Tropical Journal of Pharmaceutical Research January 2022; 21 (1): 87-92
ISSN: 1596-5996 (print); 1596-9827 (electronic)
© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i1.14

Original Research Article

Anti-asthmatic effect of fusarubin in an ovalbumin-induced asthmatic rat model

Jiajing Hong[1], Fuchun Wang[1], Dongyu Yang[2], Junming Kan[3]*
[1]College of Acupuncture and Massage, [2]Department of Traditional Chinese Medicine, The Affiliated of Changchun University of Traditional Chinese Medicine, [3]College of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Changchun, Jilin 130117, China

For correspondence:* ***Email:* *394327974@qq.com;* ***Tel/Fax:*** *0086-0431-86172513*

Sent for review: 16 November 2019 *Revised accepted: 24 December 2021*

Abstract

Purpose: *To investigate the anti-asthmatic effect of fusarubin in an ovalbumin (OVA)-induced asthmatic rat model*
Methods: *A rat model of asthma was employed in this investigation. Ovalbumin (OVA) was used to sensitise rats, and the rats were then treated with fusarubin before the onset of asthma. Histology-based examinations were conducted to assess the impact of fusarubin on mucus secretion and migration of eosinophils into the lungs.*
Results: *The results showed that fusarubin significantly decreased ovalbumin-induced airway hyperresponsiveness {AHR levels of inflammatory cells and eosinophils in bronchoalveolar lavage fluid (BALF)}, when compared to the control group ($p < 0.05$). Furthermore, fusarubin markedly downregulated levels of OVA-stimulated pro-inflammatory cytokines in BAL fluid ($p < 0.05$).*
Conclusion: *Fusarubin may be used as a potential novel compound for the management of asthma.*

Keywords: *Asthma, Hypersensitivity, Fusarubin, Inflammatory cells, Eosinophil, Airway hyperresponsiveness, Bronchoalveolar lavage, Cytokines*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Asthma is a complex health problem characterised by lung hypersensitivity, irregular impediment to airflow, and airway inflammation. The major factors responsible for asthma are respiratory tract infections (RTI) due to viruses in infants [1], and exposure to inflammatory agents in adults [2]. The severity of symptoms may be accentuated in sensitized individuals due to exposure to viral infections, irritants, allergens, exercise, and consumption of non-steroidal anti-inflammatory agents (NSAIDS). Asthma treatment primarily involves evaluation of severity and determination of long term-consequences [3]. However, the most efficient therapies do not provide ideal control of asthma for lots of patients all over the world [3]. This shortcoming in treatment outcome should be addressed by evolving novel therapies and treatment concepts. Asthma is commonly found in low- and middle-income countries where it usually starts in childhood. The number of affected persons was estimated to be 339 million in 2018, with mortality

© 2022 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

of 1000/day [4]. The characteristic features of asthma are infiltration of eosinophils, inflammation and overproduction of mucus in the pulmonary system. There are excessive secretions of immunoglobulin E and AHR, due to goblet cells [5]. It has been reported that eosinophils, mast cells and lymphocytes are involved in the exacerbation of asthmatic symptoms in sensitized individuals [6].

Previous studies indicated that eosinophilia in the airways are regulated by the effect of cytokines such as interleukin (IL)- 4, IL- 5 and IL- 13 secreted by type 2 T helper (Th2) cells [7]. On the other hand, cytokines and chemokines from eosinophils escalate the symptoms of asthma [8]. Thus, the main focus of asthma treatment involves regulation of cytokines, chemokines and other mediators so as to improve the efficiency of clinical treatment methods [9].

