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ANALYSIS OF MORPHOMETRIC PARAMETERS OF LUNG TISSUE UNDER CONDITIONS OF EXPOSURE TO THE VENOM OF THE VIPERS VIPERA BERUS BERUS AND VIPERA BERUS NIKOLSKII

Lasavutz V.S. ✉ Analysis of morphometric parameters of lung tissue under conditions of exposure to the venom of the vipers Vipera berus berus and Vipera berus nikolskii.
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ABSTRACT. Background. According to the Ministry of Health of Ukraine, since the beginning of 2020, 36 cases of snake bites have been registered in Ukraine, including six children. Their significant prevalence is registered in the southern and eastern regions, and only one species is in the western and northern regions. The venom of vipers consists of about 25 proteins and peptides with enzymatic activity. The latter mainly exhibit hemotoxic effects, but neurotoxic, myotoxic, etc., have also been studied. **Objective.** Analysis of morphometric indicators of rat lung tissue under the influence of the venom of the vipers Vipera berus berus and Vipera berus nikolskii. **Methods.** Experimental studies were conducted on white, nonlinear male rats. The animals were conditionally divided into control and experimental groups, 10 individuals each. In saline, experimental rats were intraperitoneally injected with a semi-lethal dose (LD_{50}) (1.576 $mg \cdot g^{-1}$) of Vipera berus berus and Vipera berus nikolskii venom. Control group animals were intraperitoneally injected with saline only. Rats were removed from the experiment 24 hours after exposure to the venom and euthanised by cervical dislocation. Morphometric studies were performed using a system for visual analysis of histological preparations. Images from histological preparations were displayed on a computer monitor from a MICROmed SEO SCAN microscope using a Vision CCD Camera video camera. Morphometric studies were performed using SEO ImageLabBio, ImageJ, and STATISTICA 10.0 programs. The studies were performed within the specified experimental periods in preparations stained with hematoxylin and eosin. **Results and conclusion.** The introduction of the venom of the viper Vipera berus berus to rats was accompanied by a significant increase in the average value of the area of blood vessels by 1.31 times compared to the group of intact animals. A significant decrease in the average value of the respiratory department was noted to 0.8. In this group of animals, the indicators of dysatelectasis, atelectasis and emphysematous lung tissue also significantly increased by 6.03, 7.15 and 2.0 times compared to the control rats. When exposed to the venom of the viper Vipera berus nikolskii, the experimental animals showed a significant increase in the average value of the area of blood vessels by 1.55 times compared to the group of intact rats. A significant decrease in the average value of the respiratory department was found to be 0.74. The relative areas of dysatelectasis, atelectasis, and emphysematous areas of lung tissue significantly increased by 6.64, 8.80, and 2.92 times compared to the control group.
Key words: vipers, lungs, venom, respiratory tract, rats.

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Introduction

According to the Ministry of Health of Ukraine, since the beginning of 2020, 36 cases of snakebites have been registered in Ukraine, including six children. The cases were recorded in the Transcarpathian, Lviv, Donetsk, and Zhytomyr regions. It should be noted that from 2019 to 2020, there were no deaths due to snake toxins. Only poisonous vipers are found in Ukraine, namely the common viper (Vipera berus berus), steppe viper (Vipera ursinii), Nikolsky's viper (Vipera berus nikolskii), Renard's viper (Vipera renardi), and snub-nosed viper (Vipera ammodytes).

Their significant distribution is recorded in the southern and eastern regions, and only one species in the western and northern areas.

According to the literature, Vipera berus berus venom comprises about 25 proteins and peptides with enzymatic activity. The latter mainly exhibit hemotoxic effects. Hemotoxins of this species of snake can be classified according to the mechanism of action into the following groups: activators of blood clotting factors, anticoagulants, inhibitors and activators of platelets; agents affecting fibrinolysis; haemorrhaging [1, 2]. Proteins of the first group act on blood clotting factors or directly on fibrinogen. Most of them cause the formation of fibrinopeptides A or B or, in rare cases, both types. The thrombi formed in this case are unstable and prone to endogenous or venom-induced fibrinolysis. The group of anticoagulants includes serine proteases, protein C activators, and phospholipase A_2 (PLA_2). Platelet-activating proteins belong to the lectin-like proteins of type C and thrombin-like enzymes [3]. In turn, platelet deactivators are represented by disintegrins and metalloproteinases. The group of proteins responsible for fibrinolysis includes proteins that directly destroy fibrin and plasmin activators. The last in the list are haemorrhaging or cytolysins, which cause vascular damage and haemorrhage and are represented mainly by metalloproteases [4-8].

