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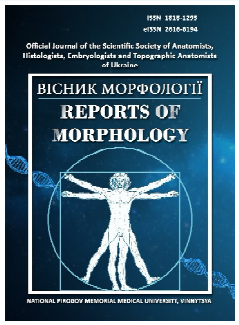

Histological organization of the spleen of rats under conditions of acute intoxication with viper venom Vipera berus berus

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The immune system plays an important role in the body's response to numerous environmental factors, including snake venom toxins. The composition of snake venom and the specifics of its effect on the human body are quite diverse. The predominance of one or another component of the toxin becomes decisive for the appearance of complications or even death. The study aims to study the peculiarities of the histological organization of the spleen of rats under conditions of acute intoxication with Vipera berus berus viper venom. Experimental studies were carried out on white, non-linear male rats. The animals were conditionally divided into a control and an experimental group of 10 individuals. Experimental rats were injected intraperitoneally with a semi-lethal dose (LD_{50}) (1.576 mg/g^{-1}) of Vipera berus berus venom in a physiological solution. Animals of the control group were injected intraperitoneally with only a physiological solution. Rats were removed from the experiment 24 hours after exposure to the poison and anaesthetized by cervical dislocation. Spleen samples were taken for microscopic examination. Fixation of the material and preparation of paraffin blocks were carried out according to generally accepted methods. Histological preparations of the spleen were stained with hematoxylin and eosin. Histological preparations were studied using an SEO SCAN light microscope. Acute intoxication with Vipera berus berus viper venom was associated with disorganization of the white pulp of the spleen of experimental animals, which manifested itself in the form of a loss of clarity and orderliness of the placement of its structural elements, poor visualization of the marginal zone of lymphoid nodules. An increase in the number of lymphoblasts characterized the germinal centres. Areas of spleen tissue destruction and hemorrhage were noted in the red pulp of the organ. The presence of foci of lymphocyte apoptosis, thickening of trabeculae, stasis and sludge of erythrocytes in the lumen of trabecular veins was also observed. Thus, under the influence of Vipera berus berus venom, disorganization of the white pulp is observed at the structural level of the rat spleen, and areas of organ destruction, lymphocyte apoptosis, and connective tissue proliferation are noted in the red pulp.

Keywords: *anatomy, histology, spleen, lymphoid nodules, lymphocytes, rats, vipers, poison.*

Introduction

The spleen is one of the most reactive organs, and it shows a rapid response to the action of damaging factors during the early stages of their impact. According to numerous experimental studies, it is essential to develop specific immunological reactions since multiple cell populations ensure the constancy of the body's internal environment [13, 23, 24]. To date, there is convincing evidence that the spleen can maintain the body's resistance in response to the action of physical and chemical environmental factors. However, under conditions of their chronic influence, the reserves of the immune status of the organ suffer significantly. They are depleted due to impaired morphology and functions of critical structural elements. In this regard, among today's scientific studies, more and more studies are focused on studying the pathophysiological pathways of spleen

damage under the influence of adverse factors of various genesis [3, 29, 32, 33].

Snakes use their venom to neutralise prey and to deter or weaken predators [2, 19]. The realisation of these goals is possible because toxins cause the development of muscle paralysis (by blocking the transmission of nerve impulses), cause changes in the functioning of the cardiovascular system, or cause pronounced local lesions at the site of bites [7, 18,]. Among all venomous animals, snakes attract special attention from humanity [11, 16, 17]. On the one hand, such interest is because cases of poisoning by their toxins are prevalent and are among the unsolved health problems worldwide, as they cause numerous fatal consequences [9, 22, 26]. According to WHO estimates, between 81,000 and 138,000 people die from snakebites each year, and another 400,000 victims suffer severe complications or even disability [30].

