

Para-Bromophenacyl bromide alleviates airway hyperresponsiveness and modulates cytokines, IgE and eosinophil levels in ovalbumin-sensitized and -challenged mice

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Abstract

Airway hyperresponsiveness, airway eosinophilia and increased IgE levels in serum are the important characteristic features of asthma. We evaluated the potential of *para*-Bromophenacyl bromide (PBPB), a known phospholipase A₂ inhibitor, on allergen-induced airway hyperresponsiveness in a mouse model. We sensitized and challenged mice with ovalbumin (OVA) to develop airway hyperresponsiveness as assessed by airway constriction and airway hyperreactivity (AHR) to methacholine (MCh) induced by allergen. The mice were orally treated with PBPB (0.1, 1 and 10 mg/kg) during or after OVA-sensitization and OVA-challenge to evaluate its protective or reversal effect on airway constriction and AHR to MCh. Determination of OVA-induced airway constriction and AHR to MCh were performed by measuring specific airway conductance (SGaw) using non-invasive dual-chamber whole body-plethysmography. We observed that PBPB (1 mg/kg) significantly reduced OVA-induced airway constriction and AHR to MCh ($p < 0.01$). PBPB (1 mg/kg) treatment significantly inhibited PLA₂ activity in the BAL fluid. Cytokine analysis of the BAL fluid revealed that PBPB caused an increase in interferon- γ (IFN- γ) ($p < 0.02$) and a decrease in interleukin-4 (IL-4) ($p < 0.05$) and interleukin-5 (IL-5) ($p < 0.05$) levels. The OVA-specific serum IgE levels ($p < 0.01$) and the BAL eosinophils ($p < 0.001$) were also reduced significantly. Thus, PBPB is capable of modulating allergen induced cytokine levels and serum IgE levels, and alleviating allergen induced airway hyperresponsiveness and eosinophils in mice. These data suggest that PBPB could be useful in the development of novel agents for the treatment of allergen induced airway hyperresponsiveness.

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1. Introduction

Asthma is an inflammatory disease of the airways and is characterized by airway hyperresponsiveness to

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a variety of physical and pharmacological stimuli. The development of asthma is mediated by proinflammatory cytokines, such as interleukin (IL)-4, IL-13 and IL-5 secreted primarily by CD4⁺ Th2 cells. On the other hand, interferon- γ (IFN- γ) secreted by CD4⁺ TH1 cells inhibits Th2 cell differentiation [1]. The asthmatic response is divided into early and late phase responses in man [2]. The early phase begins immediately after exposure to allergen and is often associated with mast cell degranulation [3]. The late phase response, associated with inflammation of the airways and airway hyperresponsiveness, occurs 8 to 24 h after allergen exposure [2]. This response is associated with an infiltration of eosinophils in the airways [4]. Similar early and late airway responses were observed in a mouse model after allergen provocation [5].

The incidence of allergic and asthmatic disorders is increasing rapidly all over the world [6]. The current mode of therapy is inadequate and also the majority of the prescribed drugs for these conditions have side effects [7]. Consequently, a major thrust has been given to identify non-steroidal anti-inflammatory molecules for mitigating these disorders. In view of this, we selected *para*-Bromophenacyl bromide (PBPB), a known inhibitor of PLA₂, which has been shown to inhibit various steps of inflammation such as edema [8,9] and superoxide (O₂⁻) release [10]. It has also been shown to inhibit OVA and PAF-induced contraction of guinea pig lung strips [11]. Furthermore, it inhibits the DNA-binding activity of NF- κ B [12], an important nuclear transcription factor involved in the expression of various inflammatory cytokines. Based on the fact that asthma is an inflammatory and airway hyperreactive disease, and PBPB has anti-inflammatory and anti-contraction effects, we speculated that PBPB could alleviate asthmatic features. In the present study, we evaluated the efficacy of PBPB in reducing airway hyperresponsiveness as assessed by allergen-induced airway constriction and airway hyperreactivity (AHR) to methacholine (MCh) in OVA-sensitized and OVA-challenged mice. The results demonstrate that PBPB significantly prevents OVA-induced airway constriction and AHR to MCh. We also report that PBPB is capable of inhibiting PLA₂ activity and of modulating cytokines and eosinophils in BAL fluid and IgE levels in serum.

2. Materials and methods

2.1. Animals and sensitization

BALB/c male mice were obtained from the National Institute of Nutrition, Hyderabad (India). Experimental protocols were approved by the Institutional Animals Ethical Committee. Mice, 8–10 weeks old, weighing 18–22 g were acclimatized for 1 week under the laboratory conditions (25 $\pm$ 2 °C, 60% humidity and normal day/night cycle, 12 h each approx.) before starting the experiment.

