

Effects of Ursolic Acid on the Expression of Th1–Th2-related Cytokines in a Rat Model of Allergic Rhinitis After PM2.5 Exposure

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Abstract

Background: Allergic rhinitis (AR) is a global health problem and closely related to environmental factors. Ursolic acid (UA) has potential in the treatment of allergic inflammation. The effects of UA intervention on PM2.5-induced AR remain uncertain.

Objective: To assess the effects of UA on nasal symptoms and the expression of T-helper (Th)1–Th2-related cytokines in a rat model of AR after fine particulate matter (particulate matter $\leq 2.5 \mu\text{m}$ [PM2.5]) exposure.

Methods: A total of 40 healthy female Sprague-Dawley rats were randomly divided into 4 groups: normal control group (NC group), ovalbumin (OVA)-induced AR model (AR group), PM2.5-exposed AR group exposed to 200 g/m³ PM2.5 for 30 days via inhalation (ARE group), and a group with UA intervention to the AR model after PM2.5 exposure (UA group). UA intervention was adopted after PM2.5 exposure in the UA group. Nasal symptoms and levels of Th1–Th2 cytokines in the serum were detected in each individual rat. The pathological changes and expression of Eotaxin in the nasal mucosa of each individual rat were examined by histology.

Results: PM2.5 significantly increased the number of sneezes and nasal rubs in the rats with AR, and UA alleviated these symptoms. UA decreased interleukin (IL)-4, IL-5, IL-13, Eotaxin-1, and OVA Immunoglobulin E (IgE) protein levels. In the AR group, hematoxylin and eosin staining showed disordered arrangement of the nasal mucosa epithelium, cell shedding, eosinophilic infiltration, swelling of the glands, and submucosal vascular congestion. UA group showed reduced eosinophilic infiltration and orderly arrangement of the mucosal epithelium when compared with the ARE group. Immunohistochemical results showed that the expression of Eotaxin in the UA group was lower than that in the ARE group.

Conclusion: UA could relieve nasal symptoms caused by PM2.5 exposure, the possible mechanism of which is to inhibit the expression of Th2 cytokines, eosinophilic infiltration, and specific IgE production.

Keywords

ursolic acid, allergic rhinitis, PM2.5, cytokines, exposure, Th1, Th2, intervention, immune regulation, treatment

Introduction

Allergic rhinitis (AR) is a global health problem and its prevalence has increased progressively over the last few decades in more developed countries with the development of economy, and at present, about 40% of the population worldwide are affected.¹ The disease adversely affects the quality of life and school and work performance in patients, causing immense suffering and placing a heavy burden on health-care services, and gives rise to enormous social and economic burdens.² Environmental factors play an important role in the

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fast-increasing prevalence of AR. Particulate matter (PM) is one of the most important ingredients of air pollution, of which the aerodynamic diameter of 2.5 μm or less fine PM (PM_{2.5}) has been confirmed in the scientific field to be the most harmful pollutant to health in atmospheric environment, playing an important role on the pathogenesis of AR. Our previous studies have shown that exposure to PM_{2.5} aggravates the symptoms of AR and causes an imbalance of T-helper (Th)1/Th2 cytokines, along with changes in the expression of the Th1- and Th2-related transcription factors T-bet and GATA-3 mRNA. We have confirmed that PM_{2.5} can cause the chemotaxis of eosinophils and remodeling of the nasal mucosa in AR rats, consequently aggravating injury to the nasal mucosa and allergic inflammatory responses.^{3–5}

Since PM_{2.5} plays an important role in the incidence of AR, prevention of the influence of PM_{2.5} on AR becomes a focus.^{4,5} Ursolic acid (UA) is a pentacyclic triterpene extracted from natural plants, which exists in many natural drugs such as *ligustrum lucidum*, *prunella sativa*, and loquat leaf in the form of free one or glycoside combining with sugar.⁶ Research has previously reported that UA has a variety of biological activities such as immune regulation,⁷ anti-inflammatory properties,⁸ antioxidant,^{9,10} and anti-fibrotic effects,¹¹ all of which have great potential in the prevention and treatment of allergic inflammation. However, the effects of UA intervention on PM_{2.5} inducing the occurrence and development of AR remain uncertain. Whether UA can reduce the airway inflammation of AR, and whether it has a protective effect on the immune response of AR induced and aggravated by PM_{2.5} is worth researching.

Therefore, we used a PM_{2.5}-induced rat AR model to assess the effect of UA intervention on the biological, pathological, and immunological changes and then to explore the effect of UA on the symptoms and expression of Th1–Th2-related cytokines in AR after PM_{2.5} exposure. These findings may contribute to a better understanding of the prevention effects of UA on AR after PM_{2.5} exposure.

Materials and Methods

PM_{2.5} Sample Preparation and Chemical Analysis

PM_{2.5} was prepared as previously described.^{3–5,12} Briefly, PM_{2.5} was collected from the roof (at a height of approximately 20 m) of a 5-story building located on the campus of Fudan University (31.3°N, 121.5°E), Shanghai, China. The PM was collected using Whatman 41 filters (Whatman, Maidstone, UK) with TSP/PM₁₀/PM_{2.5}–2 samplers (Dickel, Beijing, China) at a flow rate of 77.59 L/min. Before and after weighting, the filters were stabilized under constant temperature (20

$\pm 1^\circ\text{C}$) and humidity ($40 \pm 1\%$), and weighed by a Sartorius 2004MP analytical balance (Sartorius, Germany) with a precision of 10 μg . The obtained PM_{2.5} suspension was concentrated using a vacuum-freeze dry method, and the final product was weighed and stored at -80°C until the start of the experiment. Concentrated PM_{2.5} was diluted with a sterile 0.9% saline solution according to experimental concentrations. The diluted PM_{2.5} solution was stored at 4°C , and 10 mg/mL of PM_{2.5} was used in a liquid aerosol generator. PM_{2.5} samples were measured using gas chromatography–mass spectrometry (Agilent, Alpharetta, Georgia) to detect polyaromatic hydrocarbons and ICS 3000 ion chromatography (Dionex, Sunnyvale, California) to determine the inorganic ion content (F^- , CH_3COO^- , HCOO^- , Cl^- , NO_3^- , SO_4^{2-} , PO_4^{3-} , Na^+ , K^+ , NH_4^+).

