The Role of PPAR-γ on Regulating the Symptoms of Allergic Asthma

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Abstract

Allergic Asthma is a pulmonary disease that causes difficulty breathing due to airway obstruction, inflammatory infiltrates, and hypersensitivity to allergens and non-specific stimuli. There is no cure, but there are treatments, many of which cause detrimental side-effects. A ligand for the PPAR-γ has been shown to have an anti-inflammatory response. We are therefore determining whether the PPAR-γ ligand, GW1929, can affect the symptoms of allergic asthma in a murine model of the disease. We discovered that GW1929 does have some therapeutic effects on allergic asthmatic mice. This includes a slightly lower eosinophil infiltration, and a slightly better breathing ability.

Introduction

Allergic asthma is a pulmonary disease that causes difficulty breathing due to airway obstruction, inflammatory infiltrates, and hyper-reactivity to allergens and non-specific stimuli. Twenty-six million Americans have asthma; 8.6 million of those 26 million are under 18. This disease can cause many discomforts in the lives of children and adults. In many children, allergic asthma may cause behavior disorders. Children with this disease have trouble sleeping because the difficulty breathing during the night causes interrupted sleep. As a result, they are fatigued during the day and have trouble concentrating in school and interacting with fellow classmates [1]. In adult life, the disease can be impeding as well. Sleep deprivation is the common factor, but as adults, it causes difficulty concentrating at work and adds unneeded stress to the life of the patient and their family.

There is no cure for the disease, and many treatments today can cause detrimental side effects. Current treatments for allergic asthma may cause conditions such as osteoporosis, candidasis, Churg-Straus Syndrome or Cushing's syndrome [2]. Osteoporosis, Churg-Straus, and Cushing's are all caused by an excess of glucocorticoid, of which most treatments today are composed [3, 4].

The synthetic molecule GW1929 is a tyrosine based potent agonist of the Peroxisome Proliferator-Activated Receptor-γ [5]. GW1929 has a very high affinity for the receptor PPAR-γ. This high affinity creates higher potency than natural ligands which don't have this high affinity. PPAR-γ is a nuclear receptor and a transcription factor, that is activated by specific ligands. Once the ligand binds the receptor, the receptor is activated, then moves into the nucleus and becomes a transcription factor,

where it influences gene expression. Thus, activation of a target gene transcription depends on a ligand binding to the receptor. PPAR-γ agonists have been proposed to reduce cytokine production by inhibiting pro-inflammatory transcription factors, therefore modulating the inflammatory response [6, 7]. Peroxisome proliferator-activated receptor-γ agonists (ligands) have been shown to be anti-inflammatory and may be able to relieve the symptoms of allergic asthma.

In this report, we use GW1929 to determine whether a PPAR-γ ligand can affect the symptoms of allergic asthma in a murine model of this disease. The mouse is commonly used to model allergic asthma as it mimics many of the properties of the human disease. In addition, there are many immunological reagents and cell markers that are available and have been defined that make it simpler to study the inflammatory cascade in the mouse [8]. We use the mouse as it is a good species to use for measuring inflammatory responses because the immune response is very similar to that of humans [9, 10].

Materials and Methods

Mice – The mice we used were wild type Balb/c mice originally obtained from Jackson Laboratory (Bar Harbor, ME) and bred in the animal facility at Penn State University (PSU). Mice were fed water and food *ad libitum* and cared for according to institution guidelines. The experimental protocol was approved by the IACUC of PSU.

Experimental Design- The experimental design consisted of four groups of mice with five mice per group. Group I was not induced to develop allergic asthma and was not to be treated with the ligand. This was our control group and was used as a reference point to guide our data analysis. Group II was primed for allergic asthma, but was not treated with the ligand. This group was compared to group I (the control group) to determine the difference in lung function when allergic asthma is induced. This information helped us determine how effective the ligand is in relieving symptoms of allergic asthma. The third group (group III) was primed for allergic asthma and treated with the ligand. This group was compared to the control group to determine how close the lung function is to normal. They were also compared to group II to determine how much better the lungs function with the ligand than without. There was also a fourth group. This group (group IV) was not primed to develop allergic asthma and was treated with the ligand. This group was compared to the control group to determine if the ligand causes any side effects.