In the acute cases of inflammatory asthma, the major features of airway pathology are allergen-IgE-directed processes and histological elements like eosinophils, mast cells, and Th2 lymphocytes [10]. The most common cytokines responsible for these phenomena are IL-5, IL-4, IL3, IL-9, and IL-13 [11]. Mast cells release inflammatory cytokines as well as acute-phase mediators such as cysteinyl leukotrienes, thereby contributing to the initiation of asthma and the perpetuation of airway inflammatory events [12]. Lymphocyte sub-populations sensitive to Th2 profile accentuate the inflammatory process by liberating cytokines IL-13, IL-5, and IL-4. These factors regulate IgE production by recruitment of eosinophils, and finally trigger inflammation [10]. Eosinophils are typical elements of allergic inflammation [10]. These are biologically active enough to cause enhancement of airway hyperresponsiveness, followed by airway inflammation and impediment to airflow. They engage themselves in the airway under the effect of chemokines and cytokines such as RANTES and eotaxin or IL-5. They also produce inflammatory cytokines, leukotrienes, products of oxidative metabolism and growth factors [13].

Fusarubin ($C_{15}H_{14}O_7$), a red antibiotic obtained from *Fusarium solani* [14], is similar to oxyjavanicin isolated earlier from *Fusarium javanicum* [14]. It belongs to the napthoquinone class of compounds. Naphthoquinones are widely distributed in nature in actinomycetes, fungi and higher plants. These compounds possess diverse biological activities such as insecticidal, phytotoxic, fungicidal and antibacterial properties. In addition, several others exert anticarcinogenic and cytostatic effects [15].

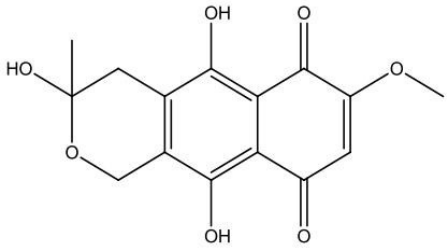

Figure 1: Structure of fusarubin

In this study, a rat model of asthma established by sensitization with ovalbumin (OVA) was used to determine the anti-asthmatic activity of fusarubin.

EXPERIMENTAL

Fusarubin

Fusarubin (Figure 1) with purity > 98.0 % was procured from Zelang Medical Technology Co. Ltd. (Nanjing, China). The compound was solubilised in dimethyl sulfoxide (Sigma- Aldrich, USA) prior to use.

Cell lines and culture

Mouse skin melanoma cells (HEK 293a), human alveolar adenocarcinoma epithelial cells (A549) and mouse melanoma cells (MDA-MB-231) were purchased from the America Type Culture Collection (ATCC). The cells were cultured overnight in DMEM containing 10 % FBS and antibiotics at 37 ^oC.

Cytotoxicity assay

The cells were seeded, each at a density of 2 x 10^5 cells in 96-well plates containing DMEM, and cultured overnight at 37 °C. Fusarubin was added to the wells at indicated doses and incubation was continued for 48 h. Then 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT; 5 mg/ml) was added to the plates and incubation was continued for additional 4 h. Then, the medium was removed, and the formazan crystals formed in the wells were solubilized in DMSO (150 µl). The absorbance of each well was read at 487 nm in a microplate reader (BioRad Laboratories, Hercules. CA).

THP_1 cell culture and measurement of cell viability

Strains of THP_1 monocytes were grown in RPMI 1640 (Sigma) containing 10 % FBS (Lonza), penicillin (100 µg /mL), streptomycin (100 µg/mL) and non-essential amino acids in 75-cm^2 filter

vent flasks (Costar) at an incubation temperature of 37 °C in a moist atmosphere of 5 % CO_2 and 95 % air. Trypan blue dye exclusion assay was used to measure the effect of fusarubin on cell viability. The cells were seeded in triplicate at a density of 2 × 10^5 cells/mL in 24-well plates, and subjected to 48-h fusarubin treatment. This was followed by cell re-suspension in 0.4 % Trypan blue and analysis of cell viability.

Assessment of cytokine inhibition

The THP_1 monocytes were fixed in 24-well plates prior to measurement of the extent of inhibition after incubation with fusarubin for 1 h. Stimulation with 10 ng/mL PMA for 48 h was followed by assay of supernatant levels of TNF-α, MCP-1 and IL-1β levels using enzyme-linked immunosorbent assay (ELISA). The absorbance of each well was read in a microplate reader at 450 nm.

