Original Article

EFFECT OF L-2-OXOTHIAZOLIDINE -4-CARBOXYLIC ACID ON MARKERS OF INFLAMMATION AND LIPID PEROXIDATION IN BRONCHOALVEOLAR LAVAGE FLUID IN MICE MODEL OF ASTHMA

Ljudmil G. Terziev,
Vanya Tsetkova,
Veneta L. Shopova[1],
Violeta Y. Dancheva[1],
Galya T. Stavreva[2],
Milena A. Atanasova[3],
Tzvetan H. Lukanov,
Angelina M. Stoyanova[4]

Sector of Clinical Immunology and Allergology
[1]Sector of Disaster Medicine
[2]Sector of Experimental and Clinical Pharmacology
[3]Sector of Biology
[4]Sector of Chemistry
Medical University
Pleven, Bulgaria

Corresponding Author:
Ljudmil G. Terziev
Clinic of Clinical Immunology and Allergology,
University Hospital –Pleven
81 Vladimir Vazov str.
Pleven, 5800
e-mail: luterzi@mail.bg

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Summary

Asthma is a serious medical and social problem, characterized by an inflammatory response and production of a large amount of reactive oxygen species. Our goal was to study the effect of a glutathione precursor on some markers of inflammation and lilpid peroxidation in an animal model of asthma. The study was carried out on 28 C_{57}B1 mice, divided into four groups: group 1 - controls; group 2 – injected and inhaled with ovalbumin (OVA); group 3 - treated with L-2-oxothiazolidine-4-carboxylic acid (OTCA) and inhaled with phosphate buffered saline; group 4 – injected with ovalbumin and OTCA, as well as inhaled with OVA. Under sodium pentobarbital anaesthesia the animals were sacrificed on hour 48 after the last inhalation to obtain bronchoalveolar lavage fluid (BALF). The total cell number and cell counting, total protein content, the levels of Il-4, Il-5 and 8-isoprostane were investigated in BALF. OVA increased the total cell number and the levels of Il-4, Il-5 and 8-isoprostane. OTCA significantly decreased the total cell number, the total protein content, as well as the levels of Il-4, Il-5 and 8-isoprostane in comparison with ovalbumine. OTCA attenuates inflammation and lipid peroxidation in asthma provoked by ovalbumin in a mouse model.

Key words: asthma, bronchoalveolar lavage fluid, inflammation, lipid peroxidation, L-2-oxo thiazolidine-4-carboxylic acid

Introduction

Over the last 10 years the incidence of bronchial asthma has increased by more than 29% in West European countries [1], and has been accompanied by increase in associated mortality rate [2]. Asthma is a chronic inflammatory disease characterized by Th_2 cytokines with a specific profile of released cytokines [3]. The upper airways are mostly affected, which leads to an interaction between the cells and mediators. This results in increased production and release of reactive oxygen species (ROS) in the airways [4]. There is a consensus about the relation between oxidants and their effects on different pulmonary diseases, including asthma [5, 6]. Bronchial inflammation plays a leading role in the clinical manifestation and pathogenesis of the disease. Impaired antioxidant capacity in asthma

patients is an evidence for increased oxidative stress [6]. Treatment with antioxidants reduces the inflammation and hyperreactivity of the airways [7]. Considering the fact that glutathione, a non enzymatic antioxidant, is 100 times more concentrated in the liquid covering the respiratory epithelium than in plasma [8], we aimed to study the effects of L-2-oxothiazolidine-4-carboxylic acid (OTCA), a glutathione precursor, on some markers of inflammation and lipid peroxidation in bronchoalveolar lavage fluid (BALF) on an allergic mice model.

Material and Methods

Chemicals

Ovalbimin, grade V, Phosphate buffered saline, L-2-oxothiazolidine-4-carboxylic acid were purchased from Sigma-Aldrich Company, Mouse IL-4 ELISA, Mouse IL-5 ELISA Kits- from R&D Systems, Mouse IgE ELISA Set Kit- from BD Biosciences, 8-Isoprostane EIA Kit- from Cayman chemicals and Inject Alum® - from Pierce Chemical Company (USA).

Animals and experimental protocol

The experiment was performed in accordance with Animal Welfare Regulations and was approved by the University Ethic's Committee.

The study was carried out on 28 mice C57Bl (weight 20±2g, 8-10 weeks old). The animals were raised at the University vivarium at a temperature of 22±2ºC and humidity of 50±10%, given a normal pelleted diet and water *ad libidum*. The mice were divided into four groups: group1, controls; group 2, injected with ovalbumin (OVA); group 3, treated with L-2-oxothiazolidine-4-carboxylic acid (OTCA) and group 4, treated with OVA and OTCA. The animals from groups 1 and 3 were injected *i.p.* with 100 µl phosphate-buffered saline (PBS)+Inject Alum® (1:1) on days 0 and 14. The animals from groups 2 and 4 were injected with a 100 µl ovalbumin solution, containing 20 µg OVA (p0012-protocol) on the same days. On days 24, 25 and 26, mice from groups 1 and 2 were given inhalation with PBS for 30 min, and those from groups 2 and 4 were given inhalation with a 1% ovalbumin solution (OVA dissolved in PBS). For the purpose, a special plexiglass chamber was used. One hour before inhalation, the animals from groups 1 and 2 were injected *i.p.* with 100 µl PBS, and those from groups 3 and 4 received a 100 µl (160mg/kg) freshly prepared OTCA solution, pH=7.2. For all injections, individual sterile needles were used.

