Original Research

Isoorientin ameliorates OVA-induced asthma in a murine model of asthma

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Impact Statement

Isoorientin is a luteolin glycoside flavonoid compound, which exists in a variety of medicinal plants. Isoorientin has a variety of pharmacological properties, such as antioxidation, inhibiting the development of inflammation, improving insulin resistance, weakening the development of liver fibrosis, and inducing apoptosis of liver cancer cells. However, its role in asthma has not been clearly studied. Our study provides a theoretical basis for the application of isoorientin in the treatment of asthma.

Abstract

Allergic asthma which is induced by ovalbumin (OVA) is a chronic airway inflammation disease. Isoorientin (Iso) is a natural C-glucosyl flavone with many biological properties. We aimed to evaluate the effectiveness of Iso on OVA-induced allergic asthma. A total of 30 C57BL/6 mice were randomly divided into five groups: control group, OVA group, Dex (dexamethasone, 10 mg/kg) group, low-dose Iso group (Iso-L, 25 mg/kg), and high-dose Iso group (Iso-H, 50 mg/kg). The serum and bronchoalveolar lavage fluid (BALF) were collected for biochemical parameters, the lung tissue was collected for hematoxylin-eosin (H&E) staining, immunohistochemistry (IHC), and western blot. The levels of IL-4, IL-5, IL-13, malondialdehyde (MDA), NO, and reactive oxygen species (ROS) in Iso-L and Iso-H groups were significantly lower than that in model group ($p < 0.05$). Simultaneously, the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity were higher than that in model group ($p < 0.05$). Iso significantly ameliorated airway hyperresponsiveness. Meanwhile, H&E staining revealed that mice treated with Iso resulted in the ameliorated inflammatory cell infiltration and a reduction in interstitial thickening. The nuclear factor erythroid 2-like 2 (Nrf2) and HO-1 protein expression in Iso-L and Iso-H groups were enhanced over that in model group, while p-NF-κB-p65 and p-IκB-α protein expression was decreased ($p < 0.05$). Our research indicated that Iso alleviated the OVA-induced allergic asthma, and this effect can be explained by the modulation of Nrf2/HO-1 and NF-κB signaling pathway; thus, the results providing a therapeutic rationale for the treatment of Iso on allergic asthma.

Keywords: Hyperresponsiveness, dexamethasone, histopathological, oxidative stress, inflammation, Nrf2/HO-1 signaling

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Introduction

Asthma represents one of the most common chronic inflammatory lung diseases. Clinical signs involve bronchial inflammation, airway hyperresponsiveness (AHR), airway edema, airflow disorder and obstruction, and increased mucus secretion.[1,2] T cells, particularly T helper Th1 and Th2, are dominant in the airways, and along with Th2 cytokines, such as IL-4, IL-5, and IL-13, these T cells have a crucial role in the pathological characteristics of asthma. Previous studies have indicated that ovalbumin (OVA)-induced asthma is manifested through the mechanisms of AHR and chronic airway inflammation.[3,4] Currently, asthma is a high-incidence common chronic respiratory disease in infants or adults, affecting millions of people worldwide.[5] A number of researchers have found that while inhaled corticosteroids (ICS) are currently used as a frontline therapy for treating this disease in the treatment of asthma, side effects are still significant for long-term treatment periods, and many patients have exhibited a diminished sensitivity to these drugs.[6,7] In recent years, the use of monoclonal antibody (mAb)-based biological agents for specific targeted treatment of severe or refractory asthma has reduced the deterioration rate and the use of glucocorticoids, while improving lung function.[8] At present, the available therapeutic targets are mainly targets related to Th2.[9] However, the side effects of mAbs are related to the body's immune response to the drug. This response may be mild, transient, subclinical, or it may be a serious and potentially fatal reaction when the immune system overreacts (hypersensitivity).[9,10] In addition, mAbs treatment is used primarily for patients with severe asthma.[11] No single cytokine is responsible for the entire pathogenesis of asthma. This is the challenging aspect of mAbs for asthma therapy.[12]

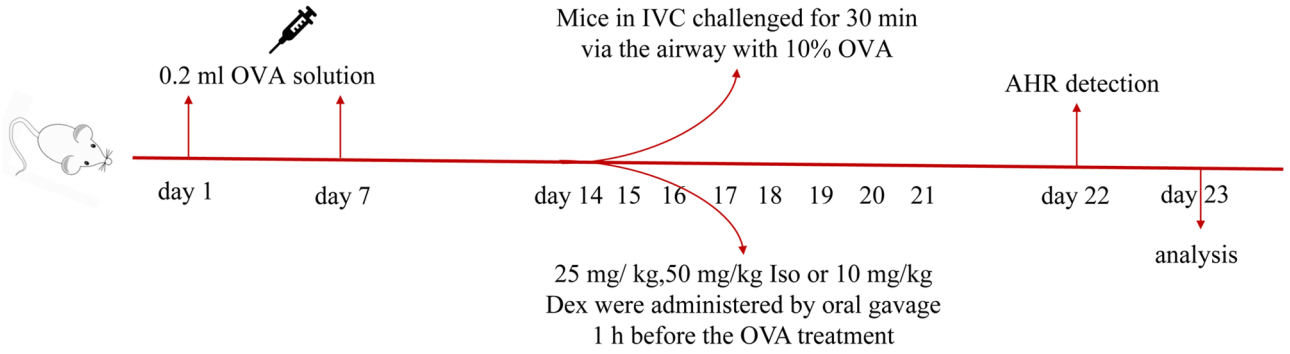

Figure 1. The experiment flow chart. (A color version of this figure is available in the online journal.)

Isoorientin (Iso) is defined as a dietary C-glucosyl flavone derived from rooibos tea, cereals and legumes and has a variety of pharmacological properties, including antioxidant, anti-inflammatory, and anticancer.[13–16] A previous study revealed that Iso inhibited inflammatory responses in endotoxemic mice and had a protective effect on inflammation-related brain injury.[17] However, the underlying effects of Iso in asthma have not been clearly defined. In this research, we established an OVA-induced asthmatic mice model to clarify the role of Iso and to provide an effective novel anti-inflammatory and antioxidant agent for asthma treatment.

