International Immunopharmacology 17 (2013) 18–25

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

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

Therapeutic potential of curcumin in experimentally induced allergic rhinitis in guinea pigs

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ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 25 April 2013
Accepted 26 April 2013
Available online 9 May 2013

Keywords:
Guinea pig model
Allergic rhinitis
Curcumin
Biochemical alterations

ABSTRACT

In the present experiments, the possible role of curcumin in ovalbumin induced allergic rhinitis in guinea pig model was investigated. Various allergic rhinitis symptoms viz sneezing, rubbing frequencies, lacrimation and nasal congestion at various humidity conditions as well as on repeated sensitization were studied. The biochemical changes like serum IgE, IL-4 and nitric oxide (NO) in nasal lavage and eosinophil peroxidase activity in nasal homogenates were determined in allergic rhinitis. Curcumin treatment significantly reduced the symptoms (sneezing, rubbing frequencies, lacrimation and nasal congestion) and improved the histopathological alterations (reduction in inflammatory cells infiltration) of nasal mucosa in allergic rhinitis. Furthermore, curcumin treatment prevented significantly elevation of serum IgE, IL-4, NO in nasal lavage and eosinophil peroxidase in nasal homogenate. In the present experimental findings, we suggest that curcumin is a promising anti-allergic agent that may be useful in the clinical management of allergic rhinitis.

1. Introduction

Allergic rhinitis is predominantly an IgE-mediated inflammation of the membranes of nasal mucosa and/or cavity, characterized by sneezing, itching, nasal congestion, rhinorrhea, post nasal drainage, lacrimation of eyes, and associated loss of smell, taste and induction of cough [1,2]. It occurs due to the interactions of resident and infiltrating inflammatory cells, in associated with inflammatory mediators viz cytokines, and neurotransmitters causing sensory nerve activation, plasma leakage, and congestion of venous sinusoids [3]. Allergic rhinitis is either seasonal or perennial depending upon the symptoms observed in response to seasonal allergen exposure or throughout the year, respectively. Common seasonal allergens are mainly pollens, grasses and weeds, while perennial allergens are derived from dust mites, molds, animal danders and other sources of occupational origin [4]. Exposure to allergens in sensitized individuals, elicits cascade of biochemical and cellular events leading to synthesis and release of IgE and subsequent allergen exposure triggers mast cell degranulation causing release of histamine, tryptase, prostaglandins,cysteinyl-leukotrienes (Cys-LT), cytokines (IL-4, IL-5, and TNF-α, etc.), and platelet-activating factor (PAF) [5].

Depending upon the symptoms of allergic rhinitis respective drug(s) has been prescribed to reduce its severity. Currently, antihistamines (Chlorpheniramine maleate, fexofenadine hydrochloride, cetirizine hydrochloride etc.), corticosteroids (betamethasone, beclomethasone, and fluticasone dipropionate), mast cell stabilizers (sodium cromoglycate), decongestants (phenylephrine, oxymetazoline, and xylometazoline), Cys-LT-receptor antagonists (Montelukast sodium, and Zafirlukast) and anticholinergics are being successfully used clinically in the management of allergic rhinitis.

Various animal models have been developed, validated and widely employed for studying the etiopathology of allergic rhinitis [6–8]. Guinea pigs are usually preferred for studying rhinitis because they mimic human rhinitis, although, rats also exhibit rhinitis symptoms on topical sensitization with known allergen [6]. This model on systemic ovalbumin administration induced symptoms which resemble human allergic rhinitis and respond to clinical pharmacotherapy. The quantification of the individual symptoms is possible in this model due to characteristic manifestation of symptoms [9].

Curcumin an active constituents *Curcuma longa*, and chemically it is [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadine-3,5-dione (diferuloylmethane)]. Various studies are documented on therapeutic benefits of curcumin in the management of diseases/disorders because of its diverse pharmacological activities, anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive, hypercholesterolemia, antioxidant, antiallergic and cardioprotection [10–17]. Curcumin elicits inhibitory effects on cytokines viz IL-1, IL-4, IL-6, and TNF-α along with activation of nuclear factor kappa B (NF-kB) [18,19]. The antiallergic activity of curcumin has been correlated with the inhibition of IgE-mediated degranulation of mast cells in Brown Norway rats [17]. Although, curcumin is known to exert its effects on IgE and IL-4, its effect on allergic rhinitis is scientifically unknown. Therefore, the present study was undertaken to investigate

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1567-5769/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.intimp.2013.04.025

the beneficial effects of curcumin in experimentally induced allergic rhinitis in guinea pig model along with the probable mechanism(s) of action.

2. Materials and methods

2.1. Animals

Dunkin Hartley guinea pigs (250–300 g) of either sex were procured from Haffkine Institute of Biopharmaceutical Sciences, Mumbai, India. Animals were maintained in our animal house at desired conditions: temperature 23 ± 1 °C, and relative humidity (RH) 50 ± 5%. The animals were provided with standard laboratory diet (Amrut Laboratory Animal Feed, Nava Maharashtra Chakan Oil Mills, and Pune, India) and water ad libitum. The animals were shifted from animal house to laboratory 2 h prior to experiments.

2.2. Institutional Animal Ethics committee approval

Experimental procedures and protocol for the present study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), constituted under the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. SIPS/IAEC/App./2011-12/14 and conformed to the Indian National Science Academy guidelines for the use and care of experimental animals in research. Animal house was registered with the Govt. of India (962/c/06/CPCSEA) dated 27 July 2006.

