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Ultrastructural changes of lung tissue under conditions of experimental obesity and smoking

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Abstract

Obesity can cause respiratory disorders inflicted by adipose tissue accumulation and the numerous cytokines adipocytes produce. Smoking is, first of all, associated with a wide range of lung diseases characterized by diffuse changes in the lung tissue and a decrease in the respiratory volume of the lungs. The study aimed to investigate the ultrastructural changes in the lungs of sexually mature male rats under conditions of experimental obesity and smoking. The total sample of experimental animals consisted of 120 rats, divided into four groups: the control group (*n*=30) – conditionally healthy rats fed on a standard diet; a group of rats subjected to isolated exposure to tobacco smoke (*n*=30); a group of experimentally obese rats (*n*=30) and a group of experimentally obese rats simultaneously exposed to tobacco smoke (*n*=30) – feeding using a high-fat diet with exposure to a chamber with tobacco smoke. The revealed ultrastructural features of the lungs in the group of rats with experimental obesity and the group of rats with experimental obesity that were simultaneously exposed to tobacco smoke did not differ qualitatively, which indicates that pathological changes in the ultrastructure of the lung tissue developed regardless of the presence or absence of a direct damaging effect on the lung tissue of passive smoking.

Keywords: lung diseases, experimental model of obesity and smoking, obesity, tobacco smoke, ultrastructural changes of lung, lung tissue.

Introduction

Obesity and smoking are strongly associated with several diseases, including coronary heart disease, cerebrovascular disease, and cancer [1–5]. At the same time, obesity is a risk factor for chronic diseases, and smoking, in turn, is associated with both chronic diseases and obesity [6].

Obesity causes several systemic complications associated with significant disorders of organs and tissues. These complications lead to respiratory disorders caused by adipose tissue accumulation and numerous cytokines produced by adipocytes [7]. Smoking, on the other hand, is primarily associated with the occurrence of a wide range of lung diseases, including diseases of the respiratory tract with vascular disorders and interstitial lung diseases, which are characterized by diffuse changes in the lung tissue and a decrease in the respiratory volume of the lungs [8].

Considering the pathogenesis of the development of lung diseases, it should also be noted that the impact of obesity on the respiratory system is often underestimated. However, the accumulation of adipose tissue in the body causes changes in the physiology of breathing with subsequent violations of various lung function parameters. Different regularities of the distribution of adipose tissue in the body differentially affect the role of the respiratory system [9].

Obesity can play a significant role in the pathogenesis of lung diseases. This impact is particularly significant given the course of the coronavirus disease 2019 (COVID-19). A study by Singh *et al.* [10] established that obesity has a direct relationship with the degree of severity of this disease and mortality from COVID-19. The results of the study showed that patients with obesity are 1.5 times more likely to suffer from severe complications and 1.09 times more likely to die compared to patients with average body weight.

In particular, this can be explained by an increase in the proinflammatory mediators produced by adipose tissue, which contributes to the development of low-grade systemic inflammation. In animal models, it has been demonstrated that inflammatory responses in the lung, in turn, influence the production of adipocytokines, leptin and adiponectin, cytokines, acute phase proteins, and other mediators produced by adipose tissue, which can promote immune responses in the lung. Increase in the mass of adipose tissue can also affect the susceptibility to lung infections, increase the inflammatory processes of the lung tissue associated with the influence of environmental factors, and increase the obstruction of the respiratory tract in case of already existing lung diseases [10]. The above determines the relevance of this study.

Aim

The purpose of this study was to determine the characteristics of electron microscopic changes in the lungs of sexually mature male rats under conditions of experimental obesity and tobacco smoke.

Materials and Methods

The study on laboratory animals was carried out in line

with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18 March 1986, Directive 2010/63/EU of the European Parliament, and the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes, the Law of Ukraine "On the protection of animals from maltreatment". The animals were kept under the conditions of an accredited vivarium based at Danylo Halytsky Lviv National Medical University, Lviv, Ukraine for four months.

Animals had free and unlimited access to food and water throughout the experiment.

An overdose of ethyl ether vapors euthanized animals. After death, lung necropsies were collected for histological examination.

