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Immunohistochemical assessment of CD20 expression in rat kidneys 1 hour after administration of *Leiurus macroctenus* scorpion venom

The aim of the work: to assess the degree of CD20 expression in the kidneys of rats 1 hour after the administration of the venom of the scorpion *Leiurus macroctenus*.

Materials and Methods. The study used 10 white male laboratory rats weighing 200 g (±10 g). The venom of scorpions from the Buthidae family, specifically the genus Leiurus and the species *Leiurus macroctenus*, was administered to rats intramuscularly (0.5 ml of a previously dissolved venom solution in saline; 28.8 μg/ml; LD_{50} = 0.08 mg/kg). To identify the subpopulation of CD20 cells in kidney tissue, rabbit recombinant primary antibody Anti-CD20 (ab64088, Abcam, USA) was used.

Results. In the kidney parenchyma of control rats, the CD20 precipitate was not detected. Immunohistochemical staining is negative in all morphological structures, particularly in the renal corpuscles, nephron tubules, and interstitium. After 1 hour of the experiment, following the administration of scorpion *Leiurus macroctenus* venom, immunohistochemical studies of the kidney revealed single CD20 cells, which are rarely found, scattered mainly in the peritubular interstitium without a tendency to group.

Conclusions. One hour after the administration of *Leiurus macroctenus* venom to rats, a low level of CD20 expression was observed in the kidney tissue of animals with single B lymphocytes in the peritubular interstitium, indicating an early stage of the humoral response.

Key words: venom; scorpions; kidneys; inflammation; rats.

Problem Statement and Analysis of Recent Research. Toxic compounds derived from animal venoms enter the bodies of their victims, disrupting the delicate balance of the internal environment. The resulting pathological changes are closely related to alterations in cell structure and function, as well as to disruptions of vital biochemical reactions. At the forefront of the body's defence against these formidable toxins are specialised cells that work tirelessly to restore homeostasis or promote specific adaptations to new conditions. Toxins from various venomous species demonstrate an extraordinary ability to influence the morphofunctional properties of cells – damaging protective membranes, creating destructive pores or disrupting the function of ion channels [1, 2]. These agents have evolved complex adaptations that enable them to penetrate target organs, triggering a cascade of pathological changes. The immune, nervous and endocrine systems play a key role in organising the body's response to the poison, activating critical signalling pathways that promote adaptation. Their harmonious interaction is essential for maintaining vital functions. Dysfunction of any of these interconnected systems can lead to serious complications or even tragic consequences after a venomous bite. This highlights the critical importance of studying how homeostasis is affected at the cellular level in such difficult circumstances [3].

To date, there is information on about 2000 species of scorpions. The vast majority of them are dangerous to humans, especially representatives of the Buthidae, Scorpionidae and Hemiscorpionidae families. According to the WHO, about 1.5 million cases of poisoning due to scorpion bites are registered in the world annually, leading to 2000-3000 deaths. Scorpion venom causes numerous adverse effects in various tissues of the body, including the kidneys, an organ with a high blood supply that is particularly vulnerable to toxic damage. Clinical manifestations of renal damage include proteinuria, hematuria, and hemoglobinuria. Inflammatory reactions and nephrotoxic side effects of the venom are also observed.

In some cases, renal failure may occur, especially after stings from scorpions of North Africa, the Middle East, the eastern Mediterranean, and South Asia. The direct pathogenetic mechanisms of renal damage due to scorpion toxin poisoning remain incompletely understood. Still, vasoconstriction of renal vessels caused by catecholamines produced by the sympathetic nervous system in response to poisoning is believed to play an important role. In turn, vasoconstriction can lead to renal ischemia and also initiate inflammatory reactions that increase tissue damage. Additionally, activation of the parasympathetic nervous system, combined with inflammatory processes, can lead to necrosis of the renal tubules. However, it remains

unclear whether necrosis is a direct consequence of the action of scorpion venom components or whether it occurs indirectly, through the activation of the sympathetic nervous system and/or the body's inflammatory response [4, 5].

The data available to date on this problem are significantly limited and require a comprehensive and detailed study. It is also worth noting that the number of new scorpion species is rapidly increasing every year, and previously unknown individual representatives are being discovered, whose venom is relevant to modern scientists for study purposes.

The aim of the work: to assess the degree of CD20 expression in rat kidneys 1 hour after administration of Leiurus macroctenus scorpion venom.

