UDC 577.151.63:577.121 doi: https://doi.org/10.15407/ubj94.03.026

INVOLVEMENT OF Cu-CONTAINING AMINE OXIDASES IN THE DEVELOPMENT OF LUNG PATHOLOGY IN OVALBUMIN-INDUCED BRONCHIAL ASTHMA IN GUINEA PIGS

O. HUDKOVA[1]✉, S. LUHOVSKYI[2], L. DROBOT[1], N. LATYSHKO[1]

[1]Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
[2]Dmitry F. Chebotarev Institute of Gerontology, National Academy of Medical Sciences of Ukraine, Kyiv;
✉e-mail: ogudkova@biochem.kiev.ua

Received: 03 August 2022; **Revised:** 15 September 2022; **Accepted:** 29 September 2022

Bronchial asthma is developed as an immune response to allergen challenges accompanied by inflammation and fibrosis implicated in airway remodeling. To reveal the causative implication of Cu-containing amine oxidases, semicarbazide-sensitive amine oxidase (SSAO), diamine oxidase (DAO), and lysyl oxidase (LOX), in bronchial asthma development we used their irreversible inhibitor, semicarbazide, and ovalbumin-induced pathology in guinea pigs. Semicarbazide was introduced to asthmatic animals via drink or inhalation. At the 16th week after disease induction, the increase in the activity of pro-inflammatory SSAO and DAO in plasma (1.6 and 2 times, respectively) was observed. The introduction of semicarbazide to asthmatic animals via drink or inhalation significantly decreased activities of these enzymes compared to the untreated asthmatic animals. A considerable increase in IL-13 content and LOX activity in the lung tissue of asthmatic animals were observed that evidenced airway inflammation and pulmonary fibrosis development. The uptake of semicarbazide by guinea pigs with bronchial asthma led to normalization of LOX activity. Histological studies confirmed that semicarbazide attenuated morphopathological changes in the lungs of asthmatic animals. Thus, the data obtained indicate the direct participation of the studied enzymes in the progression of pathological processes in atopic bronchial asthma as well as the potential use of semicarbazide as a drug in complex anti-asthmatic therapy.

K e y w o r d s: atopic bronchial asthma, semicarbazide, semicarbazide-sensitive amine oxidase, histaminase/diamine oxidase, lysyl oxidase, IL-13, nitric oxide.

Asthma is a global health problem and, despite numerous studies and activities of the Global Initiative for Asthma (GINA), whose reports have been updated annually since 2002 [1], there are many unclear points, the so-called “blank spots” in understanding the mechanisms underlying disease. Asthma, as systemic disease, results from complex interactions between inflammatory cells, their mediators, airway epithelium and smooth muscle, and the nervous system. Inflammation is the most pronounced and best studied symptom of bronchial asthma (BA) that is associated with airway hyper-responsiveness (AHR) and structural changes in the lungs, such as mucous cell metaplasia, smooth muscle thickening, and sub-epithelial fibrosis, i.e. airway remodeling [2].

According to recent data the development of atopic BA, which is a type I hypersensitivity (type I HS) reaction, proceeds in three stages: immunological (allergen sensitization, initial stage); pathobiological (allergen provocation), and pathophysiological (effector/late stage) as shown in Fig. 1. The major features of type I HS reaction are: 1) a shift in the differentiation of T-helpers towards the Th2 subpopulation capable of producing specific cytokines such as IL-4, IL-5, IL-13; 2) IL-4-induced IgE synthesis by B-lymphocytes at the initial stage; 3) IL-13-mediated regulation of AHR, mucus production, and sub-epithelial fibrosis *in viv*o in the context of allergic inflammation at the effector phase; 4) degranulation of the mast cells with the release of preformed mediators and the initiation of the produc-

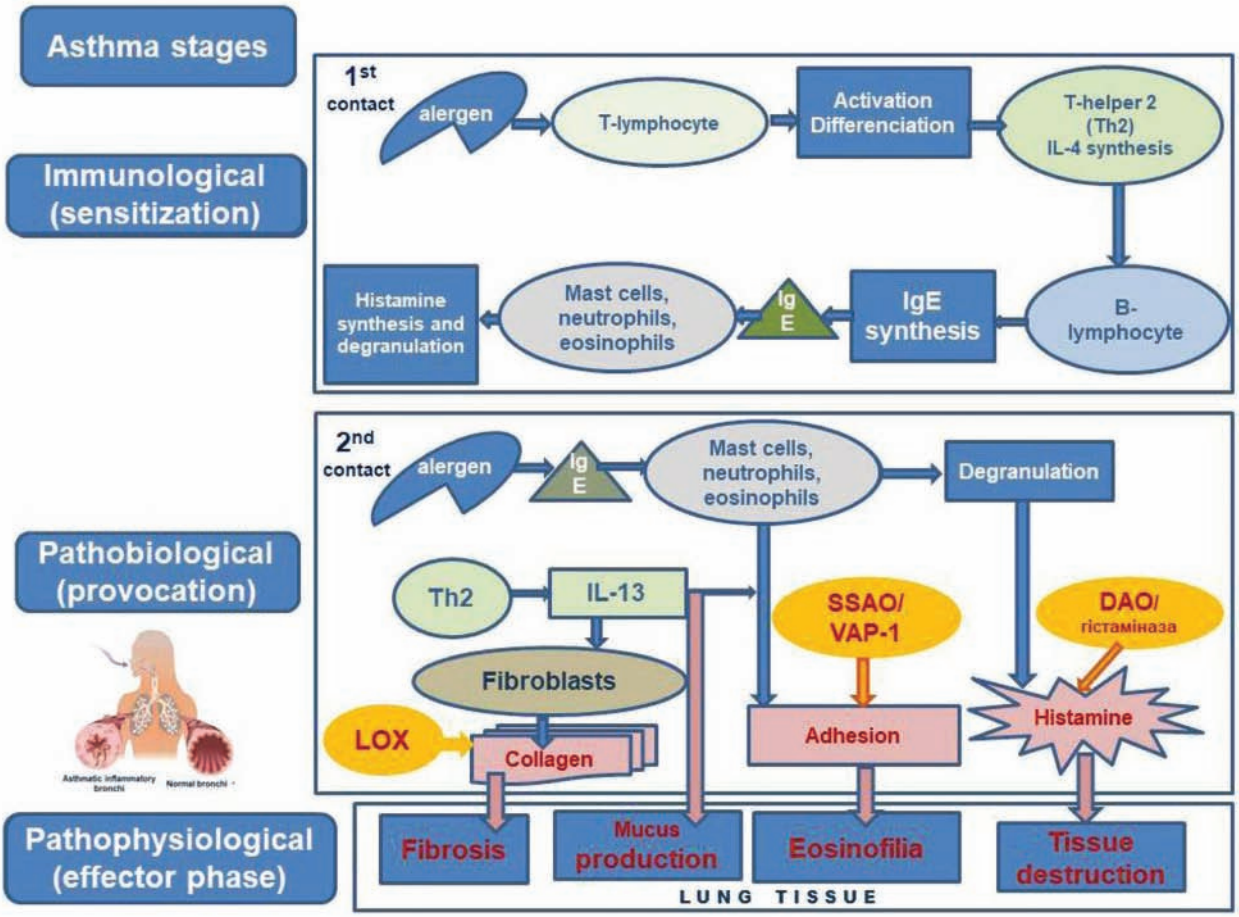


Fig. 1. Schematic representation of the pathogenesis of atopic bronchial asthma (type I hypersensitivity reaction)

tion of other mediators (chemokines and chemotactic factors) in the inflammatory cascade [3].

