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THE MORPHOLOGICAL STATE OF THE LUNGS OF RATS EXPOSED TO VIPERA BERUS NIKOLSKII VENOM

Lasavutz V.S. ✉ **The morphological state of the lungs of rats exposed to Vipera berus nikolskii venom. Bogomolets National Medical University, Kyiv, Ukraine.**

ABSTRACT. Background. Lung tissue damage is a multietiological phenomenon characterised by various structural and functional changes. Their pathogenesis is based on free radical and inflammatory processes, and the main participants are resident and migrating macrophages and neutrophils. There is a whole series of pathological conditions accompanied by respiratory complications, the ways of development of which are not sufficiently studied. Therefore, the study of this issue is timely and relevant in today's conditions. **Objective.** Detection at the light-optical level of the manifestations of damage to the lungs of rats under the influence of viper venom of the species Vipera berus nikolskii. **Methods.** To study changes in the microstructure of the structural elements of the lungs, determination of changes in the expression of markers reflecting organ damage, and assessment of the dependence of changes between structural components, we conducted histological, histochemical and morphometric studies of rat lungs. **Results and conclusion.** Under the conditions of action of Vipera berus nikolskii venom on the lungs of laboratory animals, big destructive-degenerative changes in blood vessels, bronchi, and the respiratory department were established with the development of DIC-syndrome of significant areas of inflammation, various forms of coagulopathy, and diffuse and local haemorrhages. Histologically and morphometrically, larger areas of dys- and atelectasis, zones of emphysematous expansion, zones of inflammation, and foamy macrophages were established in the respiratory department of the lungs compared to the lungs of animals bitten by Vipera berus berus.

Key words: lungs, snake venom, necrosis, haemorrhage.

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Introduction

According to the Ministry of Health, since the beginning of 2020, 36 cases of snake bites have been registered in Ukraine, including six children. Cases were recorded in Zakarpattia, Lviv, Donetsk, and Zhytomyr regions. On the territory of Ukraine, there are only poisonous vipers, namely the common viper (Vipera berus berus), steppe viper (Vipera ursinii), Nikolsky's viper (Vipera berus nikolskii), Renard's viper (Vipera renardi) and long-nosed viper (Vipera ammodytes). Their significant prevalence is registered in the southern and eastern regions, and only one species is in the western and northern areas [1, 2].

As a result of the bites of poisonous animals, including snakes, toxic stress develops in the victim's body, which becomes the cause of its induced damage to internal organs [3, 4, 5, 7, 8].

Pathology of the lung tissue in the conditions of the modern world is a widespread phenomenon. Complications from the respiratory system occur in cardiovascular, rheumatological, oncological diseases, etc. In recent years, the term "stressor lungs" has become widespread, and it characterises a whole complex of compensatory and adaptive changes in the bronchopulmonary system under the influence of factors of exo- or endogenous origin [6]. Lung tissue damage is a multietiological phenomenon characterised by various structural and functional changes. At the same time, their pathogenesis is based on free radical and inflammatory processes, and the main participants are resident and migrating macrophages and neutrophils. In addition, numerous mediators produced as a result of the activation of these cells stimulate the expression of a large number of proteases. The latter will lead to pronounced degradation of lung tissue components and strengthening of fibrotic processes. There is a whole series of pathological conditions accompanied by respiratory complications, the ways of development of which are not sufficiently studied. Therefore, the study of this issue is timely

and relevant in today's conditions.

The purpose of the research is to detect at the optical level the manifestations of damage to the lungs of rats under the influence of viper venom of the species Vipera berus nikolskii.

Materials and methods

Lung pieces were taken from pre-weighed animals of all groups for microscopic examination. The pieces were fixed in a 10% formalin solution, while the duration of exposure did not exceed 1-2 days. The applied fixing solution prevents the process of autolysis and stabilises cells and tissues for further processing and use in staining procedures. Next, the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. The prepared sections, 4-5 μm thick, were stained with hematoxylin and eosin, methylene blue, and impregnated with silver according to the Gordon-Sweets method (to identify basement membranes, reticular and immature collagen fibres). Histological preparations were studied with the help of an SEO SCAN light microscope and photo-documented with the help of a Vision CCD Camera with a system of image output from histological preparations.

Statistical processing of the obtained research results was carried out using computer programs Origin 7.0 and TotalLab 2.01. and Microsoft Excel. Differences at $p<0,05$ were considered significant.

Results and conclusions

Exposure to Vipera berus nikolskii venom leads to significant alterative, degenerative, and inflammatory changes in the bronchi, vessels, stroma, and organ parenchyma. As a result of the toxic effect of poisons, the vessels of the lungs are directly affected, which is manifested by remodelling of the wall, its thickening or local thinning, erosive and desquamative processes of the endothelium, swelling and defibration of the adventitia, and an increase in the area of inflammatory perivascular infiltrates (Fig. 1).

