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MORPHOLOGICAL CHANGES IN THE LUNG VESSELS OF LABORATORY RATS 1 HOUR AFTER ADMINISTRATION OF LEIURUS MACROCTENUS SCORPION VENOM

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One hour after the introduction of Leiurus macroctenus scorpion venom, significant morphological changes were observed in the vessels of the lungs of rats. Stagnant capillaries, petechial hemorrhages, perivascular edema and destruction of vascular membranes were noted. The formation of stasis and blood clots was observed. Analysis of semi-thin sections showed a narrowing of the lumen of arterioles, folding of the internal elastic membrane and a decrease in vascular permeability. The morphometric parameters of blood vessels indicate a tendency to decrease their diameters compared to the parameters of the control group of animals.

Key words: effect of Leiurus macroctenus scorpion venom, rats, pulmonary vessels, morphological and morphometric changes.

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МОРФОЛОГІЧНІ ЗМІНИ В СУДИНАХ ЛЕГЕНЬ ЛАБОРАТОРНИХ ЩУРІВ ЧЕРЕЗ 1 ГОДИНУ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНУ LEIURUS MACROCTENUS

Через одну годину після введення отрути скорпіону Leiurus macroctenus у судинах легень щурів спостерігались значні морфологічні зміни. Відзначались застійні капіляри, петехіальні крововиливи, периваскулярний набряк та руйнування судинних оболонок. Спостерігались утворення стазів і тромбів. Аналіз напівтонких зрізів показав звуження просвіту артеріол, складчастість внутрішньої еластичної мембрани та зниження пропускної здатності судин. Морфометричні параметри судин свідчать про тенденцію до зменшення їх діаметрів порівняно з показниками контрольної групи тварин.

Ключові слова: вплив отрути скорпіона Leiurus macroctenus, щури, судини легень, морфологічні та морфометричні зміни.

The study was conducted at the National Pirogov Memorial Medical University, Vinnytsya "Morphological changes in the lungs of rats under the influence of Leiurus macroctenus scorpion venom", state registration No. 0124U003714.

Scorpions are common on all continents of the world except Antarctica, and in one way or another come into contact with humans. Considering the venom of some species of scorpions, such contacts can lead to severe and even fatal consequences. According to the results of a systematic review, about 1.5 million cases of scorpion stings are recorded globally annually, of which approximately 1.2 million are clinically significant and require medical attention. In countries with a high level of scorpionism, such as India, Pakistan, Mexico, and Brazil, up to 100,000 cases have been reported annually. The highest rates of severe poisoning are observed in children under 5 years of age and the elderly, which indicates the vulnerability of these groups. In some tropical regions, for example in India, the frequency of severe poisoning can reach 0.4 % of the total number of cases. Mortality from scorpion bites is approximately 0.03 % in general, but this rate is much higher in countries with low levels of medical services [13].

In the northeastern Brazilian city of Campina Grande, more than 2,000 cases of scorpion stings were recorded from 2007 to 2012, with 68 % of victims having moderate symptoms and 32 % having severe symptoms. Most victims were among children under 10 years old, who accounted for 55 % of cases, and also among adults aged 20 to 40, who accounted for 35 % of cases. The average time between the bite and the start of treatment was 2 hours. Treatment included antidote therapy for 85 % of victims and symptomatic treatment for 15 % [3].

Over 100,000 cases of scorpion bites have been registered in Iran over the past two decades. The largest number of cases is observed in the southeastern regions of the country. Epidemiological data indicate that the peak incidence occurs in the summer period from June to September, when scorpion activity increases due to hot weather conditions. Victims are mostly people under the age of 20, in particular children, who make up about 40 % of all cases. Approximately 10–15 % of victims require hospitalization for specialized treatment [7].

In France from 2011 to 2020, 1,144 cases of scorpion bites were recorded. 95 % of them were in the south of France. Among the victims, 10 % had serious symptoms of poisoning that required

hospitalization, while 2 % of cases had a severe clinical picture with signs of neurological disorders. It has been established that children and persons with weakened immunity are the most vulnerable to severe forms of poisoning. Mortality from bites was less than 0.01 % of all cases [14].

And although there is no data on the lethality or number of bites of the studied scorpion Leiurus macroctenus in the scientific literature, such information is available on its "close relative", Leiurusquin questriatus. The mortality rate from the bite of this species of scorpion in the city of Luxor (Egypt) was recorded at the level of 5.5 % [1].

In this way, the problem of scorpionism does not lose its relevance, and taking into account the discovery of new species of scorpions, new experimental studies are needed.

The purpose of the study was to determine structural changes in the vascular component of the lungs of laboratory rats occurring one hour after administration of a semi-lethal dose of Leiurus macroctenus scorpion venom.

