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Original Research Article

Imperatorin inhibits allergic airway inflammatory reaction and mucin secretion in ovalbumin-induced asthmatic rats

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Abstract

Purpose: *To study the therapeutic effects of imperatorin (IPT) on allergic asthma induced by ovalbumin in rats.*
Methods: *Asthma was established in rats by injection of ovalbumin (OVA), and IPT (20, 40 and 80 mg/kg) was administered orally for 21 days. Inflammatory cells and cytokines were determined in the bronchoalveolar lavage fluid (BALF); IgE and histamine in the serum were also determined. Furthermore, MUC-5AC expression in the lung tissue was determined by Western blot assay.*
Results: *Treatment with IPT (20, 40 and 80 mg/kg) decreased inflammatory cells including eosinophils ($p < 0.01$), neutrophils ($p < 0.05$), lymphocytes ($p < 0.01$) and macrophages ($p < 0.01$). In addition, the four inflammatory cytokines {interleukin (IL) -4, IL-6, IL-13 and tumor necrosis factor (TNF)-α} were significantly decreased by treatment with IPT (20, 40 and 80 mg/kg) dose-dependently ($p < 0.01$). Furthermore, MUC5AC in lung tissues was significantly down-regulated by treatment with IPT (20, 40 and 80 mg/kg, $p < 0.01$) in a dose-dependent fashion.*
Conclusion: *The results show that IPT exerts notable therapeutic effects on allergic asthma in rats via suppression of IgE, histamine, inflammatory cells and cytokines, and also by down-regulating MUC5AC.*

Keywords: *Imperatorin, Allergic asthma, Inflammatory cytokines, MUC5AC expression, Eosinophils, Neutrophils, Lymphocytes*

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INTRODUCTION

In recent years, allergic asthma with an incidence rate of 4.3 % in the world has become a common disease affecting the normal life of millions of children and adults [1,2]. It is estimated that asthma causes over 300,000 deaths in the whole world every year [1-3]. Asthma is a chronic disease with complex pathology and characterized by serious airway inflammatory reactions, excessive phlegm and shortness of breath, etc. [4]. Currently, available drugs can temporary control or relieve the asthma symptoms, however radical cure of asthma seems very difficult. Furthermore, taking these drugs over a long term could cause several annoying adverse reactions [5,6].

Herbal medicines and natural plant-derived agents are important sources for discovering new effective drugs with low toxicity [7]. Imperatorin (IPT, Figure 1) is a natural constituent commonly existing in plants of the Umbelliferae family, such as *Peucedanum praeruptorum* and *Angelica dahurica* [8]. IPT shows a broad pharmacological activities including anti-seizure, vasodilatation, antitumor, antimicrobial, anti-inflammatory, and analgesic effects [8-10]. However, to the best of

our knowledge, there is no information on the effects of IPT on allergic asthma. Thus, in this study, the therapeutic effect of IPT on allergic asthma was evaluated based on an ovalbumin (OVA) -induced asthma model in rats, and its pharmacological mechanisms were furthermore explored.

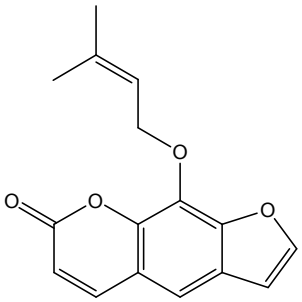

Figure 1: Chemical structure of IPT

EXPERIMENTAL

Animals

Male Sprague Dawley (SD) rats (200 ± 20 g) were purchased from the Shanghai Laboratory Animal Research Center (Shanghai, China). The animals and experimental protocols were handled according to the Declaration of Helsinki promulgated in 1964 as amended in 1996 [10], and approved by the Animal Care and Use Committee of Zhejiang University School of Medicine (approval no. An-2015-0393#).

