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ORIGINAL ARTICLE

Sinomenine ameliorates the airway remodelling, apoptosis of airway epithelial cells, and Th2 immune response in a murine model of chronic asthma

S. Işık[a,*], M. Karaman[b], S.Ç. Micili[c], Ş. Çağlayan-Sözmen[a], H.A. Bağrıyanık[c], Z. Arıkan-Ayyıldız[a], N. Uzuner[a], Ö. Karaman[a]

[a] *Dokuz Eylul University, Department of Pediatric Allergy and Immunology, Izmir, Turkey*
[b] *Dokuz Eylul University, Department of Microbiology, Izmir, Turkey*
[c] *Dokuz Eylul University, Department of Histology, Izmir, Turkey*

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Abstract
Background: Sinomenine (SIN), an alkaloid isolated from the root of *Sinomenium acutum* which has a variety of pharmacological effects, including anti-inflammation, immunosuppression and anti-angiogenesis. The present study aimed to evaluate the effects of SIN on airway remodelling, epithelial apoptosis, and T Helper (Th)-2 derived cytokine levels in a murine model of chronic asthma.
Methods: Twenty-two BALB/c mice were divided into four groups; I (control), II (placebo), III, IV. Mice in groups III and IV received the SIN (100 mg/kg), and dexamethasone (1 mg/kg) respectively. Epithelium thickness, sub-epithelial smooth muscle thickness, number of mast and goblet cells of samples isolated from the lung were measured. Immunohistochemical scorings of the lung tissue for matrix metalloproteinase-9 (MMP-9), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), terminal deoxynucleotidyl transferase-mediated dUTP nick endlabeling (TUNEL) and cysteine-dependent aspartate-specific proteases (caspase)-3 were determined. IL-4, IL-5, IL-13, Nitric oxide in bronchoalveolar lavage fluid (BALF) and ovalbumin-specific immunoglobulin (Ig) E in serum were quantified by standard ELISA protocols.
Results: The dose of 100 mg/kg SIN treatment provided beneficial effects on all of the histopathological findings of airway remodelling compared to placebo ($p < 0.05$). All cytokine levels in BALF and serum and immunohistochemical scores were significantly lower in 100 mg/kg SIN treated group compared to the placebo ($p < 0.05$).
Conclusions: These findings suggested that the dose of 100 mg/kg SIN improved all histopathological changes of airway remodelling and its beneficial effects might be related to modulating Th-2 derived cytokines and the inhibition of apoptosis of airway epithelial cells.

* Corresponding author.
E-mail address: drsakinekar83@hotmail.com (S. Işık).

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Introduction

Bronchial asthma is associated with chronic airway inflammation and progressive airway remodelling.[1] The hallmarks of the structural changes in the airways include eosinophilic airway inflammation, airway smooth muscle and goblet cell hyperplasia, collagen deposition, angiogenesis and apoptosis.[2] Current anti-inflammatory treatment of asthma is predominately based on the use of inhaled corticosteroids. Although these drugs are highly effective in preventing life-threatening consequences of asthma,[3] their effect is limited in modulating airway remodelling and they have several systemic and local side effects when used at high doses for a long time.[4] Research for the use of alternative and complementary treatments that reverse airway remodelling and have fewer side effects is continuously increasing.

Sinomenine (SIN) is an alkaloid that is isolated from the root and stem of the climbing plant *Sinomenium acutum* and demonstrates anti-inflammatory, immunosuppressive, anti-arrhythmic, analgesic and anti-rheumatic effects.[5–9] It has been successfully used for centuries in the treatment of rheumatism.[10] Only few studies in the literature have investigated the mechanism of action and efficacy of SIN on allergic inflammation.[11–13]

In our study, we firstly investigated the effects of SIN on airway remodelling, airway epithelial cell apoptosis and TH-2 immune responses in a murine model of chronic asthma in comparison with the conventional dexamethasone treatment.