Materials and methods. Based on the typical morphological characteristics of the species [11], Leiurus macroctenus scorpions were identified by Mark Stockmann and obtained from his private scorpion nursery in the city of Ibbenbüren (Germany). All scorpions were bred in captivity. For the study, 15 sexually mature individuals of both sexes were used, which were kept on a diet (1 Shelfordella lateralis cockroach per week), with access to water (distilled water was replenished weekly), in plastic containers filled with sand (Exo Terra "Desert Sand"), with ventilation (through numerous holes in the boxes), constant temperature (25–35 °C), humidity (50–60 %) and natural lighting conditions. For at least a year, only cockroaches were used as food. In case of refusal of feed, the cockroach was removed from the container after 2 days. Once a month, the sand was freed from the remains of cockroaches. Milking of 15 sexually mature Leiurus macroctenus scorpions of both sexes was performed once according to the method of Ozkan and Filazi [12], modified by Yaqoob et al. [15], 1 month after arrival from the nursery. After immobilizing the scorpion, the electrodes were directed to the cephalothorax and tail. An electric current of 24 W was then applied for 5 s to the base of the tail, while the opposite end was directed towards the sterile vial. The amount of poison released during milking was from 0.1 to 0.5 mg. The poison was stored at a temperature of -20 ºC. The venom of scorpions of the family Buthidae, genus Leiurus, species Leiurus macroctenus, was administered to rats once intramuscularly (0.5 ml of the venom solution previously dissolved in physiological solution; 28.8 μg/ml; LD50=0.08 mg/kg [6]).

The study used 10 white male laboratory rats weighing 200 g (±10 g), grown in the vivarium of the Educational and Scientific Center "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv (agreement on scientific and practical cooperation between the Taras Shevchenko National University of Kyiv Shevchenko, National Pirogov Memorial Medical University, Vinnytsya and Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine from February 1, 2021). Rats were kept on a standard diet in the conditions of an accredited vivarium in accordance with the "Standard rules for the arrangement, equipment and maintenance of experimental biological clinics (vivariums)". The experiments were conducted in accordance with the current regulatory documents regulating the organization of work using experimental animals and compliance with the principles of the "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [10]. Also, all work with animals was carried out in accordance with the Law of Ukraine dated February 21, 2006 No. 3447-IV "On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals".

Rats selected for the experiment were divided into two groups: control – 5 rats, poison was not administered, material was collected one hour after the introduction of physiological solution; experimental – 5 rats, selection of histological material 1 hour after the introduction of the poison. Rats were euthanized by inhalation of carbon dioxide. Rat lungs were isolated at 4 °C immediately after euthanasia.

For microscopic examination, pieces of the organ were taken and fixed in a 10 % formalin solution. Dehydration of the studied tissues was carried out in alcohols of increasing concentration, followed by compaction in the histoprocessor Logos ONE (MILESTONE, Italy), and poured into paraffin blocks in an automatic station for the production of paraffin blocks TEC 2800 (HESTION, Australia). Histological sections with a thickness of 4–5 μm were obtained on a rotary microtome AMR-400 (Amos Scientific Pty, Australia). Sections were stained with hematoxylin and eosin using the Azan Trichrome method.

To make semi-thin sections (1–2 μm thick), the material was fixed in a 2.5 % glutaraldehyde solution with a pH of 7.3–7.4. Postfixation was carried out with a 1 % solution of osmium tetroxide, after which dehydration was carried out in alcohols of increasing concentration and poured into a mixture of

epoxy resins with subsequent polymerization. Semi-thin sections were made on an ultramicrotome Ultrotome LKB 4801 A (Bromma, Sweden) and stained with methylene blue.

Microscopy of histological preparations was carried out using an OLIMPUS BX 41 light microscope using magnifications of 40, 100, 200 and 400 times. Image visualization and morphometry were performed using the Quickphoto micro 2.3 morphometric program. Micropreparations were described according to generally accepted pathomorphological principles. Morphometric studies of such indicators as: inner diameter of arterioles, inner diameter of venules and inner diameter of capillaries were carried out.

Statistical analysis of the obtained results was carried out in the license package "Statistica 6.0" using non-parametric estimation methods. The nature of the distributions for each of the obtained variation series, mean values and standard square deviation was evaluated. The significance of the difference in values between independent quantitative values was determined using the Mann-Whitney U-test.

Results of the study and their discussion. Rats of the control group had well-structured lungs with a well-developed microcirculatory channel. Lumens of vessels are full of blood. Regarding the morphometric parameters of the vessels of the microcirculatory bed of the lungs of control group rats, the internal diameter of arterioles was equal to 40.86±4.95 μm, capillaries – 16.55±4.78 μm, and venules – 53.94±5.11 μm.

Areas of moderately congested alveolar capillaries and petechial hemorrhages, which were characterized by the presence of erythrocytes in the interalveolar and intraalveolar spaces, were observed in the lungs of rats injected with scorpion venom after 1 hour. Perivascular edema and swelling of vessel membranes were observed (Fig. 1).

