Immunobiology 219 (2014) 565–571

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Astragaloside IV attenuates allergic inflammation by regulation Th1/Th2 cytokine and enhancement $CD4^+CD25^+Foxp3$ T cells in ovalbumin-induced asthma

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ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 10 July 2013
Accepted 12 March 2014
Available online 20 March 2014

Keywords:
Astragaloside IV
Asthma
Th1/Th2
Treg

ABSTRACT

Astragaloside IV is the chief ingredient of Radix Astragali, which has been used in the Traditional Chinese Medicine as a major component of many polyherbal formulations for the repair and regeneration of injured organ and tissues. We tested the anti-asthmatic effects of AST IV and the possible mechanisms. BALB/c mice that were sensitized and challenged to ovalbumin (OVA) were treated with AST IV (40 mg/kg and 20 mg/kg) 1 h before they were challenged with OVA. Our study demonstrated that AST IV inhibited OVA-induced increases in eosinophil count; interleukin (IL)-4 level were recovered in bronchoalveolar lavage fluid increased IFN-γ and IL-10 levels in bronchoalveolar lavage fluid. Histological studies demonstrated that AST IV substantially inhibited OVA-induced eosinophilia in lung tissue. Flow cytometry studies demonstrated that AST IV substantially increased $CD4^+CD25^+Foxp3$ T cells (Treg). Furthermore quantitative real-time (qPCR) studies demonstrated that AST IV substantially enhanced Foxp3 mRNA expression in lung tissue. These findings suggest that AST IV may effectively ameliorate the progression of airway inflammation and could be used as a therapy for patients with allergic inflammation.

Introduction

Asthma is associated with a wide range of symptoms that are characterized by airway inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR) (Galli, S.J., et al. 2008). Th2 cytokines (IL-4, IL-5, and IL-13) play critical roles in the pathogenesis of asthma. Poojary and colleagues reported that interleukin (IL)-4 and IL-2 production was suppressed in anergized Th2 cells (Venuprasad, K., et al. 2006). Of the cytokines, IL-4 and -13 stimulate immunoglobulin E (IgE) production and IL-5 enhances eosinophil accumulation, IL-5 expressed by Th2 cells is responsible for eosinophil growth, differentiation, mobilization, recruitment, activation, and survival (Sanderson, C.J. 1992; Simon, D., et al. 2004; Lopez, A.F. 1988). IL-13 plays an important role in T-cell differentiation toward a Th2 phenotype and isotype switching of B cells to immunoglobulin IgE production (Umetsu, D.T., and DeKruyff, R.H. 1997; Wills-Karp, M., et al. 1998). Th1 cells (another T helper cell) suppress Th2 immune responses by secreting interferon IFN-γ. IL-4 induces class switching in IgG1 and IgE, where as IFN-γ is involved in IgG2α class switching. Accordingly, estimation of IgE production and Th1/Th2 cytokine balance is an important tool in the evaluation of asthma (Brewer, J.M., et al. 1999).

There are evidences that $Foxp3^+$ regulatory T cells (Tregs) are much involved in the regulation of Th2-induced allergic responses (Umetsu, D.T., and DeKruyff, R.H. 2006; Venuprasad, K., et al. 2010). Treg cells expressing the forkhead/winged helix transcription factor (Foxp3) have an anti-inflammatory role and maintain tolerance to self-components through direct contact with cells or by releasing anti-inflammatory cytokines such as IL-10 (Sakaguchi, S., et al. 2006). Lee J.H. et al. (2007) found that the levels of $CD4^+CD25^+$ Tregs and mRNA expression of forkhead box protein 3 (Foxp3) were significantly low in pediatric patients with allergic rhinitis and bronchial asthma. Thus, enhancement of $Foxp3^+$ Tregs is an attractive strategy for asthma treatment.

Astragaloside IV (AST IV, Fig. 1) is one type of saponin purified from *Astragalus membranaceus*, and it is regarded as the quality standard for *A. membranaceus* injection in the Pharmacopeia of the People's Republic of China. Previous studies have shown that AST IV has a broad range of pharmacological properties, including promotion of axonal maturation, antiviral and anti-inflammatory activities, and the ability to reduce infarct size, as well as to improve postischaemic heart function (Tohda, C., et al. 2006; Zhang, W.D.,

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http://dx.doi.org/10.1016/j.imbio.2014.03.005
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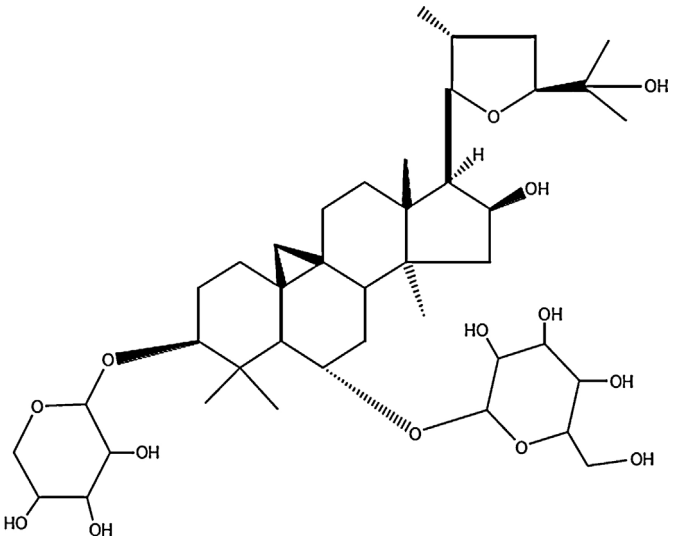

Fig. 1. Chemical structure of astragaloside IV.

et al. 2006a; Zhang, Y., et al. 2006b). In addition, AST IV may enhance immune cell proliferation and antibody production, relax cardiovascular smooth muscle and protect the ischemic/hypoxic myocardium and brain in cardiovascular and cerebrovascular diseases. Because of its multiple biological properties, in this study, we tested the anti-asthmatic effects of Astragaloside IV and the possible mechanisms.