Priming – Mice were primed with Ovalbumin (OVA) (Sigma, St. Louis, MO) complexed with Imject Alum (Alum) (Pierce, Rockford, IL) in phosphate buffered saline (PBS). We used the Ovalbumin as the antigen or allergen, and the imject alum as an adjuvant. The adjuvant enhances the immune response. The mice were primed with 222μg of 10μg Ovalbumin and 1mg imject alum solution intra-peritoneally (IP) on days 0 and 5 of the study. Mice that were not to contract the disease were given PBS and imject alum on days 0 and 5.

Challenge – On days 12 to15 mice that were primed with the OVA/Alum complex were given OVA in PBS solution intra-nasally (IN). This causes a local allergic reaction in the lungs resembling allergic asthma. Mice that were not primed to develop allergic asthma were given PBS intra-nasally on days 12 through 15. Mice primed and challenged experience increased inflammatory cell infiltration into the lung, thickening of epithelial cells in the bronchioles of the lung, mucus secretion, and increases in IgE in the serum [11]. These are hallmark symptoms of allergic asthma.

Treatment - Each mouse received 1μg of .5μgGW1929 and .5μg PBS intra-peritoneally per day on days 12 through 15. The mice that did not receive the ligand were given 1μg PBS on these days.

Collection of Data - Data was collected using a Buxco whole body plethysmograph on day 16. This machine measures lung function by calculating pressure changes inside a chamber where the live mouse is held. The machine is able to accurately distinguish between applicable breaths and breaths when the animal is just sniffing around. When the animal has made a certain number of applicable breaths the machine averages these breaths and tells us the average volume of air inspired and expired. The animal is exposed to different concentrations of aerosolized metylcholine in the chamber to gauge the reactions to the stimulant. A mouse without allergic asthma will have a weaker reaction to the methylcholine than one with allergic asthma. Bronchial responsiveness to methylcholine relates closely to the presence and severity of asthma [12]. Analyzing each mouse in the plethysmograph can take up to an hour per mouse. Since there were five mice to each group, we measured one group per day, staggering the experiment by staggering the priming and treatment. The plethysmograph was calibrated before testing each group of mice.

Histology – One lung from each mouse was harvested and fixed in formaldehyde on day 16. The lungs were embedded and sectioned to be stained with H&E for analysis. The slides were analyzed and graded by severity of the disease on a scale from one to four. Sections of the lung that were considered average for each group are displayed and discussed below.

Determination of eosinophils in lungs- For each mouse, one lung was harvested and dissociated using collagenase (150U/ml). These populations were analyzed using an Advia 1200 Hematology System (Bayer, Norwood, MA).

Results

Effect of a PPAR-γ ligand on lung pathology-A hallmark symptom of allergic asthma is leukocyte infiltration in the lungs, and thickening of the cell walls lining the bronchioles. By priming mice with OVA/Alum and challenging them with IN OVA this same response is produced in the lungs (Figure 1C). PPAR-γ ligands have been shown to reduce this infiltration [6, 7]. Therefore the lungs of the mice were harvested and one lung was fixed in formaldehyde to be embedded and sectioned then stained with H&E. These lungs were analyzed for infiltration and thickening of cell walls lining the

bronchioles. The infiltration of leukocytes in lungs of mice that were primed with OVA/Alum and challenged with IN OVA show increased infiltration in the lung, and thickening of the lining of the bronchiole walls (Figure 1C). Mice that were primed and challenged this way and treated with GW1929 show significantly less infiltration in the lung and little thickening of the cell walls lining the bronchioles (Figure 1D). Mice that were not induced to develop the disease did not show these symptoms, even when treated with GW1929 (Figure 1B).

