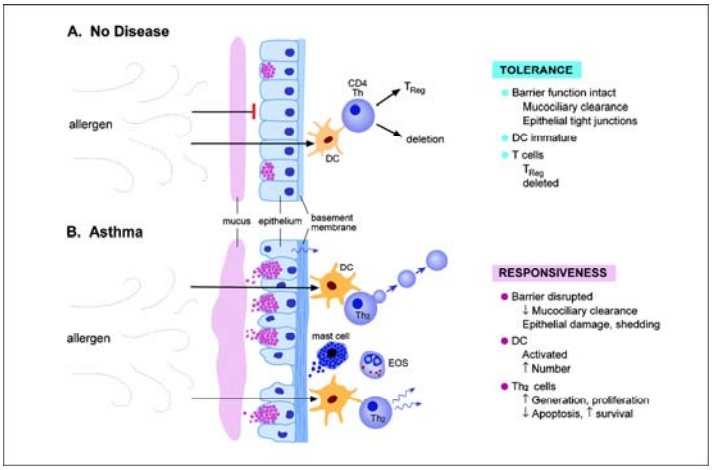

Figure 1. Fate of the airways after exposure to inhaled allergen. (A) In the absence of inflammation, inhaled allergen does not induce an inflammatory response because protective features of the respiratory tract insure immune tolerance. (B) In asthma, the inflamed airways promote immune responsiveness. Inhaled allergen stimulates further Th2 cell activation, activation of inflammatory cells, release of inflammatory mediators, and epithelial damage, thus leading to persistent inflammation and airway remodeling (6).

There is no cure for allergic asthma, but many treatments exist, most of which have very dangerous side effects. Most medications consist of glucocorticoids, which have been proven to bring on situations ranging in severity from cosmetic to life-threatening situations. Some potential side effects include gastric hemorrhage, Cushing's syndrome, glaucoma, hypertension, diabetes, growth retardation, and many more (7).

In a search for safer drugs, 3,5-Bistrifluoromethyl pyrazole (BTP) has been identified to inhibit the proliferation and gene transcription of both Th-1 and Th-2 cytokines (8). Because allergic asthma is a Th-2 mediated disease, we hypothesize that BTP should inhibit the production of the cytokines that produce the symptoms of allergic asthma, therefore reducing the symptoms of the disease.

To test this hypothesis, a murine model of the disease was used. The murine model has many similarities with the human form of the disease. Both models develop airway hyper-responsiveness and indicate the $CD4^+$ Th-2 cells are responsible for the onset of the symptoms of the disease.

Materials and Methods

Animals. T cell receptor (TCR) transgenic mice (OT-II) specific for ovalbumin (OVA) on a C57BL/6 background were used for these experiments. All guidelines set by the Institutional Animal Care and Use Committee of the Pennsylvania State University were followed.

Chemicals. Ovalbumin was obtained from Fisher Biochemicals, 3,5-bistrifluoromethyl pyrazole (BTP) was synthesized by Laurie Mottram (Department of Chemistry, Penn State University), methylcholine was from Sigma, RPMI 1640 media from Media Tech, ^{3}H-thymidine from ICN, PMA and Ionomyocin (ION), both from Sigma.

Induction of Allergic Asthma and Administration of Immunosuppressant Drug. OT-II transgenic mice were induced to develop allergic asthma as follows: Mice are anesthetized by inhaling Isoflurane, then intranasal doses of 50 μl OVA (at 5 mg/ml In PBS) were then administered. In animals treated with BTP, 10 μM of BTP in 50 μL sterile PBS was introduced intranasally to OT-II mice everyday for 4 days. Subsequent doses of 50 μl OVA were also given on these days. Twenty-four hours after the last treatment, airway hyper-responsiveness was tested via mechanical ventilation. Control mice were given 50 μl doses of OVA without BTP, but otherwise treated in the same manner as the mice given the drug.