Materials and methods

Reagents and assay kits

Astragaloside IV (pure: 99%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Dexamethasone was purchased from Xiansheng drug Store (Nanjing, China). ovalbumin (OVA) (Sigma Chemical Co., St. Louis, MO), aluminum potassium sulfate (KAl(SO4)2) (Sigma, St. Louis, MO), Fast Wright-Giemsa Stain (Nanjing Jiancheng Bioengineering institute Nanjing, China). ELISA kits ((R&D, Minneapolis, MN, USA). The compounds used in the experiments were dissolved in PBS.

Mouse asthma model and AST IV administration

Animal experiment was carried out in accordance with the Guidelines for Animal Experimentation of Nanjing University of Chinese Medicine (Nanjing, china) and the animal study was approved by the Animal Ethics Committee of the Institution. A total of 50 female BALB/c mice obtained from Experimental Animal Center of China Pharmaceutical University (Nanjing, china). All mice were kept under standard animal conditions with regulated temperature (17–25 °C), humidity (45–60%) and 12 h/12 h light/dark cycle. Free access to food and drinking water was guaranteed throughout the study period. All animals were allowed to acclimatize for 5 days prior to treatment. Mouse model of allergic asthma was developed by ovalbumin sensitization and inhalation as previously described (Tanaka, H., et al. 2001). OVA (500 μg/mL) was mixed with 10% (w/v) aluminum potassium sulfate ($KAl(SO_4)_2$) (1:1 ratio) and adjusted pH to 6.5 and incubated for 60 min at room temperature. After centrifugation at 750 × *g* for 5 min, the $OVA/KAl(SO_4)_2$ pellet was resuspended in the original volume of distilled water. All mice were intraperitoneally sensitized to OVA (0.2 mL alum-precipitated antigen containing 100 μg of OVA) and intratracheally injected with 500 μg of OVA (on days 7 and 14) on the back of the tongue. Then mice were divided into five groups ($n = 10$/group) as follows: control (normal), OVA control group, OVA + AST IV 40 mg/kg group, OVA + ASTIV 20 mg/kg group, and OVA + dexamethasone (Dex) 2 mg/kg group as a positive control. OVA inhalation was continued for next 8 weeks increasing the concentrations from 1% for 6 weeks and to 2% for 2 weeks to keep asthma condition (30 min/day, 3 days/week). AST IV or dexamethasone was orally administered 5 times/week for 6 weeks.

Measurement of airway resistance (Raw)

The forced oscillation technique was used to measure respiratory mechanics. Regular ventilation was interrupted and a computer generated volume signal that consisted of waveforms of mutually primed frequencies was delivered to the airway opening. Piston displacement and cylinder pressure were measured. Impedance values were obtained before and after the delivery of increasing concentrations of methacholine aerosols. Prior to the start of the methacholine concentration response curve, two total lung capacity breaths were delivered. Methacholine (Sigma) dissolved in saline was given via an ultrasonic nebulizer (Hudson RCI, Teleflex Medical) in increasing concentrations (0.125 mg ml^{-1}, 0.25 mg ml^{-1}, 0.5 mg ml^{-1},and 1 mg ml^{-1},). After delivery of each aerosol, forced oscillations were delivered every 15 s, over a 5-min duration. In between impedance measurements, regular ventilation was resumed. The peak response for each variable was determined.

Collection of bronchoalveolar lavage fluid (BALF)

Mice were sacrificed using an overdose of 50 mg/kg of pentobarbital 48 h after the last challenge, and tracheotomy was performed. After instilling ice-cold PBS (0.5 mL) into a lung, BAL fluid was obtained by three successive aspirations (totalvolume1.5 mL) via tracheal cannulation. BAL fluid (BALF) samples were centrifuged at 1500 rpm for 10 min at 4 °C, the supernatants were stored in −80 °C for analysis of cytokine concentrations and the pellet was resuspended in 100 μl of saline, centrifuged onto slides and stained for 8 min with Wright–Giemsa staining. The slides were quantified for differential cell count by counting a total of 200 cells/slide at 40 magnification.

Histological assessment

Animals were euthanized by a 4% isoflurane induction followed by immediate cervical dislocation before collecting lung tissues. Lung tissue slices were fixed in 10% paraformaldehyde. Epoxy-embedded sections (4 μm) of lung tissues were stained with hematoxylin and eosin (H&E), according to the modified protocol previously described (Lee, J.J., et al. 1997).

Enzyme-linked immunosorbent assay detection of BALF cytokines IL-4, IFN-γ and IL-10

BALF levels of IL-4, IFN-γ, IL-10 were measured by ELISA according to the manufacturer's instructions (R&D, Minneapolis, MN, USA). The lower detection limits of these assays were 3.9 pg/ml (IL-4), 7.8 pg/ml (IFN-γ) and 3.9 pg/ml (IL-10). All measurements were performed in duplicate.

Flow cytometric analysis of Treg

The spleen was removed from each sacrificed mouse and placed in a tube containing RPMI1640 media (Gibco). A single cell suspension was prepared by disrupting the spleen using Cell disrupter (Beckman), Cells were equally distributed into tubes and washed