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Effects of L-2-oxothiazolidine-4-carboxylic acid on the lung antioxidant defense system in an asthma mouse model

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Aim: We aimed to study the effect of a glutathione precursor, L-2-oxothiazolidine-4-carboxylic acid (OTCA), on the lung antioxidant defense system in an animal asthma model.

Materials and methods: The study was carried out on 24 female C57BL/6 mice. The mice were divided into 4 treatment groups: group 1 – control group; group 2 – injected with ovalbumin (OVA) and given an OVA inhalant; group 3 – treated with OTCA and phosphate-buffered saline inhalant; and group 4 – injected with OVA and OTCA and given an OVA inhalant. Under sodium pentobarbital anesthesia the animals were killed by exsanguination 48 h after the last inhalation to obtain a lung homogenate. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) and the content of nonprotein sulfhydryl (NPSH) groups in lung homogenate were investigated.

Results: OVA decreased the activities of SOD (P = 0.007), CAT (P = 0.004), and GP (P = 0.05) and the NPSH content (P = 0.0008) in the lung homogenate compared with the control animals. Treatment with OVA and OTCA (group 4) resulted in a significant increase in the activities of CAT (P = 0.01) and GP (P = 0.05) and the NPSH content (P = 0.002) compared to the OVA group (group 2).

Conclusion: OTCA (160 mg/kg) restored the activities of basic enzymes in the lung antioxidant defense system in an OVA-induced asthma mouse model 48 h after the last nebulization.

Key words: Asthma, L-2-oxothiazolidine-4-carboxylic acid, lung antioxidant defense system

Introduction

The development and maintenance of allergic airway inflammation depends on the complex interaction of many cytokines and chemokines. T cells involved in the proallergic response produce cytokines associated with induction of classical Th_2 response, in which there is an oversecretion of IL-4, IL-5, and tumor necrosis factor-α (TNF-α) (1). Accompanied by an increase in the mortality rate (2), bronchial asthma incidence has reached more than 29% within the past years in the West European countries (3). The development of an inflammatory immune response in numerous pulmonary diseases, including asthma, has been reported. It is described as an activation of epithelial cells and macrophages and an influx of activated neutrophils, eosinophils, monocytes, and lymphocytes into the airways. These cells can modulate the inflammatory response known to form a large amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the airways (4,5). Enzymatic and nonenzymatic antioxidants neutralize the toxic oxygen products formed under physiological conditions. However,

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under conditions of oxidative stress, the balance between oxidants and antioxidants is often disrupted due to hyperproduction of ROS or depletion of the antioxidant capacity (6,7). Glutathione, synthesized from cysteine, is an important intra- and extracellular protective antioxidant against oxidative stress (8,9). The main protective roles of glutathione against oxidative stress are: a) glutathione is a cofactor of several detoxifying enzymes, such as glutathione peroxidase and glutathione transferase; b) it participates in amino acid transport through the plasma membrane; c) it scavenges hydroxyl radical and singlet oxygen, detoxifying hydrogen peroxide and lipid peroxides by the glutathione peroxidase; and d) it is able to regenerate the most important antioxidants, vitamins C and E (10). Alterations in alveolar and lung glutathione metabolism are widely recognized as a central feature of many inflammatory lung diseases, such as asthma. The antioxidant is 100 times more concentrated in the liquid covering the respiratory epithelium than in the plasma (11). It is now well established that L-2-oxothiazolidine-4-carboxylic acid (OTCA) is a cysteine precursor that increases the plasma cysteine and glutathione concentrations (12,13). We aimed to study the effects of OTCA on the antioxidant defense system in the allergic mouse model.

Materials and methods

Chemicals

Ovalbumin (OVA; grade V), phosphate-buffered saline (PBS), and OTCA were purchased from Sigma-Aldrich Company (USA), and Imject Alum® was purchased from Pierce Chemical Company (USA).

Animals and experimental protocol

The experiment, performed in accordance with regulations for animal welfare, was approved by the university ethics committee.

The study was carried out on 24 female C57BL/6 mice weighing 20 ± 2 g at an age of 8-10 weeks old. The animals were raised in the university's animal vivarium at a temperature of 22 ± 2 °C and humidity of 50 ± 10%. They were given a normal pelleted diet and water ad libitum. The mice were divided into 4 groups: group 1 – controls; group 2 – injected with OVA; group 3 – treated with OTCA; and group 4 – treated with OVA and OTCA. The animals from groups 1 and 3 were injected intraperitoneally on days 0 and 14 with 100 µL of PBS and Imject Alum® (1:1). The animals from groups 2 and 4 were injected with a 100-µL OVA solution containing 20 µg of OVA (p0012-protocol). On days 24, 25, and 26, mice from groups 1 and 2 were given a PBS inhalant for 30 min, and those from groups 2 and 4 were given a 1% OVA solution (OVA dissolved in PBS). For this purpose, a special Plexiglas chamber was used. One hour before inhalation, the animals from groups 1 and 2 were injected intraperitoneally with 100 µL of PBS, and those from groups 3 and 4 received 100 µL (160 mg/kg) of freshly prepared OTCA solution with pH 7.2. For all injections, individual sterile needles were used.