Mechanical Ventilation. Mice were injected with pentobarbital (90 mg/Kg) until general anesthesia was reached. A cannula was placed in the trachea and secured. Mice were then placed on a mechanical ventilator to measure airway hyper-responsiveness. During the ventilation process, mice were given increasing doses of methylcholine (contractile agent) at 5-minute intervals through a nebulizer. Each dose was double the concentration of the previous dose, beginning at 1 mg/ml and ending at 100 mg/ml.

Lung Pathology. Immediately following the mechanical ventilation procedure, both lungs were removed from each mouse and placed in para-formaldehyde. Lungs were then sectioned by the Animal Diagnostic Laboratory at Penn State and stained with H&E for determination of airway inflammation. Lungs were also stained with PAS to determine production of mucous.

Spleen cell Proliferation. Splenic T cell proliferation was determined in response to OVA in vitro as follows: In protocol I, splenocytes from mice exposed to BTP were tested for proliferative response to OVA in vitro by putting cells in single cell suspension, followed by red blood cell lysis. The remaining cells were placed in a 96 well flat bottom plate (100 μl cell/media solution per well). Added to the cells are one of the following: media alone, PMA/ION, 10 μM OVA, or 100 μM OVA, each in 100 μl doses. Cells are then allowed to incubate for 72 hours. After this time, 25 μl of ^{3}H-thymidine is added to each well. Cells are allowed to incubate for another 24 hours. Cells are then cooled and harvested. T-cell counts are determined via liquid scintillation. In the second protocol, OT-II mice with no prior treatments were sacrificed; splenic T cell proliferation to OVA was determined in the presence or absence of BTP. Cells were again put in single cell

suspension and red blood cells lysed. Cell/media solution was then distributed in a 96 well flat bottom plate (100 µl of cell solution per well). The following was added to each well: media alone, PMA/ION, OVA, and/or BTP. BTP was present in four concentrations: 10 µM, 100 nM, 10 nM, and 1 nM. Proliferation was determined by ^{3}H-thymidine and scintillation counting.

Results

Effect of BTP on airway hyper-responsiveness. BTP has been shown to reduce cytokine production by T cells in vitro (8). We therefore used BTP in a murine model of the disease to test it's effectiveness in reducing the symptoms of the disease. Mice were challenged with OVA (a model allergen) and half of the mice were treated intranasally with the BTP drug, prior to being challenged with OVA. Airway hyper-responsiveness was then tested using mechanical ventilation in which the contractile agent methylcholine was used. Resulting airway responsiveness values were then plotted. The results showed that mice primed with OVA developed airways hyper-responsiveness (See Figure 2). However, mice treated with the BTP drug did not show any statistically significant difference to those not treated with the drug.

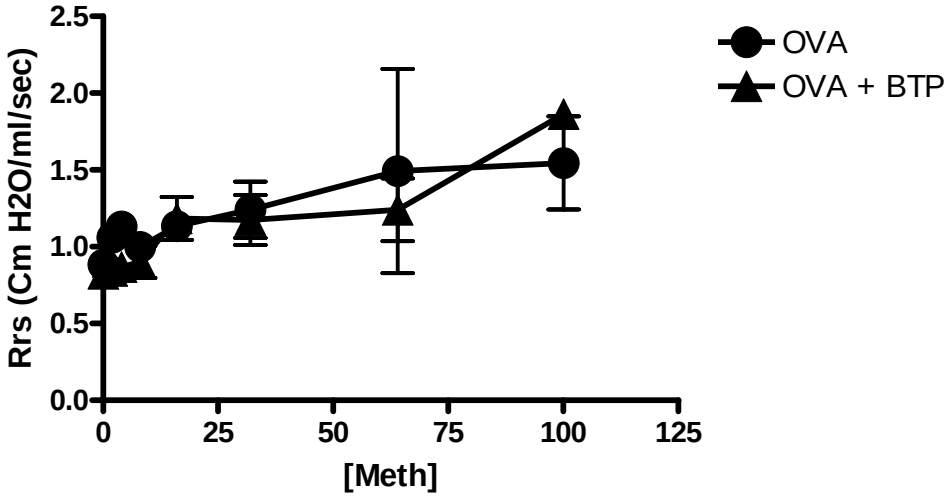

Figure 2. Effect of BTP on development of AHR. Mice were treated first with BTP and then challenged with OVA allergen for four days and compared to mice not given BTP. The BTP drug seemed to be effective when lower levels of methylcholine exposure (below 75 mg/ml) was analyzed.