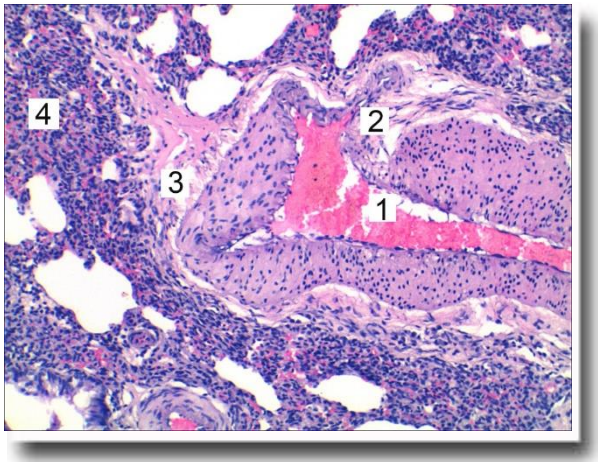

Fig. 1. Microscopic changes in a white rat's lung under Vipera berus nikolskii venom exposure. The blood-filled lumen of the artery with local desquamation of the endothelium (1), thinning and deformation of the media (2), oedema of the adventitia (3), and leukocyte infiltration of the respiratory department (4). Staining with hematoxylin and eosin. ×200.

The toxic effect of Vipera berus nikolskii venom primarily affects the vascular wall, leading to the formation of oedema and restructuring of fibrous structures and the amorphous component of mainly peribronchial and perivascular connective tissue. Hyperhydration of the connective tissue in case of poisoning with viper toxins leads to an increase in the content of sulfated glycosaminoglycans with "Hale"-positive properties and glycoproteins with PAS-positive properties (Fig. 2).

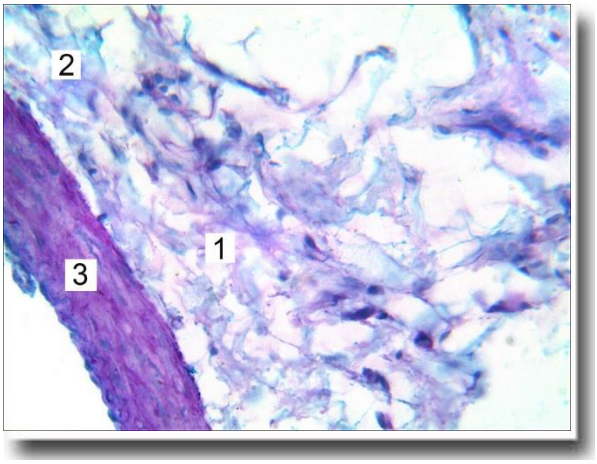

Fig. 2. Histochemical changes in a white rat's lung under exposure to Vipera berus nikolskii. Collagen fibres of the perivascular connective tissue with weakly expressed PAS-positive properties (1) and strongly expressed "Hale" positive properties of glycosaminoglycans (2) smooth myocytes of the media of the artery (3). Staining according to the Mowry method. ×400.

Hemotoxins of the venom of Vipera berus nikolskii cause significant hemodynamic disorders in the lungs of experimental animals, which is characterised by whole blood not only of veins but also of arteries, thrombus formation, sweetening of erythrocytes. In the lungs of laboratory animals after a bite of Vipera berus nikolskii, as with a bite of Vipera berus berus, loci of extravascular accumulation of fibrin are detected, which is a manifestation of coagulopathies with the course of DIC-syndrome (Fig. 3).

The direct effect of Vipera berus nikolskii venom led to significant destructive, desquamative and erosive changes in the epithelium of the bronchi in their lumen with exposure of the basement membrane (Fig. 4). Increased permeability of the walls of blood vessels and degenerative-inflammatory processes of the walls of the bronchi leads to local and diffuse haemorrhages, in particular in the lumen of the bronchus. The bronchial wall is significantly deformed, thin, and dystonic. The adventitia is deformed, and defibrillation and swelling of the amorphous component of the intercellular substance are determined. Intense diffuse histoleukocyte infiltration or local, voluminous lymphocyte conglomerates are observed in the peribronchial connective tissue.

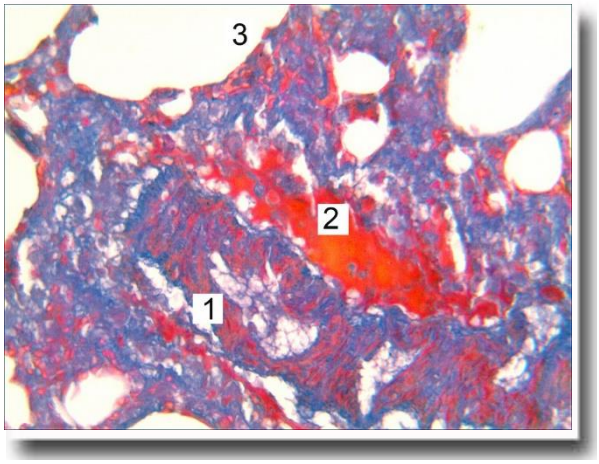

Fig. 3. Microscopic changes in a white rat's lung under exposure to Vipera berus nikolskii. Deformed media of the artery (1), accumulation of fibrin in the perivascular space (2), and alveoli of the respiratory department (2). Staining by the MSB (OKH) method. ×400.