One of the pro-inflammatory mediators of immune cells is the biogenic monoamine histamine, the concentration of which in the body is regulated by diamine oxidase/histaminase (EC 1.4.3.22, DAO), a copper- and TOPA-quinone (TPQ)-dependent oxidoreductase, which deaminates histamine to form ammonia and imidazole acetic acid. A positive correlation has been found between the severity of respiratory allergy and the level of DAO activity, so this indicator in blood serum is used as a diagnostic marker [4].

It should be noted that with an exacerbation of allergic BA, there is an increase in the concentration of biogenic amines in the blood plasma. Elevated levels of these compounds, in its turn, affect the activity of lymphocytes [5]. Thus, a persistent influx of neutrophils into the lung is characteristic of acute lung inflammation. Semicarbazide-sensitive amine oxidase (EC 1.4.3.21, SSAO), which also contains Cu^{2+} and TPQ cofactor, oxidatively deaminates aliphatic and aromatic primary amines to form the corresponding aldehydes, hydrogen peroxide and ammonia. In some mammalian tissues, especially endothelium, this enzyme also functions as a vascular adhesion protein 1 (VAP-1) promoting neutrophil migration during acute lung inflammation and leading to AHR, and subsequent tissue damage [6]. These data suggest that SSAO/VAP-1 can be directly implicated in the development of atopic asthma.

The extracellular matrix (ECM) is a key modulator of inflammation and airway remodeling. Increased ECM stiffness is associated with cross-linking of interstitial collagen and fibronectin via action of lysyl oxidase family enzymes (EC 1.4.3.14, LOX, and EC 1.4.3.13, LOXL 1-4), other members of Cu-AOs. In support of this, an increase in LOXL2 expression in asthma has recently been shown to promote airway remodeling [7]. Thus, the mechanisms of biochemical processes underlying the development of BA can be directly related to the

functioning of AOs involved in the catabolism of biogenic amines (DAO, SSAO) and post-translational modifications of ECM components (LOXs). To date, some of these aspects remain unexplored.

Numerous studies also demonstrate that endogenous and exogenous reactive oxygen and nitrogen species (ROS and RNS) play an important role in airway inflammation and are determinants of asthma severity. Elevated ROS levels can cause deleterious pathophysiological disturbances in allergic asthma [8]. According to the current hierarchical model of oxidative stress, depending on its power, activation of either the transcription factor Nrf2 or MAPK and NF-kB leads to the increased production of inflammatory cytokines, chemokines and adhesion molecules, including SSAO/VAP-1, or causes a cytotoxic response that leads to apoptotic or necrotic cell death. In inflammatory processes in the airways, excessive production of ROS and RNS, in turn, activates neutrophils, eosinophils, and mast cells, which release histamine, prostanoids, and cytokines, causing exacerbation of AHR. Among inflammatory mediators, inducible nitric oxide synthase (iNOS) has been shown to play a key role in the production of nitric oxide (NO), which acts as a powerful stimulus for allergic asthma. Previous studies have demonstrated that NO activates proinflammatory signaling and Th2 responses in atopic asthma. In addition, down-regulation of iNOS expression in the airways has been shown to reduce asthmatic responses in numerous models of asthma [9, 10]. Therefore, we also assumed a sufficient contribution of nitrosative and oxidative stress, in addition to the development of inflammation and fibrosis, to the BA pathology.

Taking into account all of the above, Cu-AOs should play a significant role in the development of BA, since they catalyze the formation of three dangerous products during the reaction of oxidative deamination of biogenic amines: ROS, reactive aldehydes (or reactive carbonyl species, RCS), and ammonia, which is a precursor in the synthesis of RNS [11]. Thus, the aim of this work was to prove the correctness of our assumption about the participation of Cu-AOs in the development of BA features by using the ovalbumin(OVA)-induced model of BA in guinea pigs and semicarbazide (SC), an irreversible inhibitor of these enzymes [12].

Materials and Methods

Materials. Ovalbumin chicken, sodium azide, semicarbazide hydrochloride, pargiline hydrochloride, methylamine hydrochloride, 2-(4-Imidazolyl) ethylamine dihydrochloride (histamine dihydrochloride), spermine hydrochloride, 1,5-diaminopentan (cadaverine), 4,5-diaminofluorescein diacetate (DAF-2DA), Bradford reagent, were obtained from Sigma Chemical Co, USA. Guinea pig IL-4, IL-13, and Nitric oxide synthase ELISA quantification kits were purchased from CUSABIO Biotech Co, China. All other reagents/chemicals were of the highest analytical grade available.

Animals. Healthy commercially available male guinea pigs were maintained on a 12 h light/dark cycle at an ambient temperature of 20–26°C, and fed with commercial pelleted chow and supplemental feed, such as hay, seed mix (sunflower seeds, oats, wheat, peas), and fresh vegetables. Following an acclimation period, animals with body weight range of 300–350 g were used for experiments. All manipulations with animals were performed in accordance with the provisions of the "General Ethical Principles of Animal Experiments" adopted by the First National Congress of Bioethics (Kyiv, 2001), the Law of Ukraine "On the protection of animals from cruelty" (from 21.02.2006), and international requirements under the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Animal grouping and induction of BA. A total of 24 outbred male guinea pigs were randomly divided into 6 groups: I – intact animals (Control, n = 4); II – animals sensitized and provoked with 0.1% OVA (Bronchial asthma, (BA), n = 4); III – animals sensitized and provoked with 0.1% OVA, which received a 0.05% solution of semicarbazide (SC) from the 1st day of provocation daily (BA+SC1, n = 5); IV – animals sensitized and provoked with 0.1% OVA, which received 0.2% SC in aerosol from the 1st day of provocation, 3 times a week for 5 min (BA+SC2, n = 4); V – animals sensitized by OVA without next provocation with the allergen (Sensitized, n = 3); VI – animals provoked with 0.1% OVA without prior sensitization (Provoked, n = 4).

Animals were sensitized by two intraperitoneal (ip) injections of 1.0 ml of sterile 0.9% NaCl containing 0.1 mg of OVA as an allergen and 10 mg of $Al(OH)_3$ as saline adjuvant, with an interval between injections of 5 days. Provocations were carried out starting from the 23rd day after the last sensitization for 12 weeks by inhalation of an OVA aerosol. For this purpose, guinea pigs were placed inside the transparent plastic chamber (V=5 L) coupled to compressor nebulizer. From the 1st to the 4th week inclusive, 2 inhalations of OVA solutions were used for 3 min with a 7-minute interval. Starting from the 5th week, 3 inhalations were performed with an increasing concentration of OVA solution (0.05%; 0.1% and 0.3%) in PBS. The exposure duration was determined by the appearance of respiratory distress signs (sneezing, coryza, and coughing, deep retraction of the thoracic wall).

Two types of SC treatment were used: taking 0.05% SC orally 5 times a week and 0.2% SC, 5 min inhalation 3 times per week.