The immune system is essential in the body's response to numerous environmental factors, including snake venom toxins. The composition of the venom and the features of its effect on the human body are pretty diverse. The predominance of one or another component of the toxin becomes decisive for complications or even death [10, 14]. Despite sufficiently thorough and large-scale studies by scientists worldwide, individual components of snake toxins, the features of their action and the pathogenesis of damage to internal organs have not yet been established. In particular, the mechanisms of spleen damage under these conditions have not been fully revealed, and information about the features of morphological changes in organs at the light-optical and submicroscopic levels has not been provided. In addition, data on the effect of V. berus berus viper toxins on the structure and function of the immune system are currently absent. Therefore, research on this issue with the aim of early diagnosis, prevention of complications, and opportunities to improve treatment methods is an urgent task.

The study aims to investigate the features of the histological organization of the spleen of rats under acute intoxication with the viper Vipera berus berus venom.

Materials and methods

The experimental study was conducted with special care and attention to the condition of the animals. The rats underwent a thorough 7-days acclimatization in a particular Taras Shevchenko National University of Kyiv animal facility. This was followed by transfer to laboratory conditions with strict adherence to temperature and light regimes [12]. During this period, the rats received standard food and water ad libitum. The experiment was conducted per 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 study was approved and confirmed by the Bioethics Commission of the Institute of Biology and Medicine of the Taras Shevchenko National University of Kyiv (protocol No. 2, dated August 19, 2021). The venom of Vipera berus berus used in our study was obtained from the V. N. Karazin Kharkiv National University. The lyophilized native venom was carefully stored at -20 °C and dissolved in saline immediately before the experiment, ensuring our study's highest scientific accuracy and reliability.

The animals were divided into two groups of 10 individuals: control and experimental. In saline, experimental rats were intraperitoneally injected with a semi-lethal dose (LD_{50}) (1.576 mg/g^{-1}) of Vipera berus berus 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 euthanized by cervical dislocation.

Spleen samples were taken from previously weighed animals of all groups for microscopic examination. The pieces were fixed in 10 % formalin solution, and the duration of exposure did not exceed 1-2 days. The applied fixative solution prevents the process of autolysis and stabilises cells and tissues for further processing and use in staining procedures. Then, the pieces were dehydrated in alcohols with increasing concentration and embedded in paraffin blocks. Histological preparations of the spleen were stained with hematoxylin and eosin [4, 18]. Histological preparations were examined on an SEO SCAN light microscope and photo-documented using a Vision CCD camera with an image output system from histological preparations.

Results

Modelling acute intoxication with the viper Vipera berus berus venom was associated with the development of histological changes in the spleen of experimental rats. Microscopic examination of the organ revealed disorganization of the white pulp structure. In particular, lymphoid nodules had indistinct contours; their marginal zone was almost not differentiated. In addition, enlarged germinative centres with a significant number of lymphoblasts were visualised, distinguished by large sizes, hypochromic nuclei and pale cytoplasm. Mature B-lymphocytes in the reactive centres of lymphoid nodules were significantly less than lymphoblasts. A moderate number of T-lymphocytes and macrophages characterized periarterial zones of lymphoid nodules. Small light and dark lymphocytes were mainly noted. The lumens of the central arteries were rounded, and their walls were somewhat thickened, mainly due to the middle and adventitious membranes. The endothelial lining of the central arteries' intima mainly was continuous, with loosening of the endothelial layer observed in some places (Fig. 1).

In the spleen's red pulp, under the administration of Vipera berus berus venom to rats, numerous erythrocytes, platelets, lymphocytes, plasma cells and macrophages were detected. In some areas of the red pulp, foci of the destruction of the spleen tissue and haemorrhage were

