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Yu.I. Kuzyk, O.Yu. Mazur[1]
Danylo Halytsky Lviv National Medical University, Lviv
[1]Communal Noncommercial Enterprise of Lviv Regional Council
"Lviv Regional Pathology Bureau", Lviv

PATHOMORPHOLOGICAL CHANGES OF THE LUNGS OF MATURE MALE RATS IN CONDITIONS OF EXPERIMENTAL OBESITY AND TOBACCO SMOKING

e-mail: olenaolga_mazur@ukr.net

The influence of obesity (with and without passive smoking) on the formation of pathomorphological changes in the lungs was studied in 4 groups of sexually mature male rats: the control group (CL), the group with isolated alimentary obesity (O), the group exposed to isolated exposure to tobacco smoke (S), the group with combined dietary obesity and exposure to tobacco smoke (OS) after 4 months of the experiment. The morphological changes found in the CL group were insignificant. In group S, the presence of changes characteristic of chronic bronchitis and focal emphysema was established. Pathomorphological changes typical for the chronic obstructive pulmonary disease were found in group O: chronic bronchitis, initial manifestations of bronchiectasis formation, pulmonary emphysema, focal pneumosclerosis, and initial manifestations of pulmonary hypertension. In the OS group, the changes were not qualitatively different from those in the O group. It was found that the changes characteristic of chronic obstructive pulmonary disease developed in rats with simulated obesity, regardless of whether the smoking model was used.

Key words: experimental obesity, smoking, rats, pathomorphological changes in the lungs

Ю.І. Кузик, О.Ю. Мазур

ПАТОМОРФОЛОГІЧНІ ЗМІНИ ЛЕГЕНЬ СТАТЕВОЗРІЛИХ ЩУРІВ-САМЦІВ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОГО ОЖИРІННЯ ТА ТЮТЮНОПАЛІННЯ

Вплив ожиріння (в умовах пасивного тютюнопаління та без такого) на формування патоморфологічних змін легень досліджувався у 4 групах статевозрілих щурів-самців: група контролю (КЛ), група, із ізольованим аліментарним ожирінням (О), група, що піддавалась ізольованій експозиції тютюнового диму (К), група, з поєднаними аліментарним ожирінням та експозицією тютюнового диму (ОК) після 4 місяців експерименту. Морфологічні зміни, виявлені у групі КЛ, були незначимими. В групі К встановлено наявність змін характерних для хронічного бронхіту та вогнищевої емфіземи. У групі О виявлено патоморфологічні зміни характерні для хронічного обструктивного захворювання легень: хронічний бронхіт, початкові прояви формування бронхоектазів, емфізема легень, вогнищевий пневмосклероз, початкові прояви легеневої гіпертензії. У групі ОК зміни якісно не відрізнялись від таких у групі О. Було встановлено, що зміни, характерні для хронічного обструктивного захворювання легень, розвивалися в щурів із модельованим ожирінням незалежно від того, чи було застосовано модель тютюнопаління.

Ключові слова: експериментальне ожиріння, тютюнопаління, щурі, патоморфологічні зміни легень

The study is a fragment of the research project "Study of pathogenetic mechanisms and pathomorphological features of diseases of the endocrine, cardiovascular, respiratory, nervous, digestive, urinary and reproductive systems with the purpose of improving their morphological diagnosis", state registration No. 0123U201668.

Preserving the functional capacity of the lungs throughout adult life is an important measure to prevent 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 significant problem of the health care system in the world today 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 organs and systems' diseases, in particular the respiratory system [13].

It has been proven that rapid weight gain, overweight, and obesity in adulthood have a negative effect on lung function. Previous population-based and occupational cohort studies have demonstrated that overweight in adulthood is associated with reduced lung function and with an increased rate of decline in lung function, independent of age and smoking status [9, 15]. The available literature on the association between the development of chronic obstructive pulmonary disease (COPD), including chronic bronchitis, emphysema, and pneumosclerosis, and obesity is contradictory.