Mice were sensitized with 0.2 ml of normal saline containing 10 μ g ovalbumin (Sigma, St. Louis, MO, USA) adsorbed on 2 mg aluminum hydroxide intraperitoneally (i.p.) on days 0, 7 and 14. Five days after final i.p. injection, the mice were subjected to aerosolized OVA (2%) inhalation for 30 min beginning from day 19 to day 23 (Fig. 1). Mice were placed in a plexiglass chamber (20 $\times$ 20 $\times$ 10 cm³) and exposed to an aerosol generated from a nebulizer (DeVilbiss, model 645, USA) with an airflow rate of 7 L/min. Control (sham-sensitized) mice received i.p. injections of 0.2 ml saline containing 2 mg alum and were challenged with saline alone.

2.2. Treatment with PBPB

Age and body weight-matched mice were randomly divided into two sets, set 1 was tested for the protective effect of PBPB (Sigma; dissolved in dehydrated alcohol) and set 2 for the reversal effect. Set 1 was divided into five (1–5) groups (6 mice in each except in group 1, which had 12 mice); mice in group 1 were sham-sensitized and saline-challenged and treated with vehicle (to six mice) or with PBPB (1 mg/kg, 20 μ l volume, to another six mice), mice in group 2 were OVA-sensitized and OVA-challenged and treated with vehicle only, mice in groups 3, 4 and 5 were OVA-sensitized and OVA-challenged and treated with 0.1, 1 and 10 mg of PBPB/kg, respectively, from day 0 to 23 once daily to evaluate the protective effect of PBPB (Fig. 1A). To examine the reversal effect, set 2 mice were first sensitized and challenged with OVA as described above. Mice with at least 40% fall in specific airway conductance (SGaw) to OVA aerosol challenge were randomly

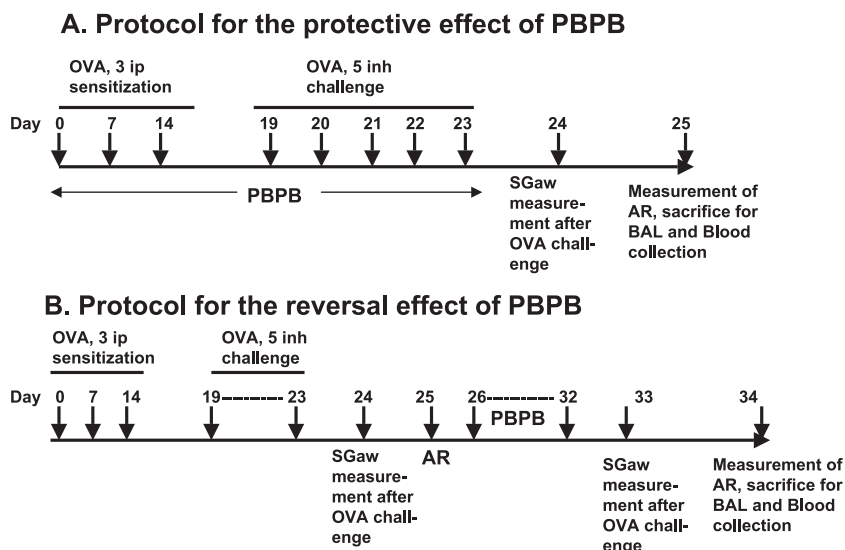


Fig. 1. Experimental protocol: Male BALB/c mice were sensitized by intraperitoneal injection of OVA on days 0, 7 and 14 and challenged for 5 days by aerosol inhalation. (A) To evaluate the protective effect, mice were given PBPB orally from days 0 to 23. On day 24, mice were challenged with OVA to measure SGaw for assessing OVA-induced airway constriction. On day 25 (after 24 h of OVA challenge), each mouse was challenged with increasing concentrations of MCh to determine airway reactivity (AR). (B) To evaluate the reversal effect, mice were sensitized and challenged with OVA and checked for airway constriction and reactivity as in protocol 'A' and then given oral PBPB for 7 days (26–32). On day 33, mice were challenged with OVA to measure SGaw for assessing OVA-induced airway constriction. On day 34 (after 24 h of OVA challenge), each mouse was challenged with increasing concentrations of MCh to determine AR. ip=intraperitoneal, inh=inhalation, OVA=ovalbumin, BAL=bronchoalveolar lavage.

selected and divided into five (1–5) groups as in Fig. 1A except that PBPB (0.1, 1 and 10 mg/kg) or vehicle (20 μ l alcohol) was administered daily from day 26 to 32 (Fig. 1B).

2.3. Measurement of airway function: specific airway conductance (SGaw)

Airway function of the mice was measured as specific airway conductance (SGaw) by using a modified dual-chamber whole body plethysmograph similar to that for guinea pigs [13,14] as described previously [15].

