International Immunopharmacology 39 (2016) 172–180

Contents lists available at ScienceDirect

International Immunopharmacology

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

Tiron ameliorates oxidative stress and inflammation in a murine model of airway remodeling

Nagla A. El-Sherbeeny [a,*], Zeinab A. Hassan [b], Hayam Ateyya [c]

[a] Department of Clinical Pharmacology, Faculty of Medicine, Suez canal University, Egypt
[b] Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt
[c] Department of Clinical Pharmacology, Faculty of Medicine, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form 13 July 2016
Accepted 25 July 2016
Available online 30 July 2016

Keywords:
Airway remodeling
Dexamethasone
Tiron
Antioxidants
TGF-β1

ABSTRACT

Airway remodeling includes lung structural changes that have a role in the irreversibility of pulmonary dysfunction shown in chronic bronchial asthmatics. The current experiment investigated the effect of the mitochondrial antioxidant, tiron in comparison with dexamethasone (DEXA) on airway remodeling in chronic asthma. Sensitized BALB/c mice were challenged with ovalbumin (OVA) aerosol for 8 weeks, OVA sensitized-challenged mice were treated with either DEXA or tiron, respectively. After that, lung tissue and bronchoaveolar lavage fluid (BALF) were used for measurement of different biological markers. Lungs were examined for histopathological changes and immunohistochemistry. Upon comparing with vehicle treated animals, trion or DEXA treatment significantly reduced eosinophils, lymphocytes, neutrophils and macrophages count in the BALF. Both drugs significantly alleviated chronic OVA-induced oxidative stress as illustrated by decreased pulmonary malondialdenhyde (MDA) and increased glutathione (GSH) and superoxide dismutase (SOD) levels. Asthmatic mice exhibited elevated levels of NOx, IL-13 and TGF-β1 that were reduced by DEXA and tiron. Histopathological changes and increased immunoreactivity of nuclear factor-Kappa B (NF-κ B) in OVA-challenged mice were minimized by tiron and DEXA treatment. In conclusion, in this model of chronic asthma DEXA and tiron ameliorated airway remodeling and inflammation in experimental chronic asthma with no difference between the effect of tiron and DEXA. Tiron has a potential role as adjuvant treatment in chronic asthma.

1. Introdution

Bronchial asthma is manifested by repeated reversible episodic airway obstruction with an ongoing process of airway inflammation [1]. Asthma occurs as a consequence of genetic and environmental factors interaction such as a family history of allergic reaction, the presence of immunoglobulin E (IgE) which is allergen-specific, viral respiratory diseases, exposure to aeroallergens such as dust mites, mold spores, seasonal pollen, animal allergens, and nonallergens such as smoke, odors, cold air, chemicals, medications, exercise, obesity, and lower socioeconomic status [2].

Airway wall remodeling and airway hyperresponsiveness are two prominent features of asthma that contribute to chronic symptoms. Remodeling of airway is characterized by a number of structural changes, including extracellular matrix component deposition like collagen, laminin, fibronectin, and tenascin, airway smooth muscle hypertrophy and hyperplasia of both muscle and goblet cells. Airway remodeling is one of the causes leading to a decrease in the effectiveness of standard therapy [3]. Airway hyperresponsiveness is a complicated process, and several factors participate in its progress. Inflammation of mucosal airway is an influx of eosinophils, activated T lymphocytes with release of Th2 cytokines (particularly IL-4, IL-5, and IL-13), eotaxin, RANTES (Regulated on Activation, Normal T Expressed and Secreted) and other proinflammatory mediators which play crucial factor in regulating the allergic inflammatory reaction that progress to airway hyperresponsiveness [4,5].

Numerous investigation have shown that airway inflammation is the principal reason for frequent attacks of airway obstruction in asthmatic patients and proved that reactive oxygen species (ROS) act a predominant function in starting and intensifying inflammation in airways of asthmatic [6]. These ROS are produced from airways of asthmatic recruited inflammatory cells. There are many antioxidants acting to overcome ROS-induced damage of several cellular molecules such as lipid membranes, proteins, and DNA [1]. On the other hand, during asthma there is an excessive production of ROS that may overwhelm these defenses causing inflammatory changes in the airways and systemic circulation. ROS-induced injury may lead to enhanced smooth muscle contraction, vascular permeability and mucus hypersecretion [7].

Designs aiming to increase endogenous antioxidants via dietary or pharmacological interference in order to restore the oxidant/

* Corresponding author at: Department of Clinical Pharmacology, Faculty of Medicine, University Road, Ismailia, Egypt.
E-mail address: n_elsherbeeny@hotmail.com (N.A. El-Sherbeeny).

http://dx.doi.org/10.1016/j.intimp.2016.07.025
1567-5769/

antioxidant balance in asthma are the modern region of investigation all over the world.

Tiron (4, 5-dihydroxy-1,3-benzene disulfonic acid) is a water-soluble cell permeable analog of vitamin E and function as a direct hydroxyl radical and superoxide scavenger [8,9]. It is both a beneficial protective antioxidant and an efficient nontoxic metal chelator that has been commonly used in oxidative stress-associated physiologic studies [10].

The utilization of tiron as antioxidant may introduce an additional remedial modality for controlling allergic asthma. Thus, this research aims at providing the role of tiron in boosting the endogenous antioxidants or scavenging excessive ROS production and examining if it can dampen/prevent the inflammatory response. The study compares the effect of tiron with the standard antiasthmatic medication DEXA in chronic asthmatic mice.