Animals and the AR Model

Forty female Sprague-Dawley rats (4–5 weeks old) weighing 180 to 200 g, which were specifically pathogen-free, were purchased from the Experimental Animal Center of Fudan University (Shanghai, China). The experimental procedures involving animals and their care were approved by the Animal Care and Use Ethics Committee of Fudan University. The rats were housed in Macrolon cages under controlled conditions of temperature (21–25°C) and humidity (40%–60%) and were provided food and water ad libitum (free from ovalbumin [OVA]). The rats were allowed to acclimatize for 1 week before the experiment, with a strict 12-hour light–dark cycle. Then, they were randomized into 4 groups (10 animals per group): a negative control group (NC group), an OVA-induced AR model (AR group), a PM_{2.5}-exacerbated AR group exposed to 200 g/m³ PM_{2.5} for 30 days via inhalation (ARE group), and an UA intervention group (UA group).

The rat model of AR was made as previously detailed with slight modification⁴ (Figure 1). Sensitization and antigen challenges were performed systemically. The rats were sensitized with 3 mg OVA (Sigma-Aldrich, St. Louis, Missouri) and 10 mg calmette-guérin (Sigma-Aldrich) in 1 mL saline which was injected intraperitoneally (i.p.) once a day for 7 days (day 1, 3, 5, 7, 9, 11, and 13). The same volume of sterile saline was injected i.p. in the control group (NC) on the same day. From day 24 to 30, local sensitization for the OVA challenge was performed by exposing the rats to an intranasal instillation of 25 μL OVA solution at a concentration of 1% OVA (1% OVA in physiologic saline solution) in each nostril every day once a day through a micropipette. Sterile saline was used on the NC rats under the same conditions. UA rats were administered 20 mg/kg of UA via gavage every day from day 24 to 30 (a total of

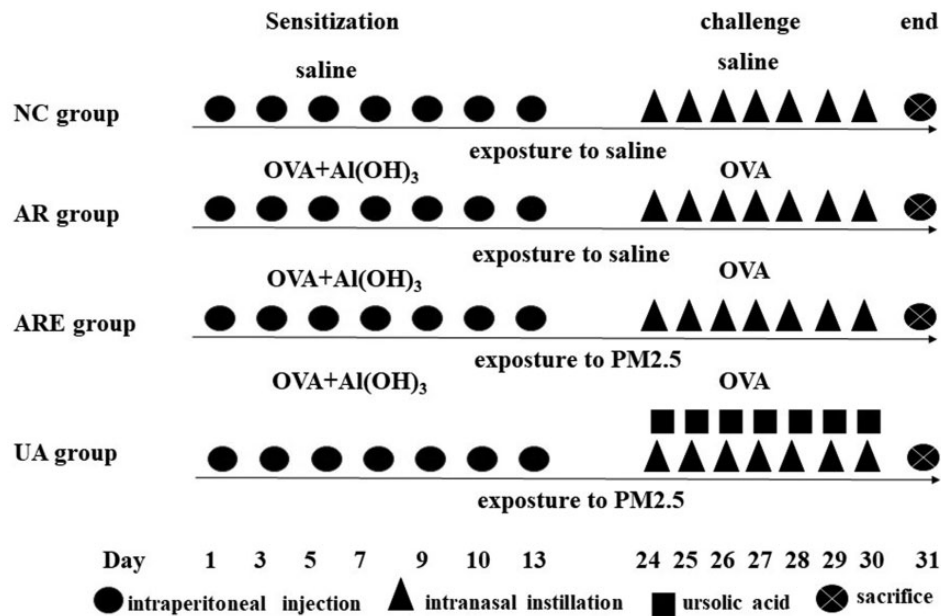


Figure 1. Experimental protocols used to induce AR in rats and PM2.5 exposure and UA intervention. The rats were randomly divided into 4 groups (10 animals per group). (1) A NC group (rats in the negative control group) which was exposed to sterile saline solution and sensitized and challenged with sterile saline solution. (2) A AR group which was exposed to sterile saline solution and sensitized with OVA + Al(OH)₃ and challenged with OVA. (3) An ARE group was rats with AR exposed to PM2.5 and sensitized with OVA Al(OH)₃ and challenged with OVA. (4) An UA group which was administrated UA via gavage every day from day 24 to 30 after PM2.5 exposure to the AR model. The sensitizations with OVA and Al(OH)₃ were administered via an intraperitoneal injection. Challenges were performed intranasally. The rats were exposed to PM2.5 on days 1 to 30. Nasal mucosa tissue was harvested on day 31. AR, allergic rhinitis; ARE, PM2.5-exacerbated AR groups exposed to PM2.5 (200 µg/m³); NC, negative control; OVA, ovalbumin; PM2.5, fine particulate matter; UA, ursolic acid.

7 consecutive days) after PM2.5 exposure in the AR model (UA was purchased from Shanghai Shengong A606646; Figure 1).

PM2.5 Inhalation Exposure System and PM2.5 Exposure

Two groups of animals (groups ARE and UA) were exposed to PM2.5 (200 µg/m³) for 3 hours/day during the sensitization stage for 30 consecutive days, from day 0 to 29 via a PM2.5 inhalation exposure system, according to previously described methods.⁴ The NC and AR groups were instead exposed to a saline solution for 3 hours/day for 30 consecutive days.