Effect of BTP on lung pathology. The lungs were sectioned and stained with H&E (for analysis of lung inflammation) and PAS (for analysis of mucous production). They were then observed under a microscope to determine the amount of airway inflammation and mucus production. In the lungs of the mice treated with OVA alone, the effect of the allergen was clear. There was a great deal of cell infiltration, airway hyper-responsiveness, and much mucus is present. The bronchiole itself looks very constricted (see Figure 3A). In the lungs of the mice treated with the drug, symptoms consistent with OVA exposure were present, but not nearly as severe as the mice who were not treated

with the drug. There were some signs of constriction, hyper-responsiveness, and mucus as well, but less bronchioles in these mice seem to be affected (see Figure 3B).

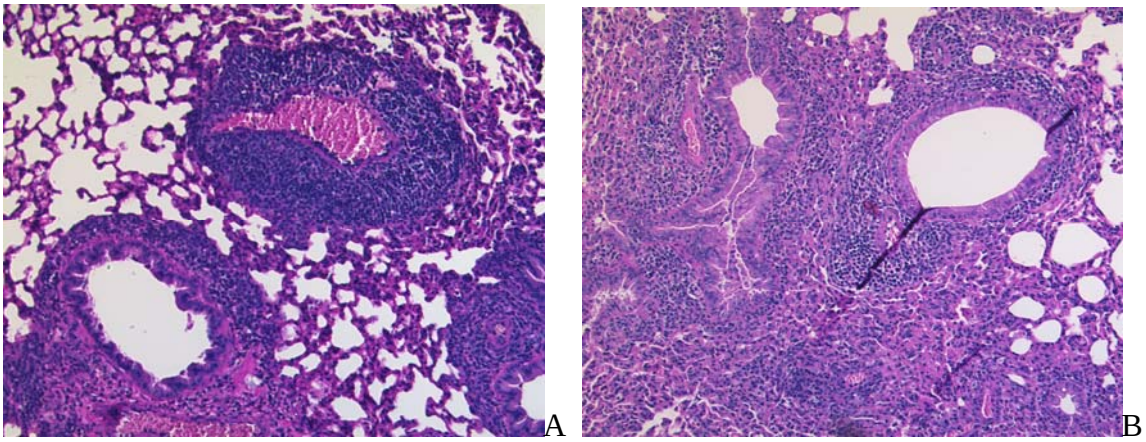

Figure 3. Effect of BTP on airway inflammation and mucous production in lungs of OVA exposed mice. Immediately following mechanical ventilation, lungs were removed. Lungs were later sectioned and stained with H&E and PBS. In mice exposed to OVA allergen (A) bronchical constriction is present. There is also a significant amount of mucus present. There is also much cell infiltration, indicating hyper-responsiveness. In mice exposed to the BTP drug (B) some constriction and mucus is also present, but at a much lesser extent than mice with allergen alone. More of the bronchioles are unaffected.

Effect of BTP on the proliferation of CD4$^+$ T cells. Allergic Asthma is caused by the proliferation of T cells when an allergen is introduced (5). We tested the effect of BTP on T cell proliferation in vitro. Mice with no prior treatments were sacrificed and spleens removed. Splenic proliferation to the OVA allergen was determined in the presence and absence of BTP. In cells treated with OVA alone, cells had high levels of proliferation. By contrast, cells treated with different concentrations of BTP did not proliferate (see Figure 4).