According to some studies, general and abdominal obesity have been found to be independent factors associated with the development of respiratory symptoms in adults. In addition, it was proved that diseases such as COPD and asthma were more common in overweight and obese patients [12]. Also, as of now, the issue of changes in lung tissue associated with obesity remains unclear, namely, the possibility of their occurrence regardless of the metabolic status of patients [3].

The purpose of the study was to investigate pathomorphological changes in lung tissue (respiratory tracts, airways, and pulmonary vessels) both under conditions of isolated exposure to obesity and smoking, and in the case of their combined exposure.

Materials and methods. Research on laboratory animals was carried out in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of March 18, 1986, Council of Europe Directive 2010/63/EU, and the Law of Ukraine "On the Protection of Animals from Cruelty". Animals were kept in the conditions of an accredited vivarium on the basis of Lviv Danylo Halytsky National Medical University for 4 months. Animals had free and unlimited access to food and water throughout the experiment. Animals were euthanized by an overdose of ether vapors. After death, lung necropsies were collected for histological examination. The total sample of experimental animals was 120 individuals. Later, 4 groups of animals were formed by a blind method. 1. Control group (CL group, n=30) – animal nutrition was carried out according to the standard diet). 2. A group of rats exposed to tobacco smoke without simulating obesity (group S, n=30) – feeding was carried out according to a standard diet with exposure in a chamber with tobacco smoke for 4 minutes twice a day, 5 days a week. 3. A group of rats with a simulation of experimental obesity (group O, n=30); eating a high-fat diet. 4. A group of rats with simulated experimental obesity exposed to tobacco smoking (OS group, n=30) – feeding a high-fat diet with exposure in a chamber with tobacco smoke for 4 minutes twice a day, 5 days a week. The groups were comparable and did not differ from each other ($p>0.05$).

The total weight of the standard diet daily portion for one individual was 43.5 g, the percentage of fat was increased monthly by 10 %, thus, for the first month of the experiment it was 30 %, for the last – 60 %, respectively. 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 assess changes in anthropometric data, body length (length from nose to anus), body weight (weighing was carried out monthly to assess growth dynamics), body mass index (BMI) and Lee's coefficient were calculated in individuals of all groups.

When analyzing the dynamics of body weight growth, the percentage increase was assessed: obesity was considered moderate when the body weight increased by 10–25 %, moderate degree – when the body weight increased by 25–40 %, and when the body weight increased by more than 40 % – severe degree [5].

BMI was calculated according to the following formula:

$BMI = m/l^2$, where l is body length (cm), m is body weight (g).

The Lee coefficient was calculated as follows:

Lee coefficient = $\sqrt[3]{m}/l$, where l is body length (cm), m is body weight (g).

The value of Lee's coefficient above 0.31 was considered sufficient to confirm the presence of obesity. The use of the passive smoking model is considered a necessary stage of the experiment since smoking is a proven unconditional risk factor for the development of pathological changes in the lungs [2].

To simulate tobacco smoking, we created a smoking chamber, the dimensions of which allowed the animals to move freely within it. Cigarettes were installed in PLA (polylactide) type plastic cigar holders made with the help of the Easy Threed 3-D printer. The experiment used cigarettes with a tar content of 6 mg and a nicotine content of 0.5 mg. Cigarette smoke was gradually drawn into the chamber by means of a pneumatic compressor.

Fixation of rat lungs and their further processing was carried out according to the standard method using hematoxylin-eosin staining.

Statistical processing of the results was carried out using the Statistica 12 software package. The mean value (M) and the error of the mean (m) were calculated.

Results of the study and their discussion. At the end of the experiment, the mean weight of rats in the Cl group, fed according to a standard diet and not exposed to tobacco smoking, was 280.20±6.62 g, body length – 21.57±0.10 cm, BMI – 0.60± 0.01, the Lee index was 0.303±0.002. During the postmortem pathohistological examination of the lung tissue of rats in this group, minor morphological changes were detected (Fig. 1).