Materials and Methods

Animals

A total of 30 C57BL/6 mice (20–23 g, 4–6 weeks) were purchased from Jinan Peng Yue Experimental Animal Breeding Co., Ltd. (Shandong, China) and divided into treatment groups as described below. The animals were housed (five mice per cage) in individual ventilated cages (IVC), renewed every 24 h, under a 12–12 h light–dark cycle at around 20–26°C, with relative humidity of 40–70%. The mice were fed on the specific pathogen-free (SPF) diet and given free access to food and water. All animal procedures performed were carried out according to the guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1996). The study was approved by Ethics committee of Yantai Yuhuangding Hospital.

Animal grouping and administration

The C57BL/6 mice were randomly assigned into five groups ($n = 6$), including control group, OVA group, Dex (dexamethasone, Dex) group, low-dose Iso (Iso-L) group, and high-dose Iso (Iso-H) group. The asthma model was established in all groups, except the control group, according to previously described procedures with minor modifications.[3] In brief, mice were immunized intraperitoneally and intradermally with 0.2 mL OVA solution (10 mg OVA [Sinopharm Chemical Reagent Co., Ltd.] and 1 g aluminum hydroxide [Xingtai Mei Xing Chemical Co., Shanghai, China] in 20 mL saline) on Days 1 and 7. Mice in the control group were injected with an equivalent amount of saline at the same injection site. The mice were placed in IVC and challenged for 30 min via the airway with 10% OVA each day from Day 14 to Day 21. Mice in the OVA treatment groups exhibited restless behavior, shortness of breath, and abdominal cramps. Mice in the control group were treated with an equivalent amount of saline at the same time.

Following model building, doses of Iso (HPLC purity ⩾98%, Sigma, USA) of 25 and 50 mg/kg were administered by oral gavage 1 h before the OVA treatment, and a dose of dexamethasone (Yao Pharmaceutical Co., Ltd., Chongqing) of 10 mg/kg was administered orally 1 h before the OVA treatment, and an equal volume of physiological saline was administered orally to the control group and the OVA group each day from Day 14 to Day 21. The Iso doses were selected to correlate to data reported elsewhere for different mice models.[18,19] The experiment flow chart is shown in Figure 1.

Determination of IL-4, IL-5, IL-13, and IgE content

All of the mice were anesthetized, then the left lung was ligatured, and the right principal bronchus was pumped with saline solution. Bronchoalveolar lavage fluid (BALF) was collected and poured out with a greater than 80% recovery rate. The supernatants were obtained by centrifuging 15,000 g for 15 min at 4°C. The levels of IL-4, IL-5, and IL-13 were measured according to the respective kits (R&D system, USA). At 24 h after the last challenge, the blood of the retro-orbital plexus in each group of mice was collected and centrifuged immediately at 3000 g for 10 min. The level of IgE in serum was detected using the IgE ELISA kit (Abcam, UK).

Cell analysis

To identify differential cell count, 100 µL BALF was put on a slide using Cytospin (Thermo Fisher Scientific, USA). Then the slides were dried, fixed, and stained by Diff-Quick staining reagent (Solarbio, Beijing, China).

Hematoxylin-eosin staining

Lung tissues in mice were removed after taking BALF. The lung tissues were fixed with 4% paraformaldehyde and fixed for 24 h, dehydrated, paraffin-embedded, and sliced (4 µm). After section, the slides were washed with phosphate-buffered saline (PBS) for three times, stained with hematoxylin for 15 min, then differentiated by 1% hydrochloride ethanol and perfused in eosin for another 15 min. The hematoxylin-eosin

(H&E) staining results were observed under the inverted microscope (Olympus, Japan). According to the severity of inflammatory injury in the lung tissue, the inflammation score ranged from 0 to 3 (0 – no inflammatory injury was observed; 1 – focal infiltration of inflammatory cells around the airway; 2 – multiple infiltration of inflammatory cells around the airway; and 3 – diffuse lesions with massive infiltration of inflammatory cells around the airways).

SOD, MDA, NO, and GSH-Px content in lung tissue detection

Lung tissues were weighed and homogenized into 10% tissue mixture, then the supernatant was obtained by centrifuging 3000 g for 10 min at 4°C. Finally, the levels of superoxide dismutase (SOD), malondialdehyde (MDA), NO, and glutathione peroxidase (GSH-Px) were measured according to the respective kits (Jiancheng Biology Engineering Institute, Nanjing, China).

ROS detection

The lung tissues were homogenized into 10% tissue with 3000 r/min for 10 min, then the supernatants were obtained. The single-cell suspension was obtained with 10^7 cells/mL. The content of reactive oxygen species (ROS) was examined by a method dependent on intracellular deacylation and oxidation of 6-carboxy-2′,7′-dichlorofluorescein diacetate (DCFH-DA, Nanjing Jiancheng Bioengineering Institute, China). DCFH-DA forms a fluorescent product, dichlorofluorescein (DCF) on oxidation with ROS. This probe is highly reactive with hydrogen peroxide and is used in evaluating the production of ROS in mammalian cells. After preincubation, the lung cells (10^7 cells/mL) in Yeast Peptone Dextrose (YPD) medium were incubated with DCFH-DA (10 µmol/L) at 37°C for 30 min, the cell suspensions (1.0 mL) were withdrawn and then washed and resuspended in 100 mL of PBS pH 7.4. Fluorescence intensity of the cell suspension (100 mL) containing 10^7cells was read with a Cytoflow 2300 fluorescence spectrophotometer (Agilent, USA) with 480 nm-excitation and 530 nm-emission. The arbitrary units were based directly on fluorescence intensity.