On some histological photographs, the destruction of the endothelial and muscular lining of arterioles and venules was noted. Stratification of the smooth myocytes of the muscle sheath was observed due to the swelling of the connective layers between the myocytes. The endothelium was detached from the basement membrane in some places. These changes provoked petechial hemorrhages, which we mentioned above. The formation of stasis and blood clots was noted (Fig. 2).

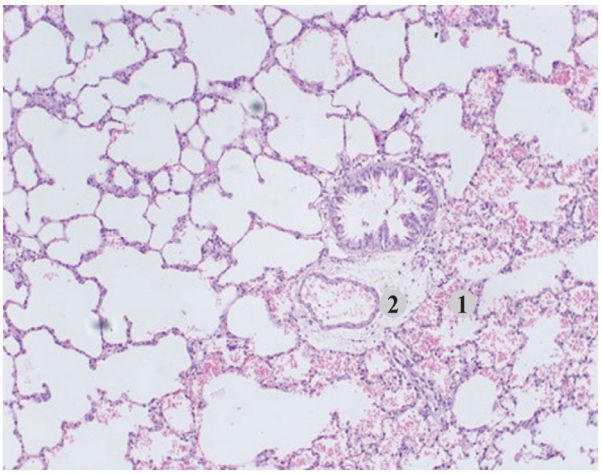

Fig. 1. A fragment of a rat lung 1 hour after the injection of scorpion venom. 1 – petechial hemorrhages; 2 – perivascular edema and swelling of vessel membranes. Hematoxylin-eosin staining. Magnification ×40.

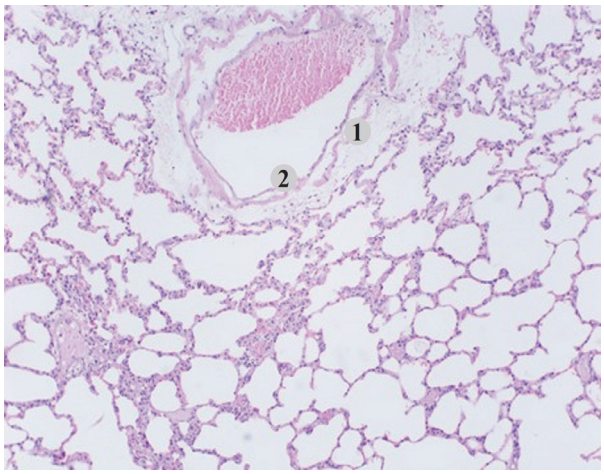

Fig. 2. A fragment of a rat lung 1 hour after the injection of scorpion venom. 1 – swelling of the adventitia membrane and connective layers between bundles of myocytes; 2 – arteriole endothelium. Hematoxylin-eosin staining. Magnification ×40.

Moderately congested alveolar capillaries and multifocal petechial hemorrhages were found, characterized by the presence of erythrocytes in the interalveolar and intraalveolar spaces, walls of individual middle bronchi (Fig. 3).

In vessels of medium and large diameter, only some venous congestion, individual diapedic extravasates, interstitial and perivascular edema, and thickening of interalveolar membranes were observed (see Fig. 3).

When examining semi-thin sections, the lumen of some arterioles was narrowed. The internal elastic membrane of the arterial vascular walls had a folded structure to the point of "corrugation", which indicates an increase in vasoconstrictor reactions in these vessels with a decrease in permeability. At the same time, their enlightenment was full-blooded. Compared to the control group of animals, an increase in the number of segmented neutrophils and lymphocytes was observed (Fig. 4).

Regarding the morphometric parameters of the vessels of the microcirculatory bed of the lungs of rats 1 hour after the introduction of scorpion venom, the inner diameter of arterioles was equal to 38.91±5.39 μm (p=0.473 compared to the control group), capillaries – 13.45±3.41 μm (p=0.112 compared to the control group), and the venule – 49.73±4.82 μm (p=0.082 compared to the control group).

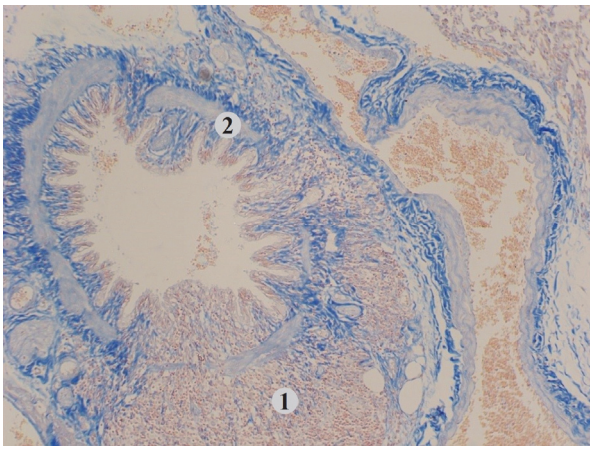

Fig. 3. A fragment of a rat lung 1 hour after the injection of scorpion venom. 1 – petechial hemorrhages; 2 – bronchus of medium order. Staining with azan trichrome. Magnification ×100.

