HISTOLOGICAL FEATURES OF RATS' NORMAL LUNG TISSUE

Dr. Koptyev M.M. [1]
E-mail: mn_koptev@ukr.net,

Dr. Pronina O.M. [1]
E-mail: elenan_pronina@mail.ru,

Dr. Danylchenko S.I. [1]
E-mail: svetlana_danilch@mail.ru,

Dr. Avetikov D.S. [1]
E-mail: davidplast@rambler.ru,

Dr. Stavitskiy S.O. [1]
stanislav_stavickii@mail.ru,

[1] Department of Emergency Medicine with Operative Surgery and Topographic Anatomy, Higher State Educational Establishment of Ukraine "Ukrainian Medical Stomatological Academy",
23 Shevchenko Str., Poltava, 36011, Ukraine

Corresponding Author:
Dr. Danylchenko Svitlana Ivanivna,
PhD, Associate Professor
Department of Emergency Medicine with Operative Surgery and Topographic Anatomy, Higher State Educational Establishment of Ukraine
"Ukrainian Medical Stomatological Academy",
23Shevchenko Str., Poltava, 36011, Ukraine
E-mail: svetlana_danilch@mail.ru

ABSTRACT

Modeling on laboratory animals is widely used for experimental morphological studies. To interpret the findings properly it is crucial to know the specific differences of their anatomical organization. The purpose of the research was the study of histological features of normal lung texture of Wistar white rats. Histological analysis showed that areas of destructive changes of alveolar walls with accumulation of alveolocytes in the alveoli lumen on different stages of destruction, as well as fibrin and individual red blood cells were observed in some of the test samples of rats' normal lung tissue. In the hollows of some alveoli few alveolar macrophages and the Type II alveolocytes were found. The special feature of hemomicrocirculatory flow of rats' normal lungs is accumulation of erythrocyte in the capillaries of alveolar septums. In the areas of erythrocyte accumulation a local damage, destruction and demolition of cytolemma of capillaries endothelium has been detected, resulted in endothelium basal membrane exposure and accumulation of pinocytic vesicles in the lumen of capillaries.

Keywords: rats, morphology, lungs

INTRODUCTION

Nowadays respiratory apparatus diseases are the most widespread pathology in the structure of morbidity of Ukrainian population. In this way, pulmonary pathology morbidity rate in 2011 constituted 17395, 6 per 100 thousand of adult population, and the spread rate constituted 24005, 4 per 100 thousand [5]. One of the reasons that facilitate the increase of morbidity rate in respiratory system is stress effect [1]. Modeling on laboratory animals is widely used for carrying out the experiments aiming at study the technique of bronchopulmonary pathology development under stresses [2]. At the same time it is extremely important to know the features of normal anatomical organization of respiratory apparatus, as they have significant specific differences [3].

PURPOSE

The purpose of the research was the study of histological features of normal lung texture of Wistar white rats.

MATERIALS AND METHODS

Study has been carried out on 20 Wistar white male rats of 240-260 g body weight and 8-10 months old, which were housed in standard conditions at vivarium of Ukrainian Medical Stomatologial Academy and were not involved in any other experiments. Before euthanasia the animals were not fed during 24 hours. The rats were killed by decapitation under thiopental anesthesia. Fragments of lungs were preserved in 10% solution of neutral formalin, and then, after appropriate alcoholization of increasing concentration, were embedded into paraffin according to standard technique. Microtome specimens were colored by hematoxylin-eosin according to Hart Van Gizon and Mallory.

The experimental part of the research has been carried out in compliance with the requirements of international principals of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 18.03.1986) and corresponding Law of Ukraine "For the Protection of Pet Animals" (No.3446-IV, 21.02.2006, Kyiv).

RESULTS AND DISCUSSION

Histological study showed that the respiratory part of rats' lung consisted of alveolus system, located on the walls of respiratory bronchioles, alveolar ducts and saccules. Alveoli were surrounded by the dense

capillary nets, forming numerous plexuses. Alveoli were separated from each other by thin alveolar septums, in which alveolar pores, i.e., junction between adjacent alveoli, became evident.