Biochemical assays in lung homogenate

The animals were sacrificed on day 28 at 48 h after the last inhalation. The chest was opened and the lungs were perfused in situ via the right heart ventricle with 10 mL of saline. The right lung was ligated at the hilum, cut, and then removed from the chest and used to prepare the lung homogenate. The tissue was homogenized with ice-cold 0.25 M sucrose in Tris HCl with pH 7.4 at a 1:10 ratio. The homogenate was centrifuged (9000 × *g*, for 30 min) and the supernatant was stored on ice. Superoxide dismutase (SOD) activity in U/mg lung tissue was determined by the method of Maral et al. (14), and catalase (CAT) activity in mcat/g tissue was assessed by the method of Koroljuk et al. (15). The activity of glutathione peroxidase (GP) in U/g lung tissue was measured by the method of Bernchnaider as modified by Pereslegina (16). Nonprotein sulfhydryl (NPSH) group content in mol/g tissue × 10^7 was measured by the method of DeLucia et al. (17).

Statistical analysis

Experimental data were analyzed using SPSS 14. When we tested for normality, 2 variables, GP and CAT, showed nonparametric distribution, and we used medians, interquartile ranges, and the Mann-Whitney test for comparison. For the rest of the variables, we applied a post hoc ANOVA test, and data are presented as mean ± standard error of mean (SEM). $P < 0.05$ was considered statistically significant.

Results

In group 2 (asthma-provoked), the activities of SOD and CAT decreased significantly in the lung homogenate by 71% (P = 0.007) and 77% (P = 0.004), respectively, in comparison with the control animals. The changes of SOD and CAT activities in group 3 (OTCA-treated) showed values close to the control group: 96% and 98%, respectively. The results were significant compared to the OVA group. The enzyme activities of SOD and CAT in group 4 were lower: 78% and 87% (P = 0.01), respectively (Table, Figures 1 and 2). Changes in GP activity showed a similar dynamic, such as a decrease in the OVA group (68%) and values approximate to those of the controls in group 4 (P = 0.05 in comparison to group 2) (Table, Figure 3). Nonprotein sulfhydryl (NPSH) group content in the lung homogenate decreased in group 2 to 68% (P = 0.0008), while in the group with OVA and OTCA the decrease was relatively lower (93%, P = 0.002) as compared to the OVA group (Table, Figure 4). The GP activity and NPSH content in group 3 did not differ significantly from those of the control group; therefore, they were significantly higher compared to group 2.

Discussion

The experimental data of our study showed that in mice, OVA can provoke asthma, a disease in which oxidative stress and decreased antioxidant capacity play a critical role (6). The activities of key antioxidant enzymes such as SOD, CAT, and GP, as well as NPSH content, decreased, suggesting an antioxidant capacity disturbance. There are many common assumptions concerning oxidative stress in asthma. In exhaled breath condensate, the levels of 8-isoprostane and CO are elevated, as well as the activities of the enzymes GP and SOD in the lung cells (18) and the products of peroxidation in the lungs, serum, and urine. The content of malondialdehyde and thiobarbituric acid reactive substances (TBARS), as markers of lipid peroxidation, is elevated in bronchoalveolar lavage fluid, urine, and sputum (7,19). Alterations in alveolar and lung glutathione metabolism are widely recognized as a central feature of many inflammatory lung diseases, such as asthma, in that either a decrease in liver glutathione stores or impaired synthesis capacity is observed (20).

Table. Effect of OTCA on the activity of some enzymes of the lung antioxidant defense system in a mouse model of asthma.

28 days after treatment (48 h after the last inhalation)

Parameters \ Groups	Control	OVA	OTCA	OVA+OTCA
SOD activity in U/g **Mean ± SEM**	31.1 ± 1.79	22.1 ± 1.94*	29.9± 1.77	24.4 ± 2.75*
CAT activity in mcat/g **Median (min-max)** **Q_3-Q_1**	38.14 (33.08-44.12) 5.07	29.48* (21.51-31.20) 4.30	37.42 (19.57-40.05) 8.14	33.14*† (30.85-36.78) 3.37
GP activity in U/g **Median (min-max)** **Q_3-Q_1**	111.22 (51.40-138.74) 29.65	76.07* (66.85-83.56) 6.38	125.97 (66.01-160.03) 66.14	101.53† (63.96-122.43) 23.15
NPSH groups in mol/g × 10^{-7} **Mean ± SEM**	0.58 ± 0.03	0.40 ± 0.03*	0.55 ± 0.03	0.54 ± 0.007†

Abbreviations: OVA, ovalbumin; OTCA, L-2-oxothiazolidine-4-carboxylic acid; SOD, superoxide dismutase; CAT, catalase; GP, glutathione peroxidase; NPSH groups, nonprotein sulfhydryl groups; SEM, standard error of mean; Q_3-Q_1, interquartile range.

*Different from control at P < 0.05; †different from group 2 (OVA) at P < 0.05.