Evaluation of Nasal Allergic Symptoms

The numbers of sneezes and nasal rubs were counted and recorded for 15 minutes immediately after the last intranasal saline or OVA challenge by 2 observers independently.

Sample Collection and Preservation

Twenty-four hours after OVA sensitization and challenge with or without PM2.5 exposure, the rats were anaesthetized with an i.p. injection of 30 mg/kg 3%

pentobarbital sodium. Blood samples were obtained from the abdominal aorta. All the blood samples were centrifuged at 4°C, 300 × g, for 20 minutes, from which the obtained serum was collected and stored at -80°C until further analysis. The rats were then sacrificed and the nasal mucosa was dissected, which was fixed in 4% paraformaldehyde for 72 hours at 20°C, and then embedded in paraffin for histological examination and immunohistochemical analysis.

Measurement of IL-4, IL-5, IL-13, IFN-γ, Eotaxin-1, and OVA-specific Serum Immunoglobulin E

The serum levels of interleukin (IL)-4, IL-5, IL-13, Interferon gamma (IFN-γ), Eotaxin-1, and OVA-specific Immunoglobulin E (IgE) were measured by enzyme-linked immunosorbent assay (ELISA, ELISA Kit for Rat IL-4, IL-5, IL-13, IFN-γ, Eotaxin-1, and OVA-sIgE, Xitang Biotech, Shanghai, China, catalog nos: F15330, F15850, F15860, F15730, F15920, F15751) according to the manufacturer's instructions.

Nasal Mucosa Histopathology

The nasal tissues were decalcified, embedded in paraffin, and sliced into 4 µm sections. We used hematoxylin and eosin (HE) staining to detect pathological changes

including eosinophil infiltration. The histopathologic changes were evaluated in tissue sections under a light microscope. Eosinophils cell counts in all stained samples were recorded as the mean of the counts for 5 non-overlapping fields in the lamina propria by light microscopy at $\times 400$ magnification. The number of eosinophils were calculated and compared. The eosinophil counts per unit area were compared in the same areas of pathological images.

Immunohistochemical Analysis

The paraffined nasal mucosal tissue sections ($4\mu\text{m}$) were deparaffinized and rehydrated for antigen retrieval. After being blocked with 3% peroxide methanol at room temperature for 10 minutes and with 2% bovine serum albumin, the sections were incubated at 4°C overnight with rabbit polyclonal antibodies against Eotaxin (1:200 dilutions, Proteintech, Chicago, Illinois). Then, sections were incubated with horseradish peroxidase-conjugated secondary antibody for 25 minutes at room temperature. Finally, sections were colored with 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Image Pro Plus 6.0 image analysis software (Media Cybernetics Company, Rockville, Maryland) was used to measure its expression semiquantitatively.

Statistical Analyses

Data were presented as the mean $\pm$ standard deviation. SPSS 20.0 software was used for statistical analysis. A one-way analysis of variance with the Tukey post hoc test was used to test for significant differences among the groups. A P value of $<.05$ was considered significant. Level of significance $\alpha = 0.05$.

Results

Analysis of PM2.5 Chemical Characteristics

The main components of PM2.5 collected in this study are as follows: acenaphthylene (20.76 ng/mg), chrysene (169.61 ng/mg), acenaphthene (18.52 ng/mg), dibenz[a,h]anthracene (11.34 ng/mg), anthracene (7.8 ng/mg), fluoranthene (254.43 ng/mg), benz[a]anthracene (167.47 ng/mg), fluorene (8.04 ng/mg), benzo[b]fluoranthene (257.52 ng/mg), indeno[123-cd]pyrene (102.33 ng/mg), benzo[k]fluoranthene (102.45 ng/mg), naphthalene (6.54 ng/mg), benzo[a]pyrene (101.53 ng/mg), phenanthrene (244.59 ng/mg), benzo[ghi]perylene (81.89 ng/mg), pyrene (201.51 ng/mg), F^- (0.8 $\mu\text{g}/\text{mg}$), CH_3COO^- (4.3 $\mu\text{g}/\text{mg}$), HCOO^- (0.2 $\mu\text{g}/\text{mg}$), Cl^- (55.16 $\mu\text{g}/\text{mg}$), NO_3^- (386.43 $\mu\text{g}/\text{mg}$), SO_4^{2-} (214.32 $\mu\text{g}/\text{mg}$), PO_4^{3-} (18.65 $\mu\text{g}/\text{mg}$), Na^+ (4.43 $\mu\text{g}/\text{mg}$), K^+ (1.72 $\mu\text{g}/\text{mg}$), and NH_4^+ (213.54 $\mu\text{g}/\text{mg}$).

AR Symptoms in Rats

After OVA sensitization and challenge, the PM2.5-exacerbated AR group of rats (ARE group) showed significantly more sneezes and rubs during the 15-minute follow-up. The number of sneezes and rubs in the AR group were significantly increased when compared with those in the NC group, the ARE group was significantly higher than those in the NC and AR groups ($P < .001$), and in the UA group after UA intervention were significantly lower than those in the ARE group ($P < .05$). However, there was no statistically significant difference between the UA and AR groups ($P > .05$; Figure 2(A) and (B)). PM2.5 aggravated the symptoms of AR in rats, and UA alleviated this reaction.