Chemicals and reagents

Imperatorin (IPT) was purchased from Chengdu Pure Chem-Standard. Co. Ltd. Chengdu, China; commercially enzyme-linked immunosorbent assay (ELISA) kits for interleukin (IL) -4, IL-13 and tumor necrosis factor (TNF)-α were purchased from Invitrogen, Carlsbad, CA, USA and ELISA kits for IgE, IL-6, and histamine were obtained from eBioscience, Shanghai, China. OVA, pentobarbital and aluminum hydroxide were purchased from Sigma-Aldrich, St. Louis, MO. U.S.A. BCA protein assay reagent and horseradish peroxidase-conjugated secondary antibody were purchased from Beyotime, Nanking, China. Primary antibodies used in western blotting assay for MUC 5AC and β-actin were purchased from Abcam, Cambridge, UK.

Animal model and experimental protocol

A total of 50 SD rats were randomly divided into five groups (n=10): normal group, control group, and IPT groups (20, 40 and 80 mg/kg). The OVA-induced asthma rats were prepared according to the method described by Lee et al with minor modification [11]. Briefly, each SD rat in control and IPT groups were immunized by intraperitoneal injection (ip) of a mixture of 20 mg OVA and 2 mg aluminum hydroxide at 1 and 7 days. Furthermore, rats in the control and IPT groups received an airway challenge with 1 % (w/v) OVA solution for 20 min using an ultrasonic nebulizer (Yuyao 402A, Nanking, China) from day 17 to day 21 after the initial sensitization. For the normal group, rats underwent the same sensitization procedures with saline instead of OVA. During this process, normal and control rats were orally treated with saline continuously from the first OVA injection to the end of the experiment (20 ml/kg), and rats in the IPT groups were administered orally with IPT at doses of 20, 40 and 80 mg/kg, respectively.

After 48 h of the final challenge, blood samples of the rats were collected under pentobarbital (50 mg/kg, ip) by abdominal aortic blood sampling, then rats were sacrificed to obtain the bronchoalveolar lavage fluid (BALF) and inflammatory cell numbers were determined by counting cells with a hemocytometer. In addition, the lung tissues were also collected for further western blot assay. All the samples were stored at -70 °C before using.

Determination of IgE and histamine in plasma

Blood was centrifuged (3000 rpm) for 10 min to obtain plasma samples. Then, the levels of IgE and histamine were determined using commercial ELISA kits according to the manufacturer's instructions.

Determination of inflammatory cytokines in BALF

The supernatants of the BALF were obtained and the levels of inflammatory cytokines in BALF including TNF-α, IL-4, IL-6 and IL-13 were determined using commercial ELISA kits according to the manufacturer's instructions.

Western blot assay

Lung tissues were homogenized and then centrifuged at 3 000 rpm for 30 min at 4 °C, and subsequently total proteins were extracted from the supernatants. After that, protein concentration was determined using a BCA protein assay reagent. Then, equal amounts of total lung proteins (30 μg) were heated at 100 °C for 5 min and subsequently loaded onto 8 % sodium dodecyl sulfate polyacrylamide gel electrophoresis gels (SDS/PAGE), followed by blotting to a PVDF membrane. Nonspecific

binding sites of the PVDF membranes were blocked with 5 % skimmed milk for 1 h. After that, the PVDF membranes were incubated with the primary antibody for MUC-5AC and β-actin at 4 °C overnight. The membranes were washed three times with TBST, and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The target protein bands on the PVDF membranes were visualized using chemiluminiscence.

Statistical analysis

Data are presented as mean ± standard deviations (SD). Statistical comparisons were made by one-way analysis of variance (ANOVA) using SPSS software (version 15.0, USA), followed by Dunnett multiple comparison test. $P < 0.05$ was set as the significance level.

RESULTS

Effect of IPT on inflammatory cells in BALF

In order to determine the inhibitory effects of IPT on airway inflammatory reactions in the development of allergic asthma, we analysed the four inflammatory cells in BALF including eosinophils, neutrophils, lymphocytes and macrophages. As can be seen from Figure 2, BALF of OVA-challenged rats exhibited significant increase in these four inflammatory cells ($p < 0.01$). However, treatment with IPT at doses of 20, 40 and 80 mg/kg decreased the inflammatory cells including eosinophils ($p < 0.01$), lymphocytes ($p < 0.01$) and macrophages ($p < 0.01$), a dose-dependent manner. In addition, IPT (40 and 80 mg/kg) also showed a significant inhibitory effects on neutrophils ($p < 0.05$, $p < 0.01$).