The sensitized guinea pigs (OVA/0.9%NaCl) were challenged with aerosolized PBS following the same schedule to exclude unspecific stimulation of the airways by the aerosol droplets. There were no animals, which failed to develop the clinical symptoms of BA. Under these conditions, none of the animals developed anaphylactic shock with a fatal outcome.

Animals were taken out of the experiment 18-20 h after the last provocation (16 weeks): they were ip injected with sodium thiopental (190 mg/kg of animal weight), as well as the analgesic lidocaine in the neck (8 mg/kg of animal weight) subcutaneous injection (sc).

Sample collection. Blood was collected from the ear veins in Eppendorf tubes containing heparin. Heparinized blood was centrifuged for 15 min at 1000 g for 30 min after collection to separate plasma for further studies. The resulting plasma was immediately used for IL-4 quantitation using an ELISA kit. Then the plasma samples were divided into aliquots and stored at -20°C until the activity of SSAO and DAO was determined (up to 24 h).

Bronchoalveolar lavage (BAL) fluid samples were collected during late asthmatic reaction, 18-20 h after the last challenge. The trachea was exposed under anesthesia and cannulated. The lungs were carefully washed 3 times with 0.9% NaCl (1 ml/100 g guinea pig weight). Approximately 77% of the BAL fluid was recovered. The resulting liquid was centrifuged at 500 g for 15 min at 4°C. The precipitated BAL cells were resuspended in sterile 0.9% NaCl. The total number of cells was determined using a Neubauer hemocytometer. NO generating cells were assessed by flow cytometry. BAL fluid (BAL cell-free supernatant) was used to determine DAO activity.

The upper lobe of the right lung was removed and placed on ice. All further steps were carried out at 0-4°C. Frozen guinea pig lungs were used to prepare samples for LOX activity as described previously [12].

For IL-13 estimation, a frozen lung portion was homogenized with liquid nitrogen and extracted with PBS followed by centrifugation at 5000 g for 5 min.

The lower lobe of the right lung was frozen in liquid nitrogen to determine the content of free radicals (FR).

The left lung was soaked in formalin solution, processed to be embedded in paraffin, and used for histological studies. It was necessary to analyze both the proximal and distal parts of the lung, since changes in the peripheral parts of the lung are involved in the pathogenesis of asthma [13, 14].

IL-4 and IL-13 levels were measured in plasma and lung samples, respectively, using a commercial enzyme-linked immunosorbent assay kits (Cusabio Biotech Co, China) following the protocols provided by the manufacturer. Absorbance spectra were determined spectrophotometrically by microplate reader μQuant (Biotek, USA) in the range of 380-600 nm with the step 5 nm. The absorbance maximum was set at 450 nm with the reference value at 570 nm. IL-4 and IL-13 content was expressed as pg/ml of plasma/lung samples. The lower detection limit was defined as 0.78 pg/ml of IL-4/13 level.

EPR spectrometry was used to determine FR content as described earlier [10].

Determination of the content of inducible nitric oxide synthase (iNOS) in animal lung tissue was performed using an ELISA kit (Cusabio, China) according to the manufacturer's protocol. The intensity of color was measured spectrophotometrically at 450 nm in a microplate reader.

The level of NO generated by inflammatory cells of guinea pig BAL was determined by flow cytometry using diaminofluorescein diacetate (DAF-2DA) (Sigma, USA). The final concentration of the fluorescent probe in the samples was 10 μM. For staining, a BAL cells suspension was used at a concentration of 5×10^6 cells/ml in sterile PBS. The sam-

ples were incubated for 30 min at 37°C in the dark. The percentage of NO-producing cells in suspension was analyzed on a Beckman Coulter flow cytometer (USA) equipped with argon laser λ_{ex} = 488 nm, determining the fluorescence intensity on the FL-1 channel, characterized by λ_{em} = 525 ± 10 nm; 10,000 events were accumulated for each measrement. Fluorescence signal alterations were defined according to fluorescence peak relative to the marker, which highlighted the autofluorescence peak indicative of unstained cells. The results were graphically presented using the FCS Express V3 program (De Novo, USA).

Histological assays. Lung specimens fixed in 10% neutral buffered formalin and embedded in paraffin were used to prepare 5-μm-thick sections, which were stained with hematoxylin-eosin (H&E) [15]. The histological assessment was carried out on a Nikon microscope (Japan) equipped with spot digital camera. Morphometry was performed by using the IMAGE J 1.45S computer program (National Institutes of Health, USA). The following parameters were determined in the lungs: the width of the entrance to the alveolus (a); alveolar depth (b); the width of the alveolar passage (c) (Fig. 2). To assess the change in the configuration of the respiratory bronchioles, we calculated such index indicators as the ratio of the width of the entrance to the alveolus to its depth (a/b; Coef.1), as well as the ratio of the width of the alveolar passage to the doubled depth of the alveolus (c/2b; Coef.2) [16].

Biochemical (enzyme activities) assays in lung, plasma, and BAL.

AOs activities. Determination of AOs activities (SSAO, DAO, and LOX) was carried out by the fluorometric method according to [12].

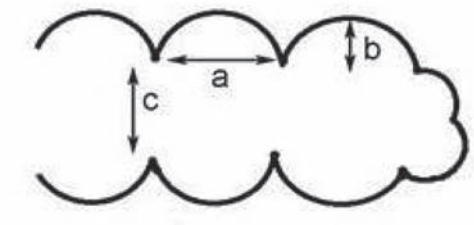


Fig. 2. Schematic representation of the respiratory bronchioles of the guinea pig lung: a – the width of the entrance to the alveolus; b – the depth of the alveolus; c – width of the alveolar passage (according to [16] with additions and changes)

Protein content estimation. The protein concentration was determined by the Bradford method [17] with bovine serum albumin as a standard.

Data analysis. All data analysis was done using Excel 2007 and Statistica 4.5. The correspondence of the experimental data to the normal distribution was checked by the Shapiro-Wilk test with a significance level of 0.05. A parametric *t*-test was used to assess the significance of the changes. The results of the experiments were presented as the M±SEM of the sample. Differences were considered significant at $P < 0.05$.

Statistical processing of morphometric data was performed using software STATISTICA 13 TIBCO Software Inc. (SN AXA905I924220FAACD-N). The results were presented by the minimum and maximum values of the indicators (min-max), median (M) and interquartile interval (25%-75%). Statistical hypotheses were tested using the Mann-Whitney test (U test) at $\alpha = 0.05$.

Results

For *in vivo* experiments, we used the well-known guinea pig model of OVA-induced asthma, which reproduces the characteristic features of allergic asthma such as airway infiltration with inflammatory cells, early and late asthmatic reactions (EAR, and LAR), AHR, to study the molecular mechanisms, underlying the pathogenesis of asthma [18, 19].

The development of BA in guinea pigs is followed by modulation of the levels of IL-4 in plasma and IL-13 in lungs. At week 16 after BA induction, IL-4 concentrations in plasma samples from all OVA-treated groups tended to increase compared to the control group, as shown in Fig. 3. At the same time, the concentration of IL-13 significantly increased ($P < 0.05$) in the lung samples of all groups of animals that were in contact with the allergen, especially in the BA group (an increase of almost two times), compared with the control group (Fig. 3). It is important to note that the levels of both indicators did not undergo statistically significant changes in both plasma and lung samples of animals that received SC (Fig. 3).