Effects of UA on the Serum Levels of IL-4, IL-5, IL-13, IFN- γ , Eotaxin-1, and OVA-specific IgE After PM2.5 Exposure

ELISA was used to detect the level of cytokines in the serum (Figure 3 (A) to (F)). The expression of IL-4, IL-5, IL-13, Eotaxin-1, and OVA IgE proteins in the AR group was increased when compared with those in the NC group, while the level of IFN- γ was decreased ($P < .05$). After PM2.5 exposure in AR rat model (ARE group), IL-4, IL-5, IL-13, Eotaxin-1, and OVA IgE protein levels increased compared with the AR group ($P < .05$), and IFN- γ protein levels decreased ($P < .05$). After UA intervention, IL-4, IL-5, IL-13, Eotaxin-1, and OVA IgE protein levels decreased in the UA group when compared with the ARE group ($P < .05$), and IFN- γ protein levels showed no significant changes ($P > .05$).

Effects of UA on Nasal Mucosa Histopathology After PM2.5 Exposure

In the AR group, HE staining of the pathological sections of the nasal mucosa showed a disordered arrangement of the nasal mucosa epithelium, cell shedding, eosinophilic infiltration of the mucosa lamina propria, swelling of the glands, and submucosal vascular congestion. The above manifestations were aggravated in the ARE group. Eosinophilic counts increased compared with the AR group ($P < .05$), and the UA group showed reduced eosinophilic infiltration and an orderly arrangement of mucosal epithelium as compared with the ARE group ($P < .05$; Figure 4).

Effects of UA on Eotaxin Expression in Nasal Mucosa After PM2.5 Exposure

Immunohistochemical analysis showed that the expression of Eotaxin in the AR group was higher than that in the NC group ($P < .05$), the ARE group was higher than the AR group ($P < .05$), and in the UA group, the

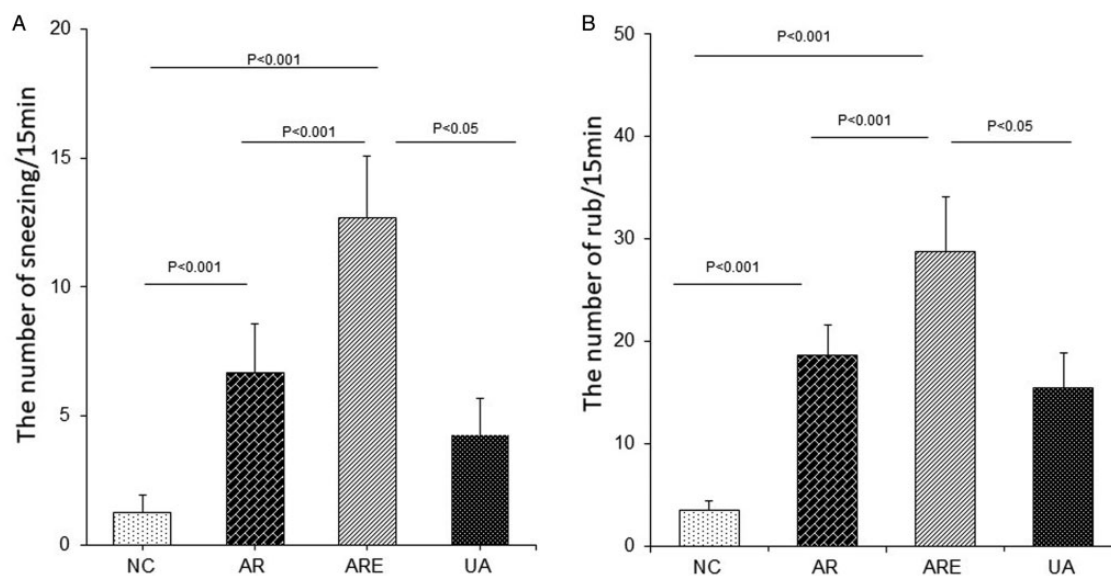


Figure 2. PM2.5 exposure aggravated the symptoms of AR in rats, and UA could alleviate these reactions ($N = 10$ per group). After the last challenge, the numbers of sneezing (A) and nasal rubs (B) in rats were observed for 15 minutes. Data are expressed as the mean $\pm$ SD, $n = 10$. The AR group was exposed to sterile saline solution and sensitized with OVA+Al(OH)₃ and challenged with OVA. The ARE group was rats with AR exposed to PM2.5 (200 $\mu\text{g}/\text{m}^3$). AR, allergic rhinitis; ARE, PM2.5-exacerbated AR groups exposed to a concentration (200 $\mu\text{g}/\text{m}^3$) of PM2.5; NC, negative control group; OVA, ovalbumin; PM2.5, fine particulate matter; SD, standard deviation; UA, ursolic acid.

expression was lower than that in the ARE group ($P < .05$), with no significant difference from other groups ($P > .05$; Figure 5).

Discussion

UA is a pentacyclic triterpene (molecular formula $\text{C}_{30}\text{H}_{48}\text{O}_3$) with a molecular weight of 456.68 g/mol, which exists in many plants. Recent studies have shown that UA has a variety of biological activities, which may play a key role through different signaling pathways and biological functions. UA is considered to target a variety of inflammatory transcription factors and provide multiple immunomodulatory activities.⁷ UA can regulate cyclin, growth factor, kinase, cytokine, chemokine, and adhesive molecules.¹³ UA inhibits the secretion of Th1-related cytokine IL-2 and IFN- γ in human peripheral blood and performs a Th1-biased immune response, which can enhance immunoregulatory function and suppress inflammation.¹⁴ UA can also promote the secretion of Th2 cytokines.¹⁵ UA has a variety of immunocompetencies, which can enhance the immune function and has no toxic side effects, and is therefore, an excellent immunoregulator. In addition, UA could prevent CCl₄-induced inflammation and hepatotoxicity based on its anti-oxidant activity and ability to modulate the mitogen-activated protein kinase (MAPK) and Nuclear factor κB (NF- κB) signaling pathways.¹⁶ Increasing studies have shown that UA has many