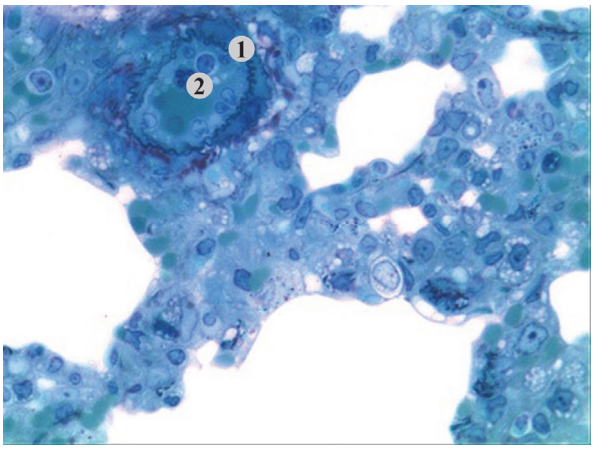

Fig. 4. A fragment of a rat lung 1 hour after the injection of scorpion venom. 1 – folded internal elastic membrane of an arteriole; 2 – a segmented neutrophil in the lumen of an arteriole. Staining with methylene blue. Magnification ×400.

Thus, 1 hour after the introduction of scorpion venom in the vascular bed of the lungs of rats, primary compensatory and adaptive elements of the modification of the vascular bed with a pronounced inflammatory component, manifested by excessive, destructive edema, an increase in the number of round cell elements in the lumen of the vessels, and a general violation of blood rheology with formation of blood clots. The results of measurement of vascular parameters 1 hour after the introduction of scorpion venom showed tendencies to decrease the internal diameter of arterioles by 4.77 %, capillaries by 18.73 %, and venules by 7.80 % compared to similar indicators of the control group.

Clinical manifestations of the effect of Leiurus macroctenus venom have not yet been investigated, while there are relevant data on the effect of Leiurusquin questriatus venom, which is genetically related to the scorpion we are studying. The authors of the study indicate pronounced local reactions in the form of redness, pain, paresthesias in the bite area, as well as systemic effects – headache, nausea, vomiting, convulsions and, in particular, severe shortness of breath, which indicates an effect on the respiratory system [1].

Bakır and co-authors [2] conducted an experimental study by injecting mice with a semi-lethal dose of Aegaeobuthus nigrocinctus scorpion venom followed by the collection of lung tissue samples for histological examination 1, 3, and 6 hours after the venom injection. After 1 hour, leukocytosis, an increased level of blood vessel filling and moderately pronounced petechial hemorrhages were found in the lungs of rats, which is consistent with the data obtained in our study.

Buthus lienhardi poison causes pronounced hemorrhages, thickening of the interalveolar membranes, emphysema; moderately pronounced pulmonary edema is also observed. At the same time, there is no mononuclear infiltration [9].

Another group of researchers studied the effect of Hottentota gentili scorpion venom [4]. Histological examinations of various organs, particularly the lungs, revealed massive hemorrhages, especially 12 hours after the injection of the poison. At the same time, the study of the degree of edema in the lungs did not reveal any significant differences between the control group and the groups of animals that were injected with poison and examined the organ after 30 minutes, 3 hours and 12 hours.

In relation to the increased number of white blood cells in the blood vessels found in our study, there are data on the number of different round cell cells in the blood of rats after the introduction of the venom of the scorpion Hemiscorpius lepturus. It was found that 2 hours after the introduction of the poison, compared to the control group of rats, there was a significant decrease in the number of white bloods, especially neutrophils, and to lesser extent lymphocytes [5]. In another study, it was found that the venom of this scorpion disrupts the normal functions of the blood, causing an increase in blood clotting time and an increase in the activity of some coagulation factors. The venom also promotes the activation of platelets, which can lead to the formation of blood clots. Different venom fractions show

different levels of these effects, indicating complex effects on the coagulation system. In general, scorpion venom leads to a violation of hemostasis [8], which was also observed in the case of Leiurus macroctenus venom.

Conclusion

As a result of the toxic effect of the venom of the scorpion Leiurus macroctenus on the lungs of rats, 1 hour after administration, instantaneous changes in the vascular component of the respiratory department occurred, which consisted mostly in the form of stagnant capillaries, petechial hemorrhages, perivascular edema, destruction of vascular membranes, and vasoconstrictor reactions with a decrease in vascular permeability, which prevents the venom from entering the lungs.

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