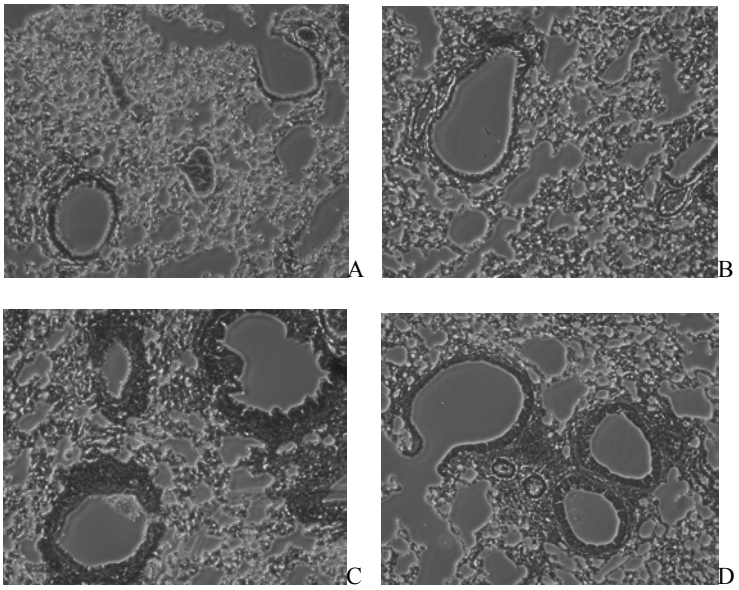

Figure 1. On day 16 mice were sacrificed and the lungs were fixed, paraffin-embedded, sectioned and stained with H&E. Balb/c mice that were primed with PBS/Alum and challenged with PBS show no infiltration or thickening of the cell wall lining the bronchioles (A). Mice primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 show increased infiltration in the lung and thickening of the epithelial walls lining the bronchioles (C). Mice that were primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 and treated with GW1929 on days 12-15 show significantly less infiltration, and little thickening of the cell walls lining the bronchioles (D). Mice that were primed with PBS/Alum and challenged with PBS and treated with GW1929 show no infiltration and no thickening of the cell walls lining the bronchioles (B).

Effect of GW1929 treatment on Airway Hyper responsiveness (AHR) – Ligands for PPARγ have been reported to reduce inflammation [6, 7]**.** This suggests that PPARγ ligands may be useful in treating allergic inflammation. We therefore used the high affinity PPARγ ligand GW1929 in a murine model of allergic asthma to determine if PPARγ ligands could affect the severity of symptoms in this disease. One hallmark of allergic asthma is difficulty exhaling. The Buxco whole body plethysmograph measures the animal's ability to exhale in response to exposure to the broncho-constrictor methycholine. This value is reported as PenH, a unit less number. Groups of mice were primed and challenged with OVA, a model allergen, and some groups were treated with .5µg GW1929 daily during intranasal exposure. Airway hyper responsiveness to exposure to methylcholine was then determined using the Buxco plethysmograph 24 hours after the final intranasal challenge. The resulting PenH values were averaged and plotted. The results show that as expected, mice that were not primed to develop allergic asthma responded to increasing concentrations of methylcholine (Figure 2). In addition,

mice primed to develop allergic asthma developed severe AHR. Treatment of mice primed to develop allergic asthma with GW1929 lead to a slight decrease in AHR. Finally treatment of mice that had not been primed resulted in normal responses to methycholine exposure.

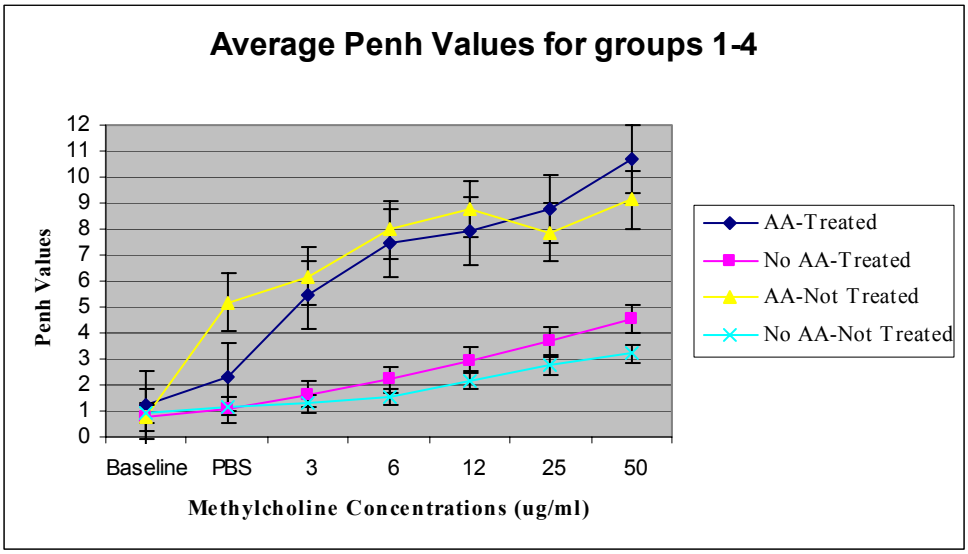

Figure 2. Mice that were primed with OVA/Alum on days 0 and 5 then challenged with IN OVA on days 12-15 show a significantly higher PenH value than mice that were primed with PBS/Alum and challenged with PBS. PenH values were measured on day 16 in a Buxco Whole Body Plethysmograph. At low levels of methylcholine concentrations mice that were treated with GW1929 and primed with OVA/Alum then Challenged with IN OVA have significantly lower PenH values than mice that were not treated.