1. Airways: focal desquamation of the bronchial epithelium, slight peribronchial sclerosis, small focal peribronchial mononuclear infiltration;

2. Respiratory departments: slight thickening of the alveolar walls (AW), single foci of emphysema, and atelectasis.

3. Pulmonary vessels: whole blood vessels of the microcirculatory bed

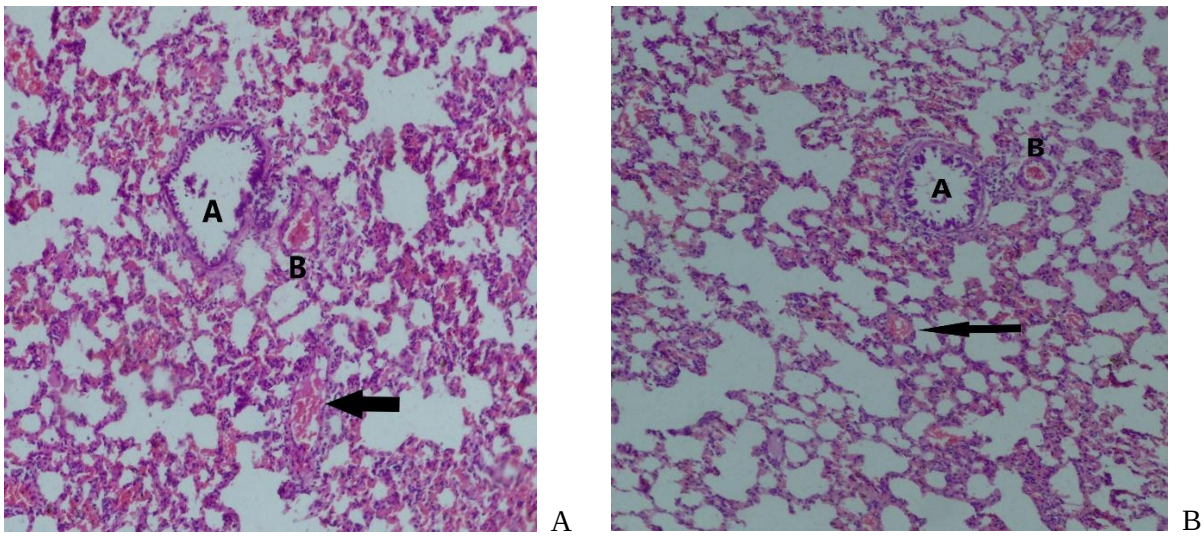

Fig. 1. Changes in the lungs of rats of the CL group: bronchi (A) and arteries (B) of medium caliber, respiratory departments with marked pleurisy (indicated by arrows). Magnification: x100, staining with hematoxylin-eosin.

The mean weight of rats in group S, fed according to the standard diet and exposed to tobacco smoke in the conditions of the experimental smoking model, at the end of the experiment was 267.87±5.59 g, body length – 21.77±0.09 cm, BMI – 0.57±0.01, the Lee index – 0.296±0.002.

During the postmortem pathomorphological examination of the lung tissue in this group of rats, the following changes were found (Fig. 2).