The total sample of experimental animals included 120 rats. Four groups of animals were formed randomly:

(*1*) The control group (CL group, n=30) – conditionally healthy rats, animals were fed according to a standard diet;

(*2*) A group of rats exposed to tobacco smoke without simulation of experimental obesity (S group, n=30) – feeding was carried out according to a standard diet with exposure in a chamber with tobacco smoke for four minutes twice a day, five days a week;

(*3*) A group of rats with the simulation of experimental obesity (O group, n=30) was kept on a high-fat diet (HFD);

(*4*) A group of rats with simulation of experimental obesity and simultaneous exposure to tobacco smoke (OS group, n=30) – feeding using a HFD with exposure in a chamber with tobacco smoke for four minutes twice a day, five days a week.

The total weight of a daily portion of a standard diet for one individual was 43.5 g, and the fat percentage was increased monthly by 10%; therefore, for the first month of the experiment, it was 30%, and for the last one – 60%, respectively. In O and OS groups, the total weight of the food portion per individual was increased by 1 g each week: during the first week of the study, one individual received 43.5 g of portioned ration, and in the last week, the weight of the portion was 58.5 g, respectively.

To evaluate changes in murinometric data, body weight (weighing was carried out monthly to assess body weight increase) and body mass index (BMI) were calculated.

BMI was calculated using the following formula: $BMI=m/l^2$, where m is body weight [g], and l is body length [cm].

Considering the capability of tobacco smoke to play an important role in the development and progression of several benign and malignant diseases of the lungs, the use of the passive smoking model was considered a necessary stage of the experiment [11].

To simulate tobacco smoke, experimental animals were placed in a smoking chamber with walls made of transparent organic glass, which allowed observing the animals and their behavior during the passive smoking process. The number of animals simultaneously placed in the chamber for the simulation of tobacco smoke depended on the body weight of the rats. Thus, the floor area per animal varied between 200 cm^2 (for the rats weighing up to 200 g) and 450 cm^2 (for those weighing over 400 to 600 g, respectively). Cigarettes were installed in polylactide (PLA)-type plastic mouthpieces made with the help of the Easy Threed 3-D printer. Winston-brand cigarettes were used in the experiment, with a tar content of 6 mg and a nicotine content of 0.5 mg. Cigarette smoke was gradually drawn into the chamber using an air pump.

The collection of lung fragments for the study was carried out after the euthanasia of the animals with ethyl ether vapors.

Fragments of white rat lungs were cut off using a blade and immediately placed in a large drop of 2% osmium tetroxide (OsO_4) solution in 0.1 M phosphate-buffered saline (pH 7.36) with sucrose. Subsequently, 0.5×0.1×0.1 cm in size strips were cut with a blade degreased in acetone and quickly transferred to another drop of fixing solution of the same composition placed on a plate of dental wax lying on an ice slab. Cube-shaped fragments (fabric blocks) with a volume of 1 mm^3 were cut from the strips. After that, the tissue blocks were fixed in a 1.5% solution of OsO_4 (SPI-Chem™, USA) in a 0.2 M solution of sodium cacodylate (Cacodylic acid sodium salt, Fluka) at pH 7.2 for 2–2.5 hours in the cold. After that, they were washed with a buffer solution of the same composition (four fresh portions for 15 minutes each). For dehydration, tissue blocks washed from fixative residues were immersed in increasing concentrations of ethanol (50°, 70°, 90°, and absolute) for 30 minutes each. They were conducted through propylene oxide (Fluka) for 10 minutes. The scheme of carrying out in solutions of ethanol: 40% – three fresh portions of 10 minutes each; 70% – three fresh portions of 10 minutes each; 96% – two fresh portions of 20 minutes each. The scheme of carrying out in acetone: "extra clean" brand acetone – six fresh portions of 15 minutes each. Subsequently, the samples were cast in Epon 812 epoxy resins (Fluka); the dehydrated fragments were placed in a mixture of Epon–Araldite epoxy resins. The tissue blocks were put in Epon–Araldite by passing through solutions of increasing resin concentration. For better percolation, the material and the resin–acetone mixture were placed in the nests of a centrifuge at 10 revolutions per minute. After that, the tissue blocks were immersed in Epon–Araldite, which was contained in glycerin capsules. Polymerization of the material was carried out in stages at 36°C, 45°C, and 60°C for 24 hours at each temperature regime [12]. Silver-stained sections were selected for the study. Sections were contrasted in a Reynolds contrast in a 1% uranyl acetate solution. Sections were examined using a TEM-100 transmission electron microscope. Photographs were taken using a Sony digital camera at magnifications of ×4000–×18 000 on the microscope screen.