2.3. Drugs and chemicals

2.3.1 . Ovalbumin

Chicken egg Grade V (Sigma, St. Louis, USA), Montelukast sodium (Cipla Ltd, Mumbai, India), aluminum hydroxide and carboxy methyl cellulose from local suppliers were procured. Purified curcumin powder was obtained as gift sample from Amsar Private Ltd. Indore, India.

2.3.2. Curcumin

The purified curcumin powder was dissolved in ethanol and quantitatively analyzed by high performance thin layer chromatography and compared with standard curcumin with scanning at 420 nm. Curcumin consisted of 3 fractions curcumin (56.99%, Rf 0.57), demethoxycurcumin (28.57%, Rf 0.39), and bisdemethoxycurcumin (14.45%, Rf 0.28). The separation of all 3 fractions was performed by preparative thin layer chromatography and analyzed for physicochemical properties and reported in our earlier experiments [16].

2.4. Oral acute toxicity study

The oral acute toxicity study of curcumin was carried out in rats as per the OECD 423 guideline. In this, animals were administered with curcumin up to 2000 mg/kg by oral route and they were observed for a period of 96 h for any clinical and behavioral symptoms. Curcumin (2000 mg/kg) did not produce clinical, toxic symptoms and no mortality; hence the doses 100 and 200 mg/kg were selected for the present studies.

2.5. Sensitization procedure

Guinea pigs of either sex in equal numbers were sensitized as per the procedure described by Underwood et al. [20] with minor modification by Bahekar et al. [9]. Briefly, Guinea pigs were randomized and divided into different groups (n = 6). They were sensitized on days 1, 7, 14 and 21 by intraperitoneal injection of ovalbumin (100 μg/animal) and aluminum hydroxide (5 mg/animal) dissolved and suspended, respectively in 0.9% saline solution. Nonsensitized animals received suspension of aluminum hydroxide in saline. After 7 days of systemic sensitization, animals were topically instilled with 60 mg/ml, 20 μl/nostril ovalbumin solutions. A week after the last sensitization, the dorsal area of animals was shaved and challenged with intradermal injection of ovalbumin solution (25 μl of 200 μg/ml). Successful sensitization was confirmed by edema and redness at the site of injection. Ovalbumin dose 60 mg/ml (20 μl/nostril) for induction of allergic rhinitis was selected which elicited the marked rhinitis symptoms [9].

2.6. Induction of allergic rhinitis

The room where experiments were performed was free of noise and strong odors and maintained at a temperature of 23 ± 1 °C and RH 50 ± 5%. Animals were allowed to acclimatize in clean perspex observation cages (26 × 26 × 26 cm) for 30 min. The curcumin (100 and 200 mg/kg) or vehicles (10 ml/kg), Montelukast (10 mg/kg), and sodium chromoglycate (100 mg/kg) were administered orally to overnight fasted animals or intranasally to non-fasted animals, for 3 days prior to intranasal ovalbumin challenge. The local ovalbumin challenge was performed by instilling ovalbumin solution into both the nostrils by using micropipette (Biosystems, India). Symptoms of rhinitis; number of sneezing, nose rubbing, eye lacrimation and difficulty in breathing were observed and scored for a period of 2 h. Sneezing was characterized by an explosive expiration just after a deep inspiration, and nose rubbing was characterized by the external perinasal scratching with either one or both forelimbs of animal [21]. Lacrimation was scored on a 3-point scale as follows: (+)—hazy eyes; (++)—lacrimation; and (+++)—lacrimation with the onset of conjunctivitis. Nasal acoustic phenomenon reflecting nasal congestion/obstruction was evaluated as (+)—impaired inspiration; (++)—nasal inflammation; and (+++)—severe breathing impairment [22]. The nasal secretion (watery fluid) discharged through nostrils or trickling on the walls of observation cages during sneezing was considered as the sign of rhinorrhea [9].

2.7. Effects of curcumin, Montelukast on ovalbumin induced rhinitis symptoms

The sensitized guinea pigs were divided into various groups (n = 6) and administered daily for 3 days with saline (20 μl/nostril), curcumin (100 and 200 mg/kg, p.o.), and Montelukast (10 mg/kg, p.o.), and 2 h after the last treatment, animals were challenged with ovalbumin (20 μl/nostril containing 1.2 mg of ovalbumin). Sneezing and rubbing frequency, lacrimation and nasal congestion were counted for 2 h and recorded. Animals were sacrificed 8 h after challenge for histopathological studies.

2.8. Histopathological studies of nasal cavity

Histopathological studies were performed as per the method described by Bahekar et al. and Yamasaki earlier [9,25]. The head was removed and lower jaw was discarded. The nasal cavity was separated from skin, muscle and soft tissues which were immersed in the freshly prepared 10% neutral buffered formalin for a period of 48 h. Thereafter, tissues were rinsed in running tap water and decalcified in 10% nitric acid solution for 5 days. After rinsing in tap water, the tissue was processed for dehydration with alcohol and embedded in paraffin. Sections (4 μm) were cut at the level of incisive papillae and first and second palatal ridges and stained with hematoxylin–eosin. Sections were examined under light microscope to observe alterations in histopathologic structure of specimen and infiltration of cells in epithelium and subepithelium. All slides were coded before the analysis and read in blind fashion to avoid observer's bias.