Materials and methods

Animals, experimental protocol and study drugs

A total of twenty-two, conventionally raised, 6–8-week-old male BALB/c mice weighing 18–20 g were used in the study. The animals were fed a commercial diet ad libitum and housed in hygienic macrolane cages in air-conditioned rooms on a 12-h light/dark cycle. All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of the Dokuz Eylul University.

Mice were divided into four groups: (I) Control ($n = 5$), (II) Asthma-untreated (placebo) ($n = 5$), (III) Asthma-100 mg/kg SIN-treated ($n = 6$), (IV) Asthma-1 mg/kg dexamethasone treated ($n = 6$). Mice in study groups except for the control group were sensitised on days 0 and 14 by an intraperitoneal (i.p) injection of 10 μg/0.1 ml chicken egg albumin (ovalbumin, grade V, ≥98% pure, Sigma, St. Lois, MO, USA) with alum as an adjuvant as described by Temelkovski et al.[14] Mice in groups II, III and IV were challenged with an aerosol of 5 ml, 2.5% ovalbumin in saline for 30 min/day for three days of the week for eight weeks beginning from the 21st day of the study (Fig. 1). The mice in the control group received normal saline with alum intraperitoneally on days 0 and 14 of the experiment and aerosolised saline without alum for 30 min per day on three days of the week for eight weeks beginning from the 21st day of the study. Exposures were carried out in a whole-body inhalation exposure system in a plexiglass chamber with 40 × 60 × 120 diameters designed for placement of cages. Temperature and relative humidity were maintained at 20–25 °C and 40–60%, respectively. A solution of 2.5% ovalbumin in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser with a flow rate of 6 L/min (Medi-cair, UK) and injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4 μm. Particle concentration was maintained in the range of 10–20 mg/mm^3 in the chamber.[15]

During the last five days of the challenge period, group II received saline, group III received SIN (Sigma Aldrich, St. Louis, MO, USA) at dose of 100 mg/kg, group IV received dexamethasone (Dekort; Deva Holding AS, Istanbul, Turkey) at dose of 1 mg/kg by orogastric tube, once a day. Animals were sacrificed by an overdose of ketamine hydrochloride (200 mg/kg) 24 h after the last drug administration.

Histopathological analysis

Two investigators who were blinded to the treatment groups interpreted the histopathology. Tissue specimens were obtained from the mid-zone of the left lung of mice. Samples were fixed in buffered 10% formalin and embedded in paraffin wax. Five-micron-thick serial sections were obtained and the first 10 samples were stained with haematoxylin and eosin (H&E). General tissue features of these samples were examined and the thicknesses of epithelium and sub-epithelial smooth muscle layers of the medium and small airways were measured. In order to evaluate the thicknesses of epithelium and sub-epithelial smooth muscle layers, measurements were performed from four points of each airway. Considering that each section contained approximately two to three airways, around 20 or more airways were evaluated for each mouse. Photomicrographs were taken by Olympus DP71 camera (Japan), which adapted on Olympus DP70 model microscope (Olympus Optical, Tokyo, Japan). Measurements were carried out with UTHSCSA Image Tool for Windows Version 3.00 software.

The consecutive 10 sections were stained with toluidine blue and the other 10 sections were stained with periodic acid-Schiff (PAS). Photomicrographs were randomly taken from five fields of each section which were stained with toluidine blue. For mast cell enumeration, a standard transparent counting frame representing an area of 16,400 μm^2 was manually used and eight fields in each photograph were examined for each mouse. Goblet cells stained with PAS were enumerated in 10 sections of each mouse. In each section, three to five randomly selected airways were photographed. Circumferences of all airways were measured and goblet cell numbers in these areas were recorded. For standardisation, goblet cell numbers in 100 μm were analysed by dividing the total goblet cell number to the total length of airway circumferences and multiplying the result by one hundred.

Measurement of cytokines in lung homogenates

Lungs were removed and washed with cold PBS three times in order to remove the blood. Lung tissues were taken into 2 ml microcentrifuge tubes and stored at −80 °C until analysis. On the study day, frozen lung tissues were thawed,

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