Animals

Thirty male Wistar rats (SPF grade, 4-week old) were obtained from the Laboratory Animal Centre, PLA General Hospital (Beijing, China). The rats were maintained for about 7 days in a specific pathogen-free environment before their use for this study. The rats were kept at 20 - 26˚C in an environment with a 12-h light/12-h dark cycle, and were allowed free access to water and feed. The rats were randomly assigned to three groups, each with 8 rats: control, fusarubin-treated and model (OVA) groups. All animal experiments were executed as per the Guidelines for Care and Use of Laboratory Animals, National Institute of Health, USA, and were approved by the institutional animal ethics committee.

Establishment of rat asthma model

Rats (8 rats/group) were infused with 20 µg OVA (Sigma-Aldrich) on days 1 and 14. Then, the rats were injected with fusarubin (10 or 20 mg/kg) or vehicle (0.5% sodium salt of carboxy methyl cellulose) on days 28, 29 and 30. After 1h, the rats were challenged with OVA (1%). The injection doses were set up as per a previous study [16].

Preparation of lymphocytes

The rats were sacrificed under carbon dioxide exposure, and their spleens were excised. The spleens were chopped into small sections and macerated with a syringe. The filtration of cells suspended in RPMI 1640 medium (Invitrogen; Thermo Fisher Scientific, Inc., USA) was carried out through a 70-µm cell strainer (BD Biosciences, USA). Disruption of red blood cells was done in 10 mM EDTA (Thermo Fisher Scientific Inc.) as described earlier [17]. Lymphocytes were separated from the spleen cells using Nyco Prep™ 1.077A (Axis-Shield PoC, Oslo) method [17]. The growth medium used for lymphocytes was RPMI-1640 enriched with 10 % fetal bovine serum. The primary spleen cells harvested from experimental rat model were challenged with 05 µg/ml concanavalin A before treating them with fusarubin.

Enzyme-linked Immunosorbent assay (ELISA)

The rats were sacrificed after euthanasia with carbon dioxide, followed by excision of the lungs. Then, bronchoalveolar lavage fluid (BALF) was collected by flushing the lungs with 0.5 ml ice-cold PBS. Commercially available ELISA kits (R&D Systems, Inc. Minneapolis, USA) were used to determine the level of chemokines and cytokines in cell BALF.

Measurement of airway hyperresponsive-ness (AHR)

Whole-body plethysmography was carried out 24 h after the final ova-albumin treatment to determine AHR in vehicle group and fusarubin-treated rats. These rats were put in chambers and exposed to aerosolized methacholine solution (Sigma-Aldrich) at doses of 20, 40, 60 and 80 mg/mL. Therefore, bronchial constriction was assessed for 5 min. The highest Penh value of each group was shown as a basal Penh value relative to the PBS-treated control.

Bronchoalveolar lavage fluid (BALF)

The rats were sacrificed using carbon dioxide euthanasia 24 h after OVA sensitization, and the lungs were excised and flushed by injecting ice-cold PBS through the airways so as to obtain BALF. Modified Wrights stain (Sigma-Aldrich) was used to stain the cytospin slides (Thermo Fisher Scientific, Inc.). The number of inflammatory cells and total corpuscles were determined using a haemocytometer (Countess II FL Automated Cell Counter; Thermo-Fisher Scientific, Inc).

Statistical analysis

Data from *in vitro* experiments are expressed as mean ± standard deviation (SD), while data from *in vivo* studies are reported as mean ± standard error of the mean (SEM). Statistical analysis was

done using Student's *t*-test. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

In vitro anticancer activity of fusarubin

The cytotoxicity of fusarubin against three cancer cell lines was measured using MTT assay. The results are shown in Table 1. Fusarubin showed potential cytotoxicity against the three cell lines. It exhibited strong cytotoxicity against A549 and MDA-MB-231, with IC_{50} values of 9.6, 1.3 µM respectively, and appreciable toxicity against HEK cell lines, with IC_{50} value of 20.3 µM.