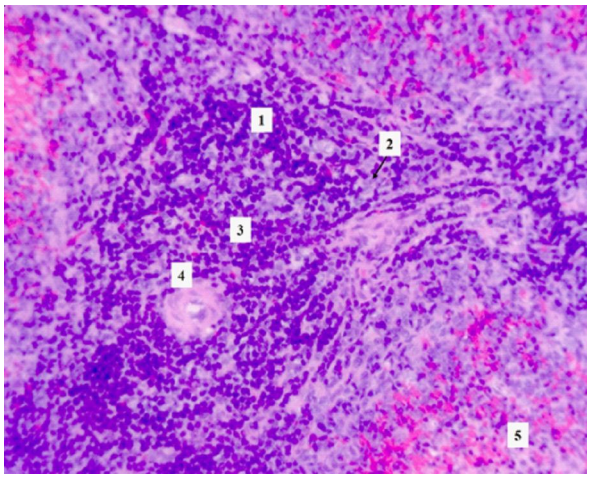

Fig. 1. Morphological changes in the spleen of rats exposed to the viper Vipera berus berus venom. Lymphoid nodule (1), lymphoblasts (2), marginal zone (3), central artery (4), red pulp (5). Staining with hematoxylin and eosin. ×400.

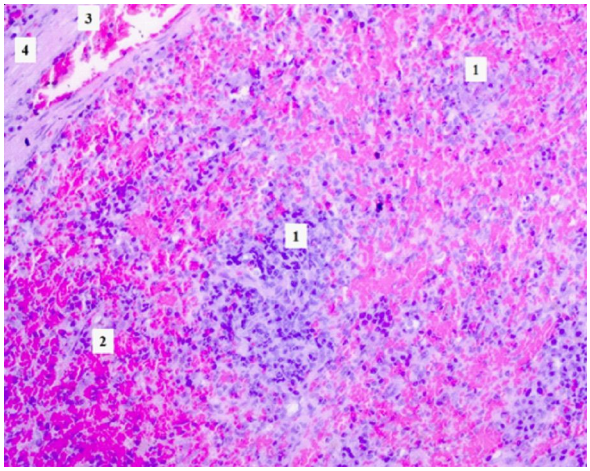

Fig. 2. Histological structure of the spleen of rats under the influence of the viper Vipera berus berus venom. Lymphoid nodules (1), the red pulp (2), the lumen of the trabecular vein (3) and splenic trabeculae (4). Staining with hematoxylin and eosin. ×400.

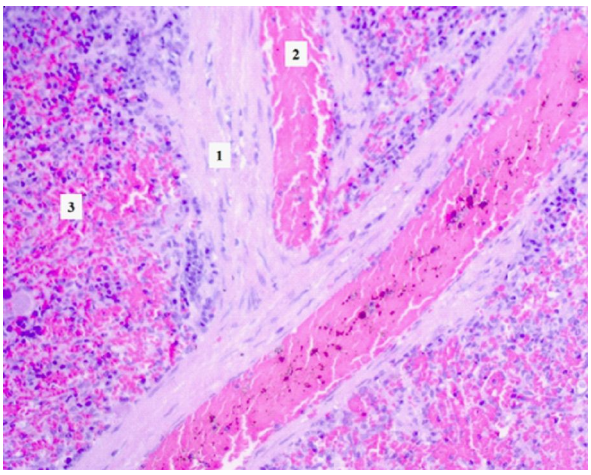

Fig. 3. Photo-optical changes in the structure of the spleen of rats under the influence of the venom of the viper Vipera berus berus. Splenic trabeculae (1), trabecular vein (2), red pulp (3). Staining with hematoxylin and eosin. ×400.

observed. Lymphocytes in the red pulp had a rounded shape. Their nuclei were hyperchromic; the cytoplasm occupied a small part of the cell, and sometimes, it was not visualized or was concentrated paranuclearly in the form of a rim. Plasma cells of the spleen had a rounded or slightly elongated shape. Their nuclei were shifted to the cell poles and were hyperchromic. In the red pulp, numerous macrophages were large and had processes. The nuclei of these cells occupied an eccentric position. The cytoplasm was sometimes illuminated, swollen, and contained remnants of phagocytised material. It should be noted that areas of lymphocyte apoptosis were observed during histological examination of the spleen of rats injected with the venom of the viper Vipera berus berus. The trabeculae of the spleen under these conditions were thickened. The smooth myocytes had elongated nuclei in their composition. The spaces between the muscle fibres were dilated, and the fibres were swollen. The trabecular veins had precise contours. Stasis and sludge of erythrocytes and adhesion of erythrocytes to the walls of the veins were detected in their lumen (Fig. 2, 3).