Data analysis

Statistical processing of the results was carried out using the Statistica 12 software package. The mean (average) value and the standard error of the mean (SEM) were calculated. Differences in average values were considered significant with a probability level of at least 95% (p<0.05).

Results

During the experiment, the body weight of rats in the CL group increased by 36.07±2.29 g (14.77% weight gain), in the S group – by 31.50±0.92 g (13.33% weight gain), in the O group – by 141.33±3.18 g (54.65% weight gain),

and in the OS group – by 132.63±0.45 g (52.51% weight gain). The most significant increase in body weight was found in the O group.

At the end of the experiment (the fourth month of the study), a significant difference in the average body weight of rats in the CL group compared to the O and OS groups was established (p<0.001). Significant differences in body weight were also found when comparing S and O, S and OS groups among themselves (p<0.001). On the other hand, the mean body weight of rats in the CL, S, O, and OS groups was not significantly different (p=0.16 and p=0.11, respectively).

Significant differences in BMI were found when comparing the CL group with the S (p=0.05), O, and OS groups, as well as when comparing the S and O, and S and OS groups, respectively (p<0.001). At the same time, BMI in the O and OS groups probably did not differ (p=0.21). Murinometric data are given in Table 1. As can be seen, murinometric measurements showed that the average weight of rats in the CL group at the end of the experiment was 280.20±6.62 g, BMI – 0.60±0.01 g/cm^2.

Table 1 – ***Murinometric data of the experimental groups***

Indicator	CL Group (1)	S Group (2)	O Group (3)	OS Group (4)	p_{1-2}	p_{1-3}	p_{1-4}	p_{2-3}	p_{2-4}	p_{3-4}
Body weight [g]	280.20±6.62	267.87±5.59	399.97±7.57	385.2±4.82	0.16	<0.001*	<0.001*	<0.001*	<0.001*	0.11
Body length [cm]	21.57±0.10	21.77±0.09	21.76±0.09	21.68±0.09	0.15	0.16	0.41	0.98	0.49	0.52
BMI [g/cm^2]	0.60±0.01	0.57±0.01	0.85±0.02	0.82±0.01	0.05*	<0.001*	<0.001*	<0.001*	<0.001*	0.21

BMI: Body mass index; CL: Control; O: Simulation of experimental obesity; OS: Simulation of experimental obesity and simultaneous exposure to tobacco smoke; S: Tobacco smoke without simulation of experimental obesity. *Statistically significant differences (p≤0.05).

Ultrastructural changes of the lung tissue in the experimental groups showed the following changes. No significant pathological changes were found in the lung tissue of the rats of the CL group (Figure 1). Figure 1 visualizes a fragment of the alveolar–capillary barrier, which includes the interalveolar membrane with clearly visualized alveolar and hemocapillary lumens. The capillary was lined by endothelial cells with distinct nuclei, a small number of round or oval mitochondria with well-defined cristae, and cisternae of the granular endoplasmic reticulum (ER) identified in the endotheliocyte cytoplasm. Slightly deformed erythrocytes have been visualized in the lumen of the capillary. The interstitial space between the epithelium and endothelium was slightly expanded. Type 1 alveolocytes with long and thin processes were located on the basal membrane of the alveolus. The components of the alveolar–capillary barrier of intact animals had a typical ultramicroscopic structure.

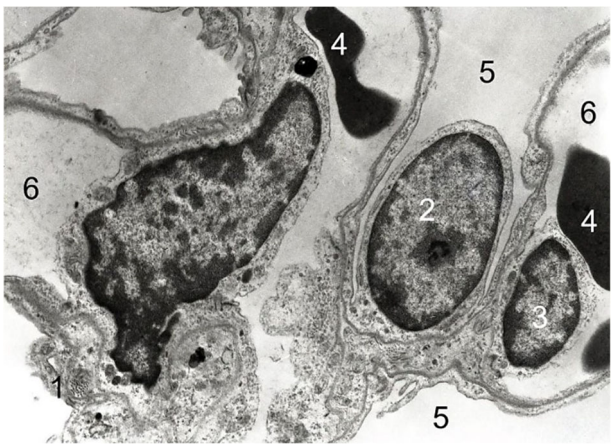

Figure 1 – ***Ultrastructural organization of a fragment of the interalveolar membrane of a white laboratory rat lung, CL group. Magnification: ×6000. 1: Single fibrils of collagen fibers in the interstitium; 2: Type 1 alveolocyte; 3: Endotheliocyte; 4: Erythrocytes; 5: Alveolar lumen; 6: Capillary lumen.***

