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Bergapten modulates ovalbumin-induced asthma

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ABSTRACT

Prior studies on the anti-inflammatory compound bergapten have shown promise in averting allergic airway hyperresponsiveness. This study sought to establish its possible role in modulating an immunologically-induced airway inflammation and hyperresponsiveness in an ovalbumin model of asthma. Asthma is characterised by inflammation and constriction of the airway passages. Therapy is aimed at bronchodilation and reducing inflammation. Histamine is an inflammatory mediator and involved in immune reactions. It is released from mast cells which are important sentinel cells of the innate immune system involved in the inflammatory response. In the asthma model, ovalbumin solution (2 mg ovalbumin) was administered i.p. as a sensitisation dose on day 1 and on day 14 as a booster dose to sensitise the immune system. This was followed by a 10-day challenge on days 21 – 30 using aerosolised ovalbumin (1% w/v dissolved in phosphate-buffered saline, PBS) at a cut-off latency of 10 min. Bergapten was administered at doses of 3 - 30 mg kg^{-1} p.o. 1 h prior to each challenge. Times to pre-convulsive dyspnoea were recorded. Guinea-pigs were sacrificed and lungs harvested for histological and antioxidant studies. In determining the effect of bergapten on histamine, an isolated guinea pig ileum preparation by the administration of bergapten (1 – 10 μg ml^{-1}) in the presence of histamine. Bergapten increased times to pre-convulsive dyspnoea in the ovalbumin challenge and reduced pathological damage to bronchial tissue. The deposition of collagen around bronchioles was minimised. Antioxidant studies showed higher catalase and superoxide dismutase activity in groups treated with bergapten. Bergapten produced a rightward shift on the Schild plot analysis with a slope of 1.4 and a pA_2 value of 9.3 was obtained in the isolated tissue experiment which confirms that bergapten acts as an antagonist on histaminic H_1 receptors. Bergapten modulates inflammation in ovalbumin-induced asthma and antagonises histamine at the H_1 receptor.

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Introduction

Asthma is a disease of the respiratory tract and is mainly characterised by hyperresponsiveness, narrowing, and inflammation of the airways [2]. It affects about 300 million people worldwide [4]. Persistent airway hyperresponsiveness causes remodelling of the bronchiolar airways due to stimulation of the inflammatory cascade and infiltration by cells of the immune system. This results in muscle hypertrophy, mucous production, and submucosal fibrosis [17]. Inflammation in asthma is mostly restricted to the large conducting airways but with disease progression, spreads to the smaller airways outside the smooth muscle. Inflammatory cells mainly implicated in asthma are mast cells, neutrophils, $CD4^+$ T lymphocytes, and eosinophils which are the most prominent cells [9].

In allergic asthma, an allergen is processed in the airways by dendritic cells in the epithelium and submucosa which express immunoglobin E (IgE) receptors on their surface. The antigen is processed and presented to T lymphocytes. There is sensitisation of the immune system to the allergen and thus a resultant immunological response on subsequent exposure to the specific allergen. T lymphocytes, predominantly Th2, migrate to the site of inflammation and expression other cytokines such as the interleukins. The cytokines expressed enhance recruitment of secondary effector cells such as macrophages, basophils, and eosinophils [[3],[9],[13]].

Mast cells on the surface of the bronchial epithelium are involved in the immediate reaction to the allergen; those located in the deeper submucosa are also involved in inflammation. Mast cells are expressed in larger numbers during chronic inflammation and are associated with the smooth muscle of both large and small airways. Through the release of autacoid mediators such as leukotriene D4, prostaglandin D2, and histamine there is a resultant remodelling of the airways [12]. Histamine is a potent constrictor of smooth muscles including bronchial smooth muscle. High concentrations of it are present in bronchiolar lavage fluid of patients suffering from asthma. Histamine H_1 receptor antagonists dilate bronchioles but are not mainstay therapy in asthma management. They are regarded as supplementary to other drugs [25].

The clinical management of asthma is mainly by the use of corticosteroids and beta-2 (β_2) adrenoceptor agonists. Short-acting β_2 agonists provide quick relief from acute exacerbations whiles patients are managed by the use of inhaled corticosteroids together with longer-acting β_2 adrenoceptor agonists. Other agents used in its management are antimuscarinics and biological agents that target protein mediators of inflammation [18]. Inhaled corticosteroids are associated with long term use side effects including oral thrush [8] while β_2 agonists may result in muscle weakness and tachycardia [16]. New agents with enhanced efficacy continue to be sought to improve the management of asthma.

Bergapten is an anti-inflammatory compound used in managing psoriasis, eczema, and dermatitis [[10],[14]]. Its anti-inflammatory actions have been explored in several in vitro studies. It suppresses the production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) (Bose et al., 2012; Yang et al., 2015). It also repressed lipopolysaccharide activation of IL-1β, prostaglandin E_2, nitric oxide (NO), and cyclooxygenase-2 (COX-2) [28]. Bergapten as demonstrated by Yang et al., [26] meaningfully reduced the recruitment of neutrophils and macrophages migrating toward the site of injury.

Prior studies by Aidoo et al., [1] demonstrated that bergapten alleviates both compound 48/80 and lipopolysaccharide mediated allergic hyperresponsiveness. Bergapten reduced death induced by compound 48/80. In the lipopolysaccharide (LPS)challenge, there was reduced cell infiltration into lung tissue and improved histological parameters (reduced oedema, congestion, and alveolar septa thickening) in animals that received bergapten. Bergapten again protected mice against anaphylactic shock. Bergapten thus demonstrated its ability in alleviating airway-induced hyperactivity. This follow-up study, therefore, sought to evaluate the effect of bergapten treatment in the ovalbumin-induced model of asthma in guinea-pigs.