Many authors in recent studies have described pronounced histological changes in the respiratory system due to the bite of Crotalus durissus terrificus. The study of lung tissue samples 2, 6 and 12 hours after subcutaneous administration of crotoxin to mice revealed such changes as a decrease in the area of alveolar sacs and an increase in the thickness of the alveolar walls. Congestive phenomena, haemorrhages, infiltration of lung tissue with polymorphonuclear leukocytes, foamy macrophages, and increased permeability of pulmonary vessels were recorded. An increase in myeloperoxidase activity was determined in homogenates. It has also been established that crotoxin from the venom of this viper species exhibits neurotoxic effects. It is a β-neurotoxin that induces blockade of impulses in neuromuscular synapses, inhibiting the release of acetylcholine from presynaptic membranes and postsynaptic desensitisation of nicotinic receptors, causing flaccid paralysis of the respiratory muscles. In addition, PLA_2 of crotoxin leads to the degradation of phospholipids of cell membranes, triggering the arachidonic acid cascade and production of prostaglandin E_2 (PGE_2), which is associated with myo-, neurotoxicity and activation of the immune response [9].

To date, there is no data describing the full range of histological changes in the lungs during bites of venomous snakes. The available studies are few and do not reveal the main pathogenetic links in developing one or another complication from the respiratory system under these conditions. In particular, the literature is completely lacking in data on the effect of Vipera berus berus and Vipera berus nikolskii venom toxins on the respiratory system's structure and function, which explains our study's importance. However, it is worth considering the results of separate thorough studies that demonstrate the characteristic features of bronchopulmonary complications in bites of different species of snakes.

The study aims to analyse the morphometric parameters of rat lung tissue under the influence of the venom of the vipers Vipera berus berus and Vipera berus nikolskii.

Materials and methods

Experimental studies were conducted on white, non-linear male rats. For preliminary acclimatisation, the animals were kept in the Taras Shevchenko National University of Kyiv animal facility for 7 days and then in laboratory conditions in compliance with temperature and light regimes. Rats received standard food and water ad libitum. All experiments were conducted according to the Recommendations of the National Institute of Health for the Care and Use of Laboratory Animals and the European Council Directive of November 24, 1986 on the Care and Use of Laboratory Animals (86/609/EEC). The studies were approved and confirmed by the Bioethics Commission of the National Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (protocol No. 2 dated August 19, 2021). Viper venom Vipera berus berus and Vipera berus nikolskii were obtained from V. N. Karazin Kharkiv National University. Lyophilised native venom was stored at -20°C and dissolved in saline immediately before the experiment.

The animals were conditionally divided into control and experimental groups containing ten individuals. In saline, experimental rats were intraperitoneally injected with a semi-lethal dose (LD_{50}) (1.576 mg· g^{-1}) of Vipera berus berus and Vipera berus nikolskii venom. Animals in the control group were intraperitoneally injected with saline only. Rats were removed from the experiment 24 hours after exposure to the venom and euthanised by cervical dislocation.

Morphometric studies were carried out using a system to analyse histological preparations visually. Images from histological preparations were displayed on a computer monitor from a MICROmed SEO SCAN microscope using a Vision CCD Camera video camera. Morphometric studies were conducted using SEO ImageLabBio, ImageJ and STATISTICA 10.0 programs. The studies were conducted within the specified experimental periods in preparations stained with hematoxylin and eosin.

They morphometrically determined the relative proportion of vessels, bronchi, lymphoid tissue and respiratory tract, in which the relative proportion of lung tissue with unchanged histostructure, atelectasis, dysatelectasis and emphysematous lung tissue was measured.

The obtained digital material was processed using

variational statistics using the Student's t-test. Arithmetic means (M), errors of arithmetic means (m), coefficients of variation, and standard deviations were calculated. Changes were considered significant at $p \leq 0.05$.