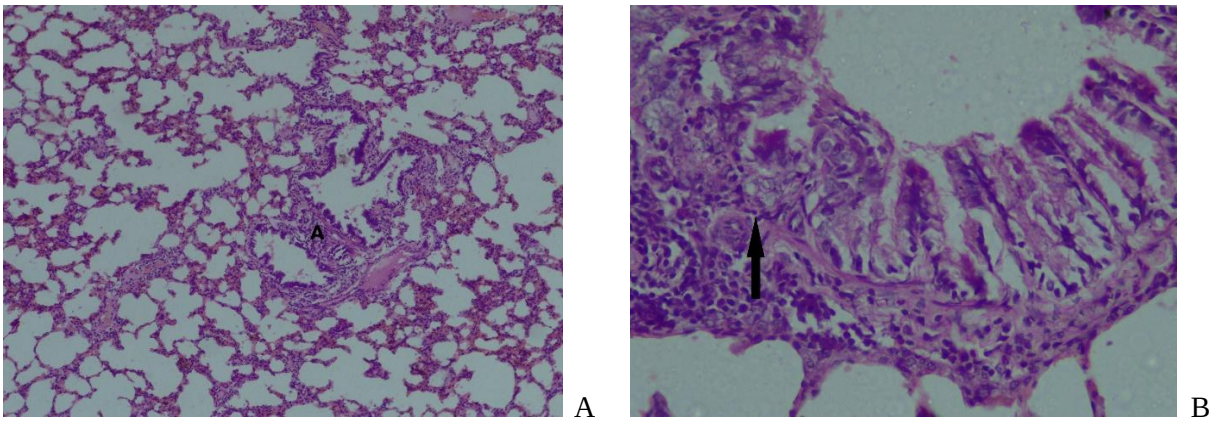

Fig. 2. Changes in the lungs of group S rats: medium (A) and large-caliber bronchi with intramural and peribronchial sclerosis, small focal peribronchial mononuclear infiltration, narrowing and deformation of the lumen, goblet cell hyperplasia (indicated by an arrow). Magnification: x40; x400, staining with hematoxylin-eosin.

1. Airways: multiple foci of narrowing and deformation of bronchial lumens of medium and small caliber were found. Mucus and desquamated epithelium were visualized in the lumens of the bronchi. There was moderate hyperplasia of goblet cells, minor intramural and peribronchial sclerosis with small focal peribronchial mononuclear (lympho-macrophagic) infiltration;

2. Respiratory departments: the lumens of the alveoli are different in shape and size, in particular with regard to focal emphysema. AW are focally thin and torn, with moderate swelling and hyperemia;

3. Pulmonary vessels: the number of vessels was moderately increased. Vascular walls with signs of arteriosclerosis, with focal perivascular sclerosis. Congestive hyperemia in the lumen.

The mean weight of rats in group O, which were fed according to a high-fat diet for the purpose of simulating experimental alimentary obesity, and were not exposed to tobacco smoke in the conditions of the model of experimental smoking, at the end of the experiment was 399.97±7.57 g, body length – 21, 76±0.09 cm, BMI – 0.85±0.02, Lee index – 0.338±0.003.

The changes detected during the postmortem pathomorphological examination of the lung tissue in group O rats are described below (Fig. 3).

1. Airways: were characterized by narrowing and deformation of bronchial lumens of all calibers. Bronchi of various calibers with irregular stellate lumens alternating with bronchi with sharply dilated lumens were found in multiple visual fields. Accumulations of mucus with casts of desquamated epithelium were visualized in the lumens themselves. The covering epithelium was focally desquamated, and

significant hyperplasia of goblet cells was noted. Significant intra- and peribronchial sclerosis was observed in all cases, which was combined with significant peribronchial mononuclear infiltration and pronounced hyperplasia of peribronchial lymphoid structures.

2. Respiratory departments: it was established that alveolar lumens are different in shape and size. Alternation of emphysematous altered alveoli with slit-like (atelectatic) narrowed ones was visualized in multiple fields of vision. AW were thickened due to pronounced edema and significant hyperemia. In addition, there was an increase in the number of vessels, significant intramural and perivascular sclerosis, multiple visualization foci of single-lumen and double-lumen vessels, and single plexiform vascular structures.