The ovalbumin-induced model of asthma has been employed in several studies to assess immune response mechanisms to asthma as well as evaluate potential anti-asthmatic agents. This chronic model would provide an appropriate study in determining further the potential use and development of bergapten in managing allergic asthma. Ovalbumin provides an IgE mediated humoral response similar to what occurs in humans, in comparison to the previously studied acute LPS-induced allergic hyperresponsiveness which is non-IgE mediated [21]. On the surface of the guinea pig ileum are expressed histamine H_1 receptors similar to those on the bronchi and this allows for the possible direct anti-histaminic effects of bergapten on isolated guinea pig ileum to be determined. Antagonism of histamine may contribute to its overall effect.

Materials

Animals

Guinea-pigs of either sex with weights between 400 – 450 g were obtained from Noguchi Memorial Institute for Medical Research, Legon, Ghana and acclimatised in the Animal House of the Department of Pharmacology, Faculty of Pharmacy and

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Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Ghana, for 7 days before experimentation. The animals were kept in cages with softwood shavings as their bedding and were provided with standard chow and distilled water ad libitum under hygienic conditions. Animals were handled appropriately throughout the experiments in accordance with Animal Welfare Regulations (USDA 1985; US Code, 42 USC § 289d) and Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011). All procedures and techniques were approved by The Ethics Committee of the Department of Pharmacology, KNUST.

Drugs and Chemicals

Bergapten and ovalbumin (OVA), were acquired from Sigma Aldrich (St. Louis, USA). Dexamethasone and Sulphasalazine were obtained from Pfizer (England, UK). Acetic acid, diethyl ether, Tween 20, chloroform, dimethyl sulfoxide (DMSO), ethanol and glacial acetic acid were obtained from VWR International (France). Dithio-bis-2-nitrobenzoicacid (DTNB) Trichloroacetic acid (TCA), Thiobarbituric acid and (TBA), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), monosodium phosphate (NaH_2PO_4), sodium bicarbonate ($NaHCO_3$) and glucose were acquired from VWR Chemicals (Chicago, USA).

Methods

Ovalbumin-Induced Asthma

Sensitisation and ovalbumin challenge

Guinea pigs were placed in 5 groups (n = 5). Sensitisation was done by i.p administration of 100 µl ovalbumin solution (2 mg ovalbumin and 10 mg aluminium hydroxide as an emulsifier to make 10 ml using normal saline). A booster dose (100 µl) of the ovalbumin solution was again administered i.p 14 days after sensitisation. The naive control group was sham-sensitised and boosted by i.p administration of 100 µl ml of normal saline. Ovalbumin challenge took place from day 21 to 30 using aerosolized ovalbumin (1% w/v dissolved in phosphate-buffered saline, PBS) daily for 10 min.

Pre-convulsive dyspnoea

Guinea pigs were pre-treated 1 h before the challenge. Animals received either normal saline (5 ml kg^{-1}), dexamethasone (3 mg kg^{-1}) or bergapten (3, 10, 30 mg kg^{-1}) p.o and the time to pre-convulsive dyspnoea determined.

Histology

Left lungs were harvested after sacrifice and dissection of guinea pigs. Left lungs were fixed in 10 $\%^{v}/_{v}$ formalin for histological processing whiles the right lungs were immediately stored in 15 ml of phosphate buffer saline (PBS).

Airway and alveolar cell infiltration. Transverse sections of the left lung of about 3µm were made and stained with haematoxylin and eosin (H & E). Evaluation of airway inflammatory cell infiltration was done as described previously by Zare et al., (2008) with a few modifications. Scores were assigned as follows: 0, no cell; 1 a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2 – 4 cell layers deep; and 4, a ring of cells >4 cell layers deep in the peribronchiolar and perivascular regions. Assessments were also made on alveolar cell infiltration as follows: 0, no infiltrate or widening septa; 1, few infiltrates with widening septa; 2; obvious infiltrates with widening septa; and 3, filled alveolar airspaces with thickened septa. Scores for peribronchiolar, perivascular, and alveolar cell infiltration were summed into an 11-point composite score.

Evaluation of collagen deposition. Segments of guinea pig left lung tissue were stained using Masson's trichrome stain. Lung remodelling was evaluated by determining the total length of the basement membrane of selected bronchioles (5 sections for each animal from the lower lung) and the corresponding peribronchiolar fibrotic section for each treatment. The extent of fibrosis was evaluated as the mean area of collagen deposition per unit length of the basement membrane. Between 5 - 7 average-sized bronchioles from each section were examined, and the average scores for each group computed.

Antioxidant studies

Right lungs were harvested and immediately stored in 15 ml of phosphate buffer saline (PBS). The right lung tissues were homogenised and stored at −80°C until use.

