International Immunopharmacology 17 (2013) 561–567

Contents lists available at ScienceDirect

International Immunopharmacology

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

Geniposide inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma

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ARTICLE INFO

Article history:
Received 24 November 2012
Received in revised form 21 June 2013
Accepted 24 June 2013
Available online 13 July 2013

Keywords:
Geniposide (Gen)
Ovalbumin (Ova)
Asthma
Hyperresponsiveness
Allergic airway inflammation
Nuclear factor-kappaB (NF-κB)

ABSTRACT

Our group recently reported the strong anti-inflammatory effects of geniposide (Gen), a bioactive iridoid glucoside derived from *gardenia jasminoides*, in a mouse acute lung injury model. Herein, we hypothesized that Gen might also have potential therapeutic benefits in treatment of asthma, which was tested in a mouse model of ovalbumin (Ova)-induced allergic airway inflammation. Ova-sensitized and -challenged BALB/c mice, as compared with control animals, displayed airway hyperresponsiveness (AHR), bronchoalveolar lavage eosinophilia, mucus hypersecretion, and increased T help 2 (Th2)-associated cytokine and chemokine amounts, as well as serum Ova-specific immunoglobulin E (IgE) level. Being compared with the Ova-induced hallmarks of asthma, intraperitoneal Gen treatment prevented eosinophilic pulmonary infiltration, attenuated the increases in interleukin (IL)-4, IL-5, and IL-13, and reduced eotaxin and vascular cell adhesion molecule 1 (VCAM-1) expression. Also, Gen significantly ameliorated the Ova-driven airway hyperresponsiveness, mucus hypersecretion, and allergen-specific IgE level, which are the cardinal pathophysiological symptoms in allergic airway diseases. In addition, the efficacy of Gen was comparable to that of dexamethasone (Dex), a currently available anti-asthmatic drug. Collectively, our findings reveal that the development of immunoregulatory strategies based on Gen may be considered as an effective adjuvant therapy for allergic asthma.

1. Introduction

Cumulative evidence revealed that the worldwide prevalence and severity of allergic asthma have increased substantially over the last decades, especially in industrialized countries [1–3]. Allergic asthma is caused by a T helper 2 (Th2)-type cell-mediated immune response to common environmental allergens and is defined as a chronic inflammatory disease characterized by aggravation of airway inflammation, mucus hypersecretion, airway eosinophilia, elevated IgE levels, and airway hyperresponsiveness (AHR) [4–6]. Although multifactorial in origin, asthma is considered an inflammatory process feature of a relative excess of Th2 cytokines during each immune response to innocuous antigens [7,5]. These Th2 responses are associated with mast cells, B lymphocytes, and eosinophils, as well as an array of inflammatory cytokines and chemokines, especially interleukin (IL)-4, IL-5, and IL-13 [8]. Furthermore, chemokine such as eotaxin is vital for the delivery of eosinophils to the airways. Accordingly, airway eosinophilia, together with Th2-type cytokines IL-4, IL-5, and IL-13, may ultimately contribute to AHR in asthma [9]. Emerging evidence has revealed that these Th2-type cytokines in turn cause AHR, vascular leakage, inflammatory cell infiltration, mucus hypersecretion, and airway remodeling including goblet cell hyperplasia and airway wall thickening, starting a cycle of events resulting in lung tissue damage which contribute to the development and aggravation of asthma [10,11]. Intriguingly, existing studies support the notion that a possible approach to treatment of asthma has evolved from anti-inflammatory strategies [12,8], although there is limited evidence pointing to therapies for asthma through controlling the process of airway inflammatory reaction.

Despite a growing understanding of the pathophysiology of asthma, its molecular regulatory mechanisms in induction of cytokines expression and activation/recruitment of inflammatory cells in asthma remain elusive [6]. However, there is a need for innovative anti-inflammatory therapeutic protocols and some previous studies

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1567-5769/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.intimp.2013.06.028

have shown that asthma is associated with persistent activation of nuclear factor κB (NF-κB) [13–15]. Also, inspired by our previous studies [12,16], remedies with anti-inflammatory properties based on modification of NF-κB signaling pathway may therefore represent a potentially useful alternative. NF-κB, a pleiotropic transcription factor, is abundant of p50 (NF-κB1)/p65 (RelA) heterodimer and plays a critical role in immune and inflammatory responses. It has been known to be present in most cell types and many of the inflammatory proteins expressed in asthmatic airway are regulated, at least partially, by NF-κB [17]. In addition, a previous investigation has reported that eosinophil migration is involved in the inflammatory process of asthma by suppressing the NF-κB pathway [18]. Therefore, there is growing recognition that NF-κB has emerged as a promising molecular target for the treatment of asthma. Likewise, growing evidence suggests that numerous components of Chinese medicinal herbs exert excellent anti-inflammatory effects through the negative regulation of NF-κB signaling pathway [19–21].

Gardenia jasminoides Ellis, which is also called Zhi-Zi in the Chinese pharmacopoeias, has been included in traditional medicine formulations for its anti-inflammatory and antioxidant properties [22,23]. Geniposide (Gen) is the major iridoid glycoside constituent of gardenia herbs and is mainly responsible for its pharmacological activities, such as the inhibition of ovalbumin-induced junction permeability and the recovery of transepithelial electrical resistance in guinea pig tracheas, which highlight the potential of Gen as an anti-asthmatic drug [24]. Our recent report has demonstrated that Gen can strongly protect mice from lipopolysaccharide (LPS)-induced acute lung injury at a dose of 80 mg/kg [19]. Also, Gen can block high glucose-induced cell adhesion through the nuclear factor-kappaB (NF-κB) signaling pathway in human umbilical vein endothelial cells [25]. There is no available study that evaluates the effectiveness of Gen for reducing airway inflammatory reactions and improving asthma symptoms.