Only in the interalveolar membranes was the presence of single disorganized fibrils of collagen fibers located in the interstitial space observed in a few sites (Figure 2). The capillary walls were lined by endothelial cells with clear nuclei and a large amount of heterochromatin near the nucleolemma, multiple mitochondria with mitochondrial cristae, and ER cisternae. A segmented neutrophil granulocyte, the nucleus of which consists of two segments, was identified in the lumen of the capillary. Its cytoplasm was rich in lysosomes (proteolytic granules). The outer cytoplasmic membrane of the cell was almost destroyed, which indicates the activation and degranulation of neutrophils. Given the absence of other signs of an immune response, inflammatory changes, necrosis, or apoptosis of alveolar–capillary barrier cells, degranulated neutrophils in the capillary lumen haven't been considered evidence of an active pathological process. Single disorganized collagen fibers in the interalveolar membrane of the focus selected for study can be interpreted as an indirect sign of a previous acute injury. This finding can be considered insignificant given this tissue fragment's small number of collagen fibers. The non-specificity of the changes and the variability of the etiopathogenetic triggers of acute damage do not allow us to determine the genesis of the findings described above confidently.

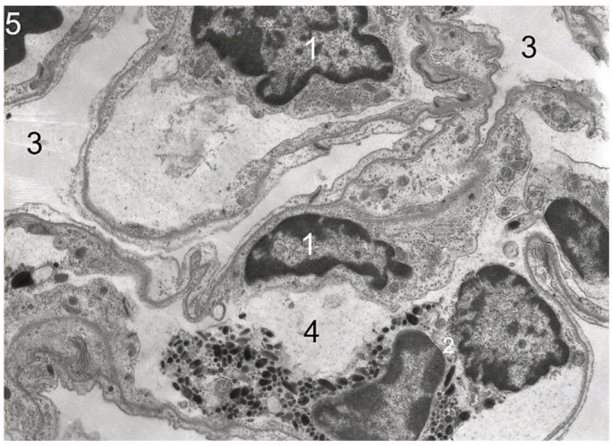

Figure 2 – ***Ultrastructural organization of a fragment of the interalveolar membrane of a white laboratory rat lung, CL group. Magnification: ×6000. 1: Endotheliocytes; 2: Degranulated segmented neutrophil with a destroyed cytoplasmic membrane; 3: Alveolar lumen; 4: Capillary lumen; 5: Erythrocyte.***

The average weight of rats in S group, exposed to tobacco smoke without obesity simulation, at the end of the experiment was 267.87±5.59 g, BMI – 0.57±0.01 g/cm^2.

Several pathological ultrastructural changes in the lung tissue were found in the rats of experimental S group. Among the pathological findings, we have noted a moderate number of collagen fibrils, which formed single bundles in the interstitium of the interalveolar membranes. Signs of destruction of alveolocytes were also visualized in combination with the initial manifestations of wall thrombus formation (Figure 3). In particular, Figure 3 showed a thin-walled capillary covered with endothelial cells. A segmented neutrophil granulocyte with multiple proteolytic granules and an erythrocyte was visualized in the lumen of the capillary. There were ultrastructural signs of degranulation of neutrophils, particularly segmental destruction of plasmalemma. The nucleus of neutrophils consists of two segments. Two plates with a fine-grained cytoplasm structure containing granules of two types – high and medium optical density – were visualized in the lumen of the capillary. The presence of the above-described blood elements in a certain fragment of the capillary lumen indicates the beginning of thrombus formation. Clear moderate collagenization with forming a small single bundle was determined in the interstitium. Destructive changes of type 2 alveolocytes were observed in the form of violation of the integrity of the cytoplasmic membrane almost along the entire length available for assessment. In the cytoplasm of type 2 alveolocytes, multiple ribosomes, lamellar bodies, mitochondria with initial signs of degradation, a visible change in electron density, and small pinocytotic vesicles were identified.