Superoxide dismutase (SOD). The estimation of SOD activity was determined by a modified method of Misra and Fridovich [19]. Ice-cold chloroform 150 μL and 750 μL ethanol (96%v/v) were added to 500 μL lung tissue homogenate vortexed for 1 min and centrifuged at 2000 rpm for 20 min. To 500 μL supernatant, 500 μL EDTA (0.6 mM), and 1ml carbonate bicarbonate buffer (0.1M, pH 10.2) was added. To establish a reaction, 50μl adrenaline (1.3 mM) was added. The absorbance was measured at 480 nm against a blank. The measurement of the quantity of enzyme needed to inhibit the auto-oxidation of adrenaline i.e. SOD activity was calculated using the equation:

$$\%inhibition = \left[\frac{\text{Absorbance test} - \text{Absorbance blank}}{\text{Absorbance test}}\right] \text{x } 100$$

SOD level was expressed in units per mg protein, where 1 unit of enzyme activity is the quantity of enzyme required to prevent the auto-oxidation of adrenaline at 25°C, and calculated with the equation:

$$units\,of\,SO\,Dactivity/mgprotein = \left[\frac{\%\ \text{inhibition}}{50\ \times\ \text{weight of protein}}\right] \times 100$$

Catalase (CAT). The method described by Sinha [24] with slight modification was used. Tissue supernatant 100 μl, was pipetted and mixed with 1 ml phosphate buffer (0.01M, pH7.0), and 400 μl H_2O_2 (1.18M), and the mixture was left to stand for 5 min. After this 2 ml of a 3:1 mixture of glacial acetic acid and dichromate (5%) was added to halt the reaction. The absorbance was measured at 620 nm with a Synergy H1 Hybrid Reader spectrophotometer (BioTek Technologies, Winooski, VT, USA). One unit of catalase activity, defined as the amount of enzymes that degrade 1 mmol H_2O_2 per min at 25°C and pH 7.0, was expressed in terms of its molar extinction coefficient, $39.4M^{-1}cm^{-1}$.

$$mUnitCAT/mgprotein = \left[\frac{\text{Absorbance 620 nm}}{394\ \times\ \text{weight of protein}}\right] \times\ 1000$$

Direct Anti-Histaminic Effect of Bergapten

The method of Dhonde et al., [5] was followed with modifications. A 400 g guinea pig was fasted overnight and sacrificed by cervical dislocation and exsanguinated by carotid artery transaction. The ileum was quickly removed and put into a Petri dish containing Tyrode's solution (NaCl 137 $mmolL^{-1}$, KCl 5 $mmolL^{-1}$, $CaCl_2$ 2 $mmolL^{-1}$, $MgCl_2$ 1 $mmolL^{-1}$, NaH_2PO_4 1 $mmolL^{-1}$, $NaHCO_3$ 12 $mmolL^{-1}$ and glucose 11 $mmolL^{-1}$) at pH 6.5. The ileum was mounted in an organ bath containing 20 ml Tyrode's solution under a basal tension of 10 mN. The Tyrode's solution was kept at 32 ± 0.5°C and continuously aerated with carbogen (95% O_2 + 5% CO_2). The tissues were allowed to equilibrate for 30 min, while the bathing solution was changed every 10 min. The contractile response of the mounted isolated guinea pig ileum to histamine (2, 20, 200, and 1000) μg ml^{-1} in the presence and absence of bergapten (1 – 10 μg ml^{-1}) was recorded.

Statistical Analysis

Results are presented as mean ± SEM using GraphPad Prism Software for Microsoft Windows, version 7.0. To ascertain the difference between groups one-way analysis of variance (ANOVA) was employed. Multiple comparisons between the treatment groups were performed using Dunnet's post hoc test value set at $p < 0.05$. The time course curves for pre-convulsive dyspnoea time was subjected to two-way (treatment × time) repeated measures analysis of variance followed by appropriate post hoc test.

Results

Effect of Bergapten on Ovalbumin-Induced-Asthma

Effect of Bergapten on Pre-convulsive Dyspnoea Times in Ovalbumin-Induced Asthma

Times to pre-convulsive dyspnoea were recorded and presented as a time course curve [Fig. 1(a)] and a two-way ANOVA repeated-measures (Treatment x time) carried out. The naïve control group which was exposed to aero solubilised saline showed no signs of dyspnoea throughout the experiment at a cut-off latency of 10 min while animals exposed to ovalbumin showed signs of dyspnoea [Fig. 1(a)]. On drug treatment with dexamethasone, there was a statistically significant difference on days 1, 2, 5, 7, 8, 9, and 10 relative to the disease control group. Bergapten 3 mg kg^{-1} showed no statistical significance on any day when compared to the disease control group. Given at 10 mg kg^{-1} it was significantly effective on day 8. When administered at 30 mg kg^{-1}, the bergapten-treated group was significantly different compared to the OVA disease control group exposed to aero solubilised ovalbumin on days 1, 2, 3, 8 and 10. The AUC for the time course curve was determined and the data presented as a column graph [Fig. 1(b)] to quantify the total response over the 10 days. In agreement with the results obtained in the time-course curves, dexamethasone and bergapten 10, 30 mg kg^{-1} were statistically significant at a p-value of $p < 0.001$ while bergapten 3 mg kg^{-1} was not statistically significant when compared to the disease control.