Considering that Gen is an ingredient of many traditional Chinese preparations and has also been widely utilized for food colorants in oriental countries for many years [26]. The potential therapeutic value and possible food intake of Gen may exert its functional benefits in asthma. Herein, we performed this study to examine the effect of Gen on an Ova-induced airway inflammation model in mice and its underlying mechanism, attempting to provide a new potential treatment for allergic asthma from traditional Chinese herbal medicine.

2. Materials and methods

2.1. Animals and materials

Female BALB/c mice, weighing approximately 18 to 20 g, were purchased from Shanghai Jingke Industrial Co., Ltd. (Certificate: SCXK2003-0003; Shanghai, China). Mice were housed in sterile microisolator cages with filtered air and autoclaved bedding, food, and water. Mice were allowed to acclimatize for 1 wk before beginning the experiments. The laboratory temperature was maintained at 24 ± 1 °C, and relative humidity was maintained at 40–80%. The experiments were approved by the Ethical Committee on Animal Research of Jilin University. All animal experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health. No mice were dead and no apparent signs of exhaustion were observed during the experimental period.

Geniposide (Gen, purity: >98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ova (Grade δ), Aluminium hydroxide adjuvant and Dexamethasone (Dex, purity: >99.6%) were provided by Sigma-Aldrich Trading Co., Ltd. (St. Louis, MO, USA). Mouse interleukin (IL)-4, IL-5, IL-13, eotaxin, and vascular cell adhesion molecule 1 (VCAM-1) ELISA kits were obtained from Biolegend (San Diego, CA, USA). The ELISA kit for immunoglobulin (Ig) E was purchased from R&D (Anniston, AL, USA). The primary antibodies including phosphorylated and non-phosphorylated forms of IκB and nuclear factor-kappaB (NF-κB) and the horseradish peroxidase-labeled IgG secondary antibodies were purchased from Cell Signaling Technology Inc (Beverly, MA, USA). β-actin was provided by Tianjin Sungene Biotech Co., Ltd (Tianjin, China). Other chemical reagents were obtained from Beijing Dingguo Changsheng Biotech Co., Ltd (Beijing, China). All these reagents were of analytical grade, unless otherwise specified.

2.2. Experimental design

2.2.1. Allergen sensitization/challenge protocol

Mice were randomly assigned to four groups (n = 8): (1) Cont group; (2) Ova group; (3) OvaGen group; (4) OvaDex. Hereafter, we used these group abbreviations to clarify the text. Mice were immunized on days 1, 7, and 14 by intraperitoneal (*i.p.*) injection of 20 μg Ova emulsified in 1 mg aluminium hydroxide adjuvant in a total volume of 0.2 mL [27]. On days 23, 24, 25, and 26 after the initial sensitization, mice were prepared for challenge by anesthetizing with an *i.p.* injection of 0.2 mL of a mixture of ketamine (0.44 mg/mL) and xylazine (6.3 mg/mL) in normal saline. Mice were placed on a board in the supine position. Subsequently, mice were intranasally challenged with 2% (w/v) Ova solution in phosphate-buffered saline (PBS, pH = 7.2), as described previously with minor modifications [28]. Control mice received equivalent volumes of PBS without Ova, *i.p.*, on days 1 and 14 and were challenged with PBS without Ova (w/v) each day from days 23 to 26 on these consecutive days. Airways hyperresponsiveness (AHR) was measured 24 h after the final Ova challenge on day 27 and then mice were sacrificed the following day to characterize the protective effects of Gen. The schematic diagram of the treatment schedule is presented in Fig. 1.

2.2.2. Administration of drugs

The dose of Gen was based on the results of our previous study [19].We administered Gen to mice at doses of 20, 40, and 80 mg/kg to evaluate the anti-inflammatory properties of Gen using a mouse model of acute lung injury (ALI). Our findings suggest that Gen at the dose of 80 mg/kg exerts strong protective effects against LPS-induced ALI. Thus, in our present study, mice were given an intraperitoneal injection of normal saline or Gen (80 mg/kg) on days 23, 24, 25 and 26, 1 h prior to each corresponding Ova administration [12]. Dex (2 mg/kg), a steroid hormone drug of the glucocorticoid class, was a potent inhibitor of airway inflammation and remodelling [29,16]. Thus, in the present study, Dex was used as a positive control.

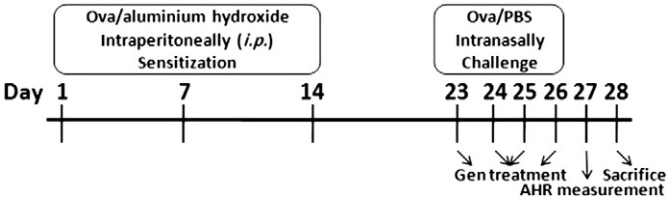

Fig. 1. Experimental protocol for development of allergic asthma and treatment with Gen or Dex using a murine model. Mice were divided into four groups (n = 8) and sensitized via an intraperitoneal injection of 20 μg Ova emulsified in 1 mg aluminum hydroxide in 200 μL PBS on days 0, 7, and 14, respectively. Subsequently, mice were given an intranasal instillation with 2% (w/v) Ova solution in PBS on days 23, 24, 25, and 26 after the initial sensitization. Mice were given an intraperitoneal injection of Gen (80 mg/kg, dissolved in normal saline) or Dex (2 mg/kg, diluted in normal saline) each day from days 23 to 26 consecutively, 1 h prior to each corresponding Ova challenge. Control mice were sensitized and challenged with equivalent volumes of PBS without drug administration. AHR assay was performed 24 h after the last Ova challenge. Then all mice were sacrificed for further experiments.