2. Material & methods

2.1. Animals

BALB/c female mice aged approximately 8 weeks were purchased from the National Research Centre, Dokki, Giza, Egypt. During the time of the experiment, mice were housed under pathogen-free conditions. Animals were kept in a room with standard controlled temperature and a 12-h dark/light cycles, and permitted free access to food and water. The experimental protocol of this study followed carefully the ethical guidelines and the principals of care, use and handling of experimental animals approved by "The research Ethics Committee", Faculty of medicine, Suez Canal University and Faculty of medicine, Cairo University, Egypt, which are in agreement with "Principles of Laboratory Animals Care" (NIH publication No. 85-23, revised 1985).

2.2. Protocol for sensitization and antigen challenge

Mice were allowed to adapt for the week preceding the beginning of the study. On days 0 and 14 sensitization of animals was achieved by intraperitoneal injection of 10 μg OVA emulsified in 500 μg of aluminium hydroxide in 200 μl saline. At 21 day, mice were exposed to OVA aerosol (1% weight/volume diluted in sterile physiological saline) for 30 min and the challenge repeated three times a week for 8 weeks. Aerosol was generated using an ultrasonic air nebulizer (Omron, Vernon Hills) attached to a cylindrical chamber (20 × 30 × 35 cm). The outlet of the chamber was connected to a vacuum pump with the flow maintained at a rate of 15 ml/min and particle size 2–6 μm adjusted by a flow meter (Hoffer Flow Controls, USA). Mice were placed in the chamber and exposed to the aerosol for 30 min during each designated challenge session.

2.3. Experimental groups

72 mice were randomly divided into groups (12 mice each):

Group I (normal group): saline treated mice.

Group II (control group): Mice alum sensitized and saline challenged. Mice received 0.5% CMC (10 ml/kg/day, orally) on the day of challenge, the dose was given 1 h before each challenge.

Group III (Tiron-control): mice alum sensitized, saline challenged and received 200 mg/kg tiron daily by gavage.

Group IV (asthmatic mice): mice OVA sensitized and challenged.

Group V (DEXA-OVA): asthmatic mice treated by daily I.P. injection of 2 mg/kg DEXA.

Group VI (Tiron-OVA): asthmatic mice given tiron.

The daily doses of treatment drugs were given 1 h before the challenge.

Twenty-four hours after the final challenge, mice were anaesthetized using I.P. urethane (2.5 mg/kg). To collect BALF for subsequent analysis, the chest of each mouse was opened, the trachea with the heart-lung package was exposed and the left main bronchus clamped. A cannula was carefully inserted into the trachea, and the right lung was lavaged three times using 1 ml PBS solution. The inferior lobe of the left lung was dissected and homogenized while the remaining part of left lung was collected for histopathology.

2.4. BALF preparation and white blood cell count

Centrifugation of BALF was carried out for 10 min at 1000 g and 4 °C. The pellet of cells was redistributed in fresh PBS (100 μl) and the supernatant was cautiously taken away and kept at −80 °C. The total number of inflammatory cells in BALF was counted using a hemocytometer. For differential white blood cell count, smears of BALF were prepared then stained with Diff-Quik solution (Dade Behring Inc., Newark, NJ, USA). Four different random locations at 40 magnifications were examined and at least 200 cells were counted in each one.

2.5. Lung homogenate preparation

The lung slices were weighed then homogenized (1:10, w/v) in 0.1 M phosphate buffer (pH 7.4) in an ice bath, then subjected to centrifugation for 20 min (3000g, 4 °C). The supernatant obtained was utilized for evaluation of the lipid peroxidation marker, MDA and antioxidants SOD and GSH concentrations.

2.6. Estimation of lipid peroxidation and antioxidants in lung tissue

Malondialdehyde (MDA) [11], superoxide dismutase (SOD) [12] and reduced glutathione (GSH) amount [13] in lung homogenates were measured (Bio-diagnostic Company, Giza, Egypt).

2.7. Estimation of nitric oxide in BALF

As an indication of nitric oxide production, the total production of nitrate/nitrite (NOx) was evaluated in BALF using NO assay kit (R&D Systems, Minneapolis, USA).

2.8. Measurement of IL-13 & TGF-β1 levels in BALF

Cytokines IL-13 and TGF-β1 concentration in BALF was determined via DuoSet ELISA kit for mouse interleukin-13 (Catalog Numbers: DY413-05 and Quantikine ELISA for TGF-β1 (Catalog Number MB100B, R&D Systems).

2.9. Tissue histopathology and immunohistochemistry

2.9.1. Histopathology of the lung

The left lungs were carefully dissected from mice. Immediately after dissection, the lungs were inflated with the fixation solution consisting of 10% neutral buffered formalin by insertion of 5 ml syringe through the opening of the trachea [14]. Sections were dehydrated and embedded in paraffin. Lung sections were sliced at 5 μm thickness and stained with hematoxylin and eosin (H&E) to determine the airway inflammatory cell infiltrate [15], Periodic Acid -Schiff (PAS), to identify goblet cells [15] or Masson's trichrome (MT) stain to determine collagen deposition/fibrosis [16]. All histological examinations were carried out by an observer blinded to the design groups.

2.9.2. Immunohistochemistry (IHC) for NF-κB expression

Lung samples were treated in 4% paraformaldehyde and rooted in paraffin wax then sectioned at 5 μm for IHC. Immunostaining was carried out via Avidin-Biotin complex (ABC) method [17]. After preparing the tissue slides, blocking was done using endogenous peroxidase with 3% H2O2. Next, the slides were incubated for the night at 4 °C with a rabbit polyclonal antibody directed against NF-κB p65 in a dilution of 1:100 (purchased from Santa Cruz Biotechnology, Santa Cruz,