Histopathology of lung tissue in ovalbumin-induced asthma

Lung tissues were stained with H & E and 10 fields for each group were viewed under a light microscope under X40 magnification. Cell infiltration scores were assigned to quantify the extent of inflammatory infiltrates. The naïve group showed normal lung histopathology with clear lumens and epithelial cell lining as well as septa. Bronchioles had a few rings of cells surrounding them with few macrophages present [Fig. 2(a)]. Ovalbumin disease control group showed narrow lumens and rings of cell infiltrate surrounding the bronchioles and blood vessels [Fig. 2(b)]. Dexamethasone reversed cell infiltration and narrowing of lumens with clear septa [Fig. 2(c)]. Bergapten at 3 mg kg^{-1} did not show any protective effect against histopathological damage and bronchioles were similar to that of the disease control. At 10 mg kg^{-1}, there was moderate protection with lesser narrowing of the bronchioles while the 30 mg kg^{-1} dose reduced cell infiltration and narrowing of

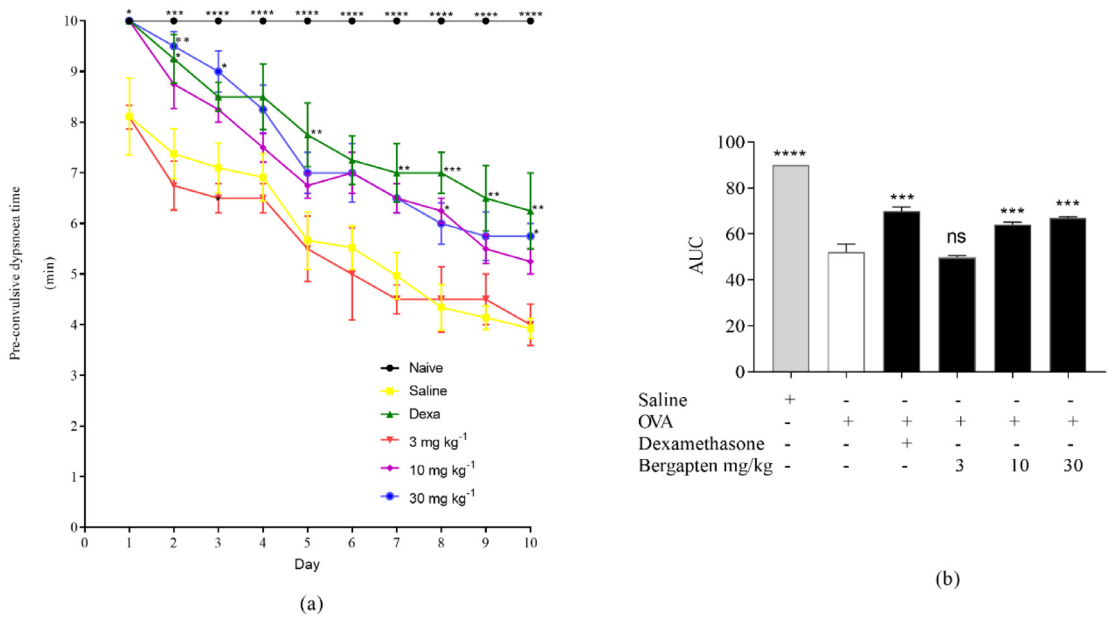


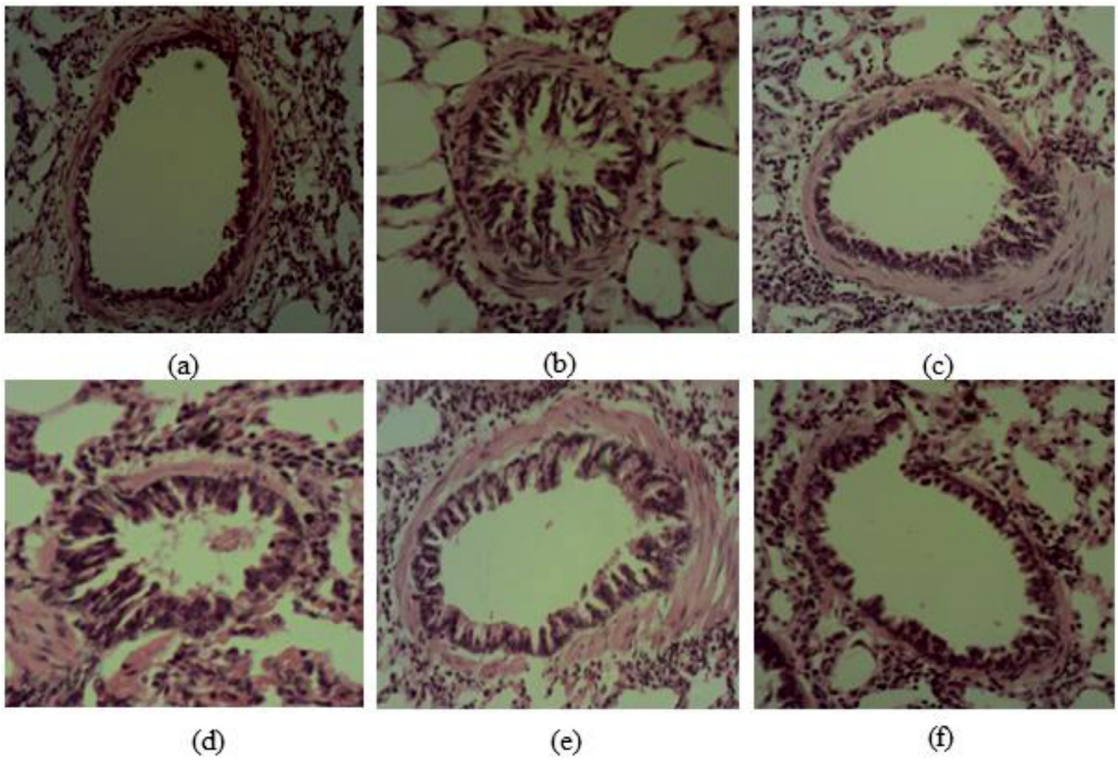
Fig. 1. Effect of bergapten on pre-convulsive dyspnoea time. Guinea-pigs were treated with either saline 5 ml kg^{-1}, dexamethasone 3 mg kg^{-1}, or bergapten 3 – 30 mg kg^{-1} and exposed to aero solubilised ovalbumin for 10 min for 10 consecutive days after a sensitisation and booster doses were administered. Pre-convulsive dyspnoea times were recorded and presented as a time course curve (a) and the AUC (b). Data is presented as mean ± S.E.M. (n = 5). ***p <0.001, ****p <0.0001 compared to the disease control group; ####p <0.0001 compared to the naive group (Two-way ANOVA repeated measures for "a") and (One-way ANOVA followed by Dunnett's post hoc test for "b")

