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ORIGINAL ARTICLE

Scrutiny of Role of Indolamine Inhibition in Asthma Using Animal Model

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ABSTRACT

Background: Asthma is a debilitating chronic inflammatory disease of airway with frequent episodes of exacerbations. It confers great burden on health systems due to associated symptoms like cough, wheeze, chest tightness and shortness of breath. We have explored the effect of an important indoleamine inhibitor on important asthmatic parameters in this study.
Aim: Exploration of an alternative pathway of indoleamine inhibition for the treatment of asthma in future.
Methods: Forty Wester rats were divided into four groups (normal control, disease control, prednisolone treated, methyltryptophan treated) with ten animals each. Blood samples were collected for eosinophil count. PCR was performed for measuring the IL-5 expression of the lung tissue.
Results:1MT significantly reduced the eosinophil levels and mRNA expression of IL-5 through indoleamine inhibition.
Conclusion: The pathway of indoleamine inhibition has appeared significant for reducing the inflammation and allergic response in asthma. Further exploration of this pathway can open new dynamics for asthma treatment.
Keywords: Asthma, indoleamines, cytokines, eosinophils, inflammation

INTRODUCTION

Asthma is a chronic inflammatory disease of airway with frequent episodes of acute exacerbations [1]. It affects nearly 300 million people worldwide and around 15000 people die from it on the continent of Europe alone every year [2]. A study conducted on Pakistani population revealed that the prevalence of allergic asthma was 19.36% in general population [3]. Common symptoms associated with this disease comprise of wheezing, cough, chest tightness and shortness of breath [1]. Pathologically, asthma involves remodeling of the airway and the bronchial walls due to intense inflammation which is followed by fibrosis of subepithelial layer, angiogenesis and hypertrophy of mucus producing glands [1].

Most of the drugs used for the treatment of asthma are centered on immune modulation and have serious adverse effects based on their long term use particularly steroids [4]. Anti asthmatic drugs include corticosteroids, β2 agonists, leukotriene antagonists, methylxanthines and IgE antibodies [5]. Inhaled and oral corticosteroids are the most frequently prescribed drugs for the treatment and maintenance therapy of asthma [6]. Despite of the fact that corticosteroids are recommended in controller treatment of asthma they are still not very effective in acute response [7]. When used in patients over a prolonged period of time, they produce wide variety of adverse effects including hyperglycemia, osteoporosis, cushing disease, hirsutism and hypertension with many others [8]. Hence, asthma is a major candidate for ongoing researches in the field of medicine owing to its significant prevalence worldwide and a treatment line with unsatisfactory safety profile. Keeping in view the above mentioned reasons we have chosen prednisolone as the comparative drug for disease model of asthma against our compound.

1 Methyl-DL-tryptophan is an important immunomodulator of increasing importance in research field and acts by inhibiting indoleamine [9]. It prevents the cytotoxic activity of T cells thus has an immunosuppressive effect[9]. 1MT has emerging protective role aginst various inflammatory conditions which has served as a background to explore its effect on asthma [10].

This study explores the effects of 1-MT in comparison to methylprednisolone upon interleukin5 and eosinophils in asthmatic model of rats. The study might help in exploration of new dimensions for the treatment of asthma by highlighting the role of 1-MT against these two important parameters.

METHODOLOGY

Chemicals: **Methylprednisolone 15mg/kg was dissolved in distilled water and administered intraperitoneally[11].**

1 methyl-dl-tryptophan(1-MT)9mg/kg was dissolved in ammonium chloride and pH was adjusted to 7 with sodium hydroxide . This drug was injected intraperitoneally too[12].

Sample size: **Sample size was calculated using following formula**

$$n=(Z_{1-\beta}+Z_{1-\alpha/2})^2(\sigma_1^2+\sigma_2^2)$$
$$(\mu_1-\mu_2)^2$$

Here n came out to be 8 hence sample size was taken as 8 per group.

Animals: Healthy male Westar rats, 6 –8weeks old weighing 180-200 g were included in the study.
Sampling technique: Simple random sampling technique was used for grouping the animals by balloting method.
Groups of Animals
Group I (Negative control group): Healthy rats, sham sensitized and challenged with PBS were given 0.5ml PBS intraperitoneally for seven consecutive days (14-21)[12].
Group II (Diseased group): Rats were sensitized on day 1 and 7 and then challenged with ovalbumin. Later they were given 0.5ml PBS intraperitoneally for seven consecutive days (14-21) [12].
Group III (Experimental group I): Rats were sensitized on day 0, 7and then challenged with ovalbumin for seven days, at the same time they were treated with methylprednisolone 15mg/kg for seven consecutive days (14-21).
Group IV (Experimental group II): Rats were sensitized on day 0,7 and then challenged with ovalbumin for 7 days ,at the same time they were treated with 1-methyl-dl-tryptophan(1-MT)9 mg/kg for seven consecutive days (1421).

Euthanization: Light ether vapor anesthesia was given to animals on day 22. Blood was collected via cardiac puncture.

Lung tissue was also preserved for RNA extraction for IL-5 in trizol [13].

Parameters Eosinophils in Blood:-
Eosinophil count in blood was determined using automated hematology analyzer[14].