SC uptake by asthmatic animals reduced elevated free radical (FR) levels in the lungs. EPR spectroscopy data also showed a significant increase in the level of FR in the lung samples of all groups of animals that were in contact with OVA, and the highest degree (more than 2.5 times) of this indica-

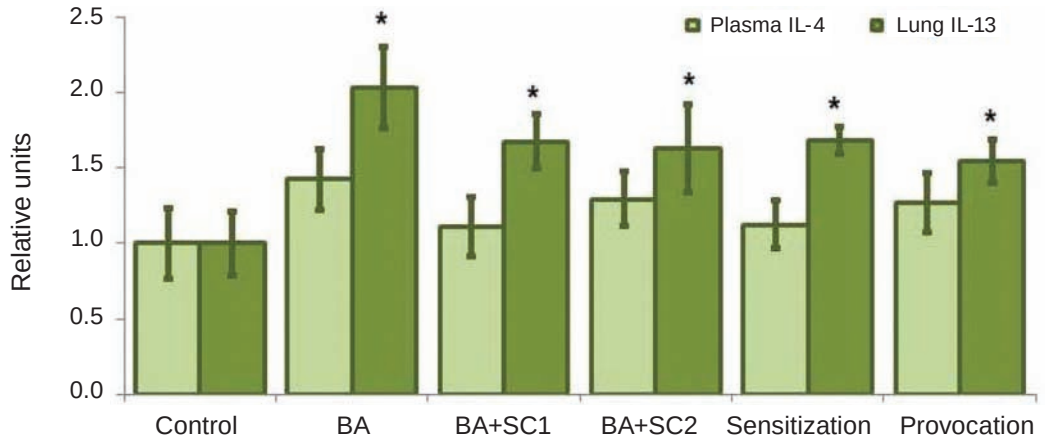


*Fig. 3.The development of BA led to a change in the levels of IL-4 in plasma and IL-13 in lungs of experimental animals (M ± SEM, n = 3-5). *P < 0.05 compared to Control*

tor was observed in the BA group compared to the control ($P < 0.05$). At the same time, SC uptake by asthmatic animals, (BA+SC1 and BA+SC2 groups) led to a significant decrease in FR levels compared to BA ($P < 0.05$) (Fig. 4).

The development of BA in guinea pigs led to an increase in nitrosative stress markers in lungs. Estimation of the content of iNOS in lungs was performed with a sandwich ELISA kit according to the manufacturer's protocol. In all experimental animal groups with OVA-induced BA (BA, BA+SC1, BA+SC2), as well as in both groups of negative control (Sensitization and Provocation) iNOS content rose almost 1.5-2 times compared to Control ($P < 0.05$) (Fig. 5, *A*).

The number of NO-generating BAL cells in guinea pigs with a high level of DAF-2DA fluorescence increased by 2-2.5 times relative to the control in all experimental groups (Fig. 5, *B* and *C*). Fig. 5, *C* shows gated plots of FL1 fluorescence intensity versus BAL cell granularity in experimental groups of guinea pigs in a model of chronic OVA-induced asthma. No statistically significant changes of this parameter were detected in the groups of asthmatic animals on the background of taking SC.

The morphopathological changes in lungs of asthmatic animals were attenuated by SC. Histopathological analysis and morphometric data revealed thinning of the walls of the alveoli and changes in the configuration of the respiratory bronchioles of guinea pigs with asthma compared to intact animals (Fig. 6, *A*). These changes developed in the peripheral areas of the lung against the background of peribronchial inflammatory infiltration,

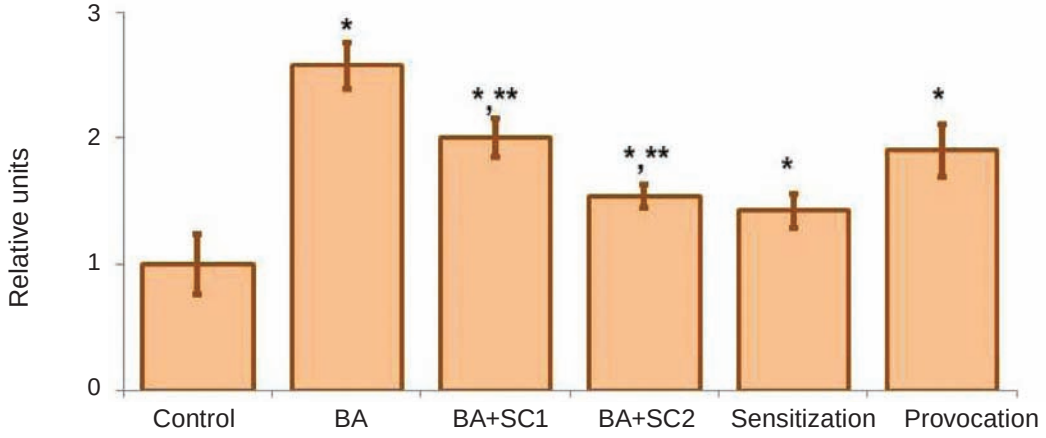
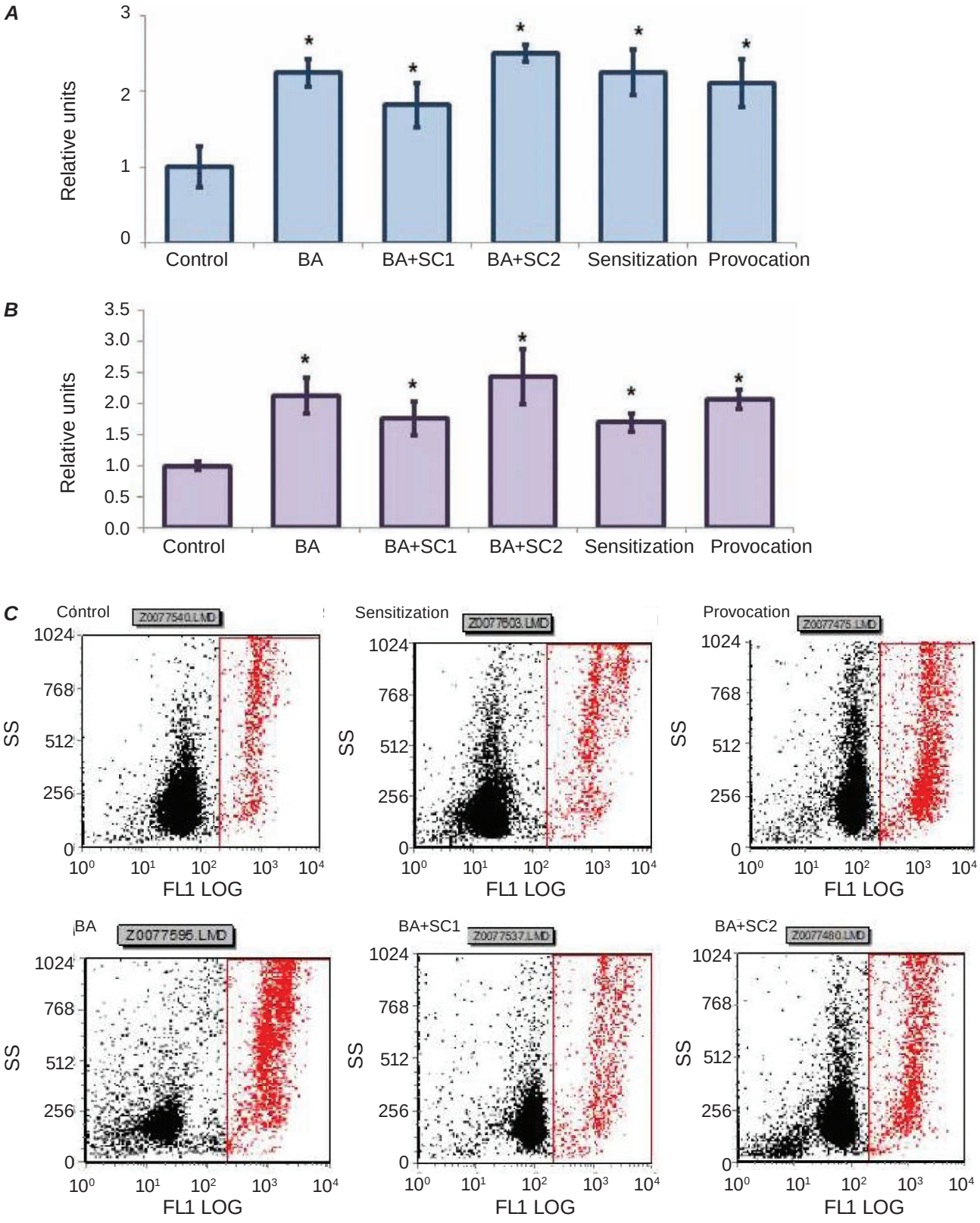