bronchiole lumens [Fig. 2(d-f)]. The mean infiltration score for dexamethasone was 2.25 ± 0.48 and that for bergapten 3, 10 and 30 mg kg^{-1} were 6.75 ± 0.48, 3.5 ± 0.65 and 2.75 ± 0.48 respectively [Fig. 2(g)]. Treatment with dexamethasone reduced infiltration scores significantly at a p-value of $p<0.001$. While bergapten at 3 mg kg^{-1} did not show any significant effect the 10 mg kg^{-1} and 30 mg kg^{-1}-treated groups were statistically significant at $p<0.01$ compared to the disease control group.

Effect of bergapten on collagen deposition in ovalbumin-induced asthma. Lungs were harvested, the tissues processed and trichome stained [Fig. 3(a-f)]. The collagen deposited around the bronchioles was stained green and this was measured to determine its area in relation to the basement membrane. Normal bronchioles in the naïve control group [Fig. 3(a)] had a normal thickness of collagen around the bronchioles while in the disease control group [Fig. 3(b)] there was an extensive deposition of collagen and narrowing around the lumen. Dexamethasone reduced collagen deposition [Fig. 3(c)] and so did bergapten at doses of 10 and 30 mg kg^{-1} [Fig. 3(e-f)]. Bergapten 3 mg kg^{-1} treated rats however, did not show reduced collagen deposited around lumens [Fig. 3(d)]. The area of collagen deposition was then quantified, and the results analysed. The naïve control animals had little collagen surrounding the bronchioles with an average area per length of the basement membrane of 0.18 ± 0.05 $\mu m^2/\mu m$. In the disease control group, there was a significantly higher deposition of collagen surrounding the bronchioles with an average per length of the basement membrane of 1.23 ± 0.09 $\mu m^2/\mu m$. Treatment with dexamethasone significantly reduced collagen deposition surrounding tissues at $p <0.001$. Bergapten at the lowest dose used did not show any significance when compared to the saline-treated OVA control group, however, the 10 and 30 mg kg^{-1} doses significantly reduced collagen deposition surrounding tissues [Fig. 3(g)].

Antioxidant effect of bergapten on lung tissue in ovalbumin-induced asthma

Oxidative stress markers were assayed in homogenized lung tissue. In the SOD assay (Fig. 4(a)), the enzymes present in the naive group were 14.00 ± 0.41 U/mg of protein while significantly lower levels of 6.75 ± 0.48 U/mg of protein was recorded for the saline-treated ovalbumin disease group. Dexamethasone maintained units of enzyme levels of 12.75 ± 0.48 U/mg of protein comparable to the naive group. Bergapten 3, 10, and 30 mg kg^{-1} treated guinea pigs had values of 7.75 ± 0.48, 9.75 ± 0.25, and 10.5 ± 0.29 U/mg of protein respectively showing a dose-dependent elevation in units of enzymes. In the catalase enzyme assay (Fig. 4(b)), a similar trend was observed with the OVA disease control having a mean score of 2.88 ± 0.15 mU/mg of protein significantly lower than levels of 5.75 ± 0.25 mU/mg of protein recorded in the naive control. Dexamethasone-treated animals had enzyme levels similar to the naive with 5.5 ± 0.29 mU/mg of protein. Bergapten 3 mg kg^{-1} treated rats were not statistically significant with an enzyme activity of 3.1 ± 0.11 mU/mg of protein while doses of 10 and 30 mg kg^{-1} were statistically significant at a p-value of $p<0.001$ with enzyme activity levels of 4.2 ± 0.11 and 4.48 ± 0.17 mU/mg of protein respectively.



