

The Possible Protective Effect of Cinnamic Acid on Ovalbumin-Induced Asthma in Mice

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Abstract

Asthma is a chronic respiratory disorder in which immunological and structural cells play a role. The limits of conventional medicines necessitate the development of innovative therapeutic techniques for asthma. In the present study, we investigated the possible protective effect of cinnamic acid (CA) on ovalbumin-induced asthma in a mouse model. Sixty albino male mice BALB/c 20-30 g were chosen at random and divided into five groups 12 animals each: Group I: PBS/liquid paraffin negative control. Group II: asthma model group. Group III: cinnamic acid control group; mice received cinnamic acid 50 mg/kg in liquid paraffin orally by gavage. Group IV: asthma model/CA 25 mg/kg; mice received 25 mg/kg in liquid paraffin orally by gavage. Group V: asthma/ (50 mg/kg) cinnamic acid group; mice received cinnamic acid 50 mg/kg in liquid paraffin orally by gavage. The experiment continued for 14 days. On day 15, broncho-alveolar lavage fluid, blood and lung tissue was collected. Total cell count, tissue TNF- α , IL-33, and serum IgE increased considerably after sensitization to ovalbumin (OVA). On the other hand, administration of cinnamic acid in 25mg and 50mg/kg has significantly decreased total WBC count, tissue TNF- α , IL-33, and serum IgE results. These findings suggest that cinnamic acid has a protective effect against OVA-induced allergic asthma in mice, possibly through its inhibitory activity on some proliferative modulating enzymes.

Keywords: Allergic asthma, Airway inflammation, Ovalbumin OVA, Cinnamic acid (CA), Chronic inflammation.

التأثير الوقائي المحتمل لحمض السيناميك على نموذج الربو المستحث في الفئران بواسطة بروتين البيض

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الخلاصة

الربو هو اضطراب تنفسي مزمن تلعب فيه الخلايا المناعية والهيكلية دورًا. تقتضي محدودية الأدوية التقليدية تطوير تقنيات علاجية مبتكرة للربو. في هذه الدراسة، قمنا بالتحقق من التأثير الوقائي المحتمل لحمض السيناميك (CA) على الربو الناتج عن البومين البيض في الفئران. تم اختيار ستين فئرا من الذكور من النوع BALB/c (20-30) جرامًا عشوائيًا وتم تقسيمها إلى خمس مجموعات 12 فئرا لكل مجموعة: المجموعة الأولى: (الطبيعية) PBS \ البارافين السائل. المجموعة الثانية: (الربو التحسسي). المجموعة الثالثة: التحكم (حمض السيناميك): تلقت الفئران حمض السيناميك 50 مجم/كجم في البارافين السائل عن طريق الفم. المجموعة الرابعة: الربو التحسسي + 25 مجم/كجم حمض السيناميك: تم إعطاء الفئران 25 مجم/كجم من حمض السيناميك في البارافين السائل عن طريق الفم. المجموعة الخامسة: الربو التحسسي + 50 مجم/كجم حمض السيناميك: تلقت الفئران حمض سيناميك 50 مجم/كجم في البارافين السائل عن طريق الفم. استمرت التجربة لمدة 14 يومًا. في اليوم الخامس عشر، تم جمع سائل غسيل القصبات الهوائية والدم وأنسجة الرئة. ارتفعت مستويات TNF- α و IL-33 و IgE بشكل كبير بعد التحسس بواسطة البومين البيض (OVA). من ناحية أخرى، أدى إعطاء حمض سيناميك 25 مجم و 50 مجم/كجم إلى انخفاض كبير في عدد كريات الدم البيضاء، وانخفاض في مستويات TNF- α و IL-33 في نسيج الرئة، ومستوى IgE في الدم، تشير هذه النتائج إلى أن حمض السيناميك له تأثير وقائي ضد الربو التحسسي في الفئران، من خلال نشاطه المثبط أو المعدل على الانزيمات التكاثرية.

