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Intranasal curcumin attenuates airway remodeling in murine model of chronic asthma

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ABSTRACT

Curcumin, phytochemical present in turmeric, rhizome of *Curcuma longa*, a known anti-inflammatory molecule with variety of pharmacological activities is found effective in murine model of chronic asthma characterized by structural alterations and airway remodeling. Here, we have investigated the effects of intranasal curcumin in chronic asthma where animals were exposed to allergen for longer time. In the present study Balb/c mice were sensitized by an intraperitoneal injection of ovalbumin (OVA) and subsequently challenged with 2% OVA in aerosol twice a week for five consecutive weeks. Intranasal curcumin (5 mg/kg) was administered from days 21 to 55, an hour before every nebulization and inflammatory cells recruitment, levels of IgE, EPO, IL-4 and IL-5 were found suppressed in bronchoalveolar lavage fluid (BALF). Intranasal curcumin administration prevented accumulation of inflammatory cells to the airways, structural alterations and remodeling associated with chronic asthma like peribronchial and airway smooth muscle thickening, sloughing off of the epithelial lining and mucus secretion in ovalbumin induced murine model of chronic asthma.

1. Introduction

Asthma has been generally recognized as a disease characterized by variable degree of chronic inflammations and structural alterations of the airways [1,2]. Airway remodeling as a potential important multicellular process represents a complex consequence that lead to structural changes involving the composition, content and organization of many of the cellular and molecular constituent of the bronchial wall. It collectively refers to structural changes of the airway wall, which may include epithelial fragility, goblet cell metaplasia, increased airway smooth muscle mass and basement membrane thickening due to extracellular matrix deposition. Apart from airway inflammation, airway remodeling has been emerging as a new therapeutic target in the management of asthma. It is a common and complex inflammatory disease of the airways that remains incurable by current available therapies which are associated with severe side-effects [3]. Earlier studies hypothesize that inflammation and remodeling may occur in parallel, beginning at the early stage of the disease [4]. Inflammation is associated with tissue eosinophilia in 40 to 60% of asthmatic patients [5] and the intensity of eosinophilia has been correlated with asthma severity [6]. Several lines of experimental evidence support the possible role of IL-5 in the pathogenesis of asthma by recruiting eosinophils to site of inflammation. First, IL-5 mRNA is identified in increased quantities in bronchial biopsies taken from asthmatics compared with non-asthmatic controls [7–9]. These data suggest that IL-5 is present in patients with asthma and is inducible by acute allergen exposure, making it a possible target for intervention. During the infiltration process, activated neutrophils are capable of producing a variety of mediators such as reactive oxygen species (ROS) and proteases [10,11]. Although detailed mechanisms underlying the neutrophil mediated airway obstruction during the late phase are unclear, earlier studies have suggested that activated neutrophils could contribute to the development of airway obstruction through induction of mucus hypersecretion and development of airway remodeling [10,11].

Here, flow cytometry (FACS) has been used to identify cells in the BALF. Analysis of large number of cell types with specific markers have been possible by using monoclonal antibodies conjugated with fluorescent dye based on forward and side scatter (FSC/SSC) channel intensity.

At present, current therapies and long term medication for the asthma management is generally limited up to their anti-inflammatory effect. Airway remodeling in established asthma is poorly responsive, such as inhalation of corticosteroids and administration of β2-agonists, anti-leukotrienes and theophylline. However, therapeutic interventions that can reverse airway remodeling are still lacking [12–15]. Thus, it is essential to look over new therapeutic alternatives for the management of the disease which can be effective in chronic cases.

The use of complementary and alternative treatments in asthma patients is increasing as an adjunct and as a substitute for effective and proven anti-inflammatory therapies [16–18]. *Curcuma longa* (turmeric) has been used as a non-toxic drug in Ayurveda for centuries for treatment of wide variety of disorders and has pharmacological

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properties including anti-inflammatory, antimicrobial, antiviral, antifungal, antioxidant, chemosensitizing, radiosensitizing, and wound healing. Immunomodulatory properties of curcumin has been reported recently [19,20]. Benefits of curcumin in chronic phase of asthma have been investigated due to the complexity and involvement of many factors in the disease. It may be possible because of having many beneficial properties and it can be used as a potential therapeutic drug for the treatment of asthma. In earlier studies effectiveness of intranasal curcumin has been investigated and therefore we hypothesized here as a potential and long term medication since it is not associated with side effects. Dexamethasone a known corticosteroid used for asthma has been used as the standard drug in the present study as a long term medication parallel to intranasal curcumin.

2. Materials and method

2.1. Animal model

Specific pathogen free Balb/c mice (6–8 weeks old; 20–22 g) were used for the experimental study. Mice were procured from Central Drug Research Institute, Lucknow, India and acclimatized for a week under standard laboratory conditions. Experimental protocols were approved by the Central Animal Ethical Committee, Banaras Hindu University, Varanasi, India.

2.2. Grouping of animals

Balb/c mice were divided into five groups (5 mice/group). Group I — Normal; Group II — OVA sensitized/OVA challenge; Group III — OVA sensitized/OVA challenge treated with DMSO; Group IV — OVA sensitized/OVA challenge with 5 mg/kg curcumin i.n.; and Group V — OVA sensitized/OVA challenge with dexamethasone (1 mg/kg, i.p.).