2.4. Measurement of OVA-induced airway constriction

OVA-induced airway constriction of mice was determined in terms of the decrease in SGaw after OVA-aerosol inhalation challenge as compared to its basal SGaw values (before sensitization on day 0). On day 24 (for protective groups) and on days 24 and

33 (for reversal groups), each mouse was given OVA (3% in saline) aerosol inhalation for 10 min in a plexiglass chamber (20 $\times$ 20 $\times$ 10 cm³) and then housed in the body plethysmograph and challenged again with OVA (3%) for 60 s and the fall of SGaw within 10 min was recorded. The decrease of SGaw from its basal value was described as airway constriction. The sham-sensitized and saline-challenged mice received saline as an aerosol inhalation in place of the OVA-challenge.

2.5. Measurement of airway reactivity to MCh

The airway reactivity to MCh (Sigma) was measured before sensitization and after 24 h of OVA-aerosol challenge. Each individual mouse was housed in the plethysmograph, acclimatized and given an aerosol of 1 ml of PBS (as vehicle) to determine the basal SGaw level. Similarly, progressively doubled concentrations of methacholine (3.1, 6.25, 12.5, 50, 100 and 200 mg/ml in PBS) were each given for 60 s until the SGaw levels decreased

approximately to 60% of the basal level. The aerosol was generated with a nebulizer (DeVilbiss model 645, USA) at 7 L/min air. A methacholine dose–SGaw response curve was prepared for each individual mouse as described by Agrawal [10] for guinea pig and the dose which produced a 35% decrease in SGaw level (MCh PD₃₅) was derived by interpolation of the curve and was used as an index of airway reactivity.

2.6. Collection of bronchoalveolar lavage (BAL) fluid and blood

After final measurements of airway constriction and airway reactivity to MCh, the mice were sacrificed (on day 25 for protective groups and day 34 for reversal groups) using an overdose of sodium pentothal (100 mg/kg, i.p.). Blood samples were collected and sera were separated by centrifugation at 3000 rpm for 10 min and kept at –70 °C until analysis for IgE. The trachea was cannulated and 0.5 ml of saline was used per lavage and repeated four times for each mouse. About 1.6 ml of BAL fluid was recovered per mouse. The BAL fluid was centrifuged (400×g, 4 °C, 6 min) and the supernatant was kept at –70 °C until analysis for cytokines. The BAL cells were washed three times with PBS and the pellet was resuspended in 200 µl cold PBS. For differential counts, cytopsin preparations were made and stained with Leishman's stain. The cells were identified and counted by standard morphology. At least 300 cells (all types) per cytopsin preparation were counted and the percentage of eosinophils was calculated.

2.7. Measurement of secretory PLA₂ (sPLA₂) activity

sPLA₂ activity was measured in the BAL fluid using a sPLA₂ kit (Assay Design, Ann Arbor, MI, USA) and the activity was reported in units/ml of the BAL fluid.

2.8. Measurement of OVA-specific IgE

OVA-specific IgE levels in sera were measured by enzyme linked immunosorbent assay (ELISA) as described by Das et al. [15]. The results were expressed in ng/ml of serum.

2.9. Measurement of IFN- γ , IL-4 and IL-5

The cytokines IFN- γ , IL-4 and IL-5 were measured in triplicate in the BALF by ELISA kits (BD Pharmingen, USA) according to the manufacturer's protocol and the results were reported in pg/ml of each sample.

2.10. Statistics

All the data were presented as mean±S.E.M. The Mann–Whitney *U*-test was applied for the difference of SGaw, MCh PD₃₅ and the percentage of eosinophils between two groups of sensitized and drug treated mice. Students *t*-test was performed for cytokines, IgE and sPLA₂ activity levels to find the level of significance. A *p* value less than or equal to 0.05 was considered to be significant.

3. Results

3.1. PBPB inhibits OVA-induced airway constriction

The effect of PBPB on OVA-induced airway constriction was examined in two separate sets of experiments to determine the protective and the reversal effects on airway hyperresponsiveness. To investigate the protective effect of PBPB on OVA-induced airway constriction, mice were treated with PBPB (0.1, 1, and 10 mg/kg) or vehicle during OVA-sensitization and OVA-challenge. The OVA-sensitized and OVA-challenged group treated with vehicle showed a fall of 48% (*p*<0.001) in SGaw as compared to their basal values, whereas sham-sensitized and saline-challenged mice showed no change (Fig. 2A). Treatment with PBPB markedly prevented the OVA-challenged induced fall in SGaw in a dose-dependent manner (Fig. 2A). A dose of 1 mg/kg of PBPB significantly blocked the fall in SGaw (up to 96% of the basal level, *p*<0.01, Fig. 2A). However, when the sham-sensitized and saline-challenged mice were treated with PBPB (1 mg/kg) alone, no difference in SGaw was observed (data not shown).