Anti-inflammatory effect of fusarubin

The anti-inflammatory effect of fusarubin against PMA-induced inflammation was evaluated at different concentrations by determining the levels of MCP-1, IL-8 and TNF-α using THP1 monocyte cells, with pioglitazone and piroxicam as reference drugs. Fusarubin showed highest anti-inflammatory activity at a concentration of 0.27 µM.

Modulation of Th_1/Th_2 balance in fusarubin-treated rats

Fusarubin treatment significantly suppressed Th1 cytokine level [IL-2 and interferon (IFN)-γ; $p < 0.001$] and markedly elevated Th_2 cytokine levels (IL-10 and TGF-β; transforming growth factor; $p < 0.001$), in a dose- dependent manner. These results indicate that fusarubin regulated balance between Th_1 and Th_2 cytokines in asthmatic rats.

Effect of fusarubin on various parameters of asthma

Airway hyper-responsiveness (AHR)

In vitro studies revealed that fusarubin modulated Th_1/Th_2 equilibrium. Thus, the effect of fusarubin was investigated in a rat model of asthma. Rats were constantly challenged with OVA for three days and infused with methacholine to trigger AHR, while the control group received PBS. The results showed that fusarubin markedly suppressed methacholine-induced AHR, relative to the vehicle control group.

Effect of fusarubin on eosinophil recruitment

Fusarubin treatment caused a significant reduction in the number of neutrophils, eosinophils and total cells in BALF, when compared to the control group ($p < 0.05$). However, fusarubin did not produce any significant effects on the number of macrophages and lymphocytes. These results are shown in Figure 1.

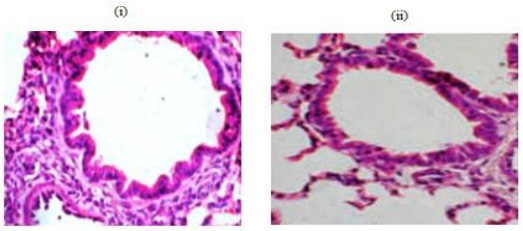

Figure 1: Photomicrographs of (a) OVA-challenged and (b) OVA-challenged and fusarubin-treated rat lung tissues

Effect of fusarubin on eosinophil migration and mucus formation

These results showed that after OVA challenge, the eosinophil recruitment and mucus formation increased significantly, when compared to the PBS group ($p < 0.05$). However, after fusarubin treatment, the inflammation and mucus score decreased from 4.8 to 2.2 ($p < 0.05$) and 3.6 to 1.9 ($p < 0.05$), respectively. These results indicate that fusarubin suppressed eosinophil migration (Figure 1; Table 2) and mucus secretion (Figure 2; Table 3) in the lungs of asthmatic rats, when compared to control group.

Levels of cytokines in BALF

The elevated levels of IL- 5, IL- 13, IL- 4 and eotaxin in BALF of OVA-challenged rats were significantly decreased by fusarubin ($p < 0.05$; Figure 3), with a minor effect on IFN-γ. Thus, fusarubin altered the levels of cytokines secreted by immune cells in the rat model of asthma.