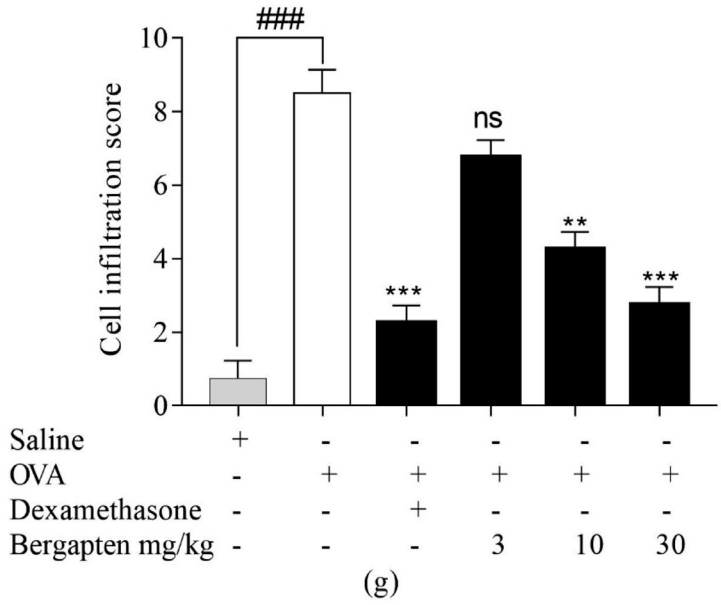
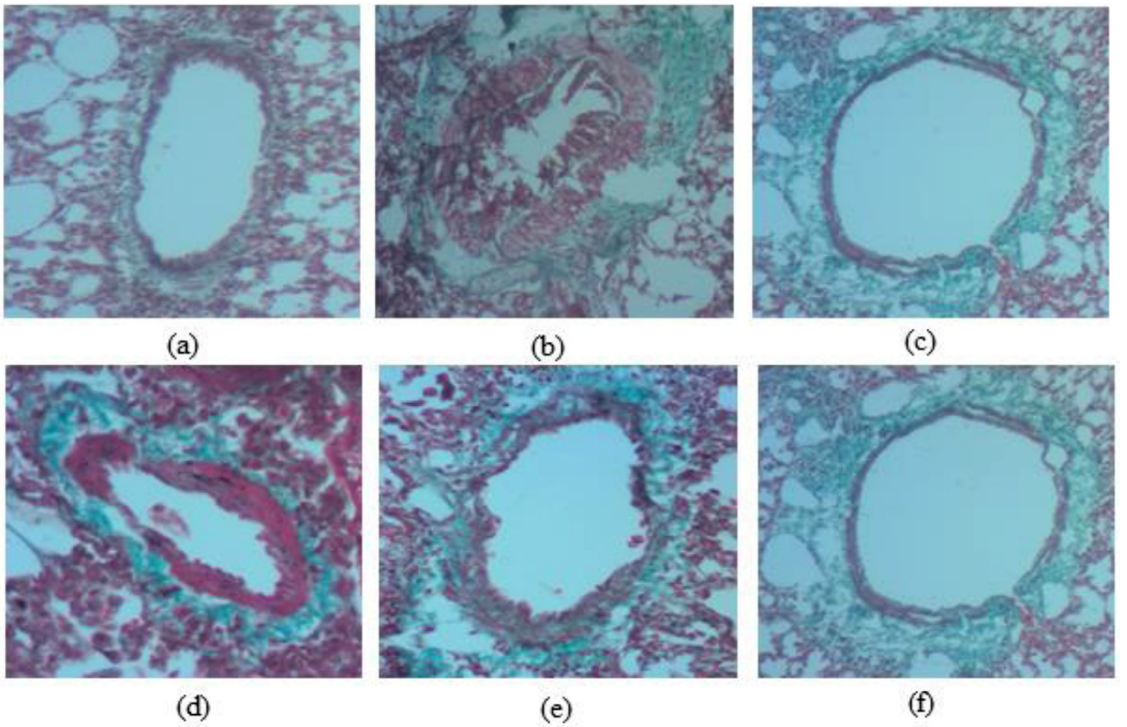


Fig. 2. Effect of bergapten on lung histopathology. Guinea pigs received either normal saline 5 ml kg^{-1}, dexamethasone 3 mg kg^{-1}, or bergapten 3-30 mg kg^{-1} p.o for 10 consecutive days during the OVA challenge. Lung tissue was stained with H & E and examined under the microscope (magnification X40 objective lens). a – naive control, b – ovalbumin disease control, c – dexamethasone (3 mg kg^{-1}), d–f bergapten (3, 10, 30 mg kg^{-1}) respectively. Cell infiltration scores were assigned (g). Data is presented as Mean ± S.E.M. $^{***}p < 0.001$ ns – not statistically significant, compared to the ovalbumin disease control group; $^{\#\#\#\#}p < 0.0001$ compared to the naive group (One-way ANOVA followed by Dunnett's post hoc test).



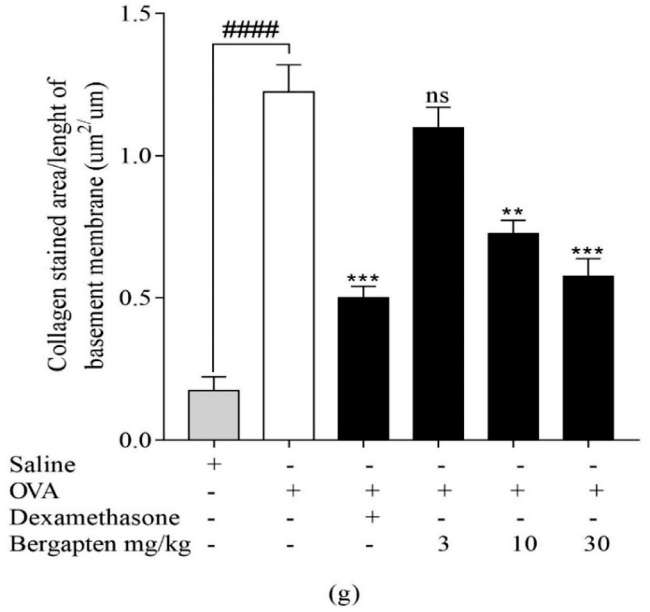


Fig. 3. Effect of bergapten on collagen deposition in ovalbumin-induced asthma. Guinea pigs received either normal saline 5 ml kg^{-1}, dexamethasone 3 mg kg^{-1}, or bergapten 3-30 mg kg^{-1} p.o during the OVA challenge. Lung tissue was harvested, processed and trichome stained and viewed under the microscope (magnification X40 objective lens). a – naive control, b – ovalbumin disease control, c – dexamethasone 3 mg kg^{-1}, d – f bergapten 3, 10, 30 mg kg^{-1} respectively. The average area per length of the basement membrane (g). Data is presented as Mean ± S.E.M. $^{**}p < 0.01$ $^{***}p < 0.001$, $^{****}p < 0.0001$ ns – not statistically significant, compared to the ovalbumin disease control group; $^{####}p < 0.0001$ compared to the naive group (One-way ANOVA followed by Dunnett's post hoc test).