To determine the reversal effect of PBPB on airway constriction, mice with OVA-induced airway constriction and AHR to MCh were treated with PBPB (0.1,

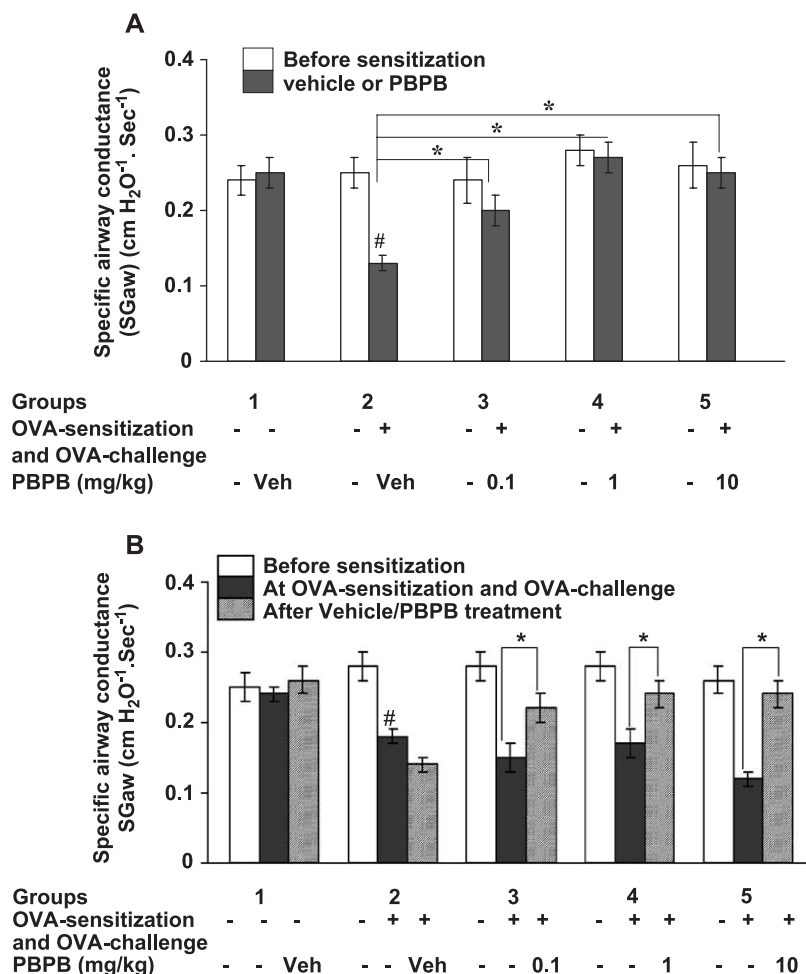


Fig. 2. PBPB inhibits airway constriction in OVA-sensitized and OVA-challenged mice. (A) Experiments were performed with five groups (1–5) ($n=6$ in each) to examine the protective effects of PBPB according to protocol in Fig. 1A. Mice in each group were sensitized with or without OVA and treated without or with different concentrations of PBPB as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.). White bar indicates the SGaw values on day 0 (before starting sensitization) and black bar indicates the OVA-induced SGaw values on day 24 after vehicle or PBPB treatment. # $p<0.001$ as compared to before sensitized SGaw level; * $p<0.01$ indicates significant difference of PBPB treated groups from that of vehicle treated group. (B) To examine the reversal effect of PBPB, experiments were done on another set of five groups (1–5, $n=6$ in each) of mice according to protocol in Fig. 1B. Sensitized and challenged mice were treated without or with different concentrations of PBPB as described in the Materials and methods. White bar indicates the SGaw values before starting the sensitization, black bar indicates the SGaw at the OVA-sensitization and OVA-challenge (on day 24), and hatched bar indicated the SGaw values after 1 week vehicle or PBPB treatment (on day 33). # $p<0.001$ as compared to before sensitized level of SGaw; * $p<0.01$ indicates significant difference of PBPB treatment from that of the previous sensitized and challenged SGaw level.

1, and 10 mg/kg) for 1 week (days 26–32) as described in the Materials and methods. Interestingly, the SGaw values measured on day 33 after PBPB administration reversed in a dose dependent manner. PBPB at a dose of 1 mg/kg was found to reverse OVA-induced fall in SGaw (up to 90% of basal level, $p<0.01$, Fig. 2B).

