ORIGINAL ARTICLE

The effect of methyl palmitate on treatment of experimental asthma

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Abstract

Objective: To determine the effects of methyl palmitate on murine model of chronic asthma.

Methods: The experimental study was conducted in the animal laboratory of DokuzEylul University, Turkey, from October to December, 2012, and comprised BALB/c mice whowere divided into four equal groups: three experimental and one control group. All groups except the control group were sensitised and challenged with ovalbumin. Mice with experimentally-induced asthma in Group I received saline; Group II dexamethasone 1mg/kg; Group III methyl palmitate300mg/kg intraperitoneally three times per week in the last four weeks of the study period. Animals were sacrificed 24h after the last administration of study drugs. Histological findings of airways were evaluated by light microscopic examination. Blood samples from vena cava inferior were taken for measurement of interleukin-5 levels. SPSS 15 was used for statistical analysis.

Results: The 28 female mice in the study were divided into 4 groups of 7(25%) each. The age range of the animals was 6-8weeks, and the weight range was 18-20g. All histological parameters and interleukin-5 levels of asthma in the Group III were significantly ameliorated compared to the Group I ($p<0.05$)). All histological parameters and interleukin-5 levels were similar between Group III and Group II.

Conclusion: Methyl palmitate exhibited anti-inflammatory effects by resolving the histological changes and reducing the interleukin-5 levels in murine model of chronic asthma.

Keywords: Anti-inflammatory, Chronic asthma, Mouse, Interleukin-5, Methyl palmitate, Airway remodeling. (JPMA 65: 632; 2015)

Introduction

Asthma is one of the most common chronic respiratory diseases in industrialised countries and imposes high social and economic costs. Additionally, disease outcomes remain suboptimal despite current effective treatment modalities.[1] Asthma is characterised by reversible airway obstruction, airway inflammation and remodelling. Airway remodelling consists of progressive structural changes in the composition, content and organisation of the cellular and molecular constituents of the airway wall.[2] Although current asthma therapies are effective in reducing inflammation, airway remodelling is poorly responsive to current therapies, such as inhaled corticosteroids, antileukotrienes, and theophylline.[3] For this reason, new therapeutic options are required.

Methyl palmitate (MP, methylester of palmitic acid) is one of fatty acid methyl esters which are endogenous compounds.[4] MP is a promising inhibitor of IκB (inhibitor of kappa B protein) phosphorylation and was shown to suppress Kupffer cell function as measured by colloidal carbon clearance and latex bead uptake.[5] MP has been proven as a general macrophage inhibitor in several invitro studies as evidenced by inhibition of isolated Kupffer cells, rat peritoneal macrophages[6] and RAW cells (macrophages of alveolar origin).[7] MP inhibition of the phagocytic activity is accompanied by differential expression of tumour necrosis fctor-alpha (TNF-α), interleukin-10 (IL-10), nitric oxide (NO) and Cyclooxygenase-2 (COX-2).[6] MP can attenuate the severity of oxidative stress (OS).[8] Also, MP was shown to prevent lung fibrosis induced by bleomycin in rats.[7] Consequently, MP may have potential efficacy in controlling inflammatory diseases. The possible benefits of MP in asthma treatment have not yet to be thoroughly investigated. Therefore, the current study was planned to investigate the efficacy of MP on lung histopathology in a murine model of chronic asthma.

Materials and Methods

The experimental study was conducted at Dokuz Eylul University, Izmir, Turkey, from October 1 to December 15, 2012, and comprised specific pathogen-free female

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BALB/c mice. They were kept in pathogen-free, hygienic macrolene cages in air-conditioned rooms and allowed food and water ad libitum on a 12-hour light/12-hour dark cycle. All experimental procedures complied with the requirements of the institutional Animal Care and Ethics Committee. The mice were randomly divided into four equal groups: I, II, III, and control group. Mice were selected at random from each group. All groups except control group were sensitised and challenged with ovalbumin. Mice with experimentally-induced asthma in Group I received saline; Group II dexamethasone 1mg/kg; Group III MP 300mg/kg; intraperitoneally three times per week in the last four weeks of the challenge period. Intraperitoneal doses of MP (Sigma-Aldrich) and dexamethasone 1mg/kg were chosen on the basis of literature.[7,9]