الكلمات المفتاحية: الربو التحسسي، التهاب الشعب الهوائية، البومين البيض OVA، حمض السيناميك (CA)، الالتهاب المزمن.

Introduction

Asthma is a chronic inflammatory respiratory illness characterized by wheezing, chest tightness, dyspnea, and cough, which are all symptoms of airway obstruction. They can occur on their own, in the early morning or late at night, following exposure to an allergen or physical activity ^(1,2). Depending on where you reside, asthma has a broad variety of prevalence, mortality, and severity. Although high-income countries have a higher incidence of asthma, asthma mortality is greatest in low- and middle- income countries.

Adults have greater prevalence of asthma-related mortality and healthcare consumption than children, despite the fact that children have a higher frequency and incidence of asthma ⁽³⁾. Acute reversible airway obstruction, persistent airway hyperresponsiveness (AHR), and airway inflammation are all characteristics of allergic asthma in humans, which is described as a chronic inflammatory illness of the airways that affects the respiratory system ^(4,5).

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Structure alterations in the airway include subepithelial and airway wall fibrosis, goblet cell hyperplasia/metaplasia, smooth muscle thickening, and enhanced vascularity⁽⁶⁾. 'Airway modifications' or 'remodeling' are the consequence of repeated allergen exposure that causes recurring or continuing inflammation in the airways⁽⁷⁾. Chronic inflammation and structural alterations are hypothesized to contribute to asthma symptoms by having functional effects. Airway remodeling, or structural changes in the airways associated with asthma, is a pathogenic aspect of chronic asthma that contributes to the disease's clinical presentations⁽⁸⁾. T-Helper 2 (TH-2) cells release different pro-inflammatory cytokines that play essential roles in the development of allergic asthma, including a specific profile of interleukin (IL)-4, IL-5, and IL-13, and this model replicates the clinical features of allergic asthma. Furthermore, they can cause the creation of mucus, as well as the release of allergen-specific immunoglobulin (IgE), chemokines, and eosinophils⁽⁹⁾. Hydroxycinnamic acids (HCA) are a family of phytochemicals, or plant-derived chemicals, with a variety of health benefits, but they are not considered essential nutrients. This group of phenolic compounds is among the most often encountered in nature. Phenolic substances are plant metabolites that are common in plant-based meals and drinks, according to research. They aid in defense against UV radiation and disease assaults in plants, and they also play a vital role in growth and development^(10,11).

Since cinnamic acid is a natural chemical with an anti-inflammatory action as a regulator of particular enzyme activity, and stop cells from multiplying.

The purpose of this study was to see whether cinnamic acid could protect against OVA-induced allergic asthma.

Material and Methods

Materials

Cinnamic acid and OVA were purchased from SIGMA-ALDRICH CHEMIE GmbH. Diethyl ether supplied from ROMAN pure chemistry, UK. Paraffin oil supplied from Applichem GmbH/Germany. ELIZA (enzyme linked immunosorbent assay) kits for IL-33, TNF- α , and IgE were supplied by SHANGHAI YEHUA Biological Technology Co.Ltd, China.

Experimental animal

Sixty adult albino male BALB/c mice (weighing between 20 and 30 g) obtained from and kept in the animal house of the College of Pharmacy/University of Baghdad under specific pathogen-free conditions, provided with water and food ad libitum during a 12-hour light-dark cycle and kept during the study with regular room temperature (18 -21°C). Animals were handled throughout this process in accordance with the

Helsinki Declaration, which was approved by the University of Baghdad's ethical committee for the use of animals in research.

Experimental protocol for allergic asthma model

For preparation of allergic asthma model, OVA is used which is the main protein found in egg white, which is not essentially immunogenic and therefore it must be systemically injected. Multiple systemic allergen injections are often required in acute sensitization procedures. Intraperitoneal administration of 1 ml of 10% OVA on day 1, then exposed to an aerosol containing of (1% OVA) for 14 successive days, 30 min /day, in an aerosol chamber built for this purpose from a portable nebulizer and a plastic food container as a nebulizing chamber^(12,13).