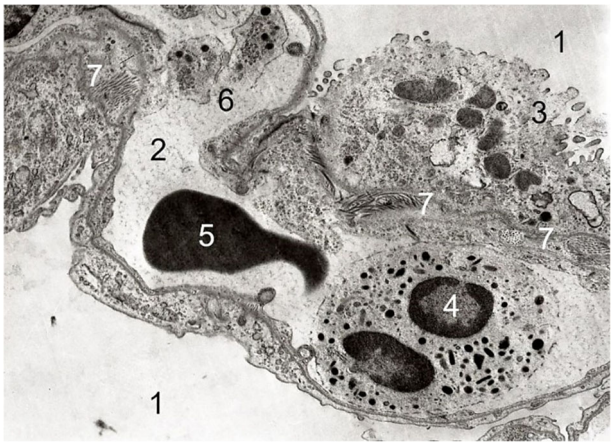

Figure 3 – ***Ultrastructural organization of a fragment of the interalveolar membrane of a white laboratory rat lung, S group. Magnification: ×6000. 1: Alveolar lumen; 2: Capillary lumen; 3: Type 2 alveolocyte; 4: Segmented neutrophil; 5: Erythrocyte; 6: Platelets; 7: Bundle of collagen fibers.***

An increased number of secretory granules of the bronchial epithelium, significant tortuosity of the own lamina of the bronchi and bronchioles, which was probably associated with bronchospasm, a moderate number of disorganized collagen fibrils without signs of the formation of fibrous structures. In some places, plasma cells were detected, which may indicate a weak immune response (Figure 4). A moderately increased number of secretory granules and multiple mitochondria with clearly visible mitochondrial cristae were visualized in the bronchial epithelium. The *lamina propria* was tortuous, with individual indistinct fibrils of collagen fibers. Smooth muscle cells were defined under the *lamina propria*. Below is a fibroblast with an apparent nucleus surrounded by collagen bundles. In the lower edge of Figure 4, there was a marked fragment of a plasma cell with clearly visible cisterns of the ER with a significant number of ribosomes.

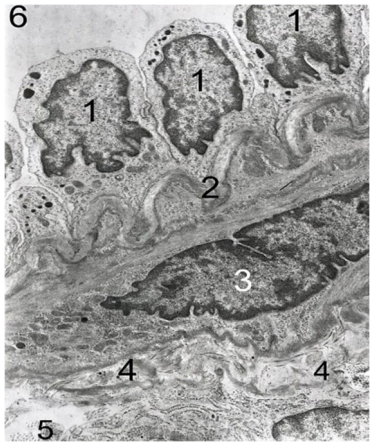

Figure 4 – ***Ultrastructural organization of a fragment of the bronchial wall of a white laboratory rat lung, S group. Magnification: ×6000. 1: Bronchial epithelium; 2: Lamina propria; 3: Fibroblast; 4: Disorganized fibrils of collagen fibers; 5: Plasma cell; 6: Bronchial lumen.***

At the end of the experiment, the average weight of rats in O group was 399.97±7.57 g, BMI – 0.85±0.02 g/cm^2. Significant pathological changes were found in the lung tissue of experimental animals of O group: thickening of the interstitium of interalveolar membranes with pronounced collagenization, formation of multiple collagen bundles (Figure 5); signs of a significant increase in the secretory activity of the bronchial epithelium, fibrotization of the *lamina propria* with the accumulation of collagen bundles (Figure 6).

Figure 5 partially shows the elements of the alveolar–capillary barrier: alveolar lumen, capillary, and interalveolar septum with significant collagenization, evidenced by the accumulation of an excessive number of collagen fibers with a tendency to form medium-sized bundles. In the interstitium, there were fibroblasts with areas of loss of the plasmalemma membrane and migration of organelles into the interstitial space.