*Fig. 4. SC caused a decrease in the level of FR in the lungs of asthmatic animals. The amount of FR was estimated by EPR spectroscopy (M ± SEM, n = 3-5). *P < 0.05 compared to Control; **P < 0.05 compared to BA*



FL1LOG – the logarithm of the DAF-2DA fluorescence intensity; SS – cell granularity expressed as arbitrary units

Fig. 5. The development of BA in guinea pigs led to the induction of nitrosative stress in lungs. Markers of nitrosative stress: ***A*** *– iNO-synthase;* ***B*** *– BAL (NO-generating cells);* ***C*** *– representative scatter dot plots of BAL cells (M ±S EM, n = 3-5). *P < 0.05 compared to Control*

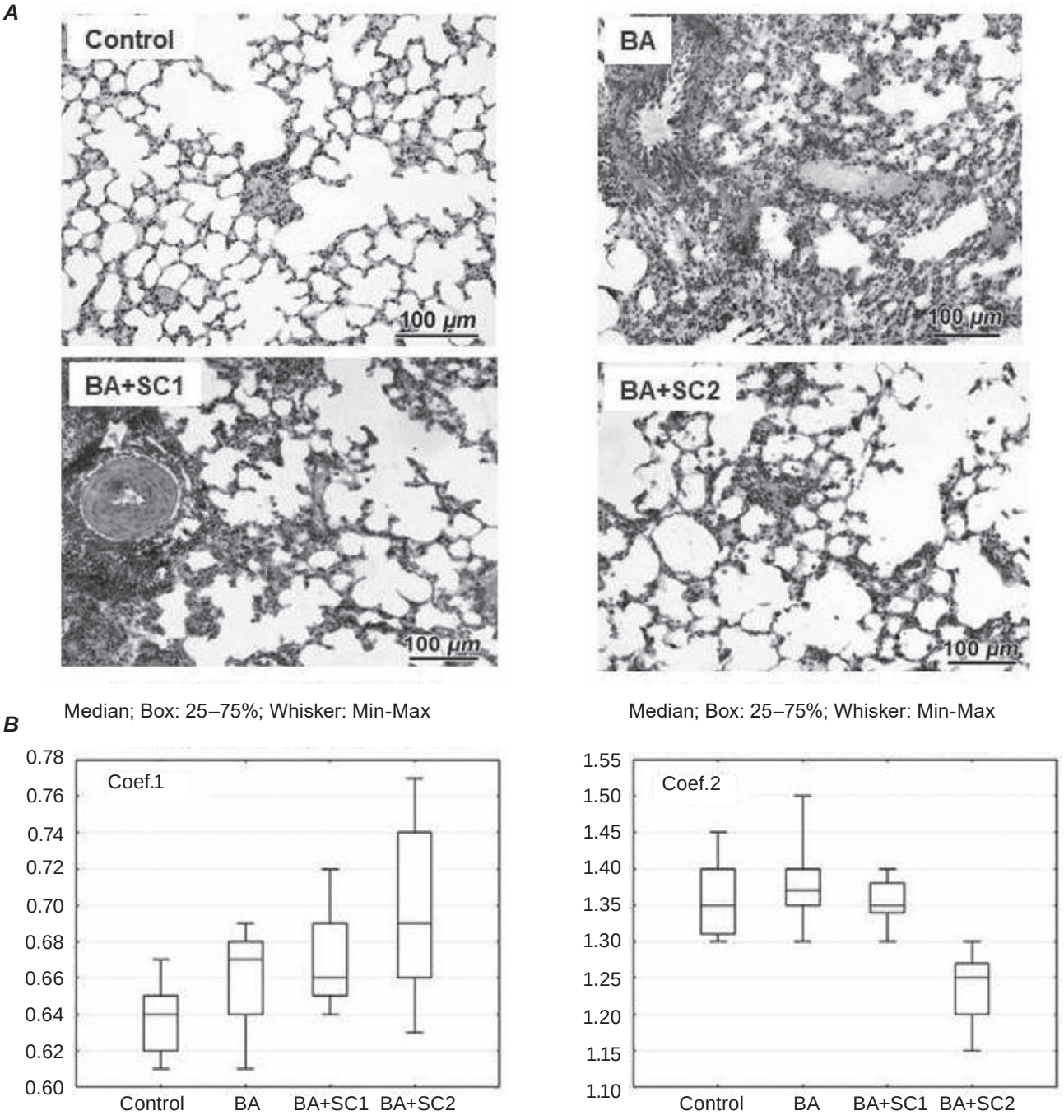


Fig. 6. The SC uptake by asthmatic animals led to an improvement in the morphofunctional state of the lungs. ***A*** *– Histology. Lungs were removed and processed for paraffin embedding, sectioned, and stained with H&E. The representative photographs of transverse sections are shown (100X original magnification);* ***B*** *– morphometric parameters of the respiratory bronchioles of the airways*

where eosinophils and neutrophils predominated, and were accompanied by obstruction of the bronchi of medium and small caliber due to hyperplasia of the bronchial epithelium, as well as pneumosclerosis (excessive growth of connective tissue of the lungs).