Study design

60 mice were divided randomly into 5 groups 12 animals each:

- 1) Group I: healthy control (phosphate buffer saline (PBS) / liquid paraffin).
- 2) Group II: asthma model (OVA-challenged).
- 3) Group IV: asthma model with cinnamic acid group: mice received (25 mg/kg) in liquid paraffin orally by gavage.
- 4) Group V: asthma model with cinnamic acid group: mice received cinnamic acid (50 mg/kg) in liquid paraffin orally by gavage.
- 5) Group III: cinnamic acid control group: mice received cinnamic acid (50 mg/kg) in liquid paraffin orally by gavage.

On day 15, mice were sacrificed; samples of blood were collected from the retro-orbital vein and centrifuged for 20 min at 3000g. Subsequently, the serum was preserved at -20°C to determine IgE levels. Six mice from each group were assigned for bronchoalveolar lavage (BALF), while the remaining six were sacrificed by cervical dislocation to retrieve lung tissue.

Bronchoalveolar lavage fluid (BALF) collection

Following blood collection, six animals from each group were euthanized under deep anesthesia. To collect BALF, the lungs were lavaged three times with 1 ml of normal saline. A total of around 2 ml of liquid was retained⁽¹⁴⁾. Centrifugation was used to separate the BALF samples at 400 g for 7 min at 4°C, with the cell-free supernatant samples retained at 20°C awaiting for experiments, ice is used to preserve the sediment for total WBC counting.

Total WBC counting

BALFs were collected and centrifuged for 7 min at 400 rpm/ min/ 4°C to separate entire cells into pellets. The pellet of entire cells was separated on ice after the supernatant was removed. The Coulter Cellular Analysis System® was used to distinguish the WBC count on the same day.

Lung homogenate preparation

Phosphate buffer saline (PBS pH 7.4) at 4°C was used to wash the post-raval and right

inferior lobe tissue to eliminate any remaining blood and other debris. The tissue was then dried and weighed. For each 100 mg of lung tissue, 0.9 ml of cold PBS was added in an Eppendorf tube. After that the Eppendorf holding the tissue was placed in an ice-filled beaker to keep it cool, the lung tissue was homogenized for 1 min at speed three on the homogenizer machine. The homogenate was centrifuged for 20 min at 4° C and 3000 rpm in a refrigerated centrifuge. The supernatant was extracted using a micropipette and kept at - 20°C until IL- 33 and TNF- α were analyzed.

Measurement of inflammatory cytokines levels

IL-33 and TNF- α levels were quantified using lung tissue homogenate supernatants, and serum levels of IgE were determined using ELISA. A microplate spectrophotometer was used to measure absorbance values at 450 nm (Human, Germany). Standard curves were used to calculate the content of IgE and inflammatory cytokines.

Histological sample preparation of lung tissue

The middle and right superior lobes were selected for histopathological evaluation. Lung tissue excised and fixed in neutral buffered formalin 10% (v/v), dried, immersed in paraffin, cut into 4- μ m slices, deparaffinized with xylene, and stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) (15). A light microscope (Optika Microscope, Italy) was used to analyze tissue sections. On a scale of 0 to 4, the severity and the presence of inflammation and goblet cell hyperplasia in the lungs were evaluated (16).

Statistical analysis

Graphpad Prism 7® was used to analyze the data. One-way analysis of variance (ANOVA) was used to compare the means of the groups. All data were reported as mean and were considered significant when the *P*-value was less than 0.05.

Results

Effects of CA administration on WBC count in BALF

In the OVA-challenged allergic asthma model; total WBC count in BALF elevated considerably when compared to control group mice. In comparison with the OVA-challenged allergic asthma model group, pretreatment with CA at a dose of 50 mg/kg and 25 mg/kg resulted in a significant decrease in total WBC count in BALF fluid. Administration of CA alone had no impact on blood cell infiltration to BAL fluid (Figure 1).