Measuring AHR to methacholine

Measurements of AHR to inhaled methacholine (Sigma-Aldrich, USA) were performed after 24 h by the FlexiVent system (SCIREQ Inc., Canada). Mice were first anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg), tracheostomized, and ventilated, then intubated with an 18-gauge stainless steel cannula. The following ventilator settings were used: tidal volume (10 mL/kg), frequency (150/min), and positive end-expiratory pressure (3 cm H_2O). The dynamic airway resistance was determined using SCIREQ software in mice exposed to nebulized PBS and Methacholine (MCh) (0.75, 1.5, 3, 6, 12, 24, 48 mg/mL) by a matched nebulizer. After receiving different doses, the response was measured by applying 2-s perturbations at 10-s intervals for a total of 3 min. The dose-response curves for each group were determined and the total respiratory system resistance (Rrs) values were recorded.

Western blotting

The lung tissues were homogenized with a tissue lysis/extraction reagent (Sigma-Aldrich, USA). BCA Protein Assay Kit (Beyotime Shanghai, China) was used to determine the content of protein in each group. Proteins (40 µg) were electrophoresed by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and then transferred to a polyvinyl difluoride membrane (PVDF, Millipore, USA). The membranes were blocked with 5% skimmed milk for 1 h and incubated with the following primary antibodies at 4°C overnight: Nrf2 antibody (1:1000; ab137550, Abcam), HO-1 antibody (1:2000; ab13243, Abcam), p-NF-κB-p65 (1:1000; ab76302, Abcam), NF-κB-p65 (1:1000; ab16502, Abcam), p-IκB-α (1:1000, cell signaling technology, USA), and IκB-α (1:1000, cell signaling technology, USA). After the membrane was washed three times with tris-buffered saline (TBS), the horseradish peroxidase (HRP)-conjugated secondary antibody sheep anti-rabbit IgG (1:5000; ab97095; Abcam) was incubated for 1 h at 37°C. Membranes were washed again, and binding was detected using an Enhanced chemiluminescence kit (ECL, Thermo-Scientific, USA). Protein band was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000; ab37168; Abcam) and evaluated using Image J software version 1.46 (NIH, USA).

Statistical analyses

Data were expressed as means ± SD. SPSS 22.0 software was used to analyze statistical differences. Differences between groups were compared using one-way ANOVA followed by the Tukey's *post hoc* test. A p-value less than 0.05 was considered to represent a statistically significant difference.

Results

Effect of Iso on levels of IL-4, IL-5, IL-13 in BALF, and IgE in serum

As presented in Figure 2, the levels of IL-4, IL-5, IL-13, and IgE in the OVA group were significantly increased compared to that in the control group ($p < 0.05$). Simultaneously, the levels of IL-4, IL-5, IL-13, and IgE in the Iso-L and Iso-H groups were significantly decreased compared to that in the OVA group ($p < 0.05$). The effect on the Iso-H group was greater than on the Iso-L group, while there was no significant difference between the Dex and Iso-H groups.

Changes of inflammatory cells

We assessed the effect of OVA-induced inflammatory cell infiltration in BALF. The results showed that the total number of cells, macrophages, neutrophils, eosinophils, and lymphocytes in the OVA group significantly increased compared to the control group ($p < 0.01$). However, Dex or Iso significantly attenuated OVA-induced inflammatory cells in BALF (Figure 3).

Characteristics of lung tissues by H&E staining

The morphology of the lung tissue in the control group demonstrated the normal alveolar space, regular thickening of the alveolar septa, and substantial integrity of the alveolar