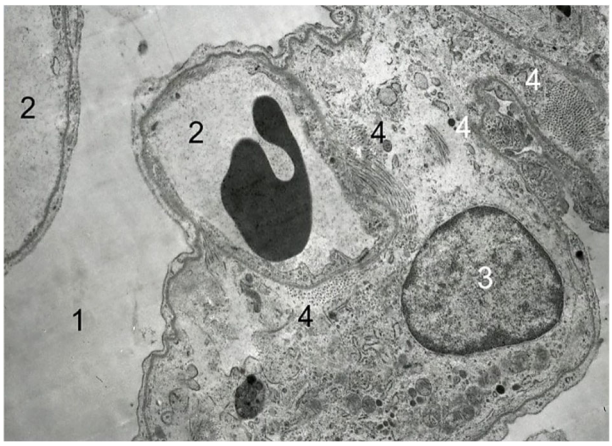

Figure 5 – ***Ultrastructural organization of a fragment of the interalveolar membrane of a white laboratory rat lung, O group. Magnification: ×6000. 1: Alveolar lumen; 2: Capillary lumen; 3: Fibroblast; 4: Collagen bundles.***

Bronchial epithelium with a significantly increased number of enlarged secretory granules was visualized on the basal membrane. The basement membrane and *lamina propria* were pathologically altered due to excessive collagenization, as evidenced by multiple collagen bundles. Smooth muscle cells with multiple glycogen inclusions can also be found on the lower edge of Figure 6.

At the end of the experiment, the average weight of rats in the OS group was 385.2±4.82 g, BMI – 0.82±0.01 g/cm^2.

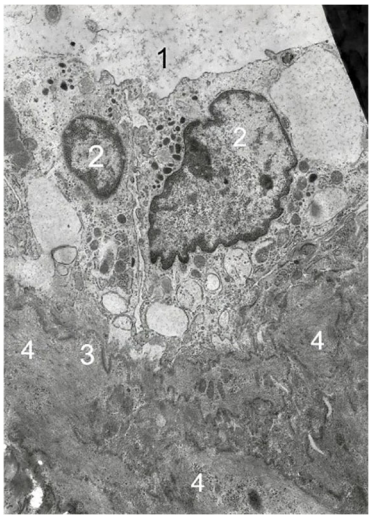

Figure 6 – ***Ultrastructural organization of a fragment of the bronchial wall of a white laboratory rat lung, O group. Magnification: ×6000. 1: Bronchial lumen; 2: Bronchial epithelium; 3: Basal membrane; 4: Bundles of collagen fibers.***

The most noticeable pathological changes in the lung tissue in the OS group were noted. They were characterized by signs of diffuse collagenization of interalveolar membranes and fragments of bronchial walls (Figures 7 and 8). These changes can be seen in Figure 7, which shows the lumen of the interalveolar membrane's alveoli, capillaries, and interstitium. The capillary lumen typically had deformed erythrocytes, optically granular blood plasma, and a platelet fragment. An excessive amount of collagen fibers forms large bundles in the interalveolar membrane's interstitial space. Single fibrils of collagen fibers were not visualized.

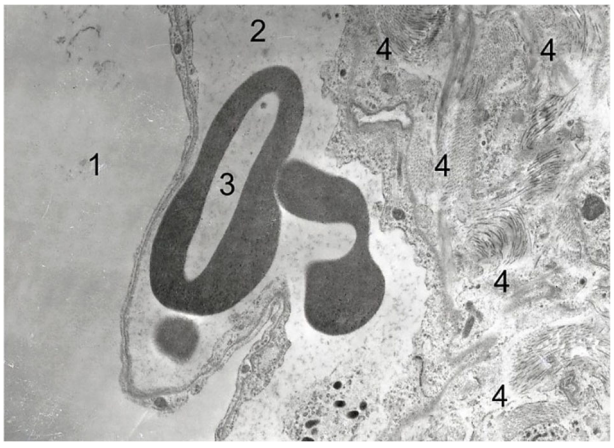

Figure 7 – ***Ultrastructural organization of a fragment of the interalveolar membrane of a white laboratory rat lung, OS group. Magnification: ×6000. 1: Alveolar lumen; 2: Capillary lumen; 3: Erythrocyte; 4: Diffuse collagenization of the interstitium.***

Bronchial epithelium with signs of fragmentary desquamation of the apical surface of cells was observed. The destruction of the cytoplasmic membrane was determined by an excessive number of significantly increased secretory granules that occupy almost the entire area of the cells. The elastic membrane, basement membrane, and smooth muscle cells were characterized by excessive collagenization of the *lamina propria* with the formation of multiple large collagen bundles (Figure 8).

