ORIGINAL ARTICLE

Immunomodulatory Effect of Betulinic Acid in Ovalbumin Induced Airway Inflammation in Male Mice

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ABSTRACT

Objective: To compare the effects of Betulinic Acid and Dexamethasone on proinflamatory markers in ovalbumin induced airway inflammation in male mice.

Study Design: Experimental Randomized Control trial.

Place and Duration of study: This study was conducted in Pharmacology department at Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad over a period of one year from May 2017 to April 2018.

Materials and Methods: Forty (n=40) healthy BALB/c mice weighing between 30-50 grams were recruited in this study. They were divided into four groups, each group comprising of 10 healthy mice. Group I (Normal control), Group II (Asthmatic control), Group III (Ovalbumin + Betulinic Acid) and Group IV (Ovalbumin + Dexamethasone). Group I was kept under normal conditions for 10 days without giving medications. Asthma was induced in group II by sensitizing mice at day 0 by intraperitoneal injection of Ovalbumin 10µg and then challenged intranasally for one week. In-group III and IV same method was used for asthma induction. In-group III, Betulinic Acid was given orally at a dose of 5mg/kg for next 7 days. In-group IV Dexamethasone was given orally at a dose of 0.5-2.0 mg/kg for next 7 days.

IL-13, IL-18, TLC and DLC were estimated in all groups and compared after one week. The data was entered and analyzed using SPSS 22.0 (Statistical Package for Social Sciences). All data was shown as Mean ± S.E.M. One way ANOVA was applied to observe group mean differences. For the comparison of mean difference between groups, Post Hoc Tuckey test was applied. A p-value of ≤0.05 was considered as statistically significant.

Results: Our result showed a major ($P < 0.001$) raise in proinflammatory markers in Asthmatic Control group as compared with Normal Control group. Treatment with Betulinic Acid and Dexamethasone considerably ($P < 0.001$) reduced proinflammatory markers as compared with Asthmatic Control group respectively

Conclusion: Betulinic acid lowers pro-inflammatory mediators as compared to Dexamethasone in ovalbumin induced airway inflammation in mice.

Key Words: *Asthma, Betulinic Acid, 1L-13, 1L-18.*

Introduction

Asthma is an allergic disease of the lungs associated with airway inflammation on and hyper responsiveness to broncho constricting agents. The cross-linking of high-affinity surface-bound IgE receptors on mast cells initially drives the pathophysiology, which results in degranulation of mast cells and of inflammatory mediators like histamine. The pathogenesis of asthma reflects, in part, the activity of T cell cytokines. Murine models support participation of interleukin-13 (IL 13) and the IL 13 receptor in asthma. IL 18, a member of the Interleukin 1 (IL-1) family, a pro-inflammatory cytokine is known to play an important role in Th1/Tc1 polarization, but it also promotes Th2 cytokine (e.g. IL-4, IL-5, IL-9, and IL-13) production from T cells, NK cells, basophils, and mast cells.[1,2]

The prevalence of asthma showed variations globally with higher rates typically seen in developed and affluent societies. According to data collected by Global Initiative for Asthma (GINA), the estimated number of people affected with asthma was 300

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million worldwide and it is anticipated that this number would increase to 400 million by 2025, which is an alarming situation.[3]

According to the data shared by the Global Initiative for Asthma (GINA), prevalence of asthma in Pakistan is 4-5%.[4,5] Around 20 million of Pakistan's adult population is suffering from asthma. With an annual increase of 5%, the prevalence of asthma is increasing on a daily basis. Chronic Respiratory Diseases like asthma is among the non-communicable diseases, which are estimated to lead to over 36% to total deaths in pakistan.[6] At present asthma cannot be restored totally and just be controlled and coped with the goal that patient can carry on with a usual routine life by avoiding potential risk and being vigilant about the sickness.[7]

According to the national and international guidelines on asthma management of adults and children, the inhaled corticosteroids (ICS) are the most operative and active medications, which may be used alone or associated with other controller therapies, in a stepwise, control-based approach. Although the most endorsed therapy based on inhaled corticosteroids may fail to reach control in more than one third of patients, especially adults and in these lung function and quality of life may gradually deteriorate leading to morbidity and eventually mortality. Novel biologic medications have been tried with patients of asthma which at first appear to guarantee in diminishing annual exacerbation rates and steroid use in glucocorticoid-dependent cases, yet when it comes to the long term management, there is still a room for discovery of such compounds which offer more benefits with less side effects.[8] Betulinic acid (BA) is a pentacyclictriterpene found in the stem bark of the plant white birch. BA and its derivatives have been subjected to intense research focusing on their anticancer properties, anti-HIV, anti-bacterial, anti-inflammatory, antimalarial, anti-helminthic, and other pharmaceutical activities.[9] As inflammation is the backbone of asthma, the focus of treatment must be a substance that tends to target the inflammation and its mediators. Betulinic acid is diverse in this aspect that it knockouts the inflammation and happens to reduce the pro inflammatory markers, thereby improving the disease condition of patient.[10]