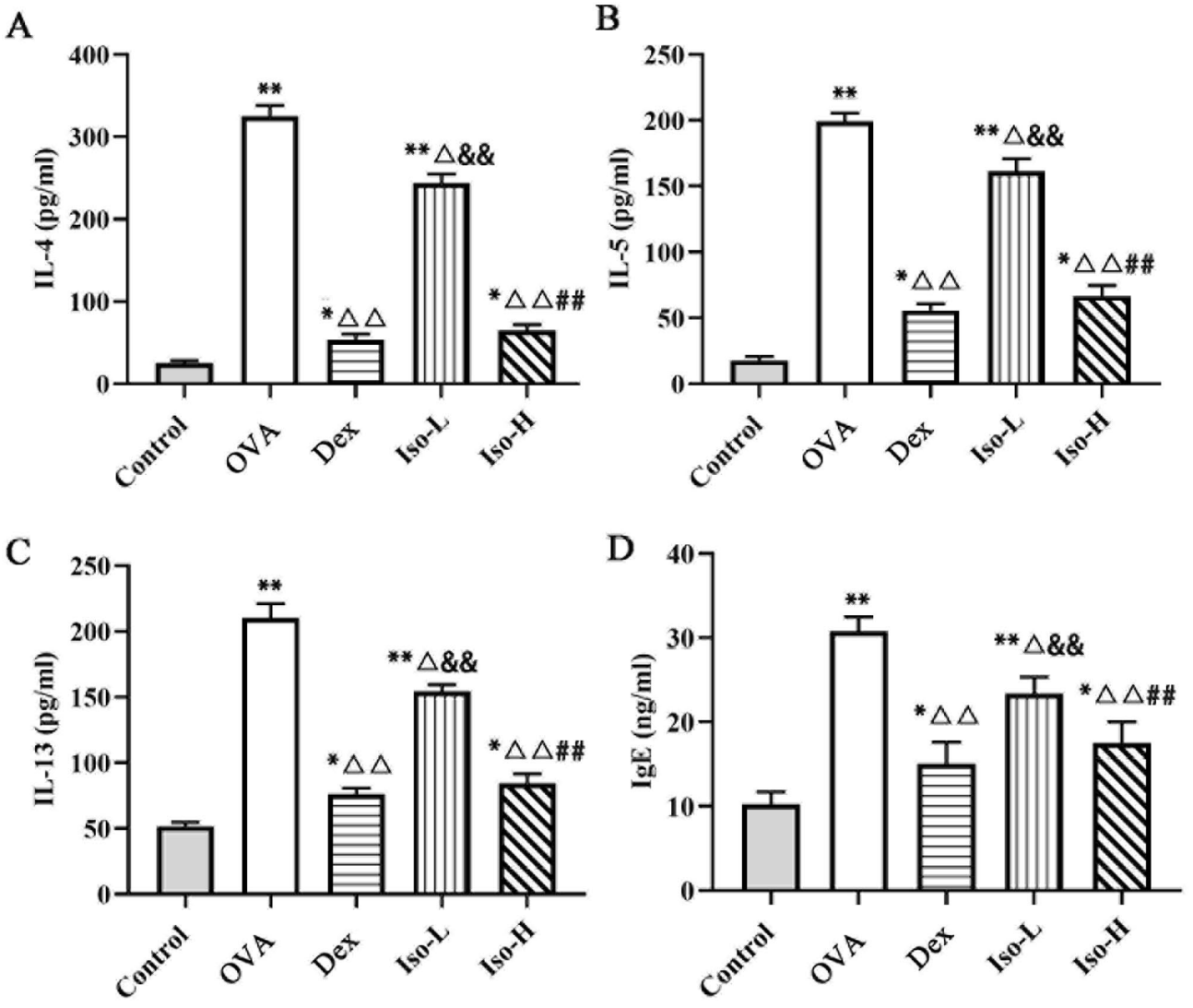

Figure 2. Iso treatment decreases the levels of IL-4, IL-5, IL-13 in BALF, and IgE in serum. (A) The level of IL-4; (B) the level of IL-5; (C) the level of IL-13; and (D) the level of IgE versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&\&}p < 0.01$; versus the Iso-L group, $^{\#\#}p < 0.01$.

epithelial structure. The airway epithelium was intact, and there was no shedding of airway epithelial cells (Figure 4(A)). However, the lungs of the mice in the OVA group illustrated marked alveolar inflammation with extensive thickening of interalveolar septa, a small number of fibroblasts and the matrix hyperplasia, and dense interstitial infiltration by macrophages, neutrophils, and plasma cells. The structure of the bronchus was disordered. The trachea wall became thick, there was infiltration of eosinophils and other inflammatory cells around the trachea, and the airway became narrowed. Mice treated with Iso and dexamethasone produced a moderate amelioration of macrophage, lymphocyte, and plasma cell infiltration, together with a reduction in interstitial thickening, compared with the model group. Mice in the Iso and dexamethasone groups revealed a normal structure of alveolar and alleviative alveolar inflammation. Meanwhile, the inflammatory infiltration around the trachea was reduced, the trachea wall showed thickening, and the airway stenosis was reduced. Both doses of Iso decreased the lung inflammatory score significantly (Figure 4(B)), and the effect on Iso-H was greater than in the Iso-L group.

Iso reduced the levels of MDA and NO while enhancing the levels of SOD and GSH-Px in lung tissue

In Figure 5, the levels of MDA and NO in lung tissue were significantly increased ($p < 0.05$), and the levels of SOD and GSH-Px were significantly reduced ($p < 0.05$) in the OVA group compared to that in the control group, indicating that the antioxidative activity was reduced in the lung tissues of the OVA group ($p < 0.05$). Simultaneously, compared with the OVA group, the levels of MDA in the Iso-L and Iso-H groups were reduced while levels of SOD, and GSH-Px were improved significantly ($p < 0.05$), indicating that Iso could reduce the level of MDA and enhance the levels of SOD and GSH-Px markedly. In addition, the effect in the Iso-H group was significantly better than that in the Iso-L group. The

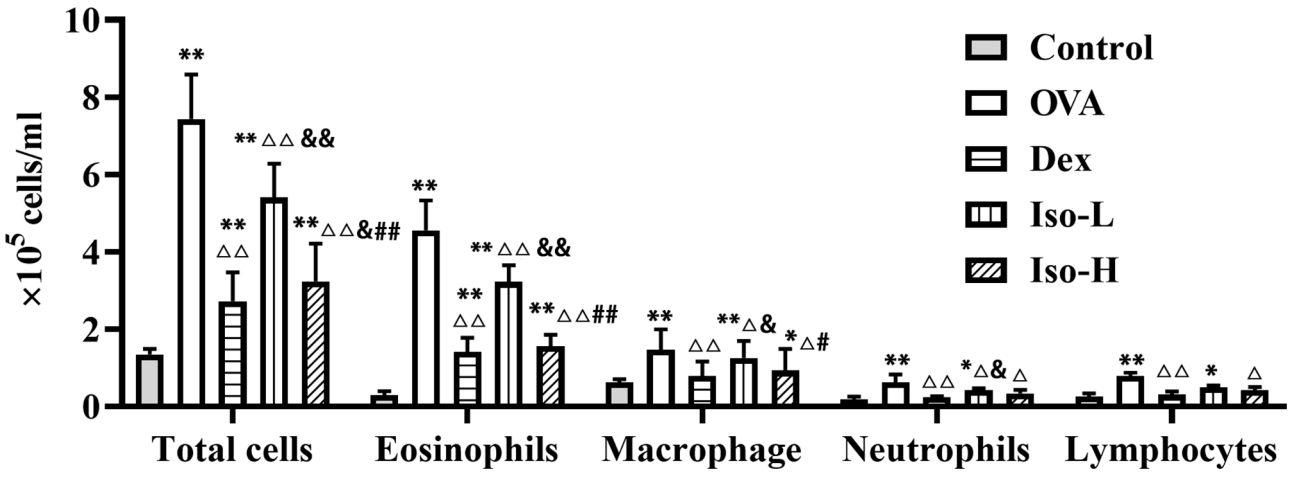

Figure 3. Iso diminished OVA-induced inflammatory cells count in the BALF versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$; versus the Iso-L group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