3.2. PBPB reduces AHR to MCh

To evaluate the protective effect of PBPB on OVA-induced airway hyperreactivity to MCh, we determined MCh PD₃₅ values after 24 h of OVA-challenge as described in the Materials and methods. As shown in Fig. 3A, there was a significant

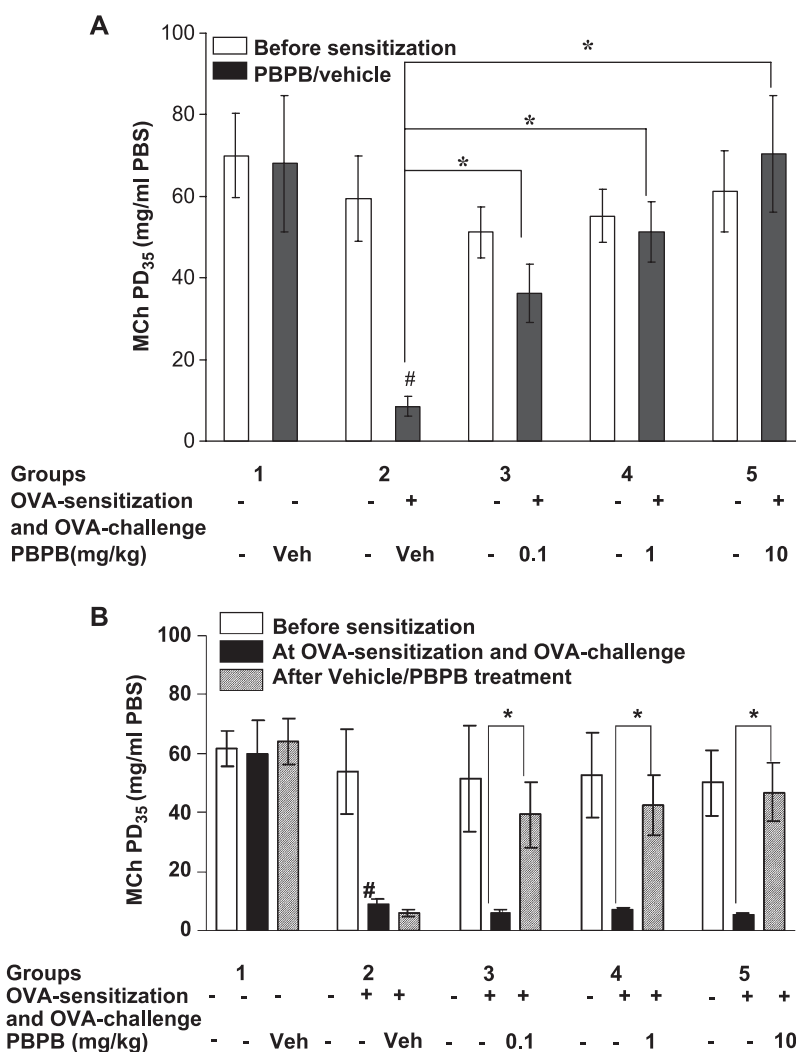


Fig. 3. PBPB inhibits airway reactivity to methacholine. (A) Experiments were performed as described in Fig. 2A and MCh PD₃₅ values were measured on day 25 to examine the protective effect of PBPB on airway reactivity of mice as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.). White bar indicates MCh PD₃₅ before sensitization (before starting sensitization) and black bar indicates the MCh PD₃₅ values on day 25 after vehicle or PBPB treatment. # p <0.001 as compared to before sensitized MCh PD₃₅; * p <0.01 indicates significant difference of PBPB treated groups from that of vehicle treated group. (B) Experiments were performed as described in Fig. 2B and MCh PD₃₅ values were measured on day 34 to examine the reversal effect of PBPB on airway reactivity of mice as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.). White bar indicates MCh PD₃₅ before sensitization, black bar indicates MCh PD₃₅ after sensitization and challenge, and hatched bar indicates MCh PD₃₅ after 1 week treatment with PBPB (on day 34). # p <0.01 as compared to before sensitization level of MCh PD₃₅; * p <0.01 indicates significant difference of PBPB treated groups from that of the respective MCh PD₃₅ after sensitization and challenge.

decrease in the MCh PD₃₅ values in OVA-sensitized and OVA-challenged mice as compared to sham-sensitized and saline-challenged mice (p <0.01). This demonstrates that OVA-sensitized and OVA-challenged mice had airway hyperreactivity to MCh.

Treatment with PBPB (0.1, 1 and 10 mg/kg) during the period of OVA-sensitization and OVA-challenge markedly prevented the decrease of MCh PD₃₅ in a dose-dependent manner, showing attenuation of AHR to MCh. PBPB at a dose of 1 mg/kg increased MCh

PD₃₅ values upto 93% ($p<0.01$) of the basal level (Fig. 3A).

In addition, when the mice were first OVA-sensitized and OVA-challenged and then treated with PBPB (0.1, 1 and 10 mg/kg) for 1 week, MCh PD₃₅ values increased significantly ($p<0.01$) (Fig. 3B). These results suggest that PBPB inhibited the airway hyperreactivity to MCh. PBPB at a dose of 1 mg/kg, increased MCh PD₃₅ values up to 81% of the basal level (Fig. 3B, $p<0.01$). PBPB at a dose of 10 mg/kg was found to increase the MCh PD₃₅ value further (94%) (Fig. 3B).