Determination of mRNA expression levels of IL-5
Total RNA was extracted from lung tissue using commercially available kit. RNA concentration was quantified by measuring optical density and the integrity of RNA was confirmed by NANO Drop. cDNA was prepared and used to perform PCR. Suitable primer was designed by using PRIMER-3 software [15].

Statistical Analysis: Statistical analysis was done by Graph Pad version 6. Mean ± SD was given for quantitative variables. One-way ANOVA was applied to determine any significance differences between the means. Post hocTukey's test was applied to determine which specific groups differed from each other. P-value ≤ 0.05 was considered as statistically significant.

RESULTS

Eosinophils levels: Prednisolone was able to reduce the number of eosinophils found in the blood significantly however the reduction caused by 1-methyltryptophan was not significant.

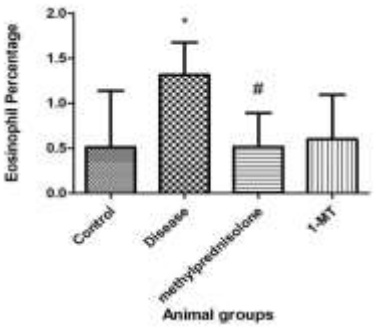

Figure 1: Eosinophil percentage in all the four groups. No outlier was removed from the data. * shows P <.05, significantly different from the normal control. # shows P < .05, significantly different from disease control. Data was analyzed by one way ANOVA followed by post hoc Tukey's test.

Interleukin-5: Both methylprednisolone and 1-methyl tryptophan had significantly reduced the interleukin-5

levels. The reduction caused by 1-MT was as significant as that caused by methylprednisolone.

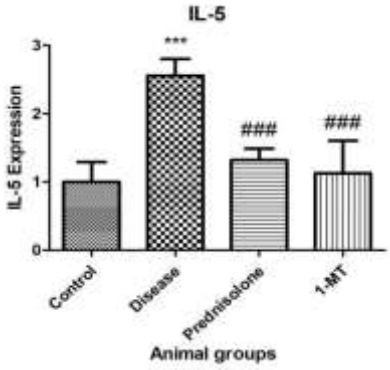

Figure 2: Fold change of interleukin 5 among all the four groups. No outlier was removed from the data. * shows P <.05, significantly different from the normal control. # shows P < .05, significantly different from disease control. Data was analyzed by one way ANOVA followed by post hoc Tukey's test.

DISCUSSION

Asthma is a prevalent disease worldwide and confers a major burden on health systems on Pakistan as well[3]. It has been treated with corticosteroids for a long period of time in the OPDs as well as emergency settings of our hospitals[3]. Corticosteroids are well known for their long term adverse effects on human body[5]. They need to be replaced by drugs with minimal adverse effects and long term safety profile which is the main idea behind choosing the disease as target for the study.

In our study, 1-methyltrypytophan has shown reduction in the eosinophil count found in the blood of rats with asthma like inflammation. However the statistical analyses did not present it to be significant when compared with prednisolone. The levels of interleukin-5 were however remarkably reduced by 1-MT and were significant in comparison to prednisolone. Eosinophil count increases during asthma in humans and so was expected in a rat model of asthma [16]. The potential of prednisolone in reducing the eosinophil count is well established and has been reestablished in our study[17]. However the efficacy of 1-MT was explored during this study. This effect might be due to slight ability of 1-MT to cause eosinophilia-myalgia in humans in some patients [18]. The inflammatory cascade of inhibition seen due to 1-MT might have reduced some number of eosinophilic cells but a major reduction was not documented. The crucial role of interleukin-5 (IL-5) in the biology of eosinophils, its considerable specificity regarding this leukocyte subset, and involvement in the majority of eosinophilic conditions makes it a very promising target for treatment of eosinophil related disorders [19]. More importantly, interleukin-5 has a limited set of cellular targets in humans, like only eosinophils, basophils and a subset of mast cells are considered to express the IL-5Rα (CD125) chain [19]. Advancement in the understanding of the role of eosinophils in multiple chronic inflammatory conditions, most importantly allergic asthma, has led to the development of monoclonal antibodies specifically targeting the surface receptors involved in eosinophil activation and proliferation [19]. These facts promote the idea of targeting IL-5 as a candidate for molecular targeting to discover new parameters for treatment of asthma. The evidence of reduction of its levels by prednisolone in human tissues is well supported due to plethora of research work. Our compound of interest, has shown reduction in the fold change for interleukin-5 which serves as a base for its potential in the treatment of asthma. The reduction of interleukin-5 might help in reduction of inflammatory allergic response generated in asthma. A longer treatment might also bring about a reduction in the percentage of eosinophils but this idea still needs more studies to support this hypothesis.

Further molecular data or parameters were not explored due to financial constraints however this hypothesis can help in exploration of molecular targets of 1-methyltryptophan in relation to asthma including more cytokines and inflammatory cells like macrophages.

CONCLUSION

1-Methyl-dl-tryptophan reduces the expression of interleukin-5 in rat model of asthma significantly and thus can help to reduce the inflammatory changes associated with increased expression of interleukin-5.

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