BALB/c mice are high responders to ovalbumin.[9] The mice in groups I, II and III were sensitised via two intraperitoneal injections, on days 0 and 14 of the experiment, of 10 mikrogram/0.1 mL chicken egg albumin (ovalbumin, grade V, ≥98% pure; Sigma, St. Louis, MO, USA) with alum as an adjuvant. The mice in groups I, II, III were then exposed to aerosolised ovalbumin for 30 min per day on three days of a week for eight weeks, beginning from the 21st day of the study. Exposures were carried out in a whole body inhalation exposure system. Temperature and relative humidity were maintained between 20-25°C and 40-60%, respectively. A solution of 2.5% ovalbumin in normal saline was delivered by aerosolisation via compressed air to a sidestream jet nebuliser injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4m. Particle concentration was maintained in the range of 10-20 mg/mm.[10] The mice in the control group were administered normal saline with alum intraperitoneally on days 0 and 14 of the experiment and exposed aerosolised saline for 30 min per day on three days of the week for eight weeks, beginning from the 21st day of the study.[10]

Animals were sacrificed 24 hours after the last administration of study drugs. Histological findings of airways were evaluated by light microscopic examination. Blood samples from venacava inferior were taken for measurement of IL-5 levels. Mid zone lung tissues were obtained and fixed in buffered 10% formalin and embedded in paraffin wax. Five-micron-thick serial sections were obtained and the first 10 sampleswere stained with haematoxylin and eosin (H&E). In these samples, general tissue features were examined and the thicknesses of epithelium and subepithelial smooth muscle layers of the medium and small airways were measured. In order to evaluate the thicknesses of epithelium and subepithelial smooth muscle layers, measurements were performed from four points of each airway. Considering that each section contained approximately two to three airways, around 20 or more airways were evaluated for each mouse. Photomicrographs were taken by Olympus DP71 camera (Japan), which adapted on Olympus DP70 model microscope (Olympus Optical, Tokyo, Japan). Measurements were carried out with University of Texas Health Science Center at San Antonio (UTHSCSA) Image Tool for Windows Version 3.00.

Then consecutive 10 sections were stained with toluidine blue and the other 10 sections with Periodic Acid-Schiff (PAS). Photomicrographs were taken randomly from five fields of each section which were stained with toluidine blue. For mast cell enumeration, a standard transparent counting frame representing an area of 16,400 µm2 was used manually and eight fields in each photograph were examined for each mouse. Goblet cells stained with PAS were enumerated in 10 sections of each mouse. In each section, three to five randomly selected airways were photographed. Circumferences of all airways were measured and goblet cell numbers in these areas were recorded. For standardisation, goblet cell numbers in 100µm were analysed by division of total goblet cell number to the total length of airway circumferences and multiplying the result by one hundred.

All statistical procedures were performed using SPSS 15.All values were expressed as the mean ± standart deviation (SD). Kruskal Wallis (among all groups) and Mann-Whitney Utests (for two groups) were used to compare staining intensity values. $P<0.05$ was considered significant.

Results

The 28 female mice in the study were divided into 4 groups of 7(25%) each. The age range of the animals was 6-8weeks, and the weight range was 18-20g. In light microscopic examination, lung tissues of the control group showed normal histological features (Figure-1). In the chronic asthma group, epithelial and subepithelial smooth muscle thicknesses and the numbers of goblet and mast cells were significantly higher than control and treatment groups ($p<0.001$) (Table-1). When asthma group was compared with steroid and MP-treatment groups, significant decrease was observed in the epithelial thicknesses and subepithelial smooth muscle as well as the numbers of goblet and mast cells ($p<0.001$). There was no significant difference between steroid (dexamethasone) and MP-treated groups in all

Table: Comparison of binary groups.

	Epithelial thickness P-value	Subepithelial thickness P-value	Mast cell number P-value	Goblet cell number P-value
Control-Asthma	0.001	0.001	0.001	0.001
Control-Steroid	0.640	0.009	0.013	0.030
Control-Palmitate	0.140	0.640	0.004	0.018
Asthma-Streoid	0.001	0.001	0.001	0.001
Asthma-Palmitate	0.001	0.001	0.001	0.001
Streoid-Palmitate	0.557	0.087	1.000	0.087

p<0.05 was regarded as significant.