Results and conclusions

Morphometrically, it was established that the average value of the relative area of the respiratory tract of intact animals is (82.96 ± 3.84)%, the average value of the relative location of the vessels is (4.82 ± 0.21)%, and the average value of the relative area of the bronchi is (5.47 ± 0.26)%.

Table 1

Correlation of structural components of animal lungs after exposure to the venom of Vipera berus berus and Vipera berus nikolskii (M±m)

Indicator	Control group	Vipera berus berus	Vipera berus nikolskii
Vessels, %	4,82 ± 0,21	6,33 ± 0,25 ***	7,48 ± 0,32 ***
Bronchi, %	5,47 ± 0,26	12,24 ± 0,46 ***	13,63 ± 0,58 ***
Lymphoid tissue, %	6,75 ± 0,23	15,05 ± 0,55 ***	17,40 ± 0,74 ***
Respiratory department, %	82,96 ± 3,84	66,38 ± 2,89 ***	61,49 ± 2,71 ***

Note: * – values that are statistically significantly different from the indicators of the control group of animals (* – p<0.05, ** – p<0.01, *** – p<0.001).

In the adventitia and fibrocartilaginous membrane of the bronchi, less often in the adventitia of the vessels, there is an accumulation of lymphocytes that usually form follicles and provide, together with other immunocompetent cells and structures of the lungs, powerful immune protection. The average area of the lymphoid tissue of the lungs of the intact group is (82.96 ± 3.84) % (see Table 1).

In the respiratory department of animals of the intact group, lung tissue with unchanged histostructure prevails; however, dys- and atelectasis and emphysema are also detected within the physiological norm. Accordingly, the average area of lung tissue with unchanged histostructure is (82.82 ± 3.91)%, dysatelectasis - 5.28 ± 0.24%, atelectasis - (3.55 ± 0.15)%, emphysematous lung tissue - (8.35 ± 0.39) (Table 2).

Table 2

Correlation of structural components of the respiratory tract of animals exposed to the venom of Vipera berus berus and Vipera berus nikolskii (M±m)

	Indicators			
	The relative proportion of lung tissue with unchanged histostructure, %	Relative proportion of atelectasis, %	Relative proportion of dys-atelectasis, %	Relative proportion of emphysematous lung tissue, %
Control group	82,82 ± 3,91	3,55 ± 0,15	5,28 ± 0,24	8,35 ± 0,39
Vipera berus berus	26,06 ± 1,25 ***	25,38 ± 1,23 ***	31,85 ± 1,49 ***	16,71 ± 0,81 ***
Vipera berus nikolskii	9,33 ± 0,43 ***	31,24 ± 1,46 ***	35,04 ± 1,68 ***	24,39 ± 1,20 ***

Note: * – values that are statistically significantly different from the indicators of the control group of animals (* – p<0.05, ** – p<0.01, *** – p<0.001).

In animals injected with Vipera berus berus venom, a significant increase in the average area of blood vessels was 1.31 times (p<0.001) compared to the intact group. The average area of bronchi and lymphoid tissue also progressively increased by 2.24 and 2.23 times (p<0.001) compared to the average values of the intact group. Accordingly, the average respiratory tract index significantly decreased to 0.8 compared to the intact value (see Table 1). Morphometrically, it was found that under the influence of Vipera berus berus toxins in the lungs of experimental animals, the relative proportions of dys- and atelectasis, emphysematous lung tissue significantly increased by 6.03; 7.15 and 2.0 times (p<0.001) compared to the intact group. Accordingly, the average value of lung tissue with unchanged histostructure progressively decreases, which is 0.31 compared to the value of the intact group (see Table 2).

In animals that received the venom of Vipera berus nikolskii vipers, morphometric calculations revealed a significant increase in the average area of blood vessels by 1.55 times (p<0.001) compared to the value of the intact group. Along with the vessels, the average values of the area of bronchi and lymphoid tissue also increased by 2.49 and 2.58 times

(p<0.001), respectively, compared to the values of the intact group. Therefore, the average value of the respiratory department significantly decreases to 0.74 compared to the intact indicator (see Table 1).