Internally, rats' alveoli were covered by the layer of epithelial cells, interconnected by dense contacts. Epithelium was located on the basal membrane, blended with endothelium basal membrane in some areas and somewhere was separated from it by fissures. Such fissures contained reticular and elastic fibers and cells. Among the epithelial cells, covering alveoli, the majority of them were represented by the Type I respiratory alveolocytes and the minority were represented by the Type II secretory alveolocytes. Apart from these cells, occurrence of alveolar macrophages and the Type III brush alveolocytes were also evident.

The Type I alveolocytes are plane cells of irregular oblong shape with very thin cytoplasm and nucleus with off-center positioning. The thickness of respiratory alveolocytes expands in the nucleate portion of the cell. On the free surface of the cytoplasm, faced the alveolus cavity, wide cytoplasmic processes, which extend the contact area of the epithelium surface with air, are detected. Pinocytotic vesicles are detected in the cytoplasm. Anucleate areas of endothelial cells of capillaries are adjacent to the anucleate areas of the Type I alveolocytes. Here the basal membrane of alveolocytes, approaching to the basal membrane of endothelial of capillaries, blends with it. Due to this the aerohematic barrier is extremely thin. It is formed by the layer of epithelial cells, covering the alveoli, basal membranes of alveoli epithelium and endothelium of capillaries and cytoplasm of endothelial capillaries.

The Type II alveolocytes are bigger than respiratory ones. They are cells with cubic form, which are projected into the alveoli lumen. Due to dense contacts the secretory alveolocytes are joined with respiratory ones. On the surface, faced the alveolus cavity, secretory alveolocytes have microvilli, and their cytoplasm contains secretory granules.

Apart from the Type II alveolocytes, another rather large orbicular cells, i.e., alveolar macrophages, project into the alveoli lumen from the alveolar walls. Cytolemma of alveolar macrophages has numerous folds, containing phagocytic particles. Cytoplasm of alveolar macrophages is of basophil origin and contains numerous phagosomes, lysosomes and pinocytotic vesicles. Nuclei are small and of orbicular, bean-shaped or of irregular form and contain large lumps of chromatin.

The Type III alveolocytes, similar to secretory ones, contain microvilli on the free surface of the cytoplasm, and their cytoplasm contains numerous vesicles.

The areas of destruction of cytoplasmic processes of the Type I alveolocytes, as well as micropinocytotic vesicles, accumulated in them, were detected in some of the test samples of rats' normal lung tissue. Rejection of destroyed respiratory alveolocytes leads to exposure of basal membrane and alternation of damaged areas of alveoli epithelium with invariable ones. In the lumen of alveoli near damaged areas numerous cells on different stages of destruction, fibrin and individual red blood cells have been observed. In the hollows of some alveoli few destroyed and undamaged alveolar macrophages and the Type II alveolocytes were also found.

According to our observations, histological structure of rats' intrapulmonary bronchi varies, depending on the bronchus size, but has common features. The wall of intrapulmonary bronchi consists of four membranes: mucous, submucous, fibro- cartilaginous and adventitious. The less is the bronchus size, the less sizes are cartilages in the fibro- cartilaginous membrane. Only smooth myocytes are detected in the wall of bronchial tubes.

Epithelium of mucous membrane of bronchial tubes is presented by double-row ciliary epithelium and in bronchial tubes of smaller size by the one-row ciliary epithelium. In the bronchial tubes among epithelial cells, except ciliary, goblet, endocrine and basal ones, the Clara's secretory cells are detected, as well as brush cells.

Proper lamina of mucous membrane of bronchial tubes is thin and contains collagenous and elastic fibers, few cellular elements. Muscle plate of mucous membrane of bronchial tubes of small size is significantly evident as compared with thickness of the entire wall. It is less developed in bronchial tubes as compared with bronchi of bigger diameter.

In the mucous membrane of rats' bronchial tubes elements of local immune barrier, i.e., lymphocytes and lymphoid nodules have been detected.