Effect of cinnamic acid (CA) administration on IL- 33 levels in lung tissue homogenate

The levels of IL-33 in OVA-challenged model group were considerably higher than in the control group. When compared to the OVA-challenged model group, pretreatment with CA at both doses had significant influence on IL-33 levels. CA treatment in the non-sensitization

animals didn't produce a substantial change in IL-33 level (Figure 2A).

Effect of cinnamic acid (CA) administration on TNF- α level in lung tissue homogenate

Figure 2B shows that OVA challenging revealed a significant increase in levels of TNF- α , a proinflammatory cytokine, compared to the control group. Pretreatment with CA at a dose of 50 mg/kg and 25 mg/kg resulted in a significant decrease in TNF- α level. On the other hand, CA treatment in the non-sensitization animals was resulted in near-control levels of TNF- α .

Effect of CA administration on serum IgE levels

Compared to the control group, the data in figure 2D demonstrates a significant and substantial increase in serum IgE levels of the OVA- challenged group. When compared to the OVA- challenged model group, pretreatment with CA at a dose of 50 mg/kg and 25 mg/kg resulted in a significant reduction in IgE serum levels. Finally, CA at a dose of 50mg/kg alone did not result in significant changes in serum IgE levels when compared to the control group.

Histopathological assessment of lung tissue sections

We carried out histological examination to illustrate the possible protective effect of CA against lung tissue remodeling in OVA-challenged group as seen in (Figure 3). The results show the significant difference between the OVA challenged group and the control group; also, a significant difference is observed between the OVA challenged / CA 50 mg group compared to the induction model (Figure 3F). Regarding the histological findings, we see the obvious protective effect of CA in the group treated with CA challenged with OVA / 50 mg (Figure 3D) compared to the control group (Figure 3A) and the group challenged with OVA (Figure 3B). Meanwhile, CA in a dose of (25 mg/kg) did not show much protection on a histological level as seen in (Figure 3C).

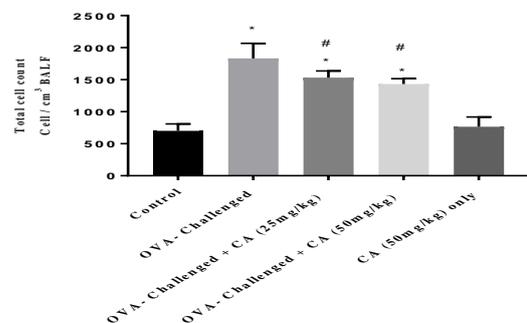


Figure 1. Effect of CA on total WBC count in BALF for OVA-induced allergic asthma in mice. (Each value represents mean \pm SD)

* Is significantly different compared with control group ($P < 0.05$).

is significantly different compared with OVA-Challenged group ($P < 0.05$).