Bronchoalveolar lavage fluid (BALF)

To obtain BALF, the animals were sacrificed on day 28 (48 hours after the last inhalation). The chest was opened and the lungs were perfused *in situ* via the right heart ventricle with saline (10-15 ml). Triple lavage of the left lung through the trachea with a total volume of 2.5 ml of saline was performed. One aliquot of the BALF was used for the purpose of total cell count. The cells were then removed by centrifugation at 300 x g for 10 min. The supernatant of BALF was used to measure interleukins and 8-isoprostane levels. The total protein content in ng/mL by the method of Lowry et al. [9], the levels of IL-4 and IL-5 in pg/mL by the ELISA method (according to protocol, R&D System), and the level of 8-isoprostane in ng/mL by the ELISA(according to assay protocol, Cayman Chemical Company, Ann Arbor, USA) method were investigated in the supernatant of BALF.

Statistical analysis

Experimental data were analysed using Statgraphics Plus for Windows 5.0, and the results were summarized as mean±SEM. Statistical analysis was based on parametric methods for the control and F-test using the criteria of Fisher (analysis of variance), $p<0.05$.

Results

The total cell number increased more than fourfold in BALF (461% as compared to the controls) in group 2 (OVA-immunized mice). The increase of the same parameter in group 4 (OVA+OTCA) was significantly lower than that in group 2 (Fig. 1). The total protein content increased in group 2, and treatment with OTCA (group 4) decreased it, compared to the OVA group (Fig. 2). OVA elevated the levels of IL-4 and 5, but in group 4 it was significantly lower than those in group 2 (Fig. 3 and 4). The 8-isoprostane level elevated in asthma group, OTCA alone did not change it, but in the OTCA + OVA group (group 4) 8-isoprostane was significantly lower in comparison with the OVA group (Fig. 5).

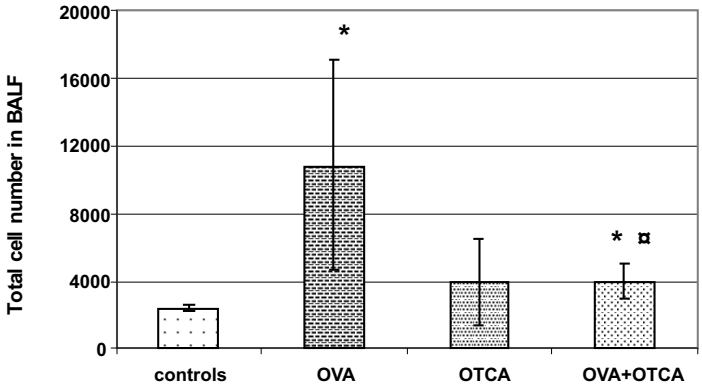

Fig. 1. Total cell number in BALF. Each point represents the mean± SE for six mice. Asterisks indicate significantly different values from those of controls. "¤" indicates significantly different values from the group 2, treated with OVA immunization and challenged ($p<0.05$ by t-test).

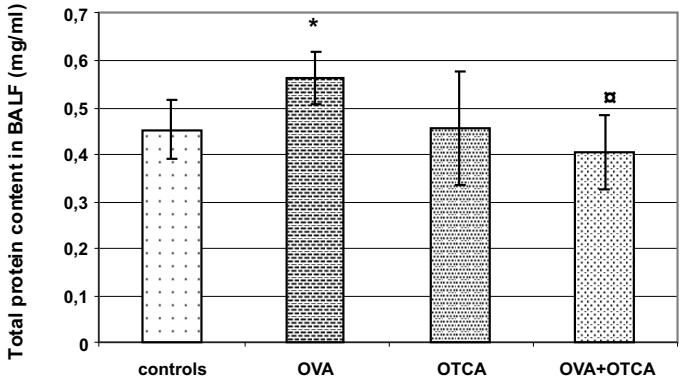

Fig. 2. Total protein content in BALF. Each point represents the mean±SE for six mice. Asterisks indicate significantly different values from those of controls. "¤" indicates significantly different values from the group 2, treated with OVA immunization and challenged ($p<0.05$ by t-test).