Table 1: *In vitro* cytotoxicity of fusarubin

Cell line	IC_{50} of fusarubin	IC_{50} of doxorubicin (standard)
MDA-MB-231	1.3 ± 0.2 µM	0.54 ± 0.07 µM
A549	9.6 ± 0.5 µM	0.58 ± 0.05 µM
HEK	20.3 ± 0.3 µM	4.2 ± 0.2 µM

Table 2: Effect of fusarubin on inflammatory cell infiltration in lung tissues of rats

Control	OVA challenged	Fusarubin treated
0.5 ± 0.01	7 ± 0.02	2.8 ± 0.01

Table 3: Effect of fusarubin on mucus secretion in lung tissues of rats

Control	OVA challenged	Fusarubin treated
0.2 ± 0.01	5.6 ± 0.05	3.0 ± 0.03

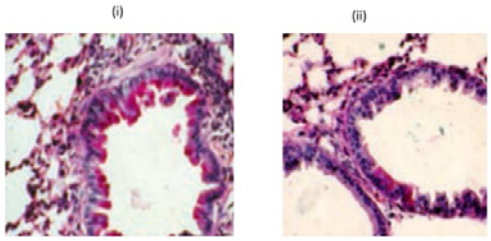

Figure 2: Effect of fusarubin on mucus secretion in rat lung tissues. Photomicrographs of (i) OVA-challenged and (ii) OVA-challenged and fusarubin-treated rat lung tissues

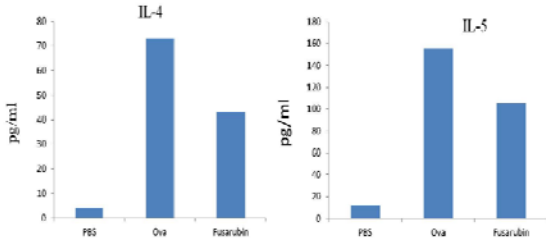

Figure 3: Effect of fusarubin on cytokine levels in rat BALF. The levels of IL-4 and IL-5 in rat BALF were determined using ELISA

DISCUSSION

eosinophils. Moreover, IL- 13 plays a major role in AHR, eosinophil recruitment, and production of mucus [20,21]. Indeed, two important chemokines i.e., RANTES and eotaxin, perform vital functions during the infiltration of eosinophils into the airways. During eosinophil recruitment, cell adhesion molecules (CAMs) are also required [22]. Airway hyper-responsiveness in asthma involves three factors: CAMs, Th_2 cytokines and chemokines [23]. In this study, fusarubin demonstrated marked downregulation of IL- 5, IL- 13, IL- 4 and eotaxin in BALF.

CONCLUSION

The findings of this study reveal that fusarubin regulates cytokine balance in OVA-sensitized rats, arrests AHR, decreases migration of eosinophils into the lungs, and reduces excessive production of mucus in a rat model of asthma. Thus, fusarubin may be a novel and potentially beneficial drug for the treatment of asthma.

Asthma is a persistent disorder of the airways. It is characterised by inflamed airways, elevated production of mucus and AHR [18]. Earlier investigations showed that the immune cells eosinophils, T, B, and mast cells, as well as chemokines and cytokines play positive roles in inflammatory reactions [18,19]. Helper type 2 cells synthesize Th_2 cytokines which are linked to B-cell maturation. The Th_2 cytokines which comprise IL- 5, IL- 13 and IL- 4 are vital in humoral immune reactions. Moreover, chemokines are involved in the recruitment of eosinophils into the pulmonary tissues. Thus, the development of prospective therapeutic strategies for asthma potentially depends on their effects on cytokines and chemokines.

Fusarubin is a chemical compound of fungal origin. Earlier studies have demonstrated its anti-tuberculosis, antimicrobial and cytotoxic activities. However, to the best of our knowledge, its anti-asthmatic activity has not been investigated till date. The present study found that fusarubin modulated Th_1/Th_2 balance in rats. Therefore, fusarubin was studied *in vivo* in asthmatic rat model to evaluate its anti-asthma effects. In the toxicity experiments, it was found that rats treated with fusarubin at a dose of 50 mg/kg survived without decrease in body weight (data not shown). These results showed that fusarubin markedly reduced AHR in the rat model of asthma, and also decreased neutrophils, eosinophils and total number of cells in BALF. The levels of cytokine and chemokines were evaluated in BALF of rats in order to know the possible mechanism of action of fusarubin. The level of Th_2 chemokines and cytokines increased significantly in rats sensitized with OVA.