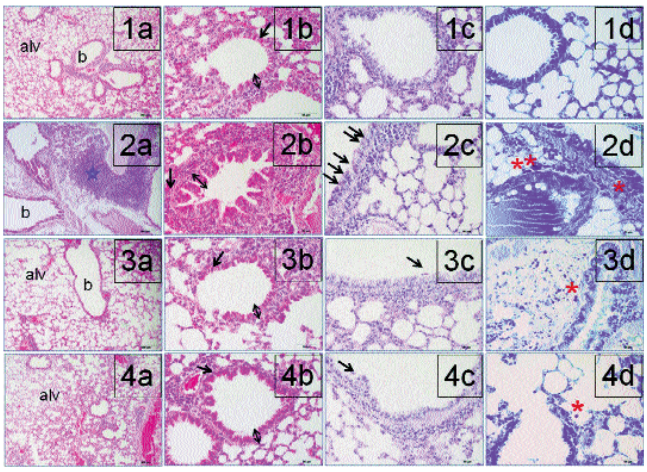

Figure-1: Light microscopic findings of groups. Lines 1 to 4 are control, saline, steroid and methyl palmitate (MP) treatment groups respectively. Lung tissues were stained with haematoxylin and eosin (H&E) (1-4a and 1-4b), Periodic Acid-Schiff (PAS) (1-4c) and Toluidine blue (1-4d) in histological images. In control group, lung histology showed normal histological structure of lung tissue (1a-d; alv: alveol, b: bronchiol). The lung histology of Group II revealed thickened epithelium (2b, duplex arrow) and thickened subepithelial smooth muscle (2b, black arrow) and inflammation (2a, blue star). In other groups, thicknesses of epithelium and subepithelial smooth muscle were similar to control group (3-4b, arrow and duplex arrow). For goblet cells, higher number in Group II (2c, arrow) and lower number in treatment groups (3-4c arrow) were seen in PAS staining. In Toluidine blue staining, higher numbers of mast cells were seen in Group II (2-4d, red stars). In comparison, MP and steroid-treated groups had no difference in light microscopic findings.

histological stainings (p>0.05). MP significantly improved epithelial and subepithelial smooth muscle thicknesses and numbers of goblet and mast cells closer to dexamethasone (p<0.05).

There was a similar pattern between histomorphometric and IL-5 measurements (Figures-1 and 2). In both analyses, steroid group had the lowest value and was followed by MP group's values. The effect of MP for IL-5 levels was closer to dexametasone.

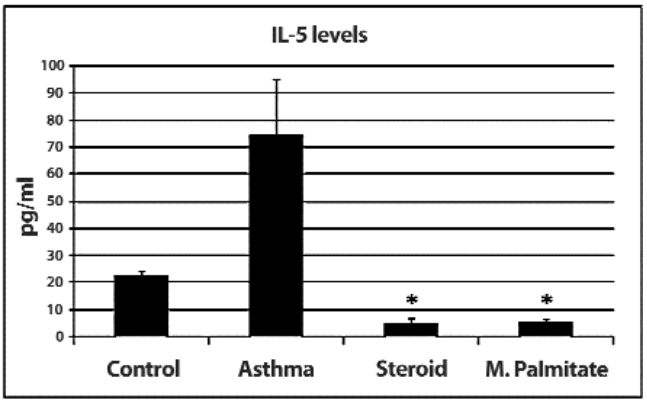

Figure-2: Effects of Methyl Palmitate and steroid on interleukin-5levels in mice with experimentally-induced asthma. Results are expressed as means ± standard deviation (n=7). Statistical significance among groups was assessed by analyusis of variancefollowed by Tukey's multiple comparison test (*p<0.001).

Discussion

Structural changes in asthmatic airways occur as a result of an injury/repair process on which there is an ongoing need for beneficial drugs.[11] MP has anti-inflammatory and anti-fibrotic effect. The current study was conducted to investigate the efficacy of MP on lung histological changes in a murine model of chronic asthma.