Changes in the configuration of the respiratory bronchioles of the airways compared with the control were detected by determining the corresponding parameters of this section of the lungs by calculating Coef.1 and Coef.2 as described in

the "Materials and Methods". A significant increase in Coef.1 ($P < 0.05$) as well as a slight increase in Coef.2 in the BA group compared with the control was shown (Fig. 6, *B*). In both groups of animals receiving SC, there was a decrease in obstruction of bronchial lumen against the background of peribronchial inflammatory infiltration and sclerosis and corresponding changes in Coef.1 and Coef.2 (Fig. 6, *B*). In particular, in animals of the BA+SC1 group, there was a significant decrease in Coef.1 in relation to the BA group ($P < 0.05$) (Fig. 6, *B*), while in the animals of the BA+SC2 group, a significant decrease in Coef.2 ($P < 0.05$) compared with BA was observed (Fig. 6, *B*). Changes in the configuration of the respiratory bronchioles in animals of the BA+SC2 group were due, on the one hand, to an increase in the width of the entrance to the alveolus, and on the other hand, to a decrease in the width of the alveolar passage, which led to significant changes in the structural manifestations of the development of obstructive pulmonary emphysema (Fig. 6, *B*). Thus, the revealed morphofunctional changes in the lungs against the background of SC administration indicate a less pronounced development of pneumosclerosis in both groups of experimental animals.

DAO/histaminase activity. As can be seen from Fig. 6, DAO/histaminase activity in plasma increased significantly by a factor of two, which corresponds to elevated histamine concentrations in the body under this pathology. Enzyme activity did not change in response to sensitization (Sensitization group), while OVA provocations (Provocation group) also caused a significant increase in activity by 2.5 times. Importantly, enzyme activity decreased to normal values when using the drug (BA+SC1 and BA+SC2), which is an irreversible inhibitor of Cu-AOs (Fig. 7).

A completely different character of the change in the level of enzyme activity in the BAL fluid was observed. In animals with developed BA, this indicator was significantly reduced by 4 times. Conditions of both sensitization without provocation and provocation without sensitization also caused a decrease in enzyme activity. SC uptake by the BA+SC1 and BA+SC2 groups of animals revealed an additional inhibitory effect on DAO activity (Fig. 7).

SSAO activity. In the blood plasma of asthmatic animals (BA group), an increase in SSAO activity by 1.5 times compared with the control was noted (Fig. 8). In both negative control groups, no changes in SSAO activity relative to control were observed. As expected, normalization of enzyme activity was detected in the animals of both groups receiving the corrective drug (BA+SC1 and BA+SC2) (Fig. 8).

LOX activity. A significant increase in LOX activity of about 2-fold was also demonstrated in asthmatic guinea pigs (Fig. 9). The introduction of SC to both BA+SC1 and BA+SC2 groups led to a significant decrease in the activity of this enzyme compared to BA. There were no significant changes in the enzyme activity in Sensitization and Provocation groups of guinea pigs (Fig. 9).

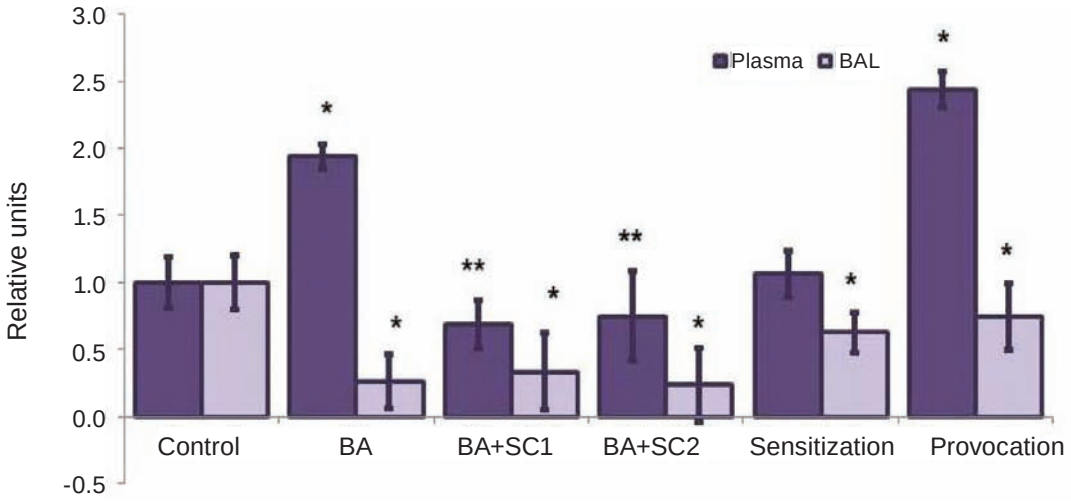


*Fig. 7. DAO activity was differently regulated in plasma and BAL of guinea pigs in all studied groups of animals (M ± SEM, n = 3-5). *P < 0.05 – compared to Control; **P < 0.05 – compared to BA*

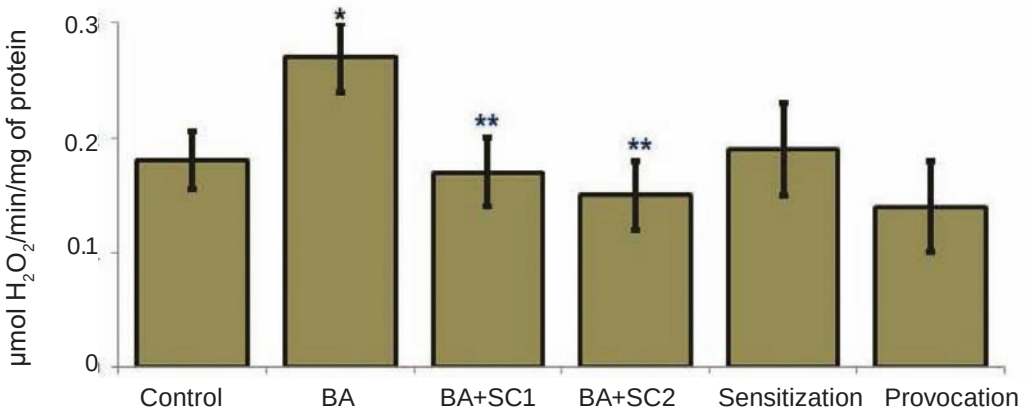


*Fig. 8. Increased SSAO activity in the plasma of asthmatic animals was down-regulated by SC to control values (M ± SEM, n = 3-5). *P < 0.05 – compared to Control; **P < 0.05 – compared to BA*

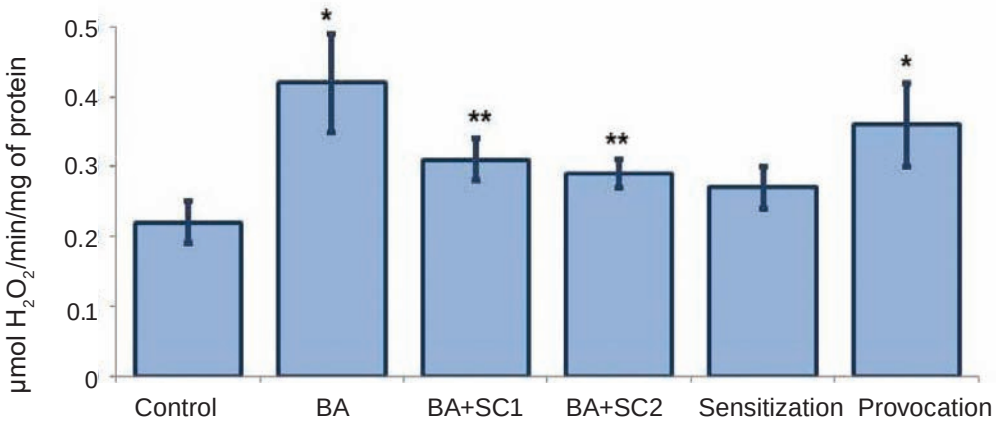


*Fig. 9. Increased LOX activity was suppressed by SC in the lungs of guinea pigs (M ± SEM, n = 3-5). *P < 0.05 – compared to Control; **P < 0.05 – compared to BA*

