International Immunopharmacology 11 (2011) 698–705

Contents lists available at ScienceDirect

International Immunopharmacology

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

Antioxidant and antiasthmatic effects of saucerneol D in a mouse model of airway inflammation

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ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 30 December 2010
Accepted 14 January 2011
Available online 2 February 2011

Keywords:
Saucerneol D
Asthma
Eosinophilia
Cytokine
Heme oxygenase-1

ABSTRACT

Chronic airway inflammation is a hallmark of asthma, which is an immune-based disease. We evaluated the ability of saucerneol D, a tetrahydrofuran-type sesquilignan isolated from *Saururus chinensis*, to regulate airway inflammation in an ovalbumin (OVA)-induced airway inflammation model. Furthermore, we determined whether heme oxygenase (HO)-1 was required for the protective activity of saucerneol D. The airways of OVA-sensitized mice exposed to an OVA challenge developed eosinophilia and mucus hypersecretion and exhibited increased cytokine levels. Mice were administered saucerneol D orally at doses of 20 and 40 mg/kg once daily on days 26–30. Saucerneol D administered orally significantly inhibited the number of OVA-induced inflammatory cells and the production of immunoglobulin E as well as Th2-type cytokines. Histopathology studies revealed a marked decrease in lung inflammation and goblet cell hyperplasia after saucerneol D treatment. In addition, saucerneol D induced HO-1 and led to a marked decrease in OVA-induced reactive oxygen species and malondialdehyde and an increase in superoxide dismutase and glutathione in lung tissues. These antioxidant effects were correlated with HO-1 induction. In our experiments, saucerneol D treatment reduced airway inflammation and suppressed oxidative stress in an OVA-induced asthma model.

1. Introduction

Chronic inflammation is recognized as a central component of the pathophysiology of asthma. Invading inflammatory cells in lung tissue release a wide variety of mediators and cytokines that contribute to the clinical characteristic of asthma [1]. Bronchial asthma is a chronic inflammatory disease of the airways characterized by airway eosinophilia and goblet cell hyperplasia with mucus hypersecretion to inhaled allergens and nonspecific stimuli [2,3]. The inflammatory process in asthma is dominated by T helper-2 (Th2) cells, which produce interleukin (IL)-4, IL-5, and IL-13 [4]. In particular, eotaxin, IL-4, IL-5, and IL-13, which are produced by Th2 cells, are all related to inflammatory changes in the airway via the activation of eosinophils and the production of immunoglobulin E (IgE) by B cells [5–7]. Recently, heme oxygenase (HO)-1 was shown to be induced in the airways of patients with asthma and chronic obstructive pulmonary disease (COPD) [8,9]. Moreover, overexpression of HO-1 decreases airway inflammation and mucus secretion in rodents [10], suggesting that HO-1 plays a critical role in protecting the host during airway inflammation. The induction of HO-1 expression by various stress stimuli, such as lipopolysaccharides and oxidants, is thought to be an adaptive mechanism that protects cells from oxidative injury [11]. Oxidative stress plays an important role in the pathogenesis of most airway diseases, particularly when inflammation is prominent [12]. There is increasing evidence that inflammation, which is characteristic of asthma, results in increased oxidative stress in the airways [13]. Reactive oxygen species (ROS) can be generated either endogenously by metabolic reactions, such as from mitochondrial electron transport during respiration or during activation of circulating inflammatory cells or phagocytes, or exogenously from air pollutants or cigarette smoke. As a result, increased levels of ROS have been shown to affect the extracellular environment, impacting on a variety of physiological processes [14,15]. Eosinophils, alveolar macrophages, and neutrophils from asthmatic patients produce more ROS than do those from normal subjects [16,17]. The overproduction of ROS or depression of protective mechanisms also results in bronchial hyperreactivity, which is characteristic of asthma [18,19]. It is proposed that ROS produced by phagocytes that have been

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doi:10.1016/j.intimp.2011.01.015

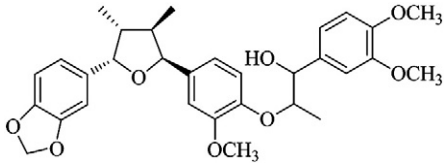

Fig. 1. Chemical structure of saucerneol D.

recruited to sites of inflammation is a major cause of the cell and tissue damage associated with many chronic inflammatory lung diseases, including asthma and COPD [20–24]. Although it is considered as one of the most important protective mechanisms against superoxide-anion-mediated injury, superoxide dismutase (SOD) activity produces an oxidant—hydrogen peroxide (H_2O_2). Alterations in the level of these enzymes have been detected in asthmatic patients [25–27]. Malondialdehyde (MDA), a major product of lipid peroxidation (LP), is generally used as the indicator of oxidative stress [21]. Cell membrane homeostasis is destroyed by the increased production of LP. Damaged and dysfunctional membranes cause loss of calcium and of other transport systems, such as a reduction in intercellular gap junction communication [28]. Proteins such as SOD and glutathione (GSH) are major enzymes in the antioxidative defense system.