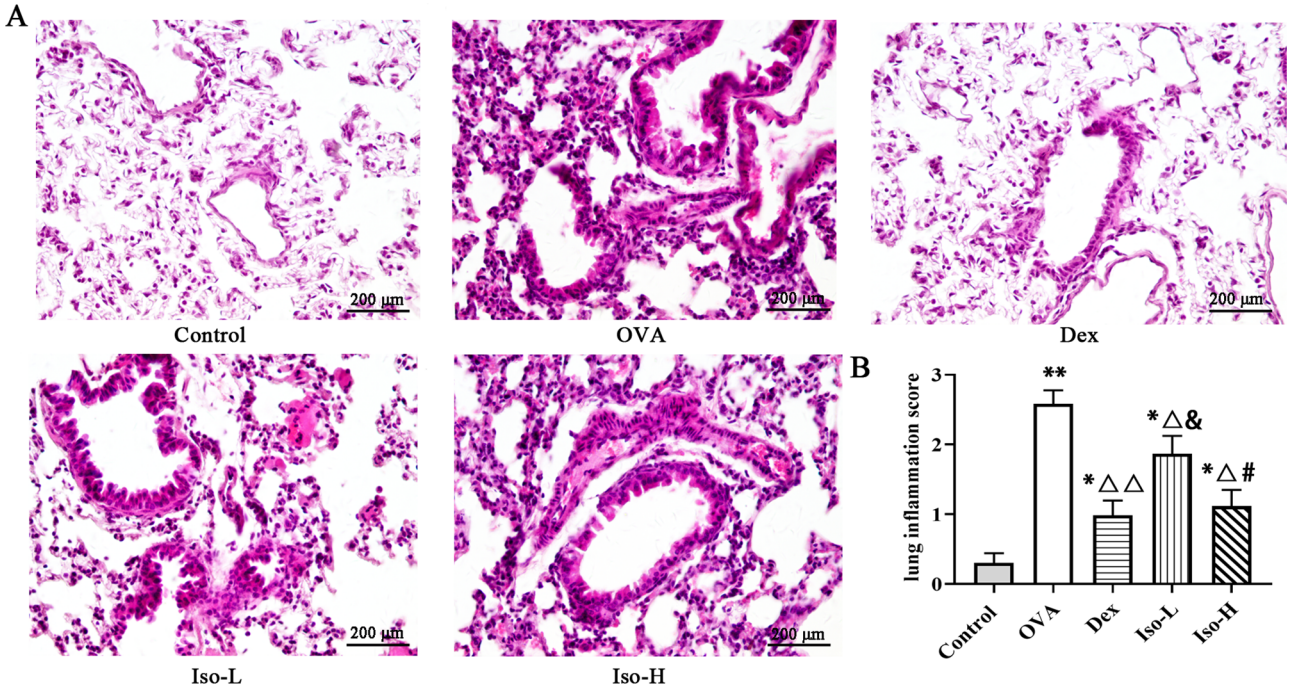

Figure 4. Histopathological observation of Iso and dexamethasone on OVA-induced allergic asthma by H&E staining. Microscopic images of 100-magnification were acquired and shown (scale bar: 200 μm). (A) H&E staining was used to investigate histopathological changes of lung tissues and (B) lung tissue inflammation score was assessed versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&}p < 0.05$; versus the Iso-L group, $^{\#}p < 0.05$.

levels of MDA, SOD, and GSH-Px in the Iso-H group had no significant difference compared to the Dex group.

Iso reduced the levels of ROS in lung tissue

Compared to the control group, the levels of ROS in Iso treatment groups were enhanced, as shown in Figure 5(E), indicating that disruption of oxidative and antioxidative system balance could lead to asthma. Compared to the OVA group, levels of ROS in Iso-L and Iso-H groups were decreased ($p < 0.05$), especially in the Iso-H group, indicating that Iso could diminish the levels of ROS.

Iso reduced the AHR in asthmatic mice

OVA mice presented a moderate increase in AHR in response to increased doses of MCh compared to mice in the control group. When the dose of MCh was low (0.75, 1.5, 3, 6 mg/mL), in OVA mice, airway resistance was enhanced in a dose-dependent manner following exposure to MCh, although not statistically significant. When the dose of MCh reached 48 mg/mL, there was a significantly diminished AHR in the Dex, Iso-L, and Iso-H groups compared to that in the OVA group (Figure 6). The results indicated that Iso effectively diminishes AHR.