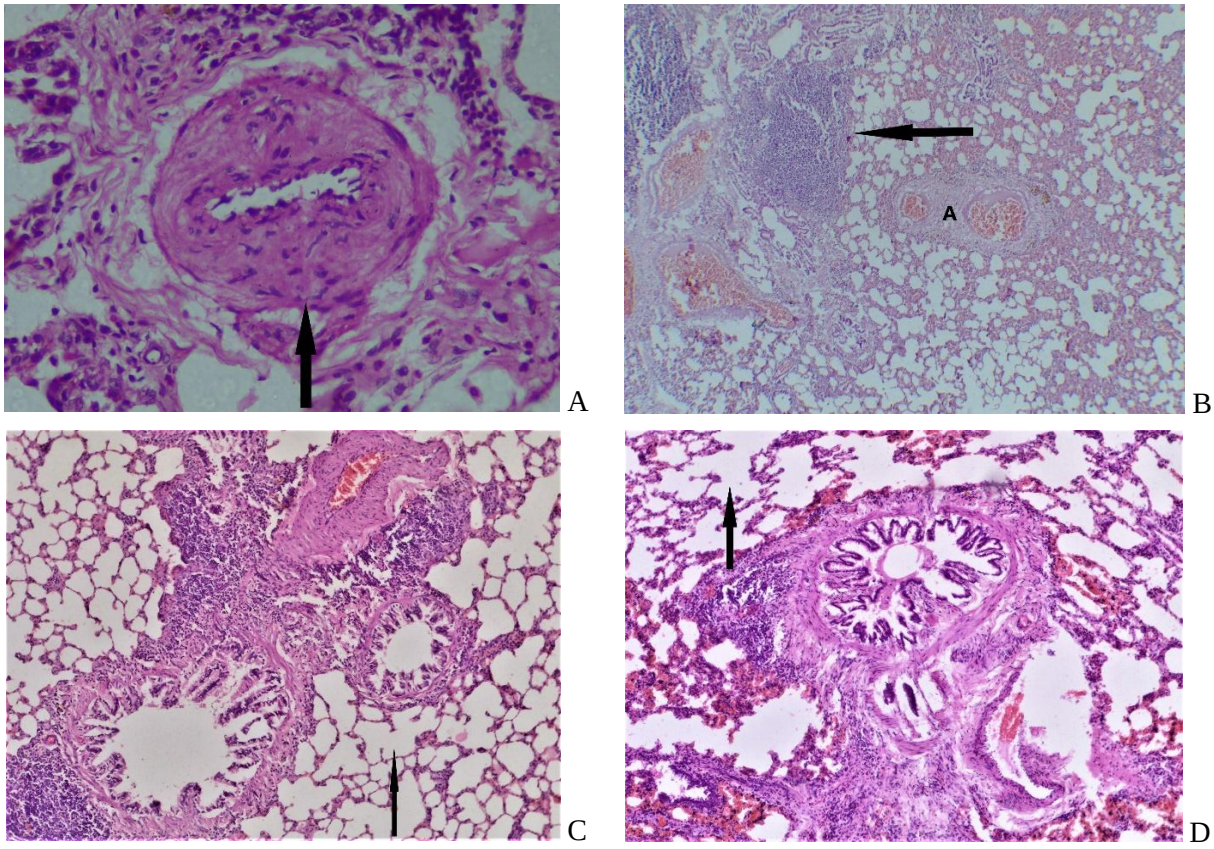

Fig. 3. Changes in the lungs of group O rats: pronounced sclerosis of arterial walls, perivascular sclerosis (marked by an arrow), formation of bicuspid vessels (A), full blood vessels, hyperplasia of peribronchial lymphoid structures (marked by an arrow). Pronounced intrabronchial, peribronchial, and perivascular sclerosis, hyperplasia of goblet cells, foci of emphysema, with thinning and rupture of AW (indicated by arrows). Magnification: x400, x40, x40; x40, staining with hematoxylin-eosin.

The mean weight of rats in the OS group, which were fed a high-fat diet to simulate experimental smoking and were exposed to tobacco smoke in the conditions of the experimental smoking model, at the end of the experiment was 385.2±4.82g, body length – 21.68±0. 09 cm, BMI – 0.82±0.01, Lee index – 0.336±0.001.