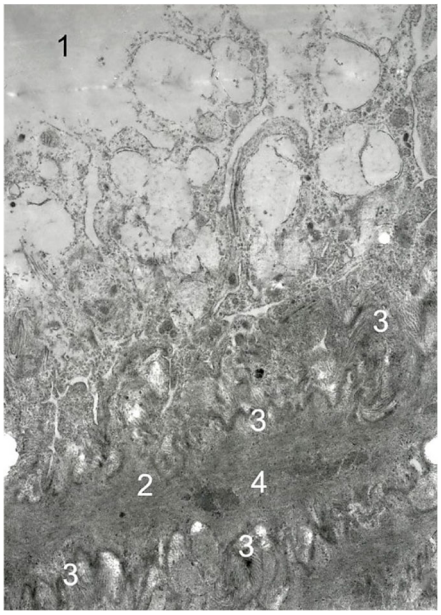

Figure 8 – ***Ultrastructural organization of a fragment of the bronchial wall of a white laboratory rat lung, OS group. Magnification: ×6000. 1: Bronchial epithelium; 2: Basal membrane; 3: Collagen bundles; 4: Smooth muscle cells.***

Discussions

An important measure is the preservation of the functional capacity of the lungs throughout adult life, preventing the development of respiratory diseases (including chronic ones), which are currently one of the most serious healthcare problems in the world [11]. However, an equally important problem of the health care system in the modern world is overweight and obesity in adults and children, since these are not only factors that significantly reduce the quality of physical and psychological health but also risk factors for the development of a significant number of diseases of organs and systems, in particular, the respiratory system [12].

It has been proven that rapid weight gain, overweight, and obesity in adulthood hurt lung function. Previous population-based and occupational cohort studies have demonstrated that the overweight in adulthood is associated with reduced lung function and an increased rate of decline in lung function, regardless of age and smoking status [9, 13]. The available literature regarding the relationship between the development of chronic obstructive pulmonary disease (COPD), including chronic bronchitis, emphysema, pneumosclerosis, and obesity, was conflicting.

According to some studies, general and abdominal obesity appeared to be independent factors associated with developing respiratory symptoms in adults. In addition, diseases such as COPD and asthma were more common

in overweight and obese patients [14]. Also, the issue of changes in lung tissue associated with obesity remains unclear today, namely, the possibility of their occurrence regardless of the metabolic status of patients [3]. It was known that the lungs are the main target organ for smoking. Chronic obstructive bronchitis of a smoker, lung cancer, smoking-associated pulmonary fibrosis, and smoker's emphysema were far from a complete spectrum of lung diseases associated with long-term smoking. Both obesity and smoking were related to the launch of proinflammatory mediators, the launch of collagenolysis under the influence of metalloproteinases, and subsequent remodeling of lung tissue with the development of fibrosis and emphysema. In addition, cigarette smoke is a recognized carcinogen that stimulates squamous cell metaplasia and the development of squamous cell carcinoma. As we can see, smoking and obesity are important issues today. At the same time, there are quite a few publications devoted to the combination of smoking and obesity on lung tissue and the role of these factors in the development of chronic lung pathology [3, 6].

Therefore, we chose experimental models of obesity and smoking to determine the influence of these factors both in isolation and in a combined effect on the lung tissue of sexually mature male rats.

Changes in the lung tissue of S group rats fed a standard diet and exposed to tobacco smoke in an experimental model of smoking have been confirmed by numerous cigarette smoke studies. We found an increase in the number of secretory granules and disorganized collagen fibrils in the *lamina propria*. The chosen experimental model of passive smoking explains the described changes.

Smoking affects lung tissue, particularly the airways, respiratory system, and pulmonary vessels, with the development of a complex set of changes characteristic of COPD [1, 12, 15]. Features of the ultrastructural changes of the lungs associated with smoking have been sufficiently studied in humans and various experimental animal models.

Magnani *et al.* (2012) described changes in the lung tissue corresponding to those we observed in this study, in particular, degenerative changes of alveolocytes, as well as the presence of thin, disorganized collagen fibrils in the interalveolar membranes with the formation of aggregates (bundles) in various areas of the interstitium [13]. According to a study by Kwon *et al.* (2012), exposure to varying volumes of tobacco smoke can cause similar changes in the respiratory tract of experimental animals. These changes include a decrease in the number of epithelial cells, destruction of these cells, uneven distribution of secretory granules, and thickening of the bronchial walls [16]. Excessive collagenization can be seen as an early sign of exposure to tobacco smoke, which can later lead to pulmonary fibrosis. As you know, pneumofibrosis and emphysema are typical pathological signs of bronchitis associated with smoking [6, 12].