Effect of IPT on levels of serum IgE and histamine

Results of the serum IgE and histamine levels in OVA-induced asthmatic rats are shown in Figure 3. The levels of serum IgE and histamine in OVA-challenged rats were increased compared with normal rats ($p < 0.01$). Furthermore, this result also demonstrated that the increased serum IgE and histamine of OVA-challenged rats could be significantly decreased by treating with IPT (20, 40 and 80 mg/kg) dose-dependently compared with the control rats ($p < 0.05$).

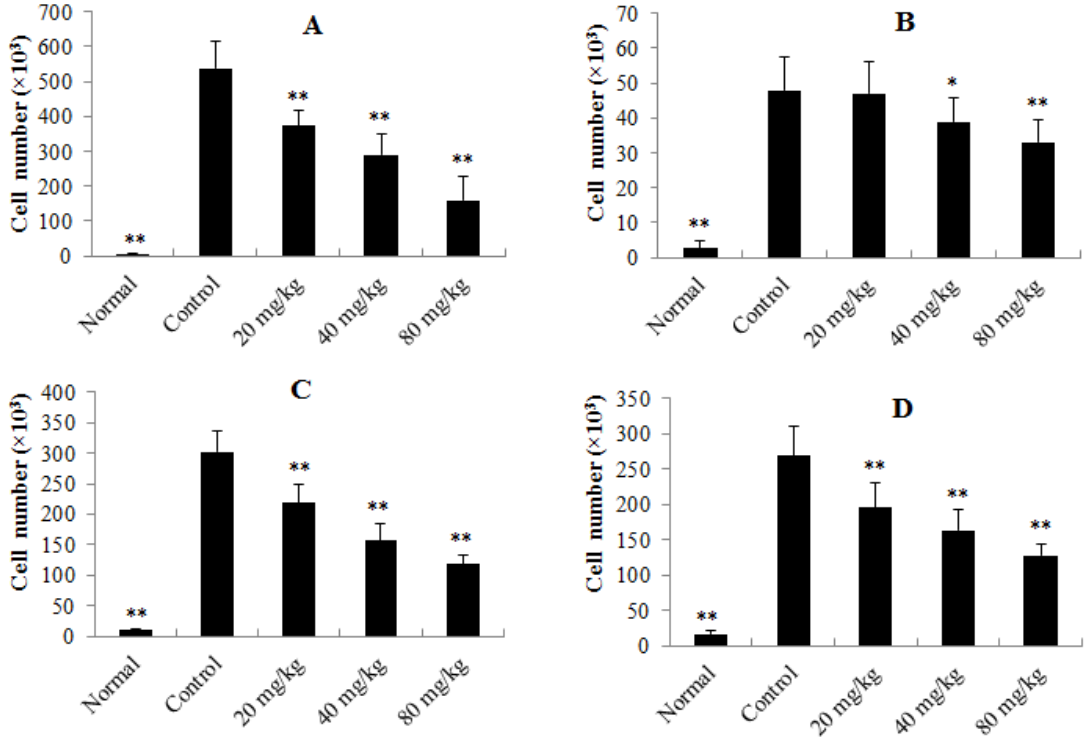

Figure 2: Effect of IPT on inflammatory cells in BALF. A-D represented eosinophils, neutrophils, lymphocytes and macrophages, respectively. Data were expressed as mean ± SD (n = 10); *$p < 0.05$, **$p < 0.01$, compared with the control group