Morphometric calculation of the respiratory tract parameters of white rats under the conditions of Vipera berus nikolskii bite showed that the values of the relative areas of dis- and atelectasis, emphysematous lung tissue significantly increased 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 and is 0.11 compared to the indicator of the intact group of animals (see Table 2).

Summary

The introduction of the venom of the viper Vipera berus berus to rats was accompanied by a significant increase in the average value of the area of blood vessels by 1.31 times compared to the group of intact animals. A significant decrease in the average value of the respiratory department was noted to 0.8. In this group of animals, the indicators of dysatelectasis, atelectasis and emphysematous lung tissue also significantly increased by 6.03, 7.15 and 2.0 times compared to the control rats.

When exposed to the venom of the viper Vipera berus nikolskii, a significant increase in the average value of the area of blood vessels was found in the experimental animals by 1.55 times compared to the group of intact rats. A significant decrease in the average value of the respiratory department was found to be 0.74. The relative areas of dysatelectasis, atelectasis, and the emphysematous regions of lung tissue significantly increased by 6.64, 8.80 and 2.92 times compared to the control group.

Prospects for further development are related to the study of submicroscopic changes in rat lung tissue under the influence of the venom of the vipers Vipera berus berus and Vipera berus nikolskii.

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. Аналіз морфометричних показників легеневої тканин за умов впливу отрути гадюк Vipera berus berus та Vipera berus nikolskii.

РЕФЕРАТ. Актуальність. За даними МОЗ в Україні з початку 2020 року зареєстровано 36 випадків укусів змій, серед них – шестеро дітей. Значна їх розповсюдженість зареєстрована в південних і східних областях і лише один вид в західних та північних. Отрута гадюк складається з близько 25 білків та пептидів з ферментативною активністю. Останні проявляють, в основному, гемотоксичну дію, але досліджено також нейротоксичну, міотоксичну дії, тощо. **Мета.** Аналіз морфометричних показників тканини легень щурів за умов впливу отрути гадюк Vipera berus berus та Vipera berus nikolskii. **Методи.** Експериментальні до-

слідження проводили на білих нелінійних щурах самцях. Тварин умовно розподіляли на дві групи – контрольну і дослідну по 10 особин в кожній. Дослідним щурам внутрішньоочеревинно вводили напівлетальну дозу (LD_{50}) (1.576 мг· $г^{-1}$) отрути Vipera berus berus та Vipera berus nikolskii на фізіологічному розчині. Тваринам контрольної групи внутрішньоочеревинно вводили лише фізіологічний розчин. Виводили щурів з експерименту через 24 години після впливу отрути, знежившюючи шляхом цервікальної дислокації. Морфометричні дослідження здійснювали, використовуючи систему візуального аналізу гістологічних препаратів. Зображення з гістологічних препаратів на монітор комп'ютера виводили з мікроскопу MICROmed SEO SCAN та за допомогою відеокамери Vision CCD Camera. Морфометричні дослідження проведені за допомогою програм SEO ImageLabBio, ImageJ та STATISTICA 10.0. Дослідження проводили у визначені терміни досліду в препаратах забарвлених гематоксиліном і еозином. **Результати і підсумок.** Введення щурам отрути гадюк *Vipera berus berus* супроводжувалось достовірним зростанням середнього значення площі судин у 1,31 рази відносно групи інтактних тварин. Відмічали достовірне зменшення середнього показника респіраторного відділу до 0,8. У даної групи тварин також достовірно підвищувались показники дисателектазів, ателектазів та емфізематозно зміненої легеневої тканини у 6,03; 7,15 та 2,0 рази відносно контрольних щурів. При впливі отрути гадюк *Vipera berus nikolskii* у дослідних тварин виявлено достовірне збільшення середнього показника площі судин у 1,55 раза порівняно з групою інтактних щурів. Встановлено достовірне зниження середнього значення респіраторного відділу до 0,74. Показники відносних площ дисателектазів, ателектазів, емфізематозно змінених ділянок легеневої тканини достовірно зростали у 6,64; 8,80 та 2,92 рази порівняно з групою контролю.

Ключові слова: гадюки, легені, отрута, респіраторний відділ, щури.