Regarding the changes found in the groups of rats that received a HFD to model experimental obesity, although the experimental model of smoking was used, it should be noted that the authors consider it appropriate to consider them together since the changes in lung tissue under the condition of using the experimental model of obesity are qualitatively and quantitatively different from the changes in the experimental conditions, which were exposed to tobacco smoke only.

It should be noted that the effect of obesity on the respiratory system is realized in several ways, including direct mechanical changes due to the accumulation of adipose tissue in the chest and abdominal cavities and around the upper respiratory tract, as well as causing systemic inflammation and a state of chronic inflammation [6, 10].

There is a growing body of research examining the relationship between COPD and obesity in light of their shared effects on disability and mortality, although the nature of these shared effects remains unclear [6]. In addition, there is strong evidence that as obesity increases, so does the risk of COPD in non-smokers [7]. Recent studies also indicate the presence of a connection between pulmonary fibrosis and dietary obesity. However, the mechanism of development of these pathological changes remains poorly understood [8].

It has been found that HFD is linked to the development of several collagen fibers in both the interalveolar membranes and the walls of the bronchi and bronchioles. This was confirmed by an experiment conducted on rats with an HFD. The results showed a significant increase in collagenization of the interalveolar membranes and bronchus walls, forming medium-sized bundles. These observations suggest that when simulating obesity, pathological changes in the lung tissue, particularly excessive collagenization, can be observed without any direct influence of damaging factors. This conclusion is supported by Saraiva *et al.* (2011), who found that patients with bronchial asthma and obesity tended to have progressive pneumofibrosis. The author suggests that rapid respiratory dysfunction in such patients could be due to an excess of leptin produced by adipocytes under the conditions of comorbid obesity, leading to the activation of proinflammatory cytokines [12]. Mancuso (2010) confirms this statement and believes that lung diseases involve the involvement of proinflammatory mediators produced in adipose tissue. This, in turn, contributes to low-grade systemic inflammation. Increased adipose tissue also increases susceptibility to lung infections, exacerbates lung inflammation associated with environmental exposures, and worsens airway obstruction in pre-existing lung disease [11]. Ultrastructural changes were observed in the group of rats with simulated alimentary obesity combined with passive smoking (OS group), including significant changes in the lung tissue: diffuse collagenization of the interstitium of the interalveolar membranes with the formation of large-sized bundles, diffuse collagenization of the bronchial walls with the formation of large-sized bundles.

The identified ultrastructural features of the lungs in O and OS groups did not differ qualitatively, which suggests that the described changes in the lung tissue developed regardless of the presence or absence of a direct damaging effect (the consequences of which were described in animals of S group) of passive smoking on the lung tissue.

It should be noted that in the CL group we found minor ultrastructural changes: slight focal expansion of the interalveolar membranes, the presence of individual disorganized fibrils of collagen fibers in the interstitium. We interpreted the detected changes as a norm variant with no special diagnostic value. These changes can probably be explained by the chosen method of death of experimental animals, which is also described by other authors [11, 13].

Conclusions

The study of experimental obesity and smoking with the determination of isolated and combined effects on lung tissue in sexually mature male rats indicates pronounced pathomorphological changes that occur already in the first months after exposure to these factors. The main ultrastructural changes observed in both obesity and smoking groups were increased collagenization of the bronchial wall plates. Distinctive changes in the experimental smoking group were pronounced destruction of type 2 alveolocytes, hemorrhages, signs of initial thrombus formation, and thickening of capillary walls. Increased secretory activity of the epithelium with the accumulation of secretory granules was observed in the experimental obesity group. The revealed ultrastructural features of the lungs in the groups of experimental obesity and a combination of obesity and smoking did not differ qualitatively. In our opinion, the described changes in the lung tissue developed regardless of the presence or absence of a direct damaging effect (the consequences of which were described in animals of the smoking group) of passive smoking on the lung tissue.

Future studies that identify how obesity and smoking affect inflammation will provide a better understanding of lung disease management and may lead to the development of new therapeutic interventions in pulmonology.

Conflict of interests

The authors declare that they have no conflict of interests.

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