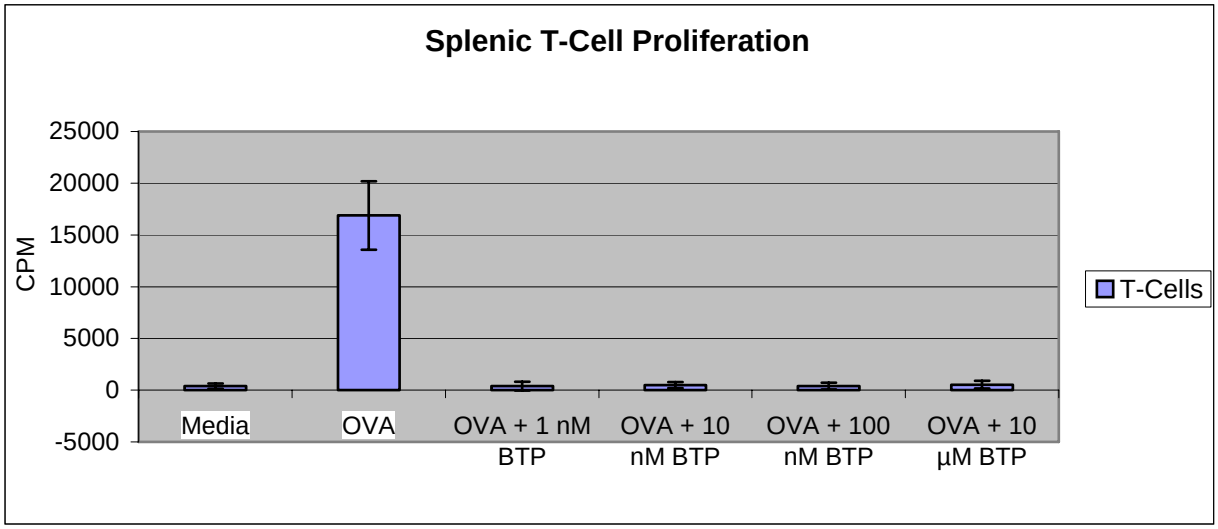

Figure 4. **Effect of BTP on antigen specific T cell proliferation.** Proliferation is higher in cells exposed to OVA alone. Proliferation was inhibited in cells treated with various concentration of BTP.

Conclusions

Reduced Airway Hyper-responsiveness. Mice exposed to OVA allergen showed increased AHR. Mice treated with the drug showed lower levels of AHR only when challenged with lower levels of methacholine. This suggests that BTP may be able to reduce the amount of AHR, even after exposure to OVA allergen. However, the amount of BTP may have to be increased to see effects at higher methacholine concentrations.

Histology. Lungs of mice exposed to the OVA allergen show much cell infiltration and mucus production. Lungs of the mice that were treated with the drug show less mucus and infiltration. This suggests that BTP improves lung condition by reducing the production of mucus and cell infiltration.

Inhibition of antigen specific T cell proliferation. Cell proliferation of T cells from mice exposed to OVA are much higher than the cells exposed to OVA and treated with the BTP drug. This demonstrates that the proliferation of the antigen specific T cells is inhibited, which would reduce the symptoms of the disease.

Discussion

This experiment shows much promise for those who are suffering from allergic asthma. This research data shows that BTP has a positive effect on the symptoms of the disease. BTP inhibits T cell proliferation, therefore greatly reducing the symptoms. These findings could lead to discovery of better and safer treatments for allergic asthma.

The results obtained from this experiment suggested that BTP could be an effective treatment for Allergic Asthma. However, in some experiments, the drug was less effective than originally anticipated. This could have been attributed to ineffective OVA or the concentration of the BTP drug.

Experiments with BTP are ongoing to further analyze its effect on the symptoms of Allergic Asthma. In the future, it would be interesting to administer the drug in different ways to determine if symptoms are affected any differently. It would also be beneficial to test the effectiveness of BTP in various concentrations to see which is most effective.

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