Discussion

The immune system plays an essential role in the body's response to numerous environmental factors that affect the ordinary course of physiological processes. An immune response accompanies any changes in homeostasis. According to the literature, the latter is also induced in the case of bites of poisonous animals, including snakes. It has been established that their toxins can activate the type II immune response; however, as recent studies have shown, the features of the pathogenesis of the interaction of this body system and viper venom are much more complex [8, 21]. Given the wide variety of enzymes and non-enzymatic components in its composition, it is unsurprising that the consequence is developing a pronounced inflammatory process. Its mechanisms are still being studied. However, the main participants are PLA2 and SVMP [15]. The high activity of PLA2 in snake venom maintains a significant concentration of arachidonic acid and associated cytokines [5]. Also, the effect of toxins triggers the production of IL-6, TNF-6, IL-1, chemokines, and lipid mediators, creating conditions for the activation and migration of leukocytes. Lipid mediators (prostaglandins, leukotrienes, thromboxanes) accompany the inflammatory process, manifesting various pathological effects (oedema, pain). It is a proven fact that snake toxins can activate endogenous PLA2. The latter affect ryanodine receptors, activate protein kinase C, release Ca^{2+}, and, thus, form a persistent humoral immune response.

Studies by Kou J. Q. [20] and Wang S. Z. [29] have shown that viper venom exerts a pronounced effect on cellular and humoral immune responses. Toxins obtained from the Naja naja atra species in mice with normal immunity increased the activity of NK cells and the proliferation of B lymphocytes. Also, in this group of animals, they suppressed the delayed-

type hypersensitivity reaction caused by dinitrofluorobenzene and inhibited the proliferation of T lymphocytes stimulated by concavalin A. In mice with moderately expressed immunodeficiency states, administering the venom of this species of viper caused an increase in the levels of Ig G and Ig M and the restoration of germinal centres in the spleen. In addition, in spleen cells examined by flow cytometry, a selective increase in Th1, Th2, IFN-r, and IL-4 levels and a decrease in IL-17 were observed.

According to scientific sources, PLA2 from snake venom activates inflammasomes. The latter are multiprotein complexes located in the cytoplasm of immune cells. They can recognise and respond to infectious agents, damage, etc. One of the most studied is the NLRP3 inflammasome (nucleotide oligomeric domain, leucine-rich protein). In the case of the interaction of PLA2, in which the catalytic domain contains lysine (at position 49) instead of aspartate and is characteristic of the venom of the snake Bothrops jararacussu, with cell membranes, NLRP3 is activated, which causes the formation of the inflammasome complex. Activated NLRP3 promotes the interaction procaspase-1 with ASC (apoptosis-associated cytosolic adaptor protein). This process leads to the formation of caspase-1, the release of IL-1β and the initiation of apoptosis [6].

Snake venoms are capable of modulating the immune system, particularly at the level of cytokine production. Venom components of some members of the Elapidae family have been shown to exhibit anti-arthritic and anti-inflammatory activity in rats, primarily through a reduction in serum levels of the pro-inflammatory cytokines IL-1β, IL-17, and TNF-α. At the same time, intraperitoneal injection of Bothrops atrox and Bothrops erythromelas venoms into mice resulted in an inflammatory response characterised by an increase in multiple serum and spleen cytokines, such as TNF-α, IL-6, IL-10, IL-12p70, and IFN-γ [25].