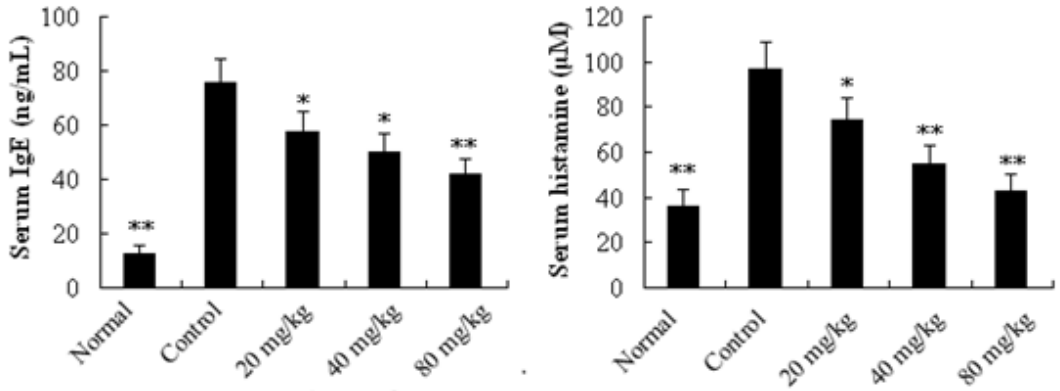

Figure 3: Effect of IPT on serum IgE and histamine. Data were expressed as mean ± SD (n = 10); *p < 0.05, **p < 0.01, compared with the control group

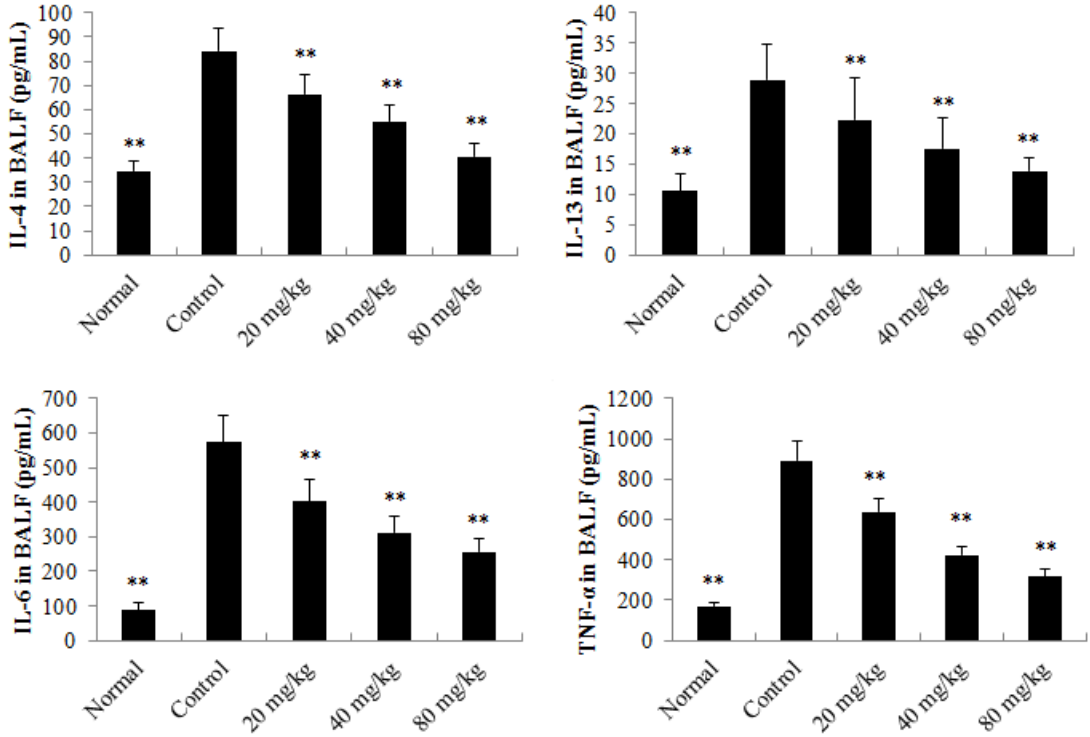

Figure 4: Effect of IPT on inflammatory cytokines in BALF. Data were expressed as mean ± SD (n = 10); *p < 0.05, **p < 0.01, compared with the control group