pharmacological effects such as anti-inflammatory and anti-oxidant activities.¹⁷

As we know, the immune system is a sensitive target for toxicologic factors in environment.¹⁸ A perfect balance of Th1 and Th2 response is essential to a normal immune response. Th2 immunoreponse is the main mechanism for AR. Th2 cells and their signature cytokines IL-4, IL-5, and IL-13 have key pathogenic roles in AR.⁴ We have researched the effects of different concentrations of PM2.5 exposure on symptoms, cytokines, and nasal mucosa pathology of AR model in rats and found that PM2.5 inhalation exposure can aggravate eosinophil infiltration in AR and can activate eosinophils to release a variety of inflammatory mediators including IL-4, IL-13, and Eotaxin-1, which in turn enhances inflammation and contributes to the damage of the nasal mucosa. Studies have found that the Th2 inflammatory response can produce IL-4 and IL-13 and promotes the binding of Eotaxin-1 to high-affinity CC chemokine receptor 3 through the MAPK signaling pathway.¹⁹ The activation of Eotaxin-1 promotes the infiltration of eosinophils, and inflammatory factors secreted by Eotaxin-1 can activate eosinophils, leading to the aggravation of the vicious cycle of inflammation. PM2.5 can aggravate the allergic inflammation of the nasal mucosa by increasing the expression of Eotaxin-1 and then the infiltration of eosinophils. In brief, our previous studies have found that PM2.5 inhalation exposure can increase IgE and aggravate AR symptoms, cause the imbalance of Th1/Th2 cytokines

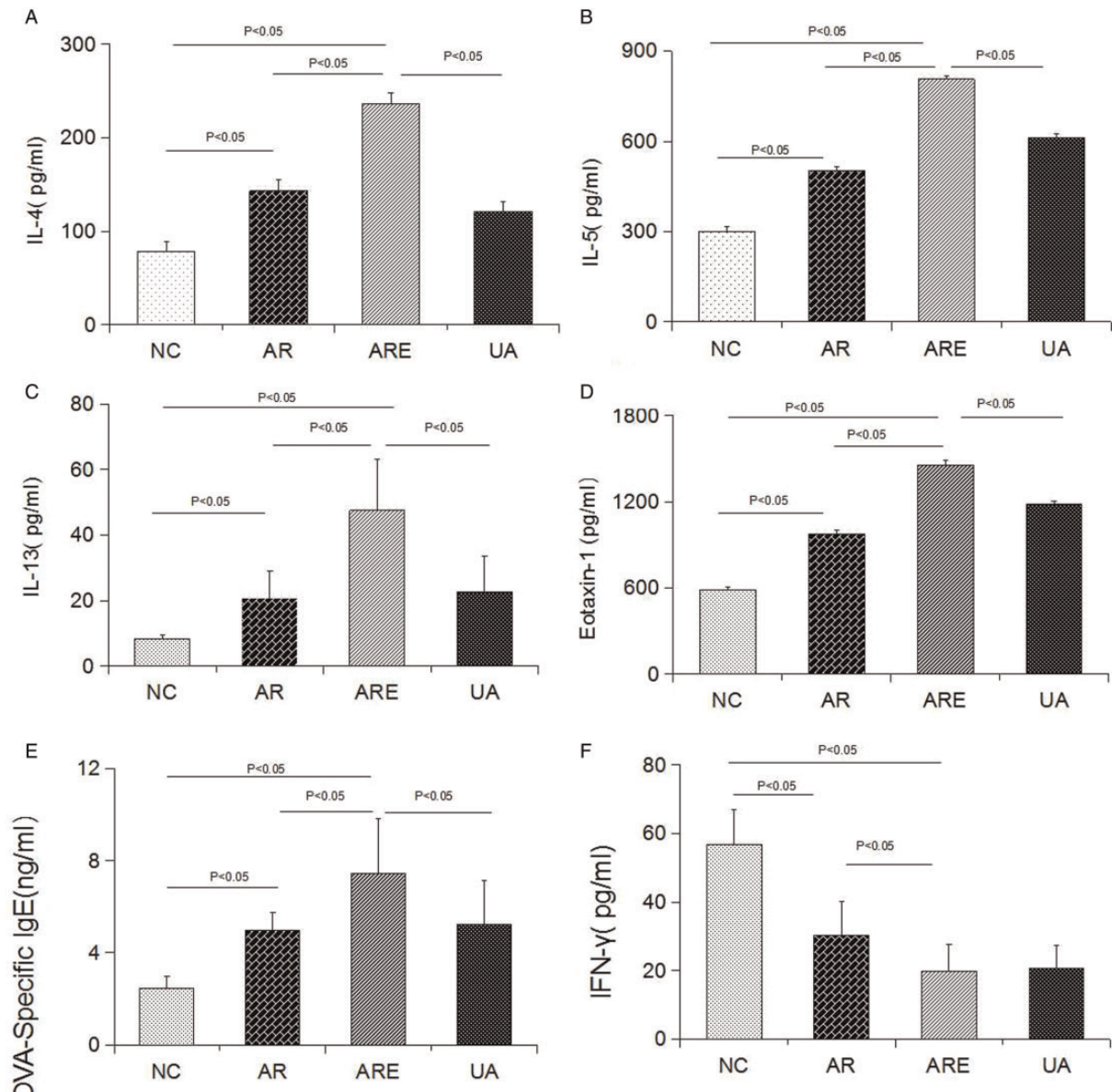


Figure 3. Effects of UA on the levels of IL-4, IL-5, IL-13, Eotaxin-1, and OVA-specific IgE (A–E), IFN- γ (F) in the serum after PM2.5 exposure (N = 10 per group). Data are expressed as the mean $\pm$ SD, n = 10. PM2.5 significantly decreased IFN- γ and increased IL-4, IL-5, IL-13, Eotaxin-1, and OVA IgE protein levels in serum, and UA decreased IL-4, IL-5, IL-13, Eotaxin-1, and OVA IgE protein levels. IFN- γ protein levels showed no significant changes compared with ARE group. AR, allergic rhinitis; ARE, PM2.5-exacerbated AR groups exposed to a concentration (200 $\mu\text{g}/\text{m}^3$) of PM2.5; IgE, immunoglobulin E; IL, interleukin; IFN- γ , interferon gamma; NC, negative control group; OVA, ovalbumin; PM2.5, fine particulate matter; UA, ursolic acid.