Saururus chinensis (Saururaceae) is used widely as a traditional medicine for the treatment of edema, jaundice, gonorrhea, pneumonia, and several inflammatory diseases [29]. Previous chemical studies of *S. chinensis* reported the presence of a large number of lignans [30], flavonoids, anthraquinones, and furanoditerpenes [31,32]. Herbal medicine is one of the main lines of complementary and alternative therapy of bronchial asthma, as it is the third most popular choice of adults and children suffering from bronchial asthma [33]. Previous investigations of saucerneol D revealed its anti-inflammatory activity in HeLa cells transfected with an NF-κB reporter [34]. However, there are no *in vivo* studies of the antiasthmatic properties of saucerneol D. Despite the wide variety of steroid and nonsteroid medications employed, all anti-inflammatory or antiasthma drugs available currently cause undesired, and possibly serious, side effects. Thus, development of new and more powerful drugs is required. Therefore, in this study, we evaluated a bronchial asthma model to examine the ability of saucerneol D to control Th2-type cytokines, IgE, oxidative stress, eosinophil infiltration, and other factors that play important roles in allergic inflammation. We hypothesized that saucerneol D would have an antiasthmatic effect on airway inflammation in a murine model of allergic asthma.

2. Materials and methods

2.1. Extraction and isolation of saucerneol D

Fresh *S. chinensis* (Saururaceae) was washed three times with tap water to remove salts, epiphytes, and sand and was stored at −20 °C. Frozen samples were lyophilized and homogenized in a grinder before extraction. Saucerneol D was isolated from the ethyl acetate extract of the roots of *S. chinensis*. The chemical structure of the isolated compound was established as saucerneol D (Fig. 1). The compound was obtained as an amorphous powder: mp, 71–72 °C; [α]D25, −75.7° (*c* 0.65, $CHCl_3$); ^{1}H- and ^{13}C-NMR data were consistent with values from the literature [34]; FABMS *m/z*, 536 [M]+. The purity of this compound was above 99.5%, based on HPLC analysis.

2.2. Animals

Specific 7-week-old, pathogen-free, inbred female BALB/c mice screened routinely serologically for relevant respiratory pathogens were purchased from Daehan Biolink Co. Ltd. (Seoul, Korea). Mice were maintained in an animal facility under standard laboratory conditions for 1 week prior to the performance of experiments, and were provided water and standard chow *ad libitum*. All experimental procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and animal handling followed the dictates of the National Animal Welfare Law of Korea.

2.3. Sensitization and airway challenge

The mice were divided into four groups and airway inflammation was induced by ovalbumin (OVA) (grade III; Sigma–Aldrich, USA) in three groups using the method described by Oh and colleagues [35]. Briefly, mice were immunized via intraperitoneal injection of 20 μg chicken OVA and 2 mg aluminum hydroxide in 200 μL PBS buffer (pH 7.4) on days 0 and 14. Mice were exposed to a 1% (w/v in PBS) OVA solution for 20 min using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) on days 28, 29, and 30 after the initial sensitization. Saucerneol D (20 or 40 mg/kg) was administered orally once daily on days 28–30. Negative and positive control mice were treated orally with PBS and dexamethasone (Dex; 3 mg/kg), respectively, once daily on days 28–30. Animals were sacrificed 48 h after the last challenge (i.e., on day 32) to characterize the suppressive effects of saucerneol D. A schematic diagram of the treatment schedule is shown in Fig. 2.

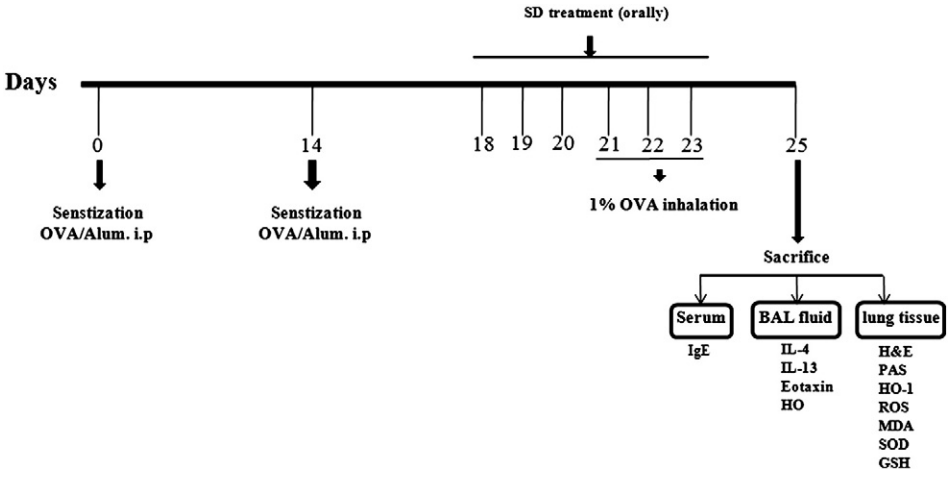

Fig. 2. Mouse model of airway inflammation and effects of treatment with saucerneol D.