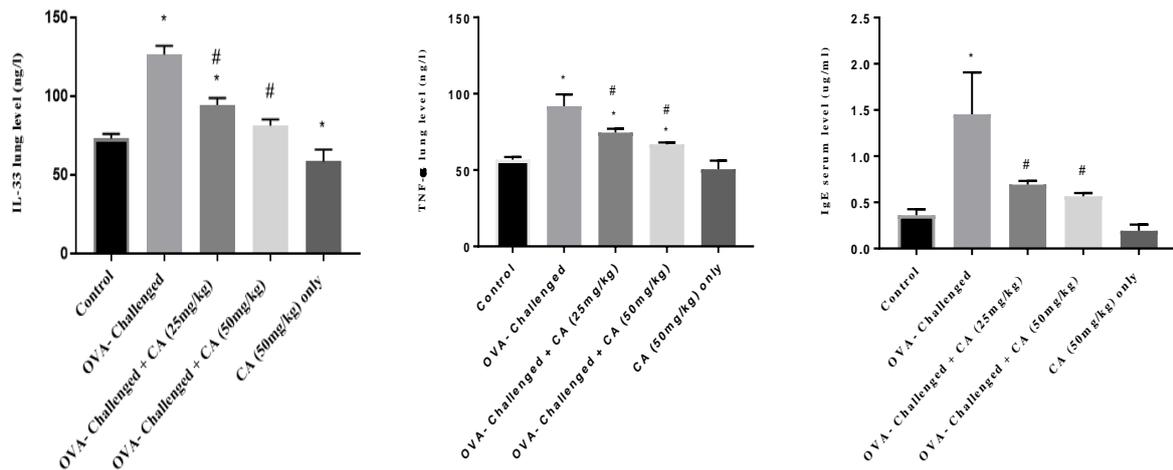


Figure 2. Effects of CA on the inflammatory contents in serum and lung tissue. IL-33, TNF- α , and IgE in serum and lung tissue for OVA-induced allergic asthma in mice. (Each value represents mean \pm SD)

* is significantly different compared with control group ($P < 0.05$).

is significantly different compared with OVA-Challenged group ($P < 0.05$).