3.3. PBPB decreases sPLA₂ activity in BAL fluid

As PBPB is a known inhibitor of secretory PLA₂ (sPLA₂), we determined its effect on the levels of sPLA₂ in the BAL fluid as described in the Materials and methods. The activity of sPLA₂ was increased significantly (50.92 ± 5.94 units/ml; $p<0.0001$) in the OVA-sensitized and OVA-challenged mice as compared to the sham-sensitized and saline-challenged mice (14.52 ± 1.57 units/ml) (Fig. 4). The oral administration of PBPB (1 mg/kg) during the period of OVA-sensitization and OVA-challenge, significantly prevented the rise in PLA₂ activity (Fig. 4, $p<0.002$). Also, when mice were first OVA-sensitized and OVA-challenged, the administration of PBPB (1 mg/kg) for

1 week significantly decreased the sPLA₂ activity in the BAL fluid (Fig. 4, $p<0.005$).

3.4. PBPB reduces ovalbumin specific IgE in sera

To investigate the effect of PBPB on serum IgE levels, we compared the OVA-specific IgE levels in mice treated with vehicle or PBPB (0.1, 1, and 10 mg/kg) (Fig. 5). The IgE levels increased markedly (384 ± 22.8 ng/ml, $p<0.0001$) in the OVA-sensitized and OVA-challenged mice as compared to the sham-sensitized and saline-challenged mice (30 ± 1.1 ng/ml) (Fig. 5). The oral administration of PBPB (1 mg/kg) during OVA-sensitization and OVA-challenge period, prevented significantly the rise in serum IgE levels (Fig. 5, $p<0.01$). Also, when mice were first OVA-sensitized and OVA-challenged and then treated with PBPB (1 mg/kg) for 1 week, there was a significant fall in the IgE levels in the serum (Fig. 5, $p<0.01$).

3.5. PBPB reduces eosinophils in BAL fluid

The number of eosinophils were markedly elevated (16%) in OVA-sensitized and OVA-challenged mice as compared to those of sham-sensitized and saline-challenged mice (1.8%) (Fig. 6, $p<0.0001$). Interestingly, PBPB (1 mg/kg) treatment during OVA-sensitization and OVA-challenge significantly ($p<0.001$)

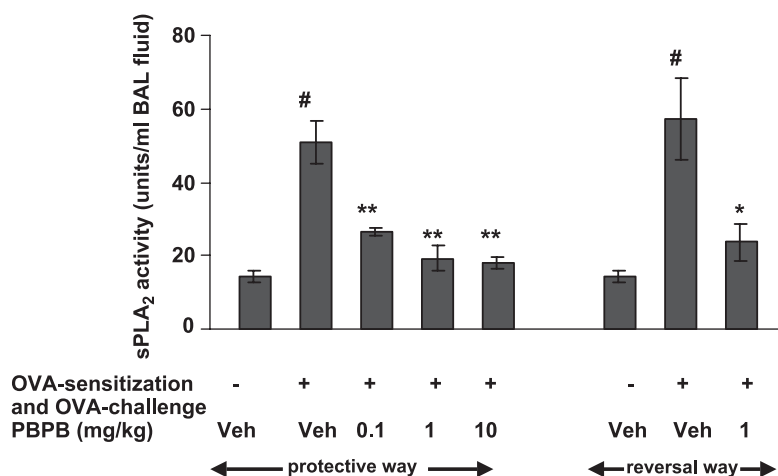


Fig. 4. PBPB inhibits sPLA₂ activity in the BAL fluid of mice. Experiments were performed as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.) ($n=6$ mice in each). # $p<0.001$ as compared to sham-sensitized and saline-challenged group; * $p<0.005$, ** $p<0.002$ indicates significant difference of PBPB treated groups from that of vehicle treated group.

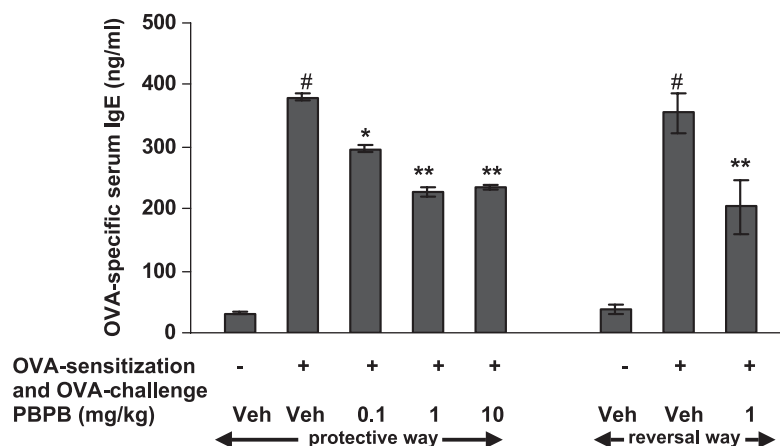


Fig. 5. PBPB reduces OVA-specific IgE in serum. Experiments were performed as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.). # $p<0.0001$ as compared to sham-sensitized and saline-challenged group; * $p<0.05$ and ** $p<0.01$ indicate significant difference of PBPB treated groups from that of vehicle treated group.