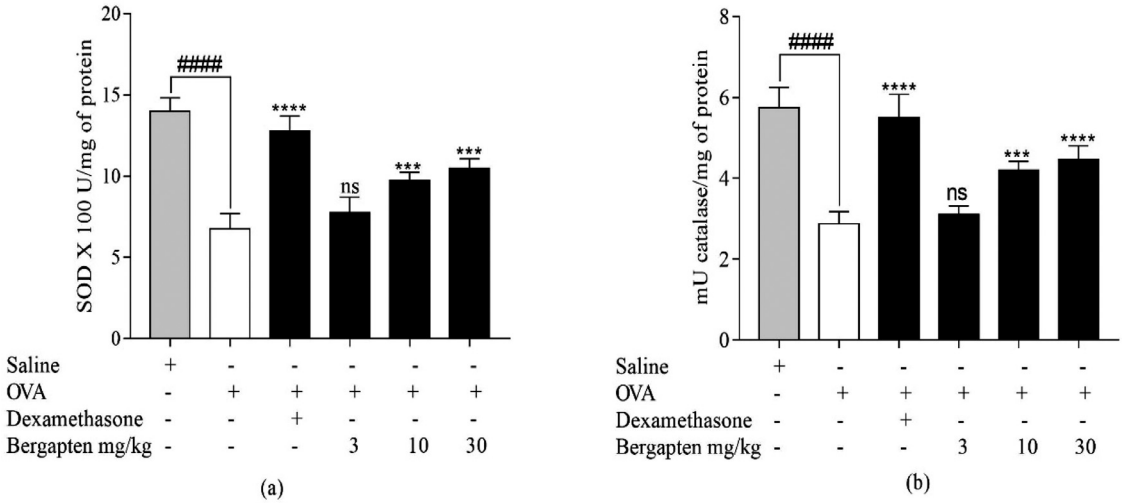


Fig. 4. Effect of bergapten on oxidative stress markers in ovalbumin-induced asthma. Guinea pigs received either saline 5 ml kg^{-1}, dexamethasone 3 mg kg^{-1}, or bergapten 3 – 30 mg kg^{-1}. Animals were exposed to aero solubilised ovalbumin and sacrificed. Lung tissues were harvested and oxidative stress markers assayed; superoxide dismutase (a), catalase (b). Data is presented as Mean ± S.E.M. (n = 4). ***p <0.001, ****p <0.001, ns – not statistically significant compared to the disease control group; ####p <0.0001 compared to the naive group (One-way ANOVA followed by Dunnett's post hoc test).

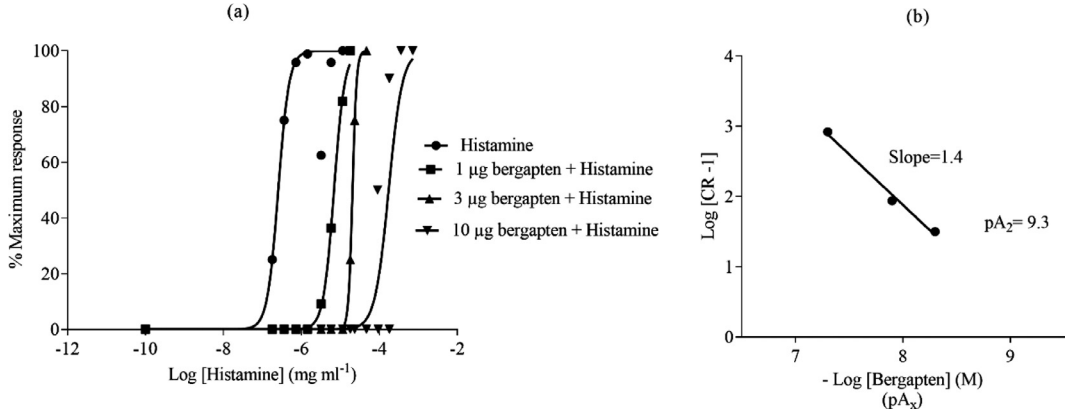


Fig. 5. Effect of bergapten on histamine-induced contractions on isolated guinea-pig ileum. Isolated guinea pig ileum preparation was set up as described in the methods. The contractile responses of the ileum to histamine (0.002 – 1 mg ml^{-1}) in the absence and presence of bergapten (1 – 10 µg ml^{-1}) were recorded. Schild plot analysis for the antagonism by bergapten in the histamine-dependent contractions of isolated guinea-pig ileum was recorded.