The changes established during the postmortem pathomorphological examination of lung tissue in the OS group are presented below (Fig. 4):

1. Airways: narrowing and deformation of bronchial lumens of all calibers, visualization foci of irregular and deformed stellate lumens, and presence of true bronchiectasis were detected. A significant amount of mucus and desquamated epithelium was observed in the lumen of the bronchi. The covering epithelium was focally desquamated, significant hyperplasia of goblet cells, pronounced intra- and peribronchial sclerosis with pronounced peribronchial mononuclear infiltration and hyperplasia of peribronchial lymphoid structures were determined.

2. Respiratory departments: multiple visualization foci of alternating emphysematous enlarged alveoli and alveoli with slit-like (atelectatic) lumens were revealed. AW were thickened due to edema and hyperemia, multiple foci of their thinning, and integrity violations were determined.

3. An increase in the number of vessels, arteriosclerosis with perivascular sclerosis, single-lumen, and double-lumen vessels were formed, as well as individual plexiform structures were determined in all individuals.

The described pathomorphological changes that were detected in the control group (CL) are explained by the chosen method of euthanasia. Thus, for example, inhalation euthanasia can cause alternative-dystrophic changes and impaired hemocirculation in lung tissue [4].

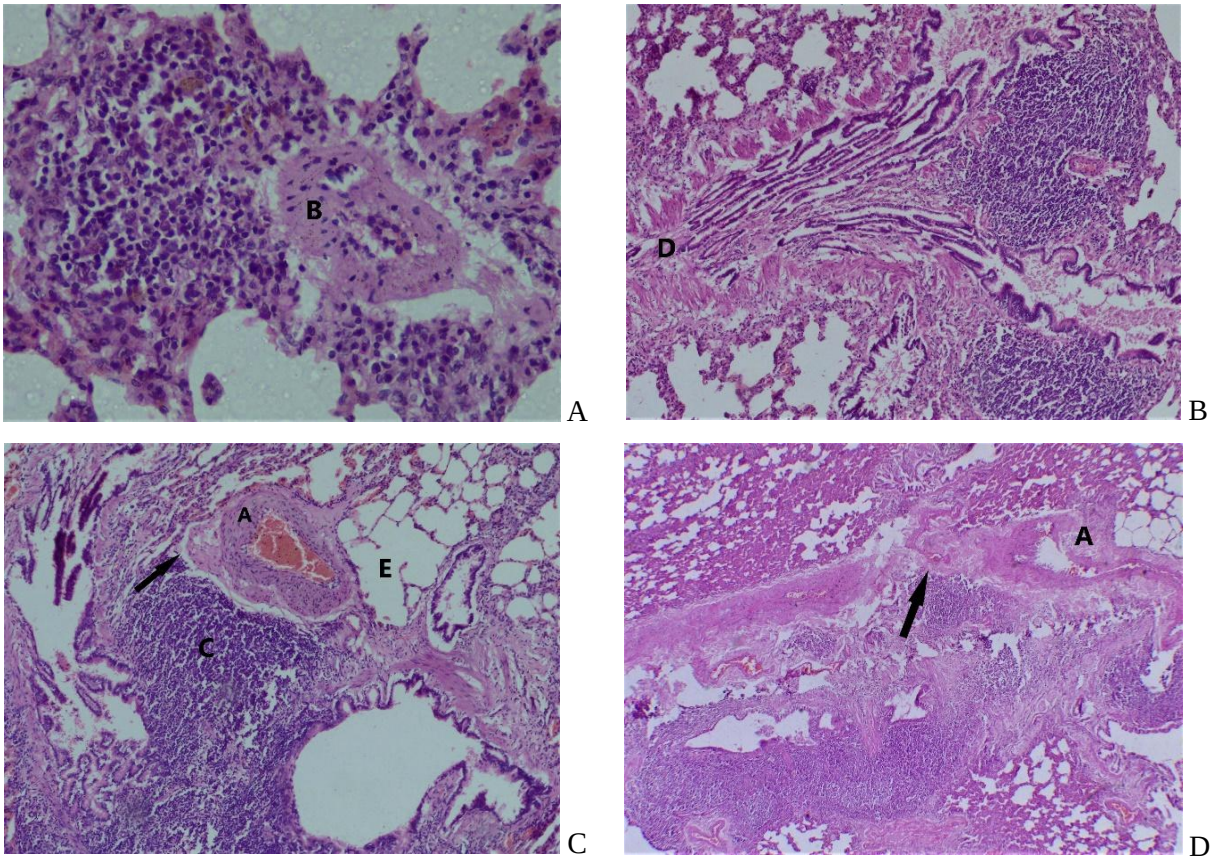

Fig. 4. Changes in the lungs of OS group rats included pronounced arteriosclerosis (A), perivascular sclerosis (indicated by arrows), formation of double-lumen vessels (B), whole blood vessels, marked hyperplasia of peribronchial lymphoid structures (C), marked intramural, peribronchial and perivascular sclerosis with the formation of bronchiectasis (D), hyperplasia of goblet cells, foci of formed emphysema with dilatation of alveoli, thinning and rupture of AW (E). Magnification: x400, x40, x40, x40 staining with hematoxylin-eosin.

Changes in the lung tissue of S rats fed a standard diet and exposed to tobacco smoke under the conditions of an experimental smoking model are confirmed by numerous studies of the cigarette smoke effects on lung tissue, in particular on the airways, respiratory departments, and pulmonary vessels with the development of a complex of changes characteristic of COPD [1, 14].

Regarding the changes found in groups of rats that were fed a high-fat diet for the purpose of simulating experimental obesity, despite the fact that the model of experimental 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 differed from those in experimental units that were exposed to tobacco smoke only.