in AR, and change the expression of Th1- and Th2-related transcription factors T-bet and GATA-3 mRNA.^{3–5}

However, the effects of UA on AR after PM2.5 exposure remained unknown. The results of this study show that the symptoms, pathology, and immunological reaction of the ARE group after PM2.5 exposure were consistent with previous studies. To further investigate whether UA can reverse the adverse

effects of PM2.5 on AR rats, we administered UA via gavage to the AR animal models after PM2.5 exposure and observed the AR symptoms, pathological, and immunological changes in rats. The study found that after UA intervention, the number of sneezing and nasal scratching's in the UA group were lower than that in the ARE group, and there was no significant difference when compared with in the AR group, indicating that UA could alleviate AR symptoms

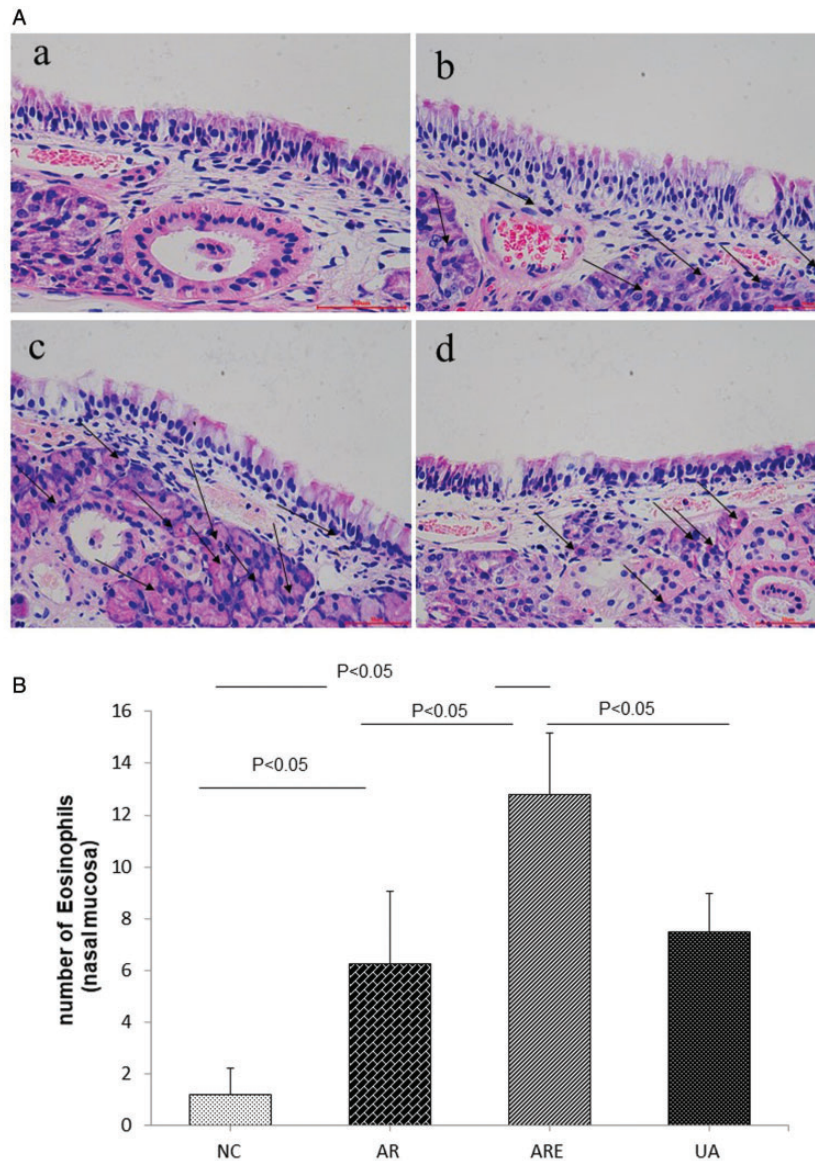


Figure 4. A, Effects of UA on nasal mucosa histopathology after PM_{2.5} exposure (N = 10 per group). HE staining showed a disordered arrangement of nasal mucosa epithelium, cell shedding, eosinophilic infiltration of mucosa lamina propria, swelling of the glands, and submucosal vascular congestion in the AR group. The above manifestations were aggravated in the ARE group, and the UA group showed reduced eosinophilic infiltration and an orderly arrangement of mucosal epithelium as compared with the ARE group. (a) NC group, (b) AR group, (c) ARE group, (d) UA group (scale bars 50 μ m, original magnification, $\times 400$). B, The number of eosinophils in nasal mucosa of each individual rat. AR, allergic rhinitis; NC, negative control group; ARE, PM_{2.5}-exacerbated AR groups exposed to a concentration ($200 \mu\text{g}/\text{m}^3$) of PM_{2.5}. UA, ursolic acid.

aggravated by PM_{2.5} exposure, but could not completely cure the allergy symptoms.

