Anti-asthma effects of synthetic salidroside through regulation of Th1/Th2 balance

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[ABSTRACT]
AIM: The aim of the study was to investigate the effect and mechanism of action of synthetic salidroside in an ovalbumin (OVA)-induced asthma model in mice.

METHOD: BALB/c mice were sensitized with an intraperitoneal injection of ovalbumin (OVA) to induce a mouse model of asthma in paracmasis. The mice were treated with dexamethasone as the positive control. At the end of the study, respiratory reactivity was detected, the numbers of various kinds of white blood cells in the bronchoalveolar lavage fluid (BALF) were counted, and the levels of IL-4 and INF-γ in BALF were determined. Quantitative PCR was used to detect the mRNA contents of IL-4 and INF-γ in lung tissue. Histologic examination was performed to observe inflammatory cellular infiltration.

RESULTS: Salidroside treatment virtually eliminated airway hyper-reactivity, markedly reduced the eosinophil percent, obviously reduced the levels of IL-4 and raised INF-γ in the bronchoalveolar lavage fluid (BALF) compared with the sham-treated group. Quantitative PCR on the mRNA content of IL-4 and INF-γ provided confirmation. Lung histologic observations showed that salidroside reduced inflammation and edema. These effects were equivalent to the effects of dexamethasone.

CONCLUSION: Synthetic salidroside exhibits an anti-asthma effect which is related to the regulation of Th1/Th2 balance. This provides a new possibility for treatment of allergic asthma.

[KEY WORDS] Synthetic salidroside; Asthma; Th1/Th2

[Introduction]
Bronchial asthma is an immune allergic disease characterized by reversible airway obstruction, airway hyper-reactivity (AHR) and airway inflammation [1]. The prevalence of allergic diseases and asthma has increased dramatically over the past decades, and has a higher incidence of morbidity and mortality [2]. The syndrome affects both adults and children, resulting in loss of work and school days. This has a significant impact on human health and lifestyle [3]. Studies for many years have indicated that Th cells play an important role in asthma. Th cells fall into two major categories, named Th1 and Th2, and the Th1/Th2 imbalance is the basis of asthma [4]. Th1 cells mainly produce IL-1 (interleukins-1), IL-2, IL-12, IL-15, IL-18, interferon-gamma (INF-γ), and tumor necrosis factor-alpha (TNF-α), while Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 [5]. Normally, Th1 cells tend to predominate, whereas in asthma patients, Th1/Th2 is out of balance, and Th2 cells tend to dominate. INF-γ is a regulator of allergic inflammation which can decrease Th2 cell-induced immune responses in the airways. When INF-γ expression decreases, the expression of IL-4, IL-5, and IL-13 increases [6-7]. These cytokines produced by Th2 cells can increase IgE production, promote the growth and differentiation of acidophilic granulocytes, and promote mucus secretion, thus inducing higher airway reactivity.

Salidroside, as one of the most effective activated constituents of Rhodiola rosea L. (Crassulaceae), has a range of pharmacological properties, such as anti-oxidation, anti-aging, anti-cancer, and immunological enhancement. Studies show that salidroside significantly reduces cardiac ischemia/reperfusion injury through regulating the activities of CK and...
Materials and Methods

Animals
A total of fifty BALB/c mice (female), 6–8 weeks of age were routinely bred in a specific pathogen-free (SPF) laboratory in the Animal Center of China Pharmaceutical University, with controlled temperature at (23 ± 2) °C and relative humidity of 50% with 12 h light/dark cycle. All experimental protocols were approved by the Animal Studies Committee of China Pharmaceutical University.

Main reagents and kits
Synthetic salidroside (Sal, provided by The Second Military Medical University, Shanghai, China; purity > 99%); dexamethasone (Dex, purchased from Xiansheng Drug Store, Nanjing, China); acetylcholine (Ach) and ovalbumin (OVA) and aluminum hydroxide (Sigma, St. Louis, MO, USA); TRIzol (Gibco BCR Inc., Shanghai, China) using a glass homogenizer and the total RNA was obtained and separated into two parts, which were respectively stored at −80 °C used for quantitative real-time PCR and 10% (V/V) neutral buffered formalin used for histological observation.

Determination of airway responsiveness (AHR)

As described previously [13-14], after the last challenge, mice were anesthetized and tracheotomies were performed. Then airway responsiveness was assessed in an unrestrained conscious state. Airway resistance (RI) in response to aerosol inhalation of increasing concentrations of methacholine (0.125, 0.5, and 1 mg·mL−1) in phosphate-buffered saline (PBS) were recorded using a whole-body plethysmograph chamber (Buxco, Sharon, CT, USA), for 3 min at each exposure level. The highest Penh value obtained during each methacholine challenge was expressed as a proportion of the basal Penh value recorded in response to the PBS aerosol.

Collection of bronchoalveolar lavage fluid (BALF)

After the assessment of AHR, mice were sacrificed with an overdose of 50 mg·kg−1 of pentobarbital. Cold PBS (0.5 mL) was injected into a lung through the trachea. BAL fluid was collected by three successive aspirations (total volume 1.5 mL) [13]. Then BALF was centrifuged and supernatant was collected and stored at −80 °C. The sedimentation was mixed with equal PBS and then placed on a slide for counting of the various kinds of cells.

Cell counting

The sediment of BALF was diluted with saline to smear and dye. The numbers of eosinophilic granulocytes, lymphocytes, macrophages, and neutrophile granulocytes in the sedimentation of BALF were counted with a hemacytometer after staining. Their numbers were counted in 200 white blood cells and their total numbers calculated in 1mL of solution according to the formula (n/200) × 107.