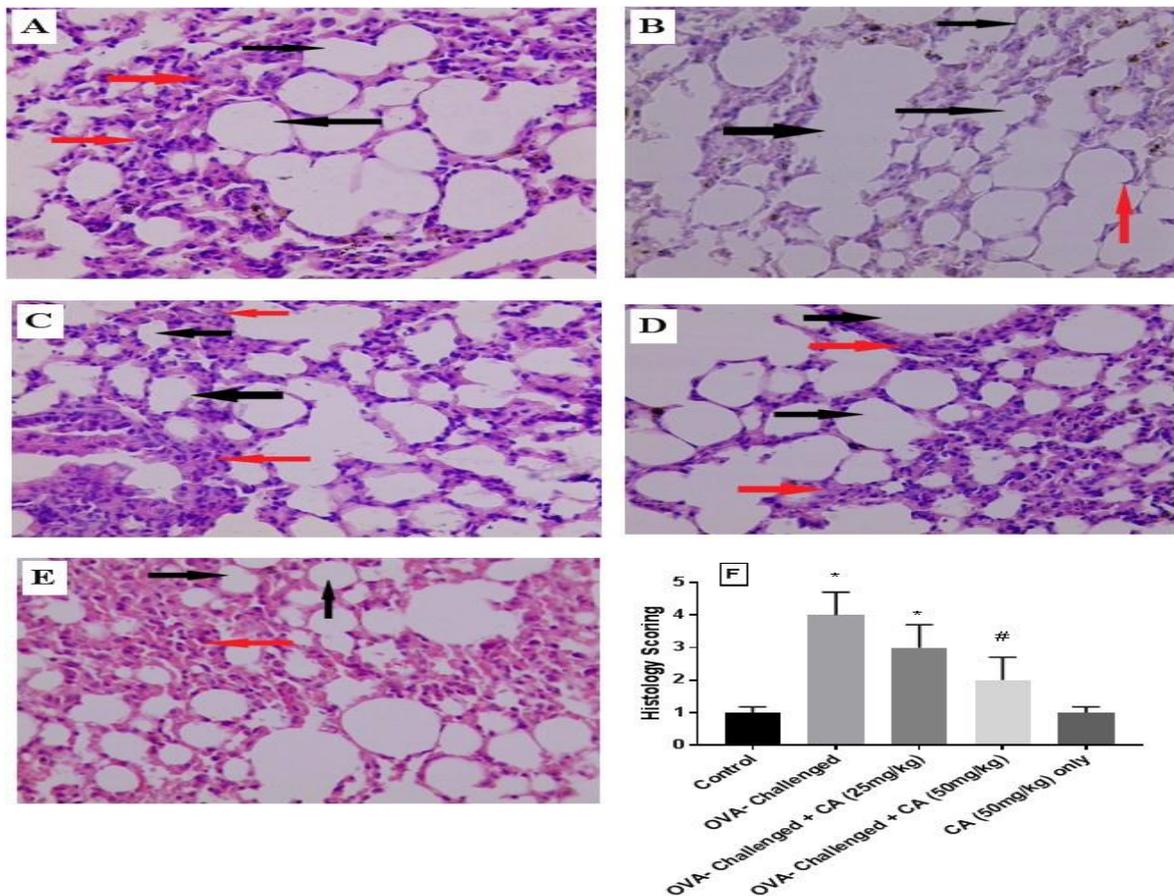


Figure 3. Effect of cinnamic acid on OVA-induced pathological pulmonary changes. Red arrow: hemorrhage or congestion. Black arrow: alveolar sack & emphysema. A: normal Lung (control); B: OVA-challenged (model); C: OVA+CA (25 mg/kg); D: OVA+CA (50mg/kg); E: CA only (50mg/kg); F: Histology scoring analysis. (Each value represents mean \pm SD)

* Is significantly different compared with control group ($P < 0.05$).

Is significantly different compared with OVA-Challenged group ($P < 0.05$).

Discussion

Ovalbumin (OVA) derived from chicken egg white is frequently used allergen that induces severe allergic lung inflammation in laboratory rodents⁽¹⁷⁾. Asthma problems have a complicated etiology. In asthma, cytokines play an important role in the immune system and inflammatory reactions. Immunomodulatory properties may be found in a variety of natural compounds, such as influencing the expression of inflammatory cytokines and controlling inflammatory cell activity, according to evidence-based research of natural medicinal herbs in asthma treatment⁽¹⁸⁾. In the present study, we found that OVA induced an allergic reaction in the lungs of mice similar to allergic asthma compared to the control group manifested by elevated levels of total WBC in the BAL fluid, high levels of IL-33 and TNF- α in the homogenate of lung tissue, increased serum IgE levels in the homogenate of tissue as shown in Figure 1 and 2. Pretreatment with CA at a dose of 50 mg / kg shows a significant alteration in results, showing a significant decrease in total WBC in BALF, also a significant decrease in IL-33 and TNF- α level in lung tissue homogenate, decreased serum IgE levels. Also, as shown in Figures 1 and 2; CA in a dose of 25 mg/kg has a similar effect, but to a lesser extent. While CA in a dose of 50 mg/kg without OVA challenging has near control values, suggesting that it only affects allergic parameter in disease state and in a dose-dependent matter. According to these observations, CA has immunomodulatory effects on total WBC, as well as IL-33, TNF-, and IgE concentrations. On a histological level, asthmatic individuals have aberrant alterations in the structure of the airway submucosa and epithelial tissue. Pathologically, asthma is defined by increased TH2 cytokine production, infiltration of inflammatory cells into epithelial tissue, metaplasia, and hyperplasia of goblet cells in epithelial tissue, smooth muscle hypertrophy, collagen deposition (collagen I, III, and V, as well as tenascin C and fibronectin), vascular congestion, and mucous glands that are larger, resulting in narrowing of airways, increased mucous Tissue remodeling is the term for these changes in airway anatomy^(19,20). As we see in (Fig 3 A, B, C, D, E and F) treatment with CA (50 mg/kg) had obvious protective effects against tissue remodeling and inflammation parameters seen in asthma.

Conclusion

The present study concluded that cinnamic acid (CA) significantly reduced the pulmonary inflammatory response and tissue damage, decreased WBC infiltration in OVA-induced allergic asthma. CA seems to inhibit the production of proinflammatory cytokines (TNF and IL33). Additionally, CA possess an immunomodulatory

effect by lowering blood IgE levels. For future work, further clinical studies are required to improve this idea for asthma management.

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