Discussion

It has been broadly recognized that asthma induced by exposure to inhaled allergens, such as OVA, is characterized by Type I HS, which lead to AHR and structural alterations in the airways. Th2-generated cytokines, IL-4 and IL-13, are responsible for airway inflammation in the initial and late (effector) stages, respectively, in asthma [20]. Because we used the OVA-induced late-stage BA (16-week onset of pathology) model, we observed a significant increase in IL-13 levels in the BA group, as expected. In addition, atopic asthma is accompanied by the development of signs of oxidative/nitrosative stress [21, 22], which were actually found in this study, namely, an increase in the level of FR, iNOS content in the lungs and the number of NO-producing cells in BAL of asthmatic animals. The data of histology and morphometry again revealed the appearance of signs of inflammation and fibrosis in the lung tissue of animals with BA, which, together with the above, indicates the development of acute respiratory hypersensitivity [23]. With an exacerbation in the plasma and BAL of asthmatics, the concentration of biogenic amines, histamine, serotonin and catecholamines (dopamine, adrenaline, and norepinephrine) increases many times, which is accompanied by a violation of adaptive processes and leads to apoptosis and cellular dystrophy. Thus, these compounds, as mediators of inflammatory cells, play an important role in the course of BA, and the level of their concentration correlates with the duration and severity of the disease. In the studied pathology, the balance between the synthesis and utilization of biogenic amines is disturbed, which is a consequence

of the absence or dysregulated activity of the corresponding enzymes [24]. Despite all the research results to date, the biochemical features of the metabolism of biogenic amines, one of the common causes of inflammatory processes, as well as the enzymes of their catabolic transformation, are not well understood.

In the model of the late stage of BA induced by OVA, we revealed an increased level of DAO/histaminase activity in the blood plasma of guinea pigs, which most likely corresponds to an increase in the concentration of histamine in this pathology. A completely different pattern was observed for this enzyme in the BAL fluid. The activity of the enzyme was significantly reduced in animals with developed asthma, although a multiple increase in the level of histamine in the BAL is known in this pathological condition. Such a mismatch between the level of enzyme activity and its substrate concentration may be due to the fact that histaminase is not directly activated in response to endogenous histamine release. It is also possible that the expression of the enzyme in the epithelium of the respiratory tract of asthmatic animals is impaired, which may be the main cause of bronchospasm activity in asthma. This assumption is supported by data from a research group that has effectively used herbal DAO in anti-asthmatic therapy. Thus, a deficiency of this enzyme may be a key link in the development of asthmatic symptoms (cough, shortness of breath, wheezing, etc.) [25].

Under conditions of severe oxidative stress in BA, activation of MAPK and NF-κB leads to the increased production of inflammatory cytokines, chemokines and adhesion molecules, including SSAO/VAP-1 [6, 26]. In the plasma of asthmatic animals, we also observed a significant increase in SSAO activity, which is consistent with published data. The release of pro-inflammatory mediators in BA causes dysfunction and apoptosis of lung epithelial and endothelial cells, which acquire the phenotypic properties of mesenchymal cells (myofibroblasts and fibroblasts). Actively proliferating fibroblasts secrete ECM components such as collagen and elastane in excess. Subsequent intermolecular cross-linking between these molecules mediated by LOX leads to the formation of fibrosis foci. Thus, pulmonary fibrosis in BA is associated with increased LOX activity [27, 28], which we observed in the present study.

As expected, we found that SC, regardless of the route of its administration (drinking/inhalation), not only significantly reduced the levels of FR and AOs activity, but also attenuated the signs of inflammation and pneumosclerosis/fibrosis observed on histological preparations [18]. At the same time, SC, as an aldehyde trap and an inhibitor of Cu-AOs, did not affect the content of pro-inflammatory mediators, IL-4 and IL-13, and nitrosative stress markers.

Conclusion. Taken together, the results obtained confirm the reliability of the development of allergic BA in guinea pigs that correlate with changes in the Cu-AOs activity profiles in the course of pathology progression. It indicates the direct involvement of Cu-AOs in pathophysiological processes associated with airway hypersensitivity and remodeling induced by the exposure to the inhaled allergen OVA as well as the ability of SC to act as a potential drug in complex anti-asthmatic therapy.

Acknowledgments. The authors express their acknowledgments to the ex-head of our research group untimely deceased Dr. Serhii Shandrenko who generated the primary research idea, planned the experiment, promoted and directed the work, as well as carried out the EPR analysis. We also thank Olena Parilova for providing an important piece of the experimental data on quantitative of ILs, iNOS, and NO-generating cells analysis

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/

Funding. The present study was supported by the National Academy of Sciences of Ukraine for R&D ‘Comparative study of the biological action of endogenous aldehydes as metabolism regulators and factors of its disorders under different pathological conditions’ (No 0110U002700).

УЧАСТЬ Cu-ВМІСНИХ АМІНООКСИДАЗ У РОЗВИТКУ ПАТОЛОГІЇ ЛЕГЕНІ ЗА УМОВ ОВАЛЬБУМІН-ІНДУКОВАНОЇ БРОНХІАЛЬНОЇ АСТМИ У МУРЧАКІВ

О. О. Гудкова[1], С. П. Луговський[2], Л. Б. Дробот[1], Н. В. Латишко[1]

[1]Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
[2]Інститут геронтології ім. Д. Ф. Чеботарьова НАМН України, Київ;
✉e-mail: ogudkova@biochem.kiev.ua

Атопічна бронхіальна астма, що розвивається як імунна відповідь організму на дію алергену, супроводжується хронічним запаленням та фіброзом легеневої тканини, процесом відомим як ремоделювання дихальних шляхів. Для підтвердження залучення у даний патологічний процес родини Cu-вмісних амінооксидаз, семікарбазидчутливої амінооксидази (SSAO), диамінооксидази (DAO) та лізилоксидази (LOX), ми використали їх необоротний інгібітор семікарбазид та модель овальбумін-індукованої бронхіальної астми у мурчаків. По завершенні 16 тижнів після ініціювання захворювання спостерігалося достовірне підвищення активності прозапальних SSAO та DAO у плазмі тварин із бронхіальною астмою (у 1,6 та 2 рази відповідно) в порівнянні з контролем. Введення семікарбазиду астматичним тваринам із питвом або інгаляційно достовірно знижувало ці показники порівняно з групою тварин, що не отримували лікування. Крім того, спостерігалось значне підвищення вмісту прозапального цитокіну IL-13 та рівня активності LOX у легеневій тканині астматичних тварин, що свідчило про розвиток запалення дихальних шляхів та легеневого фіброзу. Вживання семікарбазиду мурчаками-астматиками призводило до нормалізації активності LOX. Гістологічні дослідження підтвердили, що семікарбазид послаблював морфопатологічні зміни в легеневій тканині астматичних тварин. Таким чином, отримані дані свідчать про безпосередню участь досліджуваних ензимів у прогресуванні патологічних процесів за умов атопічної бронхіальної астми, а також про можливість використання семікарбазиду як потенційного лікарського засобу у комплексній протиастматичній терапії.