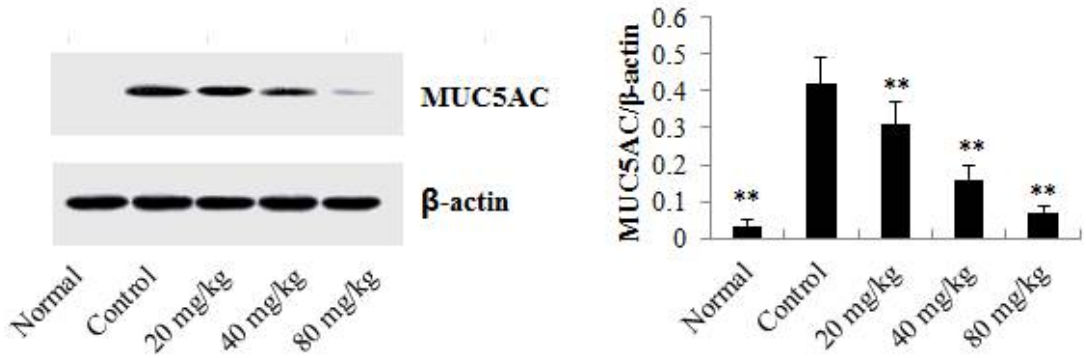

Figure 5: Effect of IPT on MUC5AC protein in lung tissues. Data were expressed as mean ± SD (n = 4); *p < 0.05, **p < 0.01, compared with the control group

Effect of IPT on inflammatory cytokines in BALF

As shown in Figure 4, after injection of OVA for 3 days, all the test inflammatory cytokines significantly increased including IL-4, IL-13, IL-6 and TNF-α ($p < 0.1$), compared with the normal rats. However, it is interesting that all the four inflammatory cytokines were significantly decreased by treatment with IPT at doses of 20, 40 and 80 mg/kg ($p < 0.01$) dose-dependently, compared with the control rats.

Effect of IPT on MUC 5AC expression in lung tissues

Furthermore, to explore the possible pharmacological mechanism, whether IPT down-regulates MUC 5AC expression in lung tissues of OVA-challenged rats was investigated using western blotting assay. As can be seen from Figure 5, MUC5AC in rats' lung tissues could be sharply up-regulated by challenging with OVA ($p < 0.01$). Furthermore, results of the present research demonstrated that MUC5AC could be significantly down-regulated by treatment with IPT at 20, 40 and 80 mg/kg ($p < 0.01$) dose-dependently, compared with the control rats.

DISCUSSION

In the present investigation, the potential therapeutic effects of IPT on allergic asthma were determined using an OVA-induced rat model for the first time. The results showed that IPT inhibited IgE, histamine, and inflammatory cells and cytokines in OVA-challenged rats, as well as down-regulated MUC5AC expression in lung tissue.

Inflammatory reaction is an important cause in the development of asthma, and previous reports also indicated that anti-inflammatory drugs could be beneficial for treating or relieving asthma symptoms [12,13]. In this study, the results showed that IPT possessed significant anti-inflammatory effects on airway inflammation induced by OVA via suppression of both inflammatory cells (eosinophils, neutrophils, lymphocytes and macrophages) and cytokines (IL-4, IL-13, IL-6 and TNF-α). Furthermore, previous researches indicated that asthma is closely related to the proliferation of allergen-specific type II T helper (Th2) lymphocyte, leading to the excessive releases of Th2 cytokines such as IL-4, and IL-13 [14,15]. Thus, suppression of IL-4 & IL-13 might be an important mechanism for the therapeutic effects of IPT.

IgE and histamine are two important reasons for the development of allergic diseases including eczema, allergic rhinitis and asthma, etc, and previous researches also indicated that IgE and histamine are also important targets for treating allergic diseases [16]. Interestingly, the present results showed that IPT could effectively decrease the serum levels of IgE and histamine. It has been reported that hypersecretion of MUC5AC in airway epithelial cells is an important characteristic of asthma and other mucus hypersecretion diseases [17,18]. Down-regulation of MUC5AC is a feasible way for the treatment of hypersecretion diseases. In addition, it has been reported that TNF-α, IL-4 and IL-13 play important roles in the release of MUC5AC [19]. The present results demonstrated that IPT could effectively suppress not only TNF-α, IL-4 & IL-13 but also MUC5AC.

CONCLUSION

The findings of the present investigation demonstrate that IPT possesses effective therapeutic effects on allergic asthma in a rat model via suppression of IgE, histamine, inflammatory cells and cytokines, as well as by down-regulation of MUC5AC in OVA-induced allergic asthmatic rats.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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