attenuated the rise in the eosinophils. When mice were first OVA-sensitized and OVA-challenged and then treated with PBPB (1 mg/kg), the number of eosinophils was also significantly reduced as compared to those of the OVA-sensitized and OVA-challenged mice treated only with vehicle ($p<0.001$).

3.6. PBPB increases IFN- γ and decreases IL-4 and IL-5 in BAL fluid

To determine the effect of PBPB on the levels of IL-4, IL-5 and IFN- γ in the BAL fluid, we measured

the cytokines by ELISA and compared the values between different groups. As shown in Table 1, the level of IFN- γ decreased ($p<0.05$) in the OVA-sensitized and OVA-challenged group of mice as compared to the sham-sensitized and saline-challenged group while those of IL-4 and IL-5 were increased ($p<0.02$ and $p<0.01$, respectively). Interestingly, PBPB (1 mg/kg) treatment increased the IFN- γ during ($p<0.02$) or after ($p<0.01$) OVA-sensitization and OVA-challenge, and decreased IL-4 ($p<0.05$) and IL-5 ($p<0.05$) levels significantly as compared to vehicle treated OVA-sensitized and OVA-challenged.

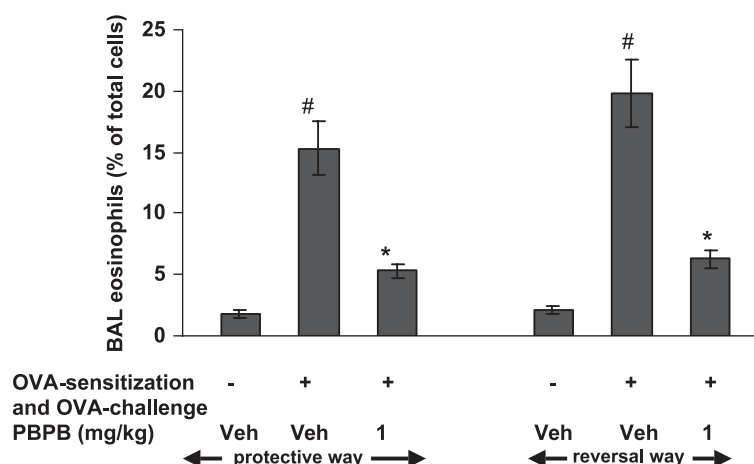


Fig. 6. PBPB reduces the number of eosinophils in BAL fluid. Experiments were performed as described in the Materials and methods. Results are expressed as mean ($\pm$ S.E.M.). # $p<0.0001$ as compared to sham-sensitized group; * $p<0.001$ indicates significant difference of PBPB treated groups from that of vehicle treated group.

Table 1
Effect of PBPB on IFN- γ , IL-4 and IL-5 levels in the BAL fluid of mice

	IFN- γ (pg/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)
Sham-sensitized and saline-challenged	495.0 $\pm$ 152.5	74.7 $\pm$ 15.1	104.8 $\pm$ 15.2
OVA-sensitized and OVA-challenged	162.0 $\pm$ 45.2*	150.1 $\pm$ 19.2**	158.7 $\pm$ 22.5***
PBPB (1 mg/kg) (protective effect)	402 $\pm$ 14.4**	82.3 $\pm$ 14.0*	75.1 $\pm$ 7.4*
PBPB (1 mg/kg) (reversal effect)	425.1 $\pm$ 54.0***	60.0 $\pm$ 17.1*	53.4 $\pm$ 2.2*

The mice were treated with PBPB (1 mg/kg) and cytokine levels in the BAL fluid were measured as described in the Materials and methods. Results are expressed as mean $\pm$ S.E.M. ($n=4$ in each group). * $p<0.05$, ** $p<0.02$, *** $p<0.01$ as compared to sham-sensitized and saline-challenged group; * $p<0.05$, ** $p<0.02$, *** $p<0.01$ as compared to OVA-sensitized and -challenged group.

However, when the sham-sensitized and saline-challenged mice were treated with PBPB (1mg/kg) alone, no significant difference in cytokine levels was observed (data not shown). PBPB, therefore, is capable of modulating the cytokine profile in OVA-sensitization and OVA-challenged mice.