Keeping in view the importance of IL-18 in terms of its association in airway inflammatory loop, we looked for the molecular aspect of IL-18 expression along with IL-13 when treated with BA. This uncovered area needs to be addressed. This experimental study was done to compare the effects of Betulinic Acid (BA) and Dexamethasone (Dex) on proinflamatory markers in ovalbumin (ova) induced airway inflammation in male mice.

Materials and Methods

This randomized control trial was conducted at Pharmacology Department and Multidisciplinary research laboratory at Islamic International Medical College Trust (IIMCT), Rawalpindi with the collaboration of National Institute of Health (NIH), Islamabad. Before starting the study, a formal approval by the Ethics Review Committee of Islamic International Medical College, Riphah International University was taken. The duration of this study was 12 months (May 2017 to April 2018). A total of forty (n=40) healthy BALB/c male mice weighing 30-50 grams were recruited in this study. All the experimental mice were divided into four groups. Group I (Normal control), Group II (Asthmatic control), Group III (Ova + B A) and Group IV (Ova + Dex). Group I was kept under normal conditions for 10 days without giving medications. Asthma was induced in group II by sensitizing mice at day 0 by intraperitoneal injection of Ovalbumin 10µg and then challenged intranasally for one week. In-group III and IV same method was used for asthma induction. IL-13, IL-18 TLC and DLC were estimated in all groups and compared after one week. IL-13 and IL-18 were estimated by PCR technique in 10^3copies/µL in all the groups. TLC and DLC were estimated by commercially available kits by Merck and 5 part Sysmex Automated Hematology Analyzer. The data was entered and analyzed using SPSS 22.0 (Statistical Package for Social Sciences). All data was shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. A p-value of ≤ 0.05 was considered as statistically significant.

Results

Our result showed a major ($P < 0.001$) raise in IL-13 and IL-18 in group II (Asthmatic Control) as compared with group I (Normal Control). Treatment with group III (Betulinic Acid) and Group IV (Dexamethasone)

considerably (P < 0.001) reduced IL-13 and IL-18 levels as compared with Asthmatic Control i.e Group II, respectively. (Table I,II & figure 1,2).

Table I: Mean ± SEM of IL-13 in All Four Groups

Mean ± SEM	Group I NC	Group II AC	Group III BA	Group IV DEX
	113.3 ± 5.221	300.6 ± 13.93	124.3 ± 7.183	136.5 ± 7.042
P value	< 0.001			

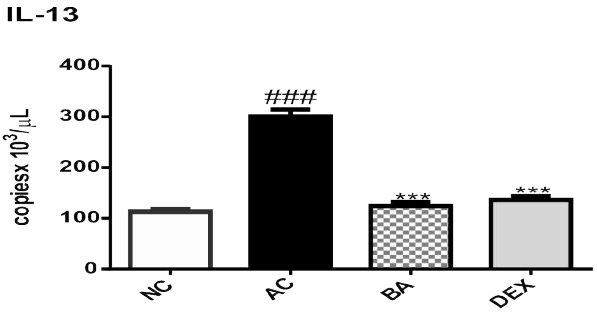

Fig 1: Graphical Presentation of IL-13 in All Groups

BA and DEX significantly decreased levels of IL-13 as compared with AC. Mean ± SEM is shown to signify the data. ### denotes P value < 0.001 when AC compared with NC whereas *** denotes P< 0.001 when BA and DEX compared with AC.

Table II: Mean ± SEM of IL-18 in All Four Groups

Mean ± SEM	Group I NC	Group II AC	Group III BA	Group IV DEX
	21.10± 1.170	75.16± 4.490	26.69± 2.648	32.40± 1.849
P value	< 0.001			

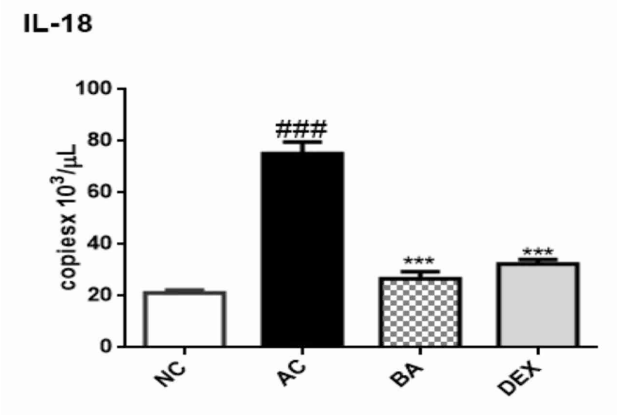

Fig 2: Graphical Presentation of IL-18 in All Groups

Mean ± SEM is shown to signify the data. ### denotes P value < 0.001 when AC compared with NC whereas *** denotes P< 0.001 when BA and DEX compared with AC.