The crude venom of Montivipera bornmuelleri has antimicrobial activity against gram-positive and gram-negative bacteria, with the most significant effect on Staphylococcus aureus and Morganella morganii, as well as against the fungus Candida albicans [1]. This species of snake has been found to contain toxic compounds that affect components of the immune system of the spleen of mice. T. Yacoub et al. [31] investigated the effect of intraperitoneal injection of multiple doses of Montivipera bornmuelleri venom on the levels of various cytokines (TNF-α, IFN-γ, IL-4, IL-10, IL-1β and IL-17) in the spleen tissue of BALB/c mice, which helped further to evaluate the potential use of the venom in immunotherapy. The study showed that Montivipera bornmuelleri venom can significantly modulate the immune system towards a pro-inflammatory Th1/Th17 response rather than an anti-inflammatory Th2/Treg response. This shift is likely mediated and maintained by an increase in the production of pro-inflammatory cytokines – IL-1β, TNF-α and IFN-γ and by the suppression of the anti-inflammatory cytokine IL-10 on the other hand, which is regulated by the ability of the venom to induce the production of IL-4. These results, together with the finding that Montivipera bornmuelleri venom is selectively cytotoxic to human keratinocyte cancer cell lines (low-grade II4) compared to the non-tumor HaCaT cell line, demonstrate that this venom is valuable in the field of immunotherapy [27, 28].

Conclusions

1. Acute intoxication with the venom of the viper Vipera berus berus leads to disorganization of the white pulp of the spleen of experimental animals, which manifests itself in a loss of clarity and orderliness in the placement of its structural elements and poor visualization of the marginal zone of lymphoid nodules. An increase in the number of lymphoblasts characterizes germinative centres.

2. In the organ's red pulp, areas of destruction of the spleen tissue and haemorrhages are noted. There are also foci of lymphocyte apoptosis, thickening of trabeculae, stasis, and erythrocyte sludge in the lumens of the trabecular veins.

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ГІСТОЛОГІЧНА ОРГАНІЗАЦІЯ СЕЛЕЗІНКИ ЩУРІВ ЗА УМОВ ГОСТРОЇ ІНТОКСИКАЦІЇ ОТРУТОЮ ГАДЮК VIPERA BERUS BERUS

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Імунна система відіграє важливу роль в реакції організму на чисельні фактори середовища, в тому числі токсинів зміїних отрут. Склад отрути змій та особливості її впливу на організм людини є досить різноманітними. Переважання того, чи іншого компоненту токсину стає визначальним для появи ускладнень чи навіть смерті. Метою дослідження є вивчення особливостей гістологічної організації селезінки щурів за умов гострої інтоксикації отрутою гадюк Vipera berus berus. Експериментальні дослідження 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 на фізіологічному розчині. Тваринам контрольної групи внутрішньоочеревинно вводили лише фізіологічний розчин. Виводили щурів з експерименту через 24 години після впливу отрути, знеживлюючи шляхом цервікальної дислокації. Для мікроскопічного дослідження забирали зразки селезінки. Фіксацію матеріалу та приготування парафінових блоків проводили за загальноприйнятими методиками. Забарвлення гістологічних препаратів селезінки здійснювали гематоксиліном та еозином. Гістологічні препарати вивчали за допомогою світлового мікроскопа SEO SCAN. Гостра інтоксикація отрутою гадюк Vipera berus berus асоціювалась з дезорганізацією білої пульпи селезінки дослідних тварин, що проявлялась у вигляді втрати чіткості та впорядкованості розміщення її структурних елементів, поганою візуалізацією крайової зони лімфоїдних вузликів. Гермінативні центри характеризувались зростанням чисельності лімфобластів. В червоній пульпі органу відмічали ділянки деструкції тканини селезінки, геморагії. Спостерігали також наявність вогнищ апоптозу лімфоцитів, потовщення трабекул, стази і сладжі еритроцитів в просвітах трабекулярних вен. Таким чином, під впливом дії отрути Vipera berus berus на структурному рівні селезінки щурів спостерігається дезорганізація білої пульпи, а в червоній пульпі відмічаються ділянки деструкції органу, апоптоз лімфоцитів та розростання сполучної тканини.

Ключові слова: *анатомія, гістологія, селезінка, лімфоїдні вузлики, лімфоцити, щури, гадюки, отрута.*

Author's contribution

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