Measurement of cytokines

The concentrations of cytokines IL-4 and IFN-γ in the supernatants of the BALF were measured by enzyme immunoassays performed using commercially available reagents according to the instructions of the manufacturer.

RNA extraction and quantitative real-time PCR

The lungs not subjected to BALF and stored at −80 °C were used to study the effect of salidroside on cytokine gene expression. Quantitative real-time PCR was performed after quantitative normalization for each gene. The lungs were homogenized in TRIzol (1 mL) (Gibco BCR Inc., Shanghai, China) using a glass homogenizer and the total RNA was extracted from the lung by the phenol-chloroform based method according to the manufacturer’s protocol. The primers for PCR were used as follows:

Gene expression was analyzed with SYBR Green PCR Mastermix (ABI), and the amount of SYBR Green was measured at the end of each cycle. The cycle time values of the genes were normalized with β-actin as the internal stan-
standard in advance. The relative differences among groups were calculated and expressed as a relative quantitative, setting the control group at 100%.

**Histological examination**

Histopathologic evaluations were performed on lungs fixed in 10% (V/V) neutral buffered formalin. After dehydration, lung tissues were sectioned, embedded in paraffin, sectioned at 4 μm thickness, and then stained with H&E solution for general morphology.

**Statistical analysis**

All data were presented as T±s. One-way ANOVA was used to compare among multiple groups. P < 0.05 was considered to be statistically significant.

**Results**

**Inhibitory effects of salidroside on airway hyperresponsiveness (AHR)**

AHR was reflected by calculating airway resistance (RI). As shown in Fig. 1, the RI level of the OVA-induced group was significantly higher than that of the PBS control group (P < 0.01) when methacholine concentrations were 0.25, 0.5, and 1 mg·mL\(^{-1}\). Mice in the Dex-treated (2 mg·kg\(^{-1}\)) group and salidroside (24 and 48 mg·kg\(^{-1}\)) groups showed an obvious decrease on RI compared with sham-treated group (P < 0.01).

**Effects of salidroside on cytokine levels in BALF**

Cytokine levels in the BALF were measured by enzyme immune-assays according to the manufacturer instructions. As shown in Fig. 3, the concentration of IL-4 produced by Th2 increased, and the IFN-γ originating from Th1 decreased in model samples compared to control mice (P < 0.01). Treatment with Dex (2 mg·kg\(^{-1}\)) or salidroside (24 and 48 mg·kg\(^{-1}\)) caused a reduction in the level of IL-4, and an increase in IFN-γ (P < 0.01).

**Effects of salidroside on mRNA in lungs**

As shown in Fig. 4, OVA aerosol challenges markedly up-regulated the lung mRNA level of IL-4, and down-regulated the mRNA level of IFN-γ (P < 0.01). Treatment with Dex (2 mg·kg\(^{-1}\)) or salidroside (24 and 48 mg·kg\(^{-1}\)) obviously lowered the expression of IL-4 mRNA, and increased the expression of IFN-γ mRNA (P < 0.01).

**Histological observation**

As compared with the control mice (Fig. 5), histological analyses of OVA-induced mice revealed typical pathologic...
features of asthma. The histological observation of OVA-induced mice showed large numbers of inflammatory cells, including eosinophils and goblet cells infiltrated around the bronchioles and vessels. There were also hemal wall thickening, vascular wall necrosis, and removal observed. Mice treated with Dex (2 mg·kg⁻¹) and salidroside (24 and 48 mg·kg⁻¹) showed obvious alleviation of the pathological changes.

**Fig. 5** Pathological changes of lung tissues observed by HE staining (light microscopy, × 200): A (control); B (model); C (dexamethasone, 2 mg·kg⁻¹); D [salidroside (24 mg·kg⁻¹)]; E [salidroside (48 mg·kg⁻¹)]

**Discussion**

Asthma, a chronic inflammatory disease of the airways, affects many individuals worldwide. The disease may cause severe morbidity, and even mortality when exacerbated. To date, a variety of drugs have been developed to treat asthma; these include steroids, leukotriene inhibitors, mast cell stabilizers, and β₂ adrenergic agonists. Many natural products were commonly used to treat asthma, but the therapeutic efficacies and modes of action of such medicines are currently unclear. Here, using an OVA-induced mouse model of asthma, it is shown for the first time that salidroside can be effective as a therapeutic drug for the treatment of allergic asthma in a mouse model.

Asthma is defined as a variable level of airway obstruction, usually accompanied by AHR. Bronchoconstriction attributable to contraction or hypertrophy of airway smooth muscle (ASM), and inflammation within the airway, leads to decreased lung function. AHR is a measure of the bronchial constriction commonly observed in individuals with asthma. The effects of salidroside on AHR were established by measuring Raw. These experiments disclosed that salidroside inhibited the OVA-induced AHR in response to inhaled methacholine.

The mechanisms of recruitment of inflammatory cells associated with, and presumably causing, AHR have been well-studied. Migration of inflammatory cells, specifically eosinophils and lymphocytes, into the lung is a major contributor to the development of allergic airway inflammation. A rise in the number of eosinophils in the BAL fluid is a characteristic of asthma. The present results clearly demonstrate that salidroside significantly reduces eosinophil numbers in the BAL fluid and lung tissue.

Previous studies documented that the underlying process that drives and maintains the asthmatic inflammatory response appears to be an imbalance of the equilibrium between the T helper cell type 1 (Th1) and T helper cell type 2 (Th2) immune response, with a bias towards an increased Th2 response. In this study, the data showed that salidroside significantly suppressed the Th2 cytokine level; and significantly increased the Th1 cytokine level. These findings support the possible consideration of salidroside as a therapeutic drug for patients with allergic asthma.

**References**


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