It should be noted that the effect of obesity on the respiratory system is implemented 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 inducing systemic inflammation and a state of chronic inflammation [6, 10].

There is a growing body of research examining the association between COPD and obesity in light of their combined effects on disability and mortality, although the nature of this combined effect remains uncertain. [6] Moreover, there is strong evidence that as obesity increases, so does the risk of COPD in never-smokers [7]. The latest studies also indicate the existence of a connection between pulmonary fibrosis and dietary obesity, however, the mechanism of these pathological changes development remains insufficiently studied today [8].

Conclusions

1. Minor pathomorphological changes were detected in the control group (CL) (plethora of microcirculatory bed, slight thickening of the alveolar walls due to plethora, foci of emphysema and atelectasis, focal desquamation of the bronchial epithelium, minor peribronchial sclerosis, small focal peribronchial mononuclear infiltration) are explained by the chosen method of inhalation euthanasia.

2. Pathomorphological changes characteristic of chronic bronchitis were found in the group of rats exposed to tobacco smoking without simulating obesity (group S).

3. In a group of rats that were fed a high-fat diet in order to simulate experimental alimentary obesity and were exposed to tobacco smoke under the conditions of an experimental smoking model (group O), pathomorphological changes characteristic of chronic bronchitis, initial manifestations of bronchiectasis formation, pulmonary emphysema, and focal pneumosclerosis, initial manifestations of pulmonary hypertension were revealed.

4. In the OS group, rats were fed a high-fat diet in order to simulate experimental alimentary obesity and were exposed to tobacco smoke under the conditions of a model of experimental smoking. The detected pathomorphological changes corresponded to those in group O and did not differ qualitatively, with the exception of isolated foci of formed bronchiectasis.

Taking into account the data obtained by us, we can state with confidence that under the conditions of applying the model of experimental alimentary obesity, pathomorphological changes in the lungs characteristic of chronic bronchitis, emphysema, focal pneumosclerosis, and signs of pulmonary hypertension developed.