Discussion

The results of the present study confirm that the airway inflammation induced by ovalbumin, is ameliorated by the both experimental agents, yet the result of therapy with betulinic acid is very impressive, as compared to the traditional corticosteroids.

Several reports described the efficacy of oral (predinisone and prednisolone, methylpredinisolone) and inhaled (triamcinolone, budesonide and fluticasone) corticosteroids in asthma management. The eosinopenic effect of corticosteroids helps in preventing the cytotoxic effect of the major basic proteins and other inflammatory mediators released from eosinophils. They prevent the increased airway reactivity associated with late bronchial reactions by blocking them.[11] BA is a recently discovered naturally occuring pentacyclic triterpenoid with anti- inflammatory and anticancer properties.[12] Current study results showed that BA can change the IL-13, IL-18, total and differential leukocytes counts when compared with the levels in mice sensitized with ovalbumin. BA reduced IL-13 levels comparable to the levels in normal controls (124.3 and 113.3 x10^3copies/µL respectively). When compared to dexamethsone, IL-13 levels were slightly lower in BA group (136.5 and 124.3x10^3copies/µL respectively). BA also reduced IL-18 levels comparable to the levels in negative controls (26.69 and 21.10x10^3copies/µL respectively). When compared to dexamethsone, IL-18 levels were slightly lower in BA group (26.69 and 32.40x10^3copies/µL respectively). BA also reduced TLC comparable to the levels in negative controls (6417 and 6500x10^3/µL respectively). When compared to dexamethsone, TLC levels were slightly lower in BA group (7033 and 6417x10^3/µL respectively). BA also reduced mean eosinophils percent comparable to dexamethasone group (1.667% and 1.5% respectively). However, it did not reduce them to the levels in normal controls (0.833%). BA also reduced mean neutrophils percent comparable to dexamethasone group (19% and 22.17% respectively). However, it did not reduce them to the levels in negative controls (12.5%). BA also reduced mean lymphocytes percent comparable to normal control group (64.3% and 63.7% respectively). However, it did not reduce them

to the levels in dexamethasone group (55%).

Several other studies reported less production of pro-inflammtory mediators in BA treated animals. In a recent study, Costa et al evaluated the effects of BA in a mouse model of endotoxic shock. They reported that pro-inflammatory mediators were produced in lesser amount by the macrophages obtained from mice treated with BA along with an increased production of IL-10 along with an increased production of IL-10 in comparison with non-stimulated macrophages from vehicle-treated mice.[13]

Authors concluded that BA has a powerful in vivo anti-inflammatory activity, which through a mechanism dependent on IL-10 protects the mice against LPS by modulating in vivo production of TNF-α by macrophages .[13]

In another study, authors determine the effects of oral administration of BA (50, 5, 0.5 mg/kg) five times at 24 hours intervals to red blood cells immunized (SRBC) and non-immunized mice. They found that, whatever the dose administered, five times administration of BA increased peripheral blood leukocyte count primarily by increasing the number of blood lymphocytes. While the administration of BA in a dose of 5 mg/kg administered 5 times at 24 hours intervals resulted in reduction of blood segmented neutrophils count on days 1 and 3 after drug administration.[14]

In another in-vitro study on human whole blood cell cultures highlighted that BA modulates the production of cytokine by Th1/Th2 cell subpopulations that results in enhancement of IL 10 formation and inhibits production of IFN-γ, resulting in lower IFN-γ/IL-10 ratio.[15]

Another study found the strongest immunomodulatory effect of BA with lowest investigated dose (0.5 mg/kg) when administered multiple times.[16]

Conclusion

Betulinic acid has better anti-inflammatory effects in asthma induced by ovalbumin as compared to dexamethasone.

Study Limitation

Study should also have involved the immunomodulatory effect of betulinic acid on other proinflammatory markers released in asthma but owing to the cost and availability issue, these parameters could not be explored.

Recommendation

1). Further research need to be directed on combined anti-inflammatory effect of corticosteroids and betulinic acid.
2). The comparison of betulinic acid with other antiasthmatic drugs should be studied.
3). The anti-inflammatory effect of other novel compounds by measuring levels of interleukin should also be explored.

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