Chronic inflammation in asthma is thought to initiate and perpetuate tissue injury and repair.[12] It seems very important to prevent airway remodelling in the chronic management of asthma[9] because, once formed, remodelling is resistant to asthma therapy.[13] Inhalation of corticosteroids and administration of beta-2 agonists, antileukotrienes and theophylline are poorly responsive to these structural changes. Inhaled corticosteroids may be effective in reducing reticular basement membrane thickness when used for a long period of time and at high doses.[2]

MP, a naturally occurring fatty acid methyl ester, can be considered a universal macrophage inhibitor as it was

shown to suppress isolated Kupffer cells, rat peritoneal macrophages and RAW cells.[6] Macrophages play a central role in the inflammatory response and serve as an essential interface between innate and adaptive immunity. It is responsible for antigen processing and presentation to antigen specific T cells.[14] Macrophages are a major cell type in lung tissue, and most of these cells exist in the pulmonary alveolus.[15] It involves in both the innate and adaptive immune responses and may be one of the major sources of IL-13 in mouse models of asthma.[16] Furthermore, activation of alveolar macrophages by the cytokines results in stimulation of the allergic immune responses, airway inflammation[17] and pulmonary fibrosis.[18]

In histological results of the present study, the structural changes observed in the asthmatic group revealed that the model was successfully established. Administration of dexamethasone and MP significantly ameliorated the histological characteristics of airway inflamation. This finding is very important because reticular basement membrane thickness is considered a hallmark for airway remodelling in asthma (Figure-1).

In order to investigate the anti-inflammatory effect of MP on airway inflamation, we examined IL-5 levels. A study showed that IL-5 plays a central role in the development of chronic inflammation of the airways and the induction of airway hyperreactivity.[19] Another study also reported that alveolar macrophages enhance IL-5 production by T cells in the airways and, as a consequence, the development of asthma in atopic individuals.[20] In our study, we observed a significant decrease for IL-5 levels in steroid and palmitate groups compared to asthma group (Figure-2). There was no significant difference on IL-5 levels between steroid and and MP-treated groups. In prevention of another model of ovalbumin-induced murine asthma, A study evaluated the anti-inflammatory effect of partially purified extract-structure viscum coloratum (PPE-SVC) and viscolin, isolated from Viscum Coloratum used in traditional Chinese medicine. Similarly, they found that both PPE-SVC and viscolin inhibited IL-5 levels, leading to reduced production and activation of eosinophils in the bone marrow.[21]

Following stimulation, macrophages culminate in the activation of two distinct downstream signalling pathways: the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and the mitogen-activated protein kinase pathway. These two pathways induce the expression of various inflammatory mediators, including NO, prostaglandins and inflammatory cytokines.[22] MP has the ability to inhibit NF-κB and downstream inflammatory cascades.[7]

NF-κB is a family of deoxyribonucleic acid (DNA)-binding protein factors that have an important role for the transcription of pro-inflammatory molecules.[23] So, NF-κB can be a basic mediator in the pathogenesis of asthma. The inhibition of NF-κB causes the reduction of allergic lung inflammation and airway hyperresponsiveness.[24] NF-κB-binding activity in bronchial mucosa biopsy samples of asthmatic patients was reduced with inhaled budesonide treatment.[25] Consequently, the inhibition of NF-κB has an important role for the control of pulmonary inflammation.[24] Our observation of reduced airway constriction and inhibited production of IL-5 in BALB/c mice could be attributed to these properties of MP (Figure-2). However, this remains to be determined in the future.

We could not perform electron microscopic evaluation. However, histology which fixes what happens in the airways of mice during ovalbumin-induced asthma and using the method described in literature in which a progressive inflammatory response replicating many features of human asthma is developed in the airways of mice increase the value of our study.[26]

Conclusion

MP exhibited anti-inflammatory effects by resolving histological changes and reducing IL-5 levels in murine model of chronic asthma.The effects produced are comparable with conventionally-used steroids. However, further studies with long-term treatments evaluating the effects of MP on lung inflammation and remodelling are needed.

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