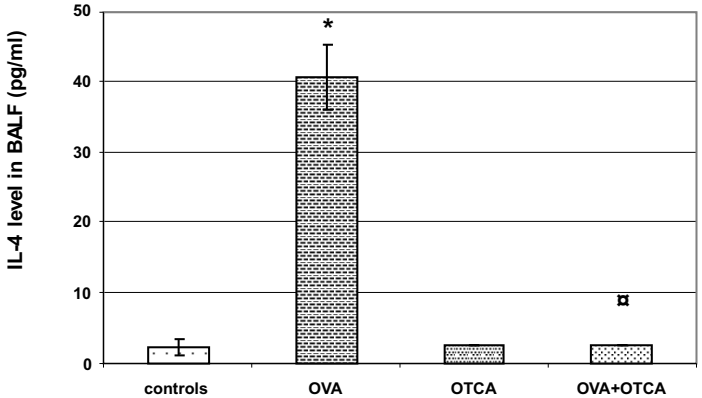

Fig. 3. IL-4 level in BALF. Each point represents the mean±SE for six mice. Asterisks indicate significantly different values from those of controls. "¤" indicates significantly different values from the group 2, treated with OVA immunization and challenged ($p<0.05$ by t-test)

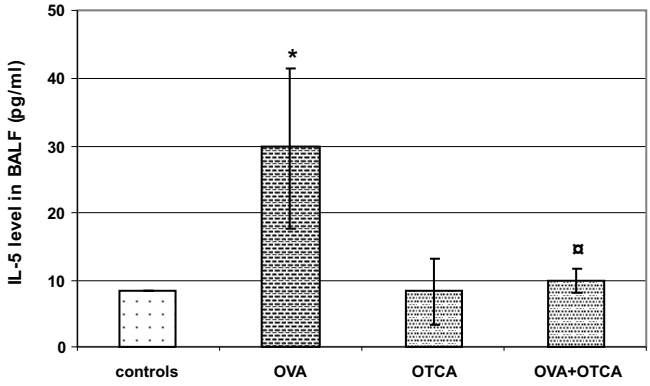

Fig. 4. IL-5 level in BALF. Each point represents the mean±SE for six mice. Asterisks indicate significantly different values from those of controls. "¤" indicates significantly different values from the group 2, treated with OVA immunization and challenged ($p<0.05$ by t-test)

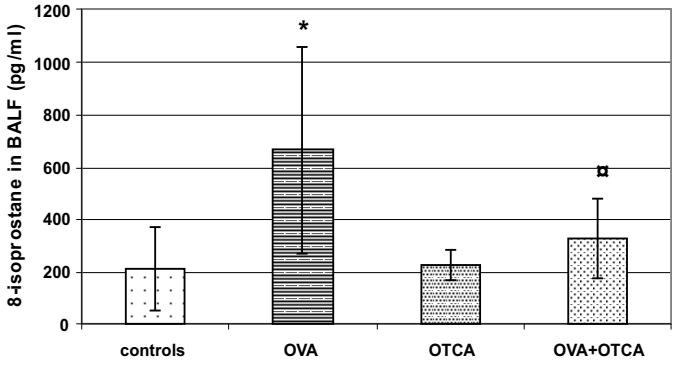

Fig.5. 8-isoprpstane level in BALF. Each point represents the mean±SE for six mice. Asterisks indicate significantly different values from those of controls. "¤" indicates significantly different values from the group 2, treated with OVA immunization and challenged ($p<0.05$ by t-test)

Discussion

The effects of OTCA on the markers investigated explain well its ability to increase the plasma concentrations of cysteine and glutathione [10, 11]. Glutathione, synthesized from cysteine, is an important intra- and extracellular protective antioxidant against oxidative stress [12, 13]. Alterations in alveolar and lung glutathione metabolism are widely recognized as a key feature of many inflammatory lung diseases, including asthma. When liver glutathione stores are depleted and/or synthesis capacity is impaired, OTCA can be successfully used [14]. This compound is easily transportable in the cells, where it is transformed into L-cysteine. OTCA is more effective than N-acetylcysteine in replenishing intracellular glutathione stores [15]. The hyperreactivity and inflammation of the respiratory airways are blocked by OTCA in asthma models [16]. It is now well known that vascular endothelial growth factor (VEGF) plays a crucial role in asthma pathogenesis [17], and enhancement of vascular permeability. This antioxidant may decrease vascular permeability by inhibiting up-regulation of VEGF expression [18]. This may explain the effect of the compound on the total protein content and the total cell number in BALF. According to Lee et al., 2004, OTCA significantly decreases the total cell number, the number of eosinophils, lymphocytes and neutrophils in BALF. Moreover, it decreases the inflammatory cell infiltration in the peribronchial and perivascular areas in the mice asthma model [16]. OTCA reduces the elevated levels of reactive oxygen species (ROS), the inflammation of the airways and the bronchial hyperreactivity in asthma [16, 18, 19]. It seems that the glutathione precursor may perform its action by inhibiting the activity of nuclear factor-kB.

Conclusion

L-2-oxothiazolidine-4-carboxylic acid (160mg/kg), administered intraperitoneally one hour before nebulization with ovalbumin, (24, 25 and 26 days) attenuates the inflammation and lipid peroxidation in the bronchoalveolar lavage fluid in the asthma mice model, provoked by ovalbumin, 48 hours after the last nebulization.

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