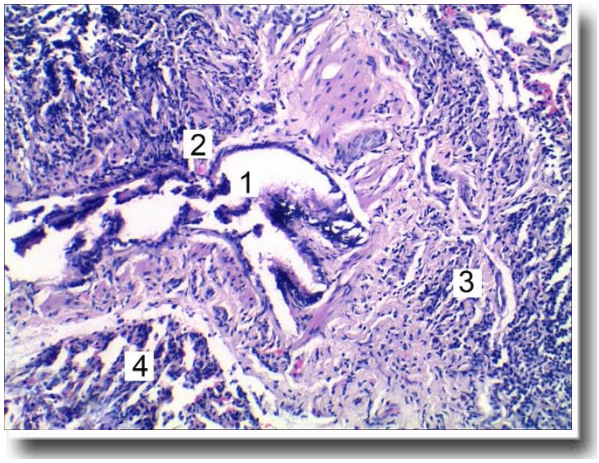

Fig. 4. Histological changes in a white rat's lung under exposure to Vipera berus nikolskii. Desquamation of the epithelium in the lumen of the bronchus with loci of erosion (1), deformation of the wall (2), histoleukocyte infiltrates of the peribronchial connective tissue (3), respiratory department (4). Staining with hematoxylin and eosin. ×200.

Desquamation-erosive processes are characteristic of the epithelium of the mucous membrane of most bronchi, both of large and medium and small diameters. The epithelial plate is fragmented; the cells are destructured, and the goblet cells are single or are found in the mucous content in the bronchus lumen and have moderately expressed PAS-positive properties. Basal epitheliocytes are single and provide complete regeneration of the epithelial plate of the mucous membrane of the bronchi (Fig. 5).

During the morphometric calculation, a significant increase in the average indicator of the area of blood vessels by 1,55 times ($p<0,001$) was established, compared to the value of the intact group. Along with the vessels, the average values of the bronchi and lymphoid tissue areas also increased by 2,49 and 2,58 times, respectively ($p<0,001$), compared to the values of the intact group. Therefore, the average value of the respiratory department is significantly reduced to 0,74 compared to the intact indicator.

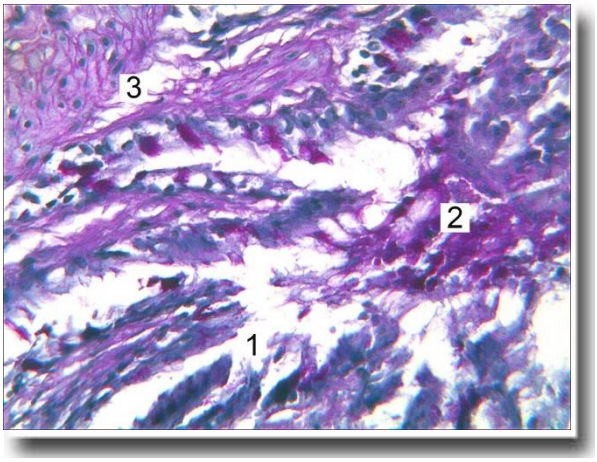

Fig. 5. Histochemical changes in a white rat's lung under exposure to Vipera berus nikolskii. Destruction and desquamation of the bronchus epithelium of medium calibre (1), serous-mucous content in the lumen of the bronchus with moderately expressed PAS-positive properties (2), destructively changed muscular plate (3). Staining according to the Mowry method. ×400.

Alteration of the respiratory department of the lungs of white rats as a result of the action of Vipera berus nikolskii venom is characterised by thickening of the walls of the alveoli and the formation of significant areas of dys- and atelectasis, which are intensively infiltrated by lymphocytes and macrophages. Increased vascular wall permeability due to the influence of hemotoxins and vascular wall destruction leads to local or extensive haemorrhages. The area of haemorrhages in the respiratory tract of the lungs increases compared to animals when bitten by a steppe viper. The accumulation of leukocytes and macrophages is determined perifocally in such loci of hemorrhages.

Mainly in the subalveolar zones, the respiratory department of the lungs is changed according to the emphysematous type, the alveoli are overstretched, and the interalveolar partitions are significantly thinned, which does not ensure the ordinary course of gas exchange.