In contrast to histological structure of submucous connective tissue layer of other bronchi, the absence of trophochrome glands and fibrocartilage membrane in the histological structure of submucous connective tissue layer of bronchial tubes has been detected.

In the outer adventitious membrane of bronchial tubes a fibrous connective tissue has been detected. Its fibers blend with interstitial connective tissue.

Histological study of rat's medium bronchi stated that its inner membrane was mucous, lined with monolayer plural-row ciliated epithelium, consisted of epithelial cells of different structure and functional purpose. Epithelial cells of mucous membrane of medium bronchi are of various height, their nuclei are located on the different levels, composing several rows. The less is bronchi size, the less is epithelium height of its mucous membrane, due to change of cells' shape – from high prismatic to low cubical ones. Among epithelial cells, except ciliated ones, goblet, endocrine and basal ones are detected. Ciliated cells have ciliated cilia, which facilitate excretion of mucus and dust particles from the bronchi lumen. Goblet cells, excreting mucin, are located between ciliated ones. Neuroendocrine cells are few and isolated; its cytoplasm contains small dense granules. Basal or cambial cells are cubical with optically dense nucleus and small amount of low-basophilic cytoplasm. They preserved the ability for mitotic division and are located in the basal layer.

One of the peculiarities of epithelium structure of bronchi mucous membrane is the location of cells' nuclei on the various levels in relation to the basal membrane. Nuclei are of orbicular shape with evenly colored chromatin and one nucleolus. One cells (ciliated, goblet ones) reach the surface of epithelial layer, and the other (basal ones) do not reach it.

Proper lamina of mucous membrane of medium bronchi, where epithelial layer is located, is indurated; the basal membrane is of sharp contour.

The middle part of the proper lamina contains numerous longitudinal elastic fibers, providing bronchi with the ability to stretch.

On the boundary of mucous membrane and submucous layer, the muscle plate has been identified, generated by obliquely circular fascicles of smooth myocytes.

Histological study of rat's medium bronchi showed that the end parts of mixed trophochrome glands were laid down here. Excretory ducts of these glands are opened on the surface of epithelium; their secretion moistens the mucosa and facilitates the excretion of foreign particles from the bronchi lumen.

On the histological sections of medium bronchi the fibrocartilaginous membrane is presented by the cartilaginous plates of irregular shape and insulae of elastic cartilaginous tissue. On the cross-sections of medium bronchi, the cartilaginous plates are of crescent or oval shape. Spaces between cartilages are filled with connective tissue. Cellular elements are not numerous, with prevailing majority of fiber component.

The outer adventitious membrane is formed by the fibrous connective tissue, which blends with interparticle tissue of lung. Among cells of hematogenous origin, lymphocytes, macrophages and mastocytes are detected, which participates in regulation of local homeostasis. Interstitial connective tissue mainly consists of elastic and collagenous fibers and amorphous substance, and also contains resident cells – fibroblasts and migratory cells – macrophages, lymphocytes, plasmacytes and mastocytes. Blood (arteries,

veins) and lymphatic vessels, as well as nerve fibers, are located here. Collagenous fibers are randomly located in different directions, are of multiple length and undulating coiled shape.

Elastic fibers are thinner than collagenous ones; on the cross-section the elastic fibers were of orbicular or oblate shape.

Blood supply of lungs is performed on two vessel systems. Venous blood is supplied to lungs on pulmonary arteries of lesser circulation. Branches of pulmonary artery accompany bronchial tree. Stenosinuous capillary network is formed at the tela of alveoli. Each alveolar duct corresponds to arteriole, from which precapillaryies are branched and bifurcated into capillaries. The latter blend with postcapillaries, gathered into venule.

Arterioles are the smallest arterial vessels of muscle type, which walls are composed of low-grade three tunics.

Inner tunic is formed by the endothelial cells, basal membrane, thin subendothelial layer and thin inner elastic membrane.

Middle tunic consists of 1-2 circular layers of smooth myocytes. Bigger arterioles have the outer elastic membrane.