Interleukin-5 is vital to the survival and infiltration of

DECLARATIONS

Acknowledgement

The authors are thankful to National Key Basic Research and Development Program (973 program, 2014CB543100), Traditional Chinese Medicine Science and Technology Project of Jilin Province (no. 2018027) and Natural Science

Foundation of Jilin Province (no. 20200201520jc) for support for this work.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

Authors declare that this is their own work and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jiajing Hong, Fuchun Wang, Dongyu Yang and Junming Kan conceived and designed the study. Mr. Jiajing Hong and Fuchun Wang collected and analysed the data and wrote the manuscript. Mr. Dongyu Yang and Dr. Junming Kan aided in analysing and writing of the manuscript. All authors approved the manuscript for publication.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. *Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008; 1; 178(7): 667–672.*
2. *Bush RK, Peden DB. Advances in environmental and occupational disorders in 2008. J Allergy Clin Immunol 2009; 123(3): 575–578.*
3. *Busse WW, Lemanske RF Jr. Expert Panel Report 3: Moving forward to improve asthma care. J Allergy Clin Immunol 2007; 120(5): 1012–1014.*
4. *Vos T, Abajobir AA, Abate KH. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017; 390(10100): 1211-59.*
5. *Elias JA, Lee CG, Zheng T, Ma B, Homer RJ, Zhu Z. New insights into the pathogenesis of asthma. J Clin Invest 2003; 111: 291-297.*
6. *Okayama Y, Ra C, Saito H. Role of mast cells in airway remodeling. Curr Opin Immunol 2007; 19: 687-693.*
7. *Pascual RM, Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: An overview. J Allergy Clin Immunol 2005; 116: 477 486.*
8. *Panettieri RA Jr. Cellular and molecular mechanisms regulating airway smooth muscle proliferation and cell adhesion molecule expression. Am J Respir Crit Care Med 1998; 158: S133-S140.*
9. *Mauad T, Bel EH, Sterk PJ. Asthma therapy and airway remodeling. J Allergy Clin Immunol 2007; 120: 997-1009.*
10. *Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma-From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000; 161(5): 1720–1745.*
11. *Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008; 8(3): 183–192. [PubMed: 18274560]*
12. *Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol 2006; 117(6): 1277–1284.*
13. *Jacobsen EA, Taranova AG, Lee NA, Lee JJ. Eosinophils: singularly destructive effector cells or purveyors of immunoregulation? J Allergy Clin Immunol. 2007; 119(6): 1313–1320.*
14. *Ruelius HW, Gauhe A. Uber Fusarubin, einen Naphthochinonfarbstoff aus Fusarien. Ann Chem 1950; 569: 38-59.*
15. *Arsenault GP. The identity of oxyjavanicin with fusarubin. Canad J Chem 1965; 43: 2423 -2424.*
16. *Arnstein HRV, Cook AH. Production of antibiotics by fungi. Javanicin, An antibacterial pigment from Fusarium javanicum. J. Chem. Soc. 1947: 1021-1028.*
17. *Medentsev AG, Akimenko VK. Naphthoquinone metabolites of the fungi. Phytochem 2005; 47: 935-959.*
18. *Liu J, Yang F, Zhang Y, Li J. Studies on the cell-immunosuppressive mechanism of Oridonin from Isodon serra. Int Immunopharmacol 2007; 7: 945 954.*
19. *Hu AP, Du JM, Li JY, Liu JW. Oridonin promotes CD4+/CD25+ Treg differentiation, modulates Th1/Th2 balance and induces HO-1 in rat splenic lymphocytes. Inflamm Res 2008; 57: 163-170.*
20. *Busse WW, Lemanske RF Jr. Asthma. N Engl J Med 2001; 344: 350 362.*
21. *Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. Nat Rev Immunol 2003; 3: 405 412.*
22. *Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003; 21: 425 456.*
23. *Lukacs NW. Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol 2001; 1: 108-116.*