Ключові слова: атопічна бронхіальна астма, семікарбазид, семікарбазидчутлива амінооксидаза, диамінооксидаза/гістаміназа, лізилоксидаза, IL-13, оксид азоту.

References

1. Boulet LP, Reddel HK, Bateman E, Pedersen S, FitzGerald JM, O'Byrne PM. The Global Initiative for Asthma (GINA): 25 years later. *Eur Respir J.* 2019; 54(2): 1900598.
2. Antwi AO, Obiri DD, Osafo N. Stigmasterol Modulates Allergic Airway Inflammation in Guinea Pig Model of Ovalbumin-Induced Asthma. *Mediators Inflamm.* 2017; 2017: 2953930.
3. Gour N, Wills-Karp M. IL-4 and IL-13 signaling in allergic airway disease. *Cytokine*. 2015; 75(1): 68-78.
4. Refaat MM, Abdel-Rehim AS, Elmahdi AR, Mohamed NA, Ghonaim SS. Diamine oxidase enzyme: a novel biomarker in respiratory allergy. *Int Forum Allergy Rhinol*. 2019; 9(12): 1478-1484.
5. Ostronosova NS, Volozhin AI. Changes in content of biologically active amines in plasma and blood cells in bronchial asthma. *Patol Fiziol Eksp Ter.* 2005; (4): 11-13. (In Russian).
6. Schilter HC, Collison A, Russo RC, Foot JS, Yow TT, Vieira AT, Tavares LD, Mattes J, Teixeira MM, Jarolimek W. Effects of an anti-inflammatory VAP-1/SSAO inhibitor, PXS-4728A, on pulmonary neutrophil migration. *Respir Res.* 2015; 16(1): 42.
7. Ramis J, Middlewick R, Pappalardo F, Cairns JT, Stewart ID, John AE, Naveed SUN, Krishnan R, Miller S , Shaw DE, Brightling CE, Buttery L, Rose F, Jenkins G, Johnson SR, Tatler AL. Lysyl oxidase like 2 is increased in asthma and contributes to asthmatic airway remodelling. *Eur Respir J.* 2022; 60(1): 2004361.
8. Vasconcelos LHC, Ferreira SRD, Silva MDCC, Ferreira PB, de Souza ILL, Cavalcante FA, da Silva BA. Uncovering the Role of Oxidative Imbalance in the Development and Progression of Bronchial Asthma. *Oxid Med Cell Longev.* 2021; 2021: 6692110.
9. Zhou DY, Du Q, Li RR, Huang M, Zhang Q, Wei GZ. Grape seed proanthocyanidin extract attenuates airway inflammation and

hyperresponsiveness in a murine model of asthma by downregulating inducible nitric oxide synthase. *Planta Med*. 2011; 77(14): 1575-1581.
10. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev.* 2004; 84(3): 731-765.
11. Hudkova O, Krysiuk I, Drobot L, Latyshko N. Rhabdomyolysis attenuates activity of semicarbazide sensitive amine oxidase as the marker of nephropathy in diabetic rats. *Ukr Biochem J*. 2022; 94(1): 23-32.
12. Hudkova OO, Krysiuk IP, Kishko TO, Popova NM, Drobot LB, Latyshko NV. Semicarbazide diminishes the signs of bleomycin-induced pulmonary fibrosis in rats. *Ukr Biochem J*. 2021; 93(5): 72-81.
13. Farah CS, King GG, Brown NJ, Downie SR, Kermode JA, Hardaker KM, Peters MJ, Berend N, Salome CM. The role of the small airways in the clinical expression of asthma in adults. *J Allergy Clin Immuno*l. 2012; 129(2): 381-387.
14. Ueda T, Niimi A, Matsumoto H, Takemura M, Hirai T, Yamaguchi M, Matsuoka H, Jinnai M, Muro S, Chin K, Mishima M. Role of small airways in asthma: investigation using high-resolution computed tomography. *J Allergy Clin Immunol*. 2006; 118(5): 1019-1025.
15. Cardiff RD, Miller CH, Munn RJ. Manual hematoxylin and eosin staining of mouse tissue sections. *Cold Spring Harb Protoc.* 2014; 2014(6): 655-658.
16. Avtandilov GG. Problems of pathogenesis and pathological diagnosis of diseases in the aspects of morphometry. M.: Medicine, 1984. 288 p. (In Russian).
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72(1-2): 248-254.
18. Almohawes ZN, Alruhaimi HS. Effect of Lavandula dentata extract on Ovalbumin-induced Asthma in Male Guinea Pigs. *Braz J Biol*. 2020; 80(1): 87-96.
19. Laboratory Animal Medicine. (Third Edition, ed. By Fox GJ, Otto GM, Whary MT, Andersen LC, Pritchen-Corning KT). Academic Press. 2015, 1750 p.
20. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. *Immunity.* 2019; 50(4): 975-991.
21. Prado CM Martins MA, Tibério IFLC. Nitric oxide in asthma physiopathology. *ISRN Allergy.* 2011; 2011: 832560.
22. Cho YS, Moon HB. The role of oxidative stress in the pathogenesis of asthma. *Allergy Asthma Immunol Res.* 2010; 2(3): 183-187.
23. Allergy Essentials. (Second Edition, ed by O'Hehir RE, Holgate ST, Hershey GKK, Sheikh A). Elsevier Inc, 2022. 323 p.
24. Sánchez-Jiménez F, Ruiz-Pérez MV, Urdiales JL, Medina MA. Pharmacological potential of biogenic amine-polyamine interactions beyond neurotransmission. *Br J Pharmacol*. 2013; 170(1): 4-16.
25. Masini E, Bani D, Marzocca C, Mateescu MA, Mannaioni PF, Federico R, Mondovì B. Pea seedling histaminase as a novel therapeutic approach to anaphylactic and inflammatory disorders. A plant histaminase in allergic asthma and ischemic shock. *Sci World J*. 2007; 7: 888-902.
26. Li H, Du S, Niu P, Gu X, Wang J, Zhao Y. Vascular Adhesion Protein-1 (VAP-1)/Semicarbazide-Sensitive Amine Oxidase (SSAO): A Potential Therapeutic Target for Atherosclerotic Cardiovascular Diseases. *Front Pharmacol*. 2021; 12: 679707.
27. Zibadi S, Vazquez R , Moore D, Larson DF, Watson RR. Myocardial lysyl oxidase regulation of cardiac remodeling in a murine model of diet-induced metabolic syndrome. *Am J Physiol Heart Circ Physiol*. 2009; 297(3): H976-H982.
28. Li S, Yang X, Li W, Li J, Su X, Chen L, Yan G. N-acetylcysteine downregulation of lysyl oxidase activity alleviating bleomycin-induced pulmonary fibrosis in rats. *Respiration.* 2012; 84(6): 509-517.