Outer tunic of arterioles consists of collagenous and elastic fibers.

Capillaries are contiguous with one alveole by one of their surface, and with adjacent alveole by another one. In the alveolar blood capillaries the red blood cells are arranged in one row, creating optimal conditions for gas exchange. Blood capillaries are covered with continuous layer of endotheliocytes, located on the basal membrane. Pre-nucleate part of endothelial cell (perikaryon) is thickened, and peripheral part is thinned. Plasmalemma of endothelial cells are covered with thin glycocalyx, has cytoplasmic processes of multiple length and protrusions inside cytoplasm, forming micropinocytic vesicles to transfer substances through endothelium. On the periphery, cytoplasm of endotheliocytes becomes thinner, stretching along the basal membrane to considerable distances, forming the thinnest portions of aerohaematic barrier.

Basal membrane of endothelium, similar to alveolar epithelium, is formed of thin intertwined fibrils, deepened into basal substance of connective tissue. In thin portions of aerohaematic barrier, the basal membranes of endothelium and epithelium are approaching each other in ways that look like generic basal membrane.

In some areas the cells with fibrils in cytoplasm, i.e., pericytes, are identified in breakdowns of basal membrane, which are probable precursors of fibroblasts and smooth myocytes and sources of synthesis of components of basal substance of connective tissue.

Capillaries on their venous end transit into postcapillary venules, and the greater diameter is, the greater number of pericytes is. Such venules flow into gathering venules, which, apart from the pericytes, have also outer tunic, which is formed from the fibroblasts and collagenous fibers. Internally, venules are covered with endotheliocytes, which in some parts are dense and in other parts fissures are formed between their membranes. Just after the endothelium the basal membrane is detected. Along or around venous endothelial tube the processes of pericytes are stretching. Pericytes and their processes are also covered by the basal membrane, on the outer surface of which the collagenous fibers are located.

The special feature of hemomicrocirculatory flow of rats' normal lungs is the occurrence of erythrocyte aggregation in the capillaries of alveolar septums. Some of the capillaries are fully filled with agglutinate erythrocytes. In the areas of erythrocyte aggregation, especially if it is accompanied by erythrocyte adhesion to cytoplasmic processes of endotheliocytes, a local damage, destruction and demolition of cytolemma of capillaries endothelium is detected. In some cases in the cytoplasm of damaged endotheliocytes the dense inclusions are identified. Rejection of destroyed endotheliocytes leads to endothelium basal membrane exposure and accumulation of pinocytic vesicles in the lumen of capillaries.

CONCLUSION

The study of structure of rats' lungs in control group concluded that rat's normal lungs had specific generic histological features. The areas of destruction of cytoplasmic processes of the Type I alveolocytes and accumulation of micropinocytotic vesicles in them were observed in some of the test samples of rats' normal lung tissue. Rejection of destroyed respiratory alveolocytes leads to exposure of basal membrane and alternation of damaged areas of alveoli epithelium with invariable ones. In the lumen of alveoli near damaged areas numerous cells on different stages of destruction, fibrin and individual red blood cells have been detected. In the hollows of some alveoli few destroyed and undamaged alveolar macrophages and the Type II alveolocytes have been found. The special feature of hemomicrocirculatory flow of rats' normal lungs is the occurrence of erythrocyte aggregation in the capillaries of alveolar septums. Some of the capillaries are fully filled with agglutinate erythrocytes. In the areas of erythrocyte aggregation, especially if it is accompanied by erythrocyte adhesion to cytoplasmic processes of endotheliocytes, a local damage, destruction and demolition of cytolemma of capillaries endothelium have been detected. In some cases in the cytoplasm of damaged endotheliocytes the dense inclusions have been identified. Rejection of destroyed endotheliocytes leads to endothelium basal membrane exposure and accumulation of pinocytic vesicles in the lumen of capillaries.

Thus, rat's normal lungs have certain specific differences of anatomical organization, which should be taken into consideration while carrying out experimental studies.

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