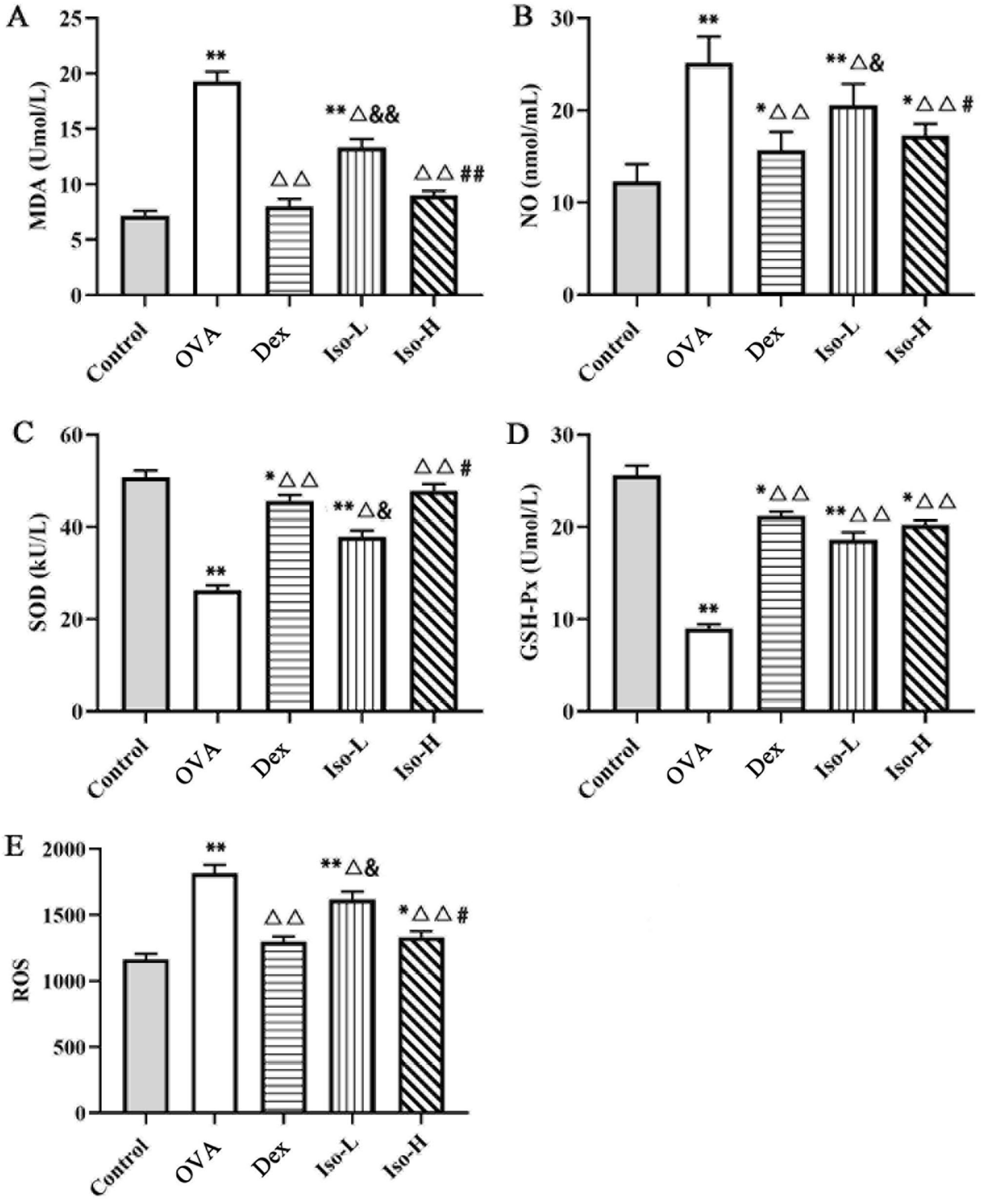

Figure 5. Iso attenuates oxidative stress in lung tissue of mice. (A) The level of MDA in lung tissues; (B) the level of NO in lung tissue; (C) the level of SOD in lung tissue; (D) the level of GSH-Px in lung tissue; and (E) the level of ROS in lung tissue versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$; versus the Iso-L group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

Iso induced the expression of Nrf2 and HO-1

To investigate the expression of Nrf2/HO-1 signaling, we detected the expression of Nrf2 and HO-1 proteins, and western blot results revealed that the expression of Nrf2 and HO-1 proteins in the Iso and dexamethasone treatment groups significantly increased compared to the control and OVA groups ($p < 0.05$). Moreover, the expression of Nrf2 and

HO-1 proteins in the Iso-H group was higher than the Iso-L group. These results indicate that Iso was able to induce the expression of Nrf2 and HO-1 (Figure 7).

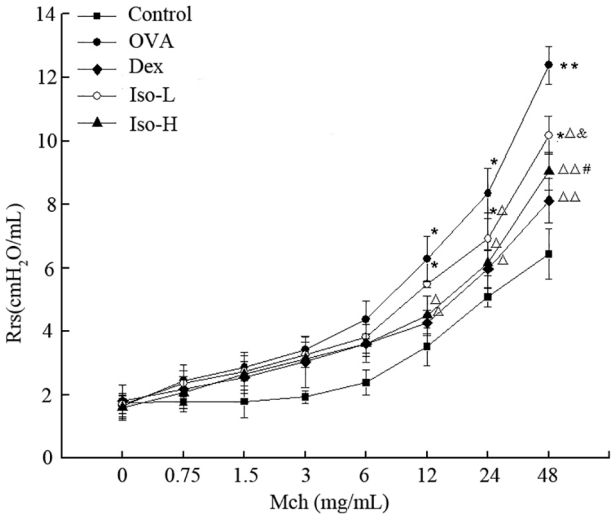

Figure 6. Iso and dexamethasone treatment reduces OVA-induced AHR versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&}p < 0.05$; versus the Iso-L group, $^{\#}p < 0.05$.

Iso inhibited NF-κB signaling

We also detected the expression of NF-κB signaling, and as shown in Figure 8, the levels of phospho-NF-κB-P65 and p-IκB-α in the Iso and dexamethasone treatment groups significantly decreased compared to the OVA group. The degradation of IκB-α decreased significantly ($p < 0.05$). The results indicate that Iso treatment diminished the activity of NF-κB signaling. The effect on the Iso-H group was greater than in the Iso-L group.

Discussion

Iso is a traditional Chinese medicine monomer with anti-inflammatory, antioxidation, and anticancer properties, representing a hotspot in the field of traditional Chinese medicine research in recent years. Iso has been reported to possess anticarcinogenic activities and inhibit inflammation responses.[14,15] Previous research has found that Iso is an effective agent for ameliorating cognitive impairments.[18] Iso also had a very favorable effect on clinical system diseases, such as acute kidney injury.[19] In this study, we found that Iso inhibited OVA-induced airway inflammation.

OVA-induced allergic asthma is related to the inflammation of the airways, which is connected with the infiltration of eosinophils or lymphocytes into the lung tissues and