4. Discussion

In the present study, we demonstrate for the first time that PBPB treatment prevents OVA-induced airway constriction and AHR to MCh in OVA-sensitized and OVA-challenged mice (Figs. 2 and 3). The decreased PLA₂ activity in the BAL fluid (Fig. 4) confirms that PBPB inhibits PLA₂ in this model and may be effective in part or in whole by PLA₂ inhibition. However, other modes of action may also operate such as modulating Th2 cytokines and decreasing IgE and eosinophil levels, which are all implicated in the manifestation of airway hyper-responsiveness. Further investigation of these possibilities is warranted.

Our findings that PBPB is effective in attenuating airway hyperresponsiveness (Figs. 2 and 3) may be explained by its inhibitory action on PLA₂ activity. PLA₂ has been found to induce airway hyperreactivity

in rat tracheal smooth muscle [16]. It has been suggested that the increased sPLA₂ breaks the membrane lipid of airways into fatty acids and lysophosphatidylcholine (LPC) [17]. Arachidonic acid metabolites such as cysteinyl leukotrienes are very potent inflammatory mediators and implicated in bronchoconstriction, eosinophil infiltration and mucus hypersecretion [18]. Furthermore, arachidonic acid metabolites [19,20] and LPC [21] are reported to cause desensitization of beta-adrenoceptors in the lung tissue/tracheal strip which are implicated in the genesis of AHR [22,23]. In our mouse experiments, we also observed a significant increase in the sPLA₂ activity in the BAL fluid of OVA-sensitized and OVA-challenged mice (Fig. 4), which could be responsible for the AHR. Interestingly, PBPB is able significantly to inhibit the increased sPLA₂ activity in OVA-sensitized and OVA-challenged mice (Fig. 4). Therefore, one of the potential mechanisms of PBPB action on AHR might be through the inhibition of sPLA₂ activity.

Moreover, some lines of evidence indicated that PLA₂ inhibition might reduce the levels of IgE and Th2 cytokines and other related parameters. Thus, recombinant, enzymatically active PLA₂ from bee venom induces IgE antibody formation in CBA/J (H-2k) mice [24]. It was suggested that this enzymatic activation of mast cells or basophils leads to IL-4 production which favors the differentiation of T-helper cells into TH2 cells, thereby leading to B-cell activation for an IgE response [24]. Most importantly, using a cPLA₂ knock out mice model, it has been demonstrated that OVA-induced bronchoconstriction is more persistent in cPLA₂^{+/+} mice than in cPLA₂^{-/-} mice [25]. It is, therefore, probable that a PLA₂ inhibitor could inhibit TH2 cytokines and IgE production, and thereby reduce allergen-induced bronchoconstriction.

Our cytokine analysis of BAL fluid showed that PBPB decreased IL-4 levels and increased IFN- γ levels (Table 1). This indicates that PBPB can switch the TH2 response towards a TH1 dominated response, and this inhibits IgE production. The early airway response is IgE dependent and is caused by activation of lung mast cells via allergen cross linking of IgE antibodies bound to the high affinity IgE receptor Fc ϵ RI [26]. In the present study, the prevalence of mast cells was not measured. However, the IgE levels

were significantly decreased by PBPB administration both protectively and reversally. It has also been reported that PBPB inhibits IgE mediated secretion of histamine and arachidonic acid from mast cells [27,28]. The attenuating effect of PBPB on airway constriction may, therefore, be attributed to its inhibitory effects on OVA-specific IgE levels (Fig. 5). Using guinea pig lung strip as a model, it was reported that PBPB was able to inhibit Paf-acether or OVA-induced contraction [11]. Synder et al. [29] also demonstrated that PBPB inhibits the PLA₂-mediated contraction of guinea pig lung pleural strips. These findings also support the ability of PBPB to reduce airway constriction as identified in the present study.

The AHR to a vasoconstrictor is mostly associated with increased IL-5 and eosinophils in the lung [30]. The terminal differentiation and proliferation of eosinophils in the bone marrow, recruitment of eosinophils in the tissue and long-term survival of eosinophils are mediated by IL-5 [31]. In the present study, PBPB effectively reduced IL-5 and eosinophils and attenuated AHR to MCh. The reduction of eosinophil counts by PBPB may be attributed to the reduced IL-5 levels in the BAL fluid (Table 1). In addition, PBPB has been found to possess an anti-adhesion property [32]. It is, therefore, possible that PBPB reduces the adhesion of leukocytes particularly eosinophils to the endothelial cells and thereby prevents their migration into the airways and hence attenuates the AHR to MCh.

In conclusion, our study demonstrates that PBPB is capable of alleviating allergen induced airway constriction and AHR to methacholine in a mouse model of asthma. It is capable of inhibiting sPLA₂ activity, modulating cytokine levels and reducing eosinophils in the BAL fluid, and IgE levels in serum. PBPB, therefore, might be useful as a novel lead in the development of new anti-asthmatic drugs.

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