ELISA showed that Th2 cytokines IL-4, IL-5, IL-13, Eotaxin-1, and OVA-specific IgE protein levels in the UA group were decreased after UA intervention when compared with those in the ARE group, but the differences were not statistically significant. IFN- γ protein levels of Th1 cytokines were not significantly changed when compared with those in the ARE group. These results suggest that UA can inhibit the expression of

Th2 cytokines and IgE in AR model serum after PM_{2.5} exposure. Th2 cells selectively produced IL-4, IL-5, and IL-13 to induce B cells to transform into plasma cells and produce IgE. At the same time, they promoted local aggregation and activation of eosinophils leading to and aggravating the allergic inflammatory reaction. Maintaining the immune balance of Th1 and Th2 plays an important role in preventing and treating allergic inflammation. PM_{2.5} aggravates AR through an imbalance of Th1/Th2 cytokines, while UA

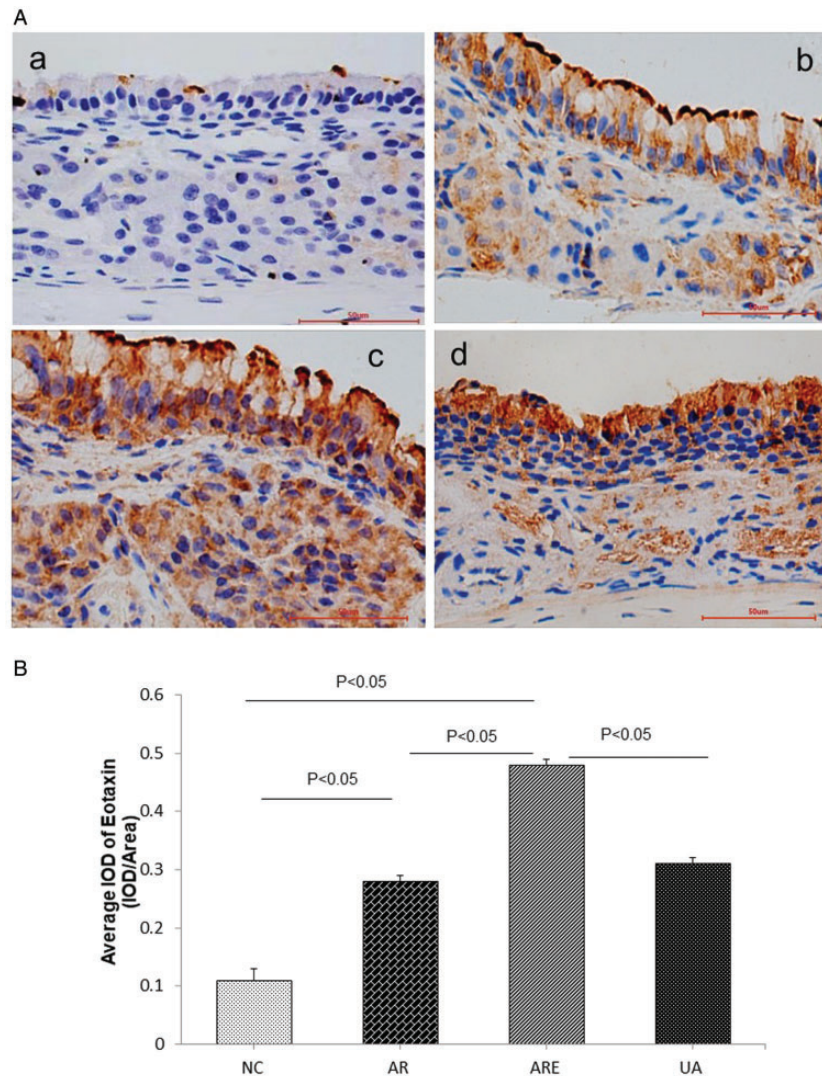


Figure 5. Effects of UA on expression of Eotaxin after PM2.5 exposure as detected by IHC (N = 10 per group). IHC staining of the nasal mucosa showed that the expression of Eotaxin in the AR group was higher than that in the NC group ($P < .05$), the ARE group was higher than the AR group ($P < .05$), and in the UA group, the expression was lower than that in the ARE group ($P < .05$). A and B, UA reduced Eotaxin expression in the nasal mucosa after PM2.5 exposure. (a) NC group, (b) AR group, (c) ARE group, (d) UA group (scale bars 50 μm, original magnification, $\times 400$). B, IHC IOD of Eotaxin expression in nasal mucosa of each individual rat. AR, allergic rhinitis; ARE, PM2.5-exacerbated AR groups exposed to a concentration ($200 \mu\text{g}/\text{m}^3$) of PM2.5; IOD, integrated optical density; NC, negative control group; UA, ursolic acid.

can inhibit the expression of Th2 cytokines, which is of great significance in preventing and controlling the deterioration of AR caused by PM2.5 exposure in the environment. ELISA was used to measure changes in the Th1 cytokines (including IL-2 and IFN- γ) and Th2 cytokines (including IL-4, IL-5, and IL-10) secreted by the spleen cells treated with UA, and the results showed that UA had a strong Th2 tendency and anti-inflammatory potential in vitro,¹⁵ which was consistent with this study.

In this study, HE staining of the rats nasal mucosa histopathological sections showed that the UA group had less eosinophil infiltration than the ARE group,

and the mucosal epithelium was arranged in a more orderly manner. Immunohistochemical results showed that the expression of Eotaxin was decreased in the UA group when compared with the ARE group. Previous studies have shown that UA is a potential peroxisome proliferator-activated receptor agonist, which suppresses ovalbumin-induced airway inflammation and Penh by downregulating IL-5, IL-13, and IL-17 in a mouse model of allergic asthma.²⁰ The results revealed that the therapeutic mechanism of UA in asthma is based on reductions in the Th2 cytokines (IL-5 and IL-13), ovalbumin-specific IgE production, and eosinophil

infiltration through the Th2-GATA-3, signal transducer and activator of transcription 6 (STAT), and IL-17-NF- κ B pathways.²⁰ This result was in line with our study. Pathological changes after PM2.5 exposure in AR rats can be improved through UA intervention, the possible mechanism of which is to inhibit the expression of Th2 cytokines, eosinophilic infiltration, and specific IgE production.