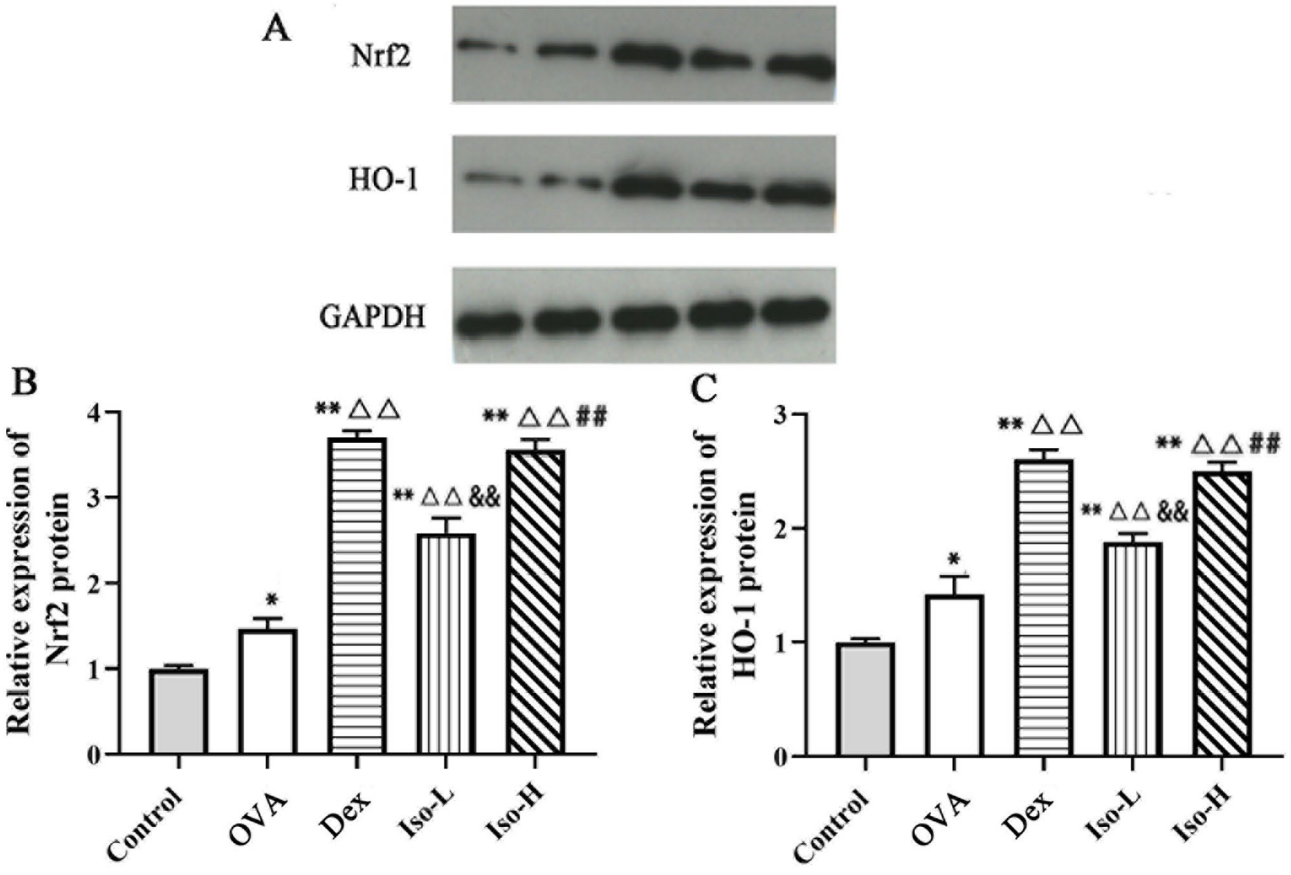

Figure 7. Iso treatment increases the expression of Nrf2 and HO-1 in lung tissue. (A) Western blot detected the expression of Nrf2 and HO-1 protein; (B) quantitative analysis of Nrf2 protein; and (C) quantitative analysis of HO-1 protein versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&\&}p < 0.01$; versus the Iso-L group, $^{\#\#}p < 0.01$.