2.2.1. Sensitization, challenge and experimental protocol

Sensitization and challenge protocols were followed as described earlier [21,22] with some modification. Briefly, all mice were sensitized with 50 μg OVA adsorbed in 4 mg aluminum hydroxide given (i.p.) on days 0, 7 and 14 days. After a week, mice were challenged with 2% OVA through nebulizer for 30 min twice a week for 5 weeks. Curcumin (Sigma Aldrich) 5 mg/kg dissolved in DMSO and given intranasal to each mice from days 21 to 55 an hour before every nebulization (2% OVA). The non-sensitized mice were nebulized by saline in similar way. All mice were sacrificed on day 56 (24 h after the last OVA challenge).

2.3. Brochoalveoler lavage fluid (BALF)

Twenty-four hours after the last challenge all experimental mice were sacrificed. The chest cavities were carefully opened, tracheas were exposed and BALF was performed by delivering 0.8 ml cold PBS into the airway through a trachea cannula and gently aspirating the fluid. The lavage was repeated three times to recover a total volume of 2–3 ml. The cells were stained with trypan blue to determine viability by using a hemocytometer. BALF was centrifuged at 3000 rpm for 10 min and supernatants were collected and stored at −80 °C for further study and pellets were resuspended in PBS for total cell enumeration by trypan blue dye exclusion.

2.4. Lung histology for airway inflammation, collagen deposition and goblet cell hyperplasia

Lungs removed from the chest cavity were fixed by injection of 1 ml of 10% formalin into the tracheal cannula. Lung sections were embedded in paraffin, cut into 5 μm sections, and stained with H&E for histological analysis. Additional sections were stained with PAS to identify mucus-containing cells. The severity of peribronchial inflammation was graded semi quantitatively for the following features: 0, normal; 1, few cells; 2, a ring of inflammatory cells 1 cell layer deep; 3, a ring of inflammatory cells 2–4 cells deep; 4, a ring of inflammatory cells of >4 cells deep on a 5 point scoring system described in Myou et al. [22]. The numerical scores for the abundance of PAS-positive mucus-containing cell in each airway were determined as follows: 0, <0.5% PAS-positive cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; and 4, >75%. To evaluate the thicknesses of epithelium and subepithelial smooth muscle layers, measurements were performed from four points of each airway at levels of 3, 6, 9, and 12 o'clock described in Karaman et al. [23] by measuring the length bar feature of Motic image plus software. Approximately 15–20 airways were evaluated for this study. For the detection of collagen deposition in peribronchial region, lung sections were stained with Masson's trichome stain and 0–3 scoring system was applied to each bronchi observed as described in Valentin et al. [24]. Briefly scoring system was; 0 — no collagen deposition; 1 — a thin layer of collagen; 2 — a cluster of collagen; and 3 — a thick layer of collagen. Lung sections were stained with periodic acid and Schiff's base (PAS) and counterstained with hematoxylin to detect mucus containing goblet cells. The mucus content in the cells were scored 0–4 according to the criteria already described [25]. Briefly 0 — no mucus-containing cells along the basement membrane; 1 — few positive cells along the basement membrane with less than 75% of the cytoplasm stained; 2 — few positive cells along the basement membrane with more than 75% of the cytoplasm stained; 3 — numerous positive cells along the basement membrane with less than 75% of the cytoplasm stained; and 4 — numerous positive cells along the basement membrane with more than 75% of the cytoplasm stained.

2.5. Detection of IgE level in serum and cytokines in BALF

OVA-specific serum IgE levels were measured by ELISA as described earlier with some modification [26]. Serum was collected by retro orbital bleeding before sacrifice from each group for IgE detection. Briefly, a 96 well microtitre plate was coated with 20 μg of OVA in 100 μl coating buffer and kept overnight at 4 °C. The wells were washed in PBS and blocked in blocking buffer (1% BSA in PBS) and kept at 37 °C for 2 h. After washing thrice with PBST (0.05% Tween 20 in PBS), 100 μl serum (diluted 1:9 in blocking buffer) was added to the wells and kept 37 °C for 2 h. The wells were washed three times with PBST and 100 μl of horseradish peroxidase labeled anti-IgE antibody (1:1000 dilution) was added and incubated for 2 h at 37 °C. Then 100 μl of substrate (3,3,5,5-tetramethylbenzedine) was added. The reaction was stopped by adding 2.4 M H_2SO_4 to each well. Absorbance was measured at 450 nm. The cytokines IL-4, IL-5 and IFN-γ, were measured in BAL fluid supernatant by ELISA kits (IL-4, IL-5, and IFN-γ from Biolegend, USA) and the results were expressed in pg/ml [26].

2.6. Determination of eosinophil peroxidase activity (EPO) in BALF

Eosinophil peroxidase (EPO) activity was determined by a specific method according to Strath M et al. [27]. Briefly 100 μl of the substrate solution [0.1 mM O-phenylene-diamine-dihidrochloride], 0.1% Triton X-100, 1 mM hydrogen peroxide in 0.05 M Tris–HCl [pH 8.0] were added to 100 μl of BALF (1:1 in PBS) and incubated for 30 min at 37 °C. The reaction was stopped by adding 50 μl of 1 M sulfuric acid, and the optical densities were read at 490 nm.

2.7. Histamine detection in BALF

Histamine was detected in BALF as previously described [28] with slight modification. Briefly 180 μl of the sample was mixed with 36 μl of 1 M NaOH and 9 μl ophthalialdehyde in 96 black well micro plates. After 10 min at room temperature, 18 μl of 3 M HCl were added to stop the reaction. Fluorescence intensity was measured using a