Conclusions

In summary, in this study, we have demonstrated that PM2.5 exposure will cause the aggravation of symptoms and an imbalance of Th1/Th2 cytokines and Th2 dominance in AR rats. While UA can reduce AR symptoms caused by PM2.5 exposure, the possible mechanism of which is to inhibit the expression of Th2 cytokines and specific IgE in serum, and decrease the infiltration of eosinophilic infiltration, and specific IgE production. AR is an allergic disease of the nose which is regulated by multiple genes and closely related to environmental factors. This study provides a novel idea for the prevention and treatment of AR, which is of major significance for the prevention of AR exacerbated by unavoidable environmental exposure to PM2.5.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

- Cheng L, Chen J, Fu Q, et al. Chinese Society of Allergy Guidelines for diagnosis and treatment of allergic rhinitis. *Allergy Asthma Immunol Res.* 2018;10(4):300–353.
- Meltzer EO. Allergic rhinitis: burden of illness, quality of life, comorbidities, and control. *Immunol Allergy Clin North America.* 2016;36(2):235–248.
- Hong Z, Guo Z, Zhang R, et al. Airborne fine particulate matter induces oxidative stress and inflammation in human nasal epithelial cells. *Tohoku J Exp Med.* 2016;239(2):117–125.
- Guo ZQ, Dong WY, Xu J, et al. T-helper type 1-T-helper type 2 shift and nasal remodeling after fine particulate matter exposure in a rat model of allergic rhinitis. *Am J Rhinol Allergy.* 2017;31(3):148–155.
- Renwu Z, Zhiqiang G, Ruxin Z, et al. Nasal epithelial barrier disruption by particulate matter $\leq 2.5\mu\text{m}$ via tight junction protein degradation. *J Appl Toxicol.* 2018;38(5): 678–687.
- Pironi AM, de Araujo PR, Fernandes MA, et al. Characteristics, biological properties and analytical methods of ursolic acid: a review[J]. *Crit Rev Anal Chem.* 2018;48(1):86–93.
- Jang, SM, Yee, ST, Choi, J, et al. Ursolic acid enhances the cellular immune system and pancreatic beta-cell function in streptozotocin-induced diabetic mice fed a high-fat diet. *Int Immunopharmacol.* 2009;9(1), 113–119.
- Kashyap D, Sharma A, Tuli HS, Punia S, Sharma AK. Ursolic acid and oleanolic acid: pentacyclic terpenoids with promising anti-inflammatory activities. *Recent Patents Inflamm Allergy Drug Discov.* 2016;10(1):21–33.
- Ding H, Wang H, Zhu L, Wei W. Ursolic acid ameliorates early brain injury after experimental traumatic brain injury in mice by activating the Nrf2 pathway. *Neurochem Res.* 2017;42(2):337–346.
- Rai SN, Yadav SK, Singh D, Singh SP. Ursolic acid attenuates oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in MPTP-induced parkinsonian mouse model. *J Chem Neuroanat.* 2016;71:41–49.
- Dong X, Liu S, Zhang L, et al. Downregulation of miR-21 is involved in direct actions of ursolic acid on the heart: implications for cardiac fibrosis and hypertrophy. *Cardiovasc Ther.* 2015;33(4):161–167.
- Zhao R, Guo Z, Dong W, et al. Effects of PM2.5 on mucus secretion and tissue remodeling in a rabbit model of chronic rhinosinusitis. *Int Forum Allergy Rhinol.* 2018;8(11),1349–1355.
- Shanmugam MK, Dai X, Kumar AP, et al. Ursolic acid in cancer prevention and treatment: molecular targets, pharmacokinetics and clinical studies[J]. *Biochem Pharmacol.* 2013;85(11):1579–1587.
- Ramos AA, Pereira-Wilson C, Collins AR. Protective effects of ursolic acid and luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells[J]. *Mutat Res/Fund Mol Mech Muta.* 2010;692(1–2):6–11.
- Ku CM, Lin JY. Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. *Food Chem.* 2013;141(2):1104–1113.
- Ma JQ, Ding J, Zhang L, et al. Ursolic acid protects mouse liver against CCl4-induced oxidative stress and inflammation by the MAPK/NF-kappaB pathway[J]. *Environ Toxicol Pharmacol.* 2014;37(3):975–983.
- Ramirez CN, Li W, Zhang C, et al. In Vitro-in vivo dose response of ursolic acid, sulforaphane, PEITC, and curcumin in cancer prevention[J]. *AAPS J.* 2017;20(1):19.
- Yuan X, Ghosh A, Jie Q, et al. Effects of desloratadine citrate disodium injection on rat models of ovalbumin-induced allergic rhinitis: involvement of T-cell responses modulation. *Int Forum Allergy Rhinol.* 2015;5:1170–1176.

19. Zhou X, Hu H, Balzar S, et al. MAPK regulation of IL-4/IL-13 receptors contributes to the synergistic increase in CCL11/Eotaxin-1 in response to TGF-beta1 and IL-13 in human airway fibroblasts[J]. *J Immunol.* 2012;188(12):6046–6054.
20. Kim SH, Hong JH, Lee YC. Ursolic acid, a potential PPAR γ agonist, suppresses ovalbumin-induced airway inflammation and Penh by down-regulating IL-5, IL-13, and IL-17 in a mouse model of allergic asthma. *Eur J Pharmacol.* 2013;701(1–3):131–143.