In the lungs of experimental animals, in the perivascular and peribronchial zones and mainly in the zones of dys- and atelectasis of the respiratory department, the accumulation of brightly "Hale"-positive macrophages-hemosiderophages is determined as a manifestation of haemorrhages and the breakdown of haemoglobin.

In the parenchyma of animals' lungs exposed to the toxic effect of Nikolsky's viper venom, foamy macrophages —cells of the macrophage system—accumulate. These macrophages are contained in the lumen of the alveoli or areas of histoleukocyte infiltrates and manifest the pathological accumulation of lipids in their cytoplasm (Fig. 6).

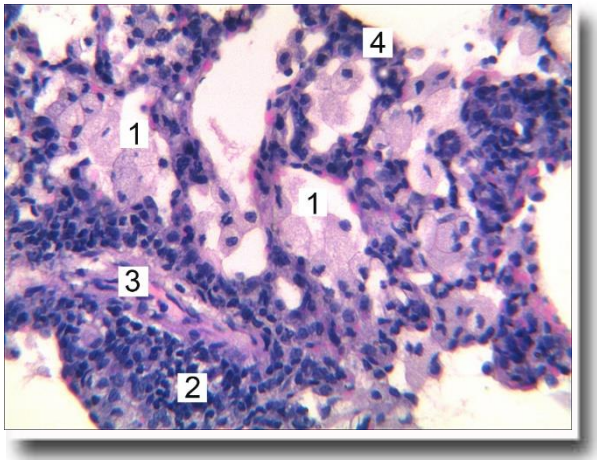

Fig. 6. Microscopic changes in a white rat's lung under exposure to Vipera berus nikolskii. Foamy macrophages (1), leukocyte perivasal infiltrates (2), vein (3). interalveolar septa (4). Staining with hematoxylin and eosin. ×200.

The morphometric calculation of the indicators of the respiratory department of the lungs of white rats under the conditions of a Vipera berus nikolskii bite showed that the values of the relative areas of dys- and atelectasis, emphysematously changed lung tissue increased reliably by 6,64; 8,80 and 2,92 times ($p<0,001$) compared to the values of the intact group. Therefore, the average value of lung tissue with unchanged histostructure decreases to 0,11 compared to the indicator of the intact group of animals.

Summary

Under the conditions of action of Vipera berus nikolskii venom on the lungs of laboratory animals, significant destructive-degenerative changes in blood vessels, bronchi, and the respiratory department were established with the development of DIC-syndrome of substantial areas of inflammation, various forms of coagulopathy, and diffuse and local haemorrhages. Histologically and morphometrically, more critical areas of dis- and atelectasis, zones of emphysematous expansion, zones of inflammation, and foamy macrophages were established in the respiratory department of the lungs compared to the lungs of animals bitten by Vipera berus berus, which indicates a more pronounced toxic effect of Vipera berus nikolskii venom compared to Vipera berus berus venom on the lung tissue of research animals.

Prospects for further development are related to the analysis of structural and functional changes in the lungs of experimental rats after the introduction of Vipera berus nikolskii venom.

Information on conflict of interest

There are no potential or apparent conflicts of interest related to this manuscript at the time of publication and are not anticipated.

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Ласавуц В.С. Морфологічний стан легень щурів при впливі отрути Vipera berus nikolskii.

РЕФЕРАТ. Актуальність. Пошкодження легеневої тканини є мультиетіологічним явищем, характеризується значною різноманітністю структурно-функціональних змін. В основі патогенезу їх лежать вільно-

радикальні, запальні процеси, а головними учасниками виступають резидентні і мігруючі макрофаги та нейтрофіли. Існує ціла низка патологічних станів, що супроводжуються респіраторними ускладненнями, шляхи розвитку яких не є достатньо вивченими. Тому дослідження даного питання є своєчасним і актуальним в умовах сьогодення. **Мета.** Виявлення на світлооптичному рівні проявів пошкодження легень щурів при впливі отрути гадюк виду Vipera berus nikolskii. **Методи.** Для вивчення змін мікроструктури структурних елементів легень; визначення змін експресії маркерів, що відображають ураження органу; оцінки залежності змін між структурними компонентами, нами було проведено гістологічне, гістохімічне та морфометричні дослідження легень щурів. **Результати та підсумок.** За умов дії отрути Vipera berus nikolskii на легені лабораторних тварин встановлено глибокі деструктивно-дегенеративні зміни судин, бронхів та респіраторного відділу з розвитком ДВЗ-синдрому значних площ запалення, різними формами коагулопатій та дифузними і локальними крововиливами. Гістологічно та морфометрично встановлено в респіраторному відділі легень більші площі дис- та ателектазів, зон емфізематозного розширення, зон запалення та пінистих макрофагів порівняно із легенями тварин при укусі Vipera berus berus.

Ключові слова: легені, отрута змій, некроз, крововилив.