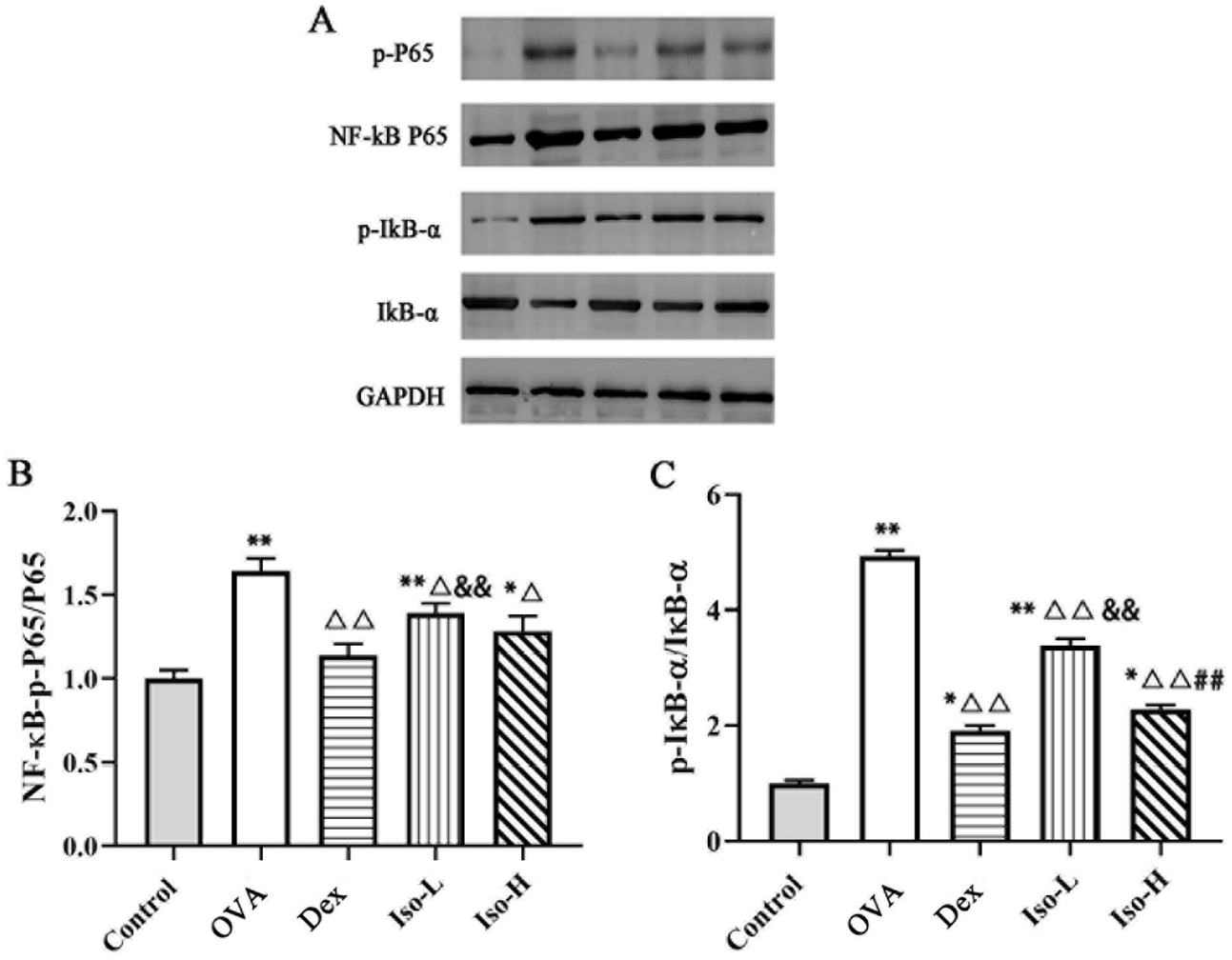

Figure 8. Iso treatment increases the expression of NF-κB signaling in lung tissue. (A) Western blot detected the expression of p-NF-κB-p65, NF-κB-p65, p-IκB-α, and IκB-α; (B) quantitative analysis of NF-κB-p65 protein; and (C) quantitative analysis of IκB-α protein versus the control group, $^{*}p < 0.05$, $^{**}p < 0.01$; versus the OVA group, $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$; versus the Dex group, $^{\&\&}p < 0.01$; versus the Iso-L group, $^{\#\#}p < 0.01$.

bronchial lumen.[20] Simultaneously, OVA-induced asthma is considered to be a disease characterized by the infiltration of inflammatory cells, involving T-lymphocytes, mast cells, neutrophils, macrophages, and eosinophils. OVA-induced asthma mice were challenged by inhalation of aerosolized antigen, which represents a similar delivery route and lung segmental allergen challenge to that of human asthma.[21] Th2 cytokines, such as IL-4, IL-5, and IL-13, play an important role in the progression of allergic asthma.[3,22] We investigated the content of IL-4, IL-5, IL-13 in BALF, and IgE in serum, and the results indicate that Iso could effectively diminish the levels of IL-4, IL-5, IL-13 in BALF, and IgE in serum. In addition, Iso significantly attenuated OVA-induced inflammatory cells in BALF. These results indicate that the anti-asthmatic effects of Iso are related to downregulation of Th2 cytokines. Inhibiting excessive Th2 cytokines is an important treatment strategy for allergic asthma.[23] Biological modifiers of Th2 ILs, such as mAbs, soluble receptors, and receptor antagonists, are a reasonable strategy to develop new treatments. However, their use needs to target patients with "active" appropriate Th2 immune pathways.[24] Compared to biological modifiers of Th2 ILs, Iso is a natural flavonoid, it has application value for patients with mild-to-moderate asthma, and we thought that the combination of Iso and Th2 may be helpful. However, this hypothesis needs to be confirmed by a large number of experiments in the future.

Free radicals are usually ROS which cause the damage of cell membranes.[25] SOD, ROS, and MDA levels indirectly indicate the radical-obliterating ability in tissues. Our research highlighted that Iso drastically inhibits asthmatic reactions by attenuating ROS and MDA levels and enhancing the ability of SOD, thereby enhancing the antioxidation ability in asthmatic mice, and a reduction of the generation of free radicals. Therefore, Iso reduced the generation of free radicals to enhance the ability of antioxidation in asthmatic mice. Simultaneously, we demonstrated that Iso reduced the airway inflammation damage and relieved asthma symptoms, which can be mediated by the levels of GSH-Px and NO. GSH-Px is defined as a kind of important regulating metabolite and antioxidant, having the redox regulating functions, clearing free radicals, and protecting the viscera from damage induced by radicals, and NO could produce cytotoxicity and free radicals.[26–28] The GSH-Px activity of asthmatic mice in low- and high-dose Iso groups was higher

than in the OVA group, indicating that Iso exerts the effects by increasing the levels of GSH-Px. The levels of NO in low and high doses of Iso were significantly lower than the OVA group. Our results indicate that Iso had a protective effect on OVA-induced asthma by enhancing GSH-Px levels and reducing NO levels. Furthermore, the high-dose Iso treatment had no significant difference from dexamethasone treatment. Meanwhile, H&E staining also demonstrated that mice treated with Iso and dexamethasone ameliorated inflammatory cell infiltration and led to a reduction of interstitial thickening.

Nrf2/HO-1 plays an important role in cell adaptation and antioxidant stress. Numerous studies have reported that the activation of the Nrf2/HO-1 signaling pathway can suppress inflammatory responses in OVA-induced asthma.[29,30] In our study, we investigated whether Iso induced antioxidative stress through the Nrf2/HO-1 pathway by western blot analysis. Our results revealed that the protein expression of Nrf2 and HO-1 was remarkably enhanced in the Iso treatment group compared with the OVA group. At the same time, the protein expressions of Nrf2 and HO-1 in the Dex group also increased, and there was no significant difference between the Iso-H group and the Dex group. We also investigated NF-κB signaling and found that the phosphorylation activity of NF-κB-p65 was decreased after treatment with Iso. A previous study proved that Iso attenuates LPS-induced pro-inflammatory responses in BV-2 microglia, and its role is related to NF-κB signaling.[31] Our results are consistent with their study.

Conclusions

This study shows that Iso plays a crucial role in the treatment of OVA-induced asthma. Iso markedly reduced the levels of IL-4, IL-5, IL-13, IgE, MDA, NO, and ROS and enhanced the ability of GSH-Px and SOD, which contributed to amelioration of asthma. Therefore, our data suggest that Iso may provide a therapeutic rationale for allergic airway diseases, such as asthma. The mechanism is related to the decrease in Th2 cytokines, activation of Nrf2/HO-1, and attenuation of the NF-κB signaling pathway.

AUTHORS' CONTRIBUTIONS

SL, YZ, and CW contributed to conception and design; GC contributed in administrative support; SL, YZ, and GC participated in provision of study materials or patients; SL, YZ, and CW participated in collection and assembly of data; SL, YZ, and CW contributed to data analysis and interpretation. All authors wrote the article and approved the final version of article. SL and YZ contributed equally to this work.

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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