Anti-histamine effect of bergapten on isolated guinea pig ileum

The effect of bergapten on histaminic H_1 - receptors was studied on a guinea - pig ileum. Dose-response curves for histamine were obtained and repeated in the presence or absence of bergapten. Administration of bergapten (1 – 10 µg ml^{-1}) causes a parallel rightward shift of the log agonist concentration-response curve with no depression of the maximum response [Fig. 5(a)]. Schild plot analysis was done and a slope of 1.4 was obtained [Fig. 5(b)]. The Schild plot analysis also produced a straight line with an intercept of pA2= (-log kd), which is a measure of the affinity of the antagonist for its receptor. From the graph, a pA2 value of 9.3 was obtained which showed that bergapten acts as an antagonist of strong potency on histamine receptor blocking activity [Fig. 5(b)].

Discussion

In this study, bergapten significantly increased pre-convulsive dyspnoea times in ovalbumin challenged animals which were also sensitised with intraperitoneal administration of ovalbumin and alum. This signifies reduced hyperresponsiveness to the allergen. There was therefore visible reduced microscopic damage, in a dose-dependent manner, in lung tissues which were stained for microscopic assessment. Collagen deposition around bronchioles was further reduced in trichome stained tissues. The reduced allergic and inflammatory effects resulted in reduced oxidative catalase and superoxide dismutase stress

markers which were measured in the homogenised lung tissues. In determining the antihistaminic effect of bergapten on histamine H_1 receptors, there was a rightward shift in the dose-response curve plotted which indicates antagonistic activity.

Asthma is a condition characterised by wheezing, shortness of breath, and inflamed bronchioles which narrows air passage through it. Ovalbumin was used as the trigger of the allergic reaction and acts by the Th2 mediated immune response mechanism which eventually result in the IgE mediated activation of mast cells and basophils. The activation of these cells releases histamine, prostaglandins, interleukins, and other cytokines which cause airway hyperresponsiveness in the early phase of asthma [11]. Bergapten has previously demonstrated anti-allergic effects and mast cell stabilisation [[1], [27]] which may explain the effects seen in the results showing reduced airway hyperresponsiveness. Guinea pigs were exposed to aerosolubilised ovalbumin for the challenge in the treatment groups to induce an asthmatic response. Times to pre-convulsive dyspnoea were increased for bergapten when administered prophylactically.

In the late/chronic phase of asthma, there is the subsequent release of eosinophils, macrophages, and other inflammatory cells which in turn enhances the release of more cytokines resulting in remodelling. Eosinophils are the major cells responsible for this. [17]. Remodelling of airway tissue is often associated with asthma because of continuous injury due to inflammatory agents and production of ROS This produces the hypertrophy and causes deposition of collagen around the septa of bronchioles [7]. Histopathological assays showed reduced hypertrophy and narrowing of bronchial airways in groups treated with bergapten 10 and 30 mg kg^{-1}. Hypertrophy and narrowing occur with persistent exposure to allergens which induces an asthmatic attack. There was reduced cell infiltration by lymphocytes and eosinophils when viewed microscopically using H & E staining. Collagen was deposited around bronchioles and blood vessels as was observed. This was intensive in the disease control group but reduced with bergapten treatment. Bergapten with its properties of reducing inflammatory cells such as eosinophils and macrophages and cytokines such as interleukin-6 may explain these effects.

Reduction in the remodelling of airways may also be attributed to the antioxidant effects of bergapten shown in the catalase and superoxide dismutase assays. Oxidative stress is usually induced with tissue injury and inflammation and studies have shown a correlation between the consumption of antioxidants and lung activity (Corradi et al., 2003). In asthma, there is a reduction in catalase, superoxide dismutase, and glutathione. Increased production of reactive oxygen species leads to increased hyperresponsiveness as well. An antigen challenge results in the immediate production of oxygen radicals [22]. A high presence of reactive oxygen species may change the immune response and result in activation of NF-kβ, a powerful inducer of pro-inflammatory genes [6]. Bergapten with its antioxidant effects would hence, have contributed to the overall reduced remodelling and protective effect against ovalbumin-induced asthma.

Histamine causes contraction via activation of histaminic H_1 receptors located on the smooth muscles of the intestine, bronchi, trachea, and capillaries [15]. We investigated the contractile response produced by histamine on isolated guinea pig ileum in the presence and absence of bergapten. The administration of bergapten caused a parallel rightward shift of the log agonist concentration-response curve with no depression of the maximum response. Schild plot analysis was done and a slope of 1.4 was obtained. Evidence from research shows that a slope that is significantly greater than 1 indicates positive cooperativity in the binding of the antagonist, depletion of a potent antagonist from the medium by receptor binding or non - specific binding, or lack of antagonist equilibrium (Boskabady & Shaikhi 2009; [23]). The Schild plot analysis also produced a straight line with an intercept of pA_2 = (-log Kd), which is a measure of the affinity of the antagonist for its receptor (antagonist potency). From the graph, a pA_2 value of 9.3 obtained showed that bergapten acts as an antagonist of strong potency on histamine H_1 receptor blocking activity.

Overall, this study shows the potential for the use of bergapten in managing allergen-induced asthma by reducing airway responsiveness and inflammation which culminates in remodelling of bronchial tissue in asthmatic patients. However, in some cases of asthma, there is glucocorticoid insensitivity or resistance and the use of oral glucocorticoids produces numerous systemic effects [20]. Bergapten with demonstrated anti-allergic effects and showing promising signs in alleviating allergen mediated asthma would be a suitable candidate to be further assessed for its possible role in managing situations of glucocorticoid insensitivity or resistance. Also, the ability of bergapten to directly block the effects of histamine may enhance its use in the management of the condition.

Declaration of Competing Interest

None. We the authors declare no conflict of interest.

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Data availability

All data in support of the findings in this study can be obtained from the corresponding author upon request.

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