However, it should be noted that with the simultaneous application of experimental obesity and smoking, the changes in the lungs did not differ qualitatively from those in the group of rats for which only the model of experimental alimentary obesity was applied. Therefore, it can be assumed that the complex pathomorphological changes typical for the chronic obstructive pulmonary disease developed in rats with simulated obesity, regardless of whether the smoking model was used.

References

1. Aghaloo T, Kim JJ, Gordon T, Behrsing HP. In Vitro Models, Standards, and Experimental Methods for Tobacco Products. Adv Dent Res. 2019 Oct;30(1):16–21. doi: 10.1177/0022034519872474.

2. Baffi CW, Wood L, Winnica D, Strollo PJ Jr, Gladwin MT, Que LG, et al. Metabolic Syndrome and the Lung. Chest. 2016;149: 1525–1534. pmid:26836925

3. CCAC guidelines on: euthanasia of animals used in science. Ottawa: Canadian Council on Animal Care, Cop; 2010.

4. Conn PM. Animal Models for the Study of Human Disease. Animal Models for the Study of Human Disease. Elsevier Inc.; 2013. 1–1089 p.

5. Dixon AE, Peters U. The effect of obesity on lung function. Expert Rev Respir Med. 2018 Sep;12(9):755–767. doi: 10.1080/17476348.2018.1506331.

6. Fuller-Thomson E, Howden KEN, Fuller-Thomson LR, Agbeyaka S. A Strong Graded Relationship between Level of Obesity and COPD: Findings from a National Population-Based Study of Lifelong Nonsmokers. J Obes. 2018 Nov 20; 2018:6149263. doi: 10.1155/2018/6149263.

7. Guo X, Sunil C, Qian G. Obesity and the Development of Lung Fibrosis. Front Pharmacol. 2022 Jan 10; 12:812166. doi: 10.3389/fphar.2021.8121668.

8. Hegewald MJ. Impact of obesity on pulmonary function: current understanding and knowledge gaps. Curr Opin Pulm Med. 2021 Mar 1;27(2):132–140. doi: 10.1097/MCP.0000000000000754.

9. Hornung F, Rogal J, Loskill P, Löffler B, Deinhardt-Emmer S. The Inflammatory Profile of Obesity and the Role on Pulmonary Bacterial and Viral Infections. Int J Mol Sci. 2021 Mar 26;22(7):3456. doi: 10.3390/ijms22073456.

10. Kiley JP, Gibbons GH. Reply: Primary and Secondary Prevention of Chronic Obstructive Pulmonary Disease: Where to Next? American Journal of Respiratory and Critical Care Medicine. 2014 Oct;190(7):840–1. doi: 10.1164/rccm.201405-0883LE

11. Kisiel M, Arnfelt O, Lindberg E, Jogi O, Andrei Malinovschi, Johannessen A, et al. Association between abdominal and general obesity and respiratory symptoms, asthma and COPD. Results from the RHINE study. 2023 Mar 1;211:107213–3. doi: 10.1016/j.rmed.2023.107213.

12. Lim Y, Boster J. Obesity and Comorbid Conditions [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574535

13. Sousa C, Rodrigues M, Carvalho A, Viamonte B, Cunha R, Guimarães S, et al. Diffuse smoking-related lung diseases: insights from a radiologic-pathologic correlation. Insights into Imaging. 2019 Jul 16;10(1). doi.: 10.1186/s13244-019-0765-z

14. Sutherland TJ, McLachlan CR, Sears MR, Poulton R, Hancox RJ. The relationship between body fat and respiratory function in young adults. Eur Respir J. 2016 Sep;48(3):734–47. doi: 10.1183/13993003.02216-2015.

15. Yeroshenko GA, Donets IM, Shevchenko KV, Ulanovska-Tsyba NA, Grygorenko AS, Sokolenko VM, et al. Restructuring of the rat pulmonary vascular bed induced by the complex of food additives. World of Medicine and Bilogy. 2023 Jan 1;19(83):197–7. doi: 10.26724/2079-8334-2023-1-83-197-202

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