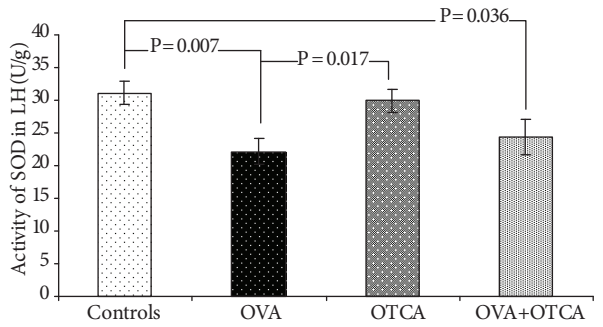

Figure 1. Activity of superoxide dismutase (SOD) in lung homogenate (LH). Each point represents the mean ± SEM for 6 mice.

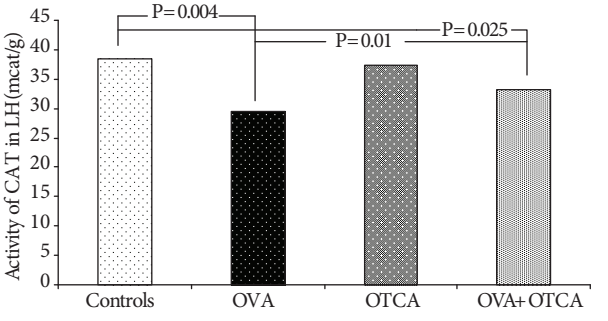

Figure 2. Activity of catalase (CAT) in LH. Each point represents the median for 6 mice.

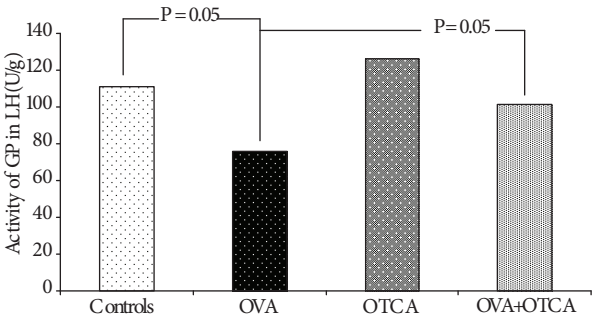

Figure 3. Activity of glutathione peroxidase (GP) in LH. Each point represents the median for 6 mice.

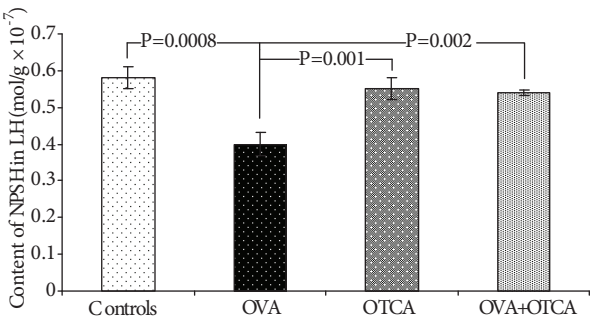

Figure 4. Content of nonprotein sulfhydryl groups (NPSHs) in LH. Each point represents the mean ± SEM for 6 mice.

The auspicious effects of OTCA on the investigated enzymes of the antioxidant defense system in the lungs can be explained by the fact that OTCA is a cysteine precursor. This compound is easily transportable into cells, where it is transformed into L-cysteine, and glutathione is synthesized from cysteine (8,9). OTCA, a 5-oxoproline analog, is therefore a substrate for the ubiquitous intracellular enzyme called 5-oxoprolinase (21,22). 5-Oxoprolinase catalyzes the reaction converting 5-oxoproline, also known as pyroglutamate, to glutamate, which is required for the first step of glutathione synthesis. The major nonenzymatic scavenger is glutathione, which has been shown to be an important component of the lung antioxidant defense (23). It is unlikely to function as a direct scavenger of H_2O_2, but it would rather serve as a cofactor for peroxidases, such as the glutathione peroxidase. OTCA can increase the plasma concentrations of cysteine and glutathione (12,13), thereby restoring the impaired balance between oxidants and antioxidants (glutathione). OTCA is more effective than N-acetylcysteine in replenishing intracellular glutathione stores (24). In case of a decrease in liver glutathione stores and impaired synthesis capacity, OTCA can be successfully used (20). It reduces the elevated levels of reactive oxygen species (ROS), the increased expression of IL-18, the inflammation of the airways, and bronchial hyperreactivity in the animal asthma model (25). It seems that the glutathione precursor may perform its action by inhibiting the activity of nuclear factor-kB (25-27).

OVA provoked the development of asthma in the mouse model, demonstrated by depletion of basic antioxidant enzymes such as SOD, CAT, and GP, as well as NPSH content in the lungs. OTCA, a cysteine precursor, had a beneficial effect in the asthma mouse model by increasing the glutathione concentrations. OTCA administered intraperitoneally 1 h before

nebulization with OVA on days 24, 25, and 26 restored the activities of basic enzymes CAT and GP in the lung antioxidant defense system and NPSH content 48 h after the last nebulization.

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