Effect of GW1929 treatment on lung Eosinophilia – Eosinophilia is a hallmark of allergic asthma. Eosinophils are attracted to the bronchial wall and lumen by an allergen IgE reaction [13]. This causes accumulation of eosinophils in the bronchial wall and lumen during allergic asthma, and is a good marker of allergic asthma [14]. The lungs of the mice from the different experimental groups were harvested and eosinophils counted using an Advia 1200 Hematology System. The averages were taken for each group and plotted below. There was a downward trend in percent eosinophils from mice that were not treated to mice that were. This suggests that less of an infiltration occurred in mice that were treated with the ligand (Figure 3).

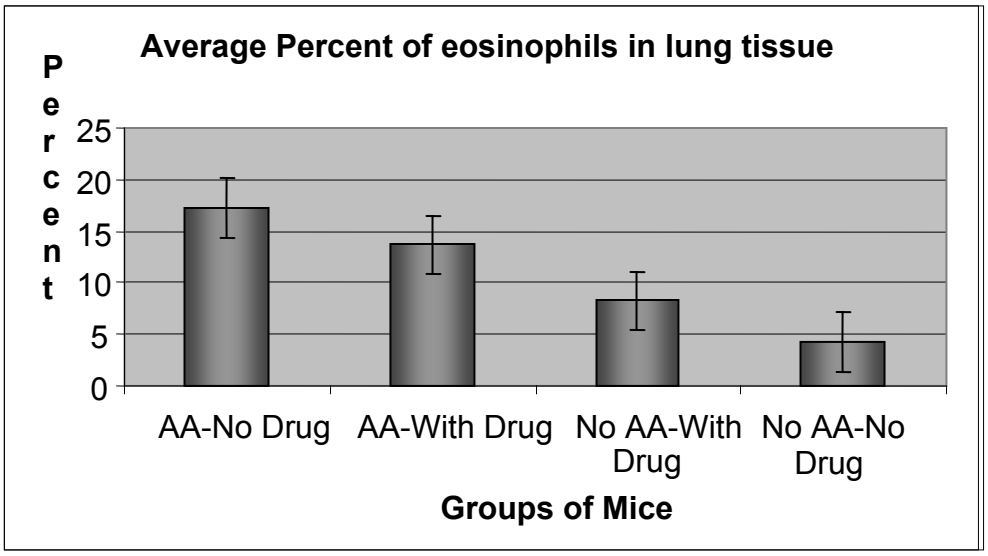

Figure 3. Mice that were primed with OVA/Alum then challenged with IN OVA have a significantly higher percent of eosinophils in the lungs than mice that were primed with PBS/Alum then challenged with PBS. The mice that were treated with GW1929, primed with OVA/Alum and challenged with IN OVA do show a downward trend in percent eosinophils in the lungs.

Conclusions

Reduced difficulty exhaling – The data above suggests that mice treated with a PPAR-γ agonist, such as GW1929, have slightly less difficulty exhaling. This implies that at least some of the symptoms of allergic asthma were relieved.

Reduced Eosinophila – Lower amounts of eosinophils in the lungs of the mice that were treated with the ligand and developed allergic asthma suggests that there was less infiltration. If there is less infiltration this would mean damage was done to the airways, therefore reducing the difficulty breathing.

Histology – The lungs of the mice that were not induced with allergic asthma clearly have no infiltration of eosinophils into the lung. Mice that were induced to develop the disease and treated with the ligand show similar lung pathology to those that were not. This suggests that the ligand helped to reduce the infiltration in the lungs.

Discussion

This experiment has many potential benefits for allergic asthma patients. This new treatment could relieve them of their allergic asthma symptoms without causing them any severe side-effects. The above data shows that GW1929 does have a therapeutic effect on allergic asthma, and therefore may hold exciting new clues to treating this disease.

We are currently conducting experiments to further analyze the effectiveness of GW1929 on relieving symptoms of allergic asthma. We are administering the treatment in different ways to determine if the symptoms are affected differently.

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