Materials and Methods. The venom of scorpions of the Buthidae family, genus Leiurus, species *Leiurus macroctenus*, was administered to rats once intramuscularly (0.5 ml of venom solution previously dissolved in saline; 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" of Taras Shevchenko National University of Kyiv (agreement on scientific and practical cooperation between Taras Shevchenko National University of Kyiv, National Pirogov Memorial Medical University and Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine dated February 1, 2021). Rats were kept on a standard diet in an accredited vivarium in accordance with the "Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria)". The experiments were conducted in accordance with the current regulatory documents regulating the organisation of work with experimental animals and compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [7]. Also, all work with animals was carried out in accordance with the Law of Ukraine No. 3447-IV of February 21, 2006, "On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals". The rats selected for the experiment were divided into two groups: a control group (5 rats), where no poison was administered, and a material was collected one hour after the administration of saline; and an experimental group (5 rats), where histological material was collected 1 hour after the administration of poison. Rats were euthanised by inhalation of carbon dioxide. Kidneys were isolated from rats at 4 °C immediately after euthanasia.

To identify the CD20+ cell subpopulation in kidney tissue, rabbit recombinant primary antibodies Anti-CD20 (ab64088, Abcam, USA) were used.

Antigens were revealed by temperature-induced reprivation in EnVision FLEX Target Retrieval Solution High pH buffer (pH 9.0) in a KOS histology processor (Milestone, Italy) at 98 °C for 20 minutes. 3% aqueous H_2O_2 was used to block endogenous peroxidase activity. Further incubation with specific primary antibodies was performed for 60 minutes using the rabbit polyclonal antibody Anti-CD68 (Cat. No. ab125212, Abcam, USA) to detect CD68+ cells [8, 9].

Visualisation of the formed immune complexes was performed using the Mouse/Rabbit PolyVue™ HRP/DAB polymer detection system (Diagnostic BioSystems, USA), which provides high sensitivity and specificity through polymer-conjugated secondary antibodies with peroxidase activity and subsequent chromogenic development with diaminobenzidine (DAB). Counterstaining of cell nuclei was performed with Mayer's hematoxylin according to a standard protocol. The preparations were examined using a MICROmed SEO SCAN light microscope, and a Vision CCD Camera was used for photodocumentation.

Results. In the kidney parenchyma of rats of the control group, the precipitate of the reaction to CD20+ is not detected (---). Immunohistochemical staining is negative in all morphological structures, particularly in the renal corpuscles, nephron tubules, and interstitium. The absence of CD20+ cells indicates that, under physiological conditions, there is no infiltration of renal tissue by B-lymphocytes. This confirms the kidney's stable immune status under norm conditions and serves as a reference point for assessing inflammatory changes in experimental conditions. Thus, the absence of the precipitate can be regarded as an indicator of the basic level of CD20 expression, which is practically zero, i.e. negative in the control group of animals (Fig. 1).

After 1 hour of the experiment, following the administration of venom from the scorpion *Leiurus macroctenus*, immunohistochemical studies of the kidney revealed single CD20+ cells, rarely found, scattered mainly in the peritubular interstitium, without a tendency to group. A weak, diffuse, dull, light-brown cytoplasmic staining is observed, with a reaction of low intensity (+--), in individual cells, indicating a low level of antigen expression (Fig. 2).

Discussion. A comprehensive review of scientific literature indicates that endothelial cells in the vascular

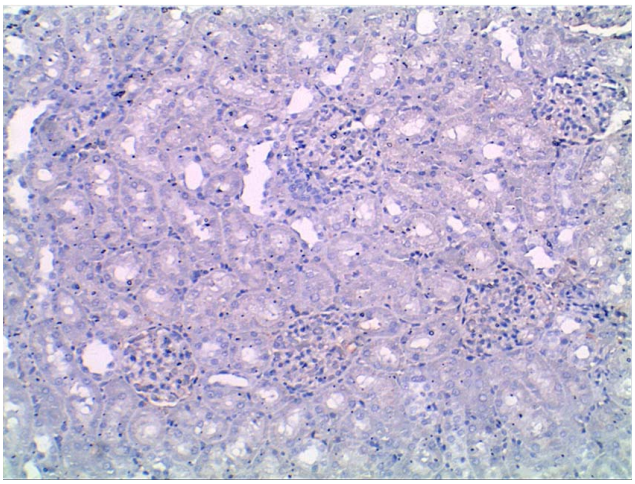

Fig. 1. Expression of CD20+ cells in the parenchyma of the renal cortex of rats in the control group. Absence of precipitate in the structural components of the kidney of rats in the intact group. Immunohistochemical staining using an antibody to CD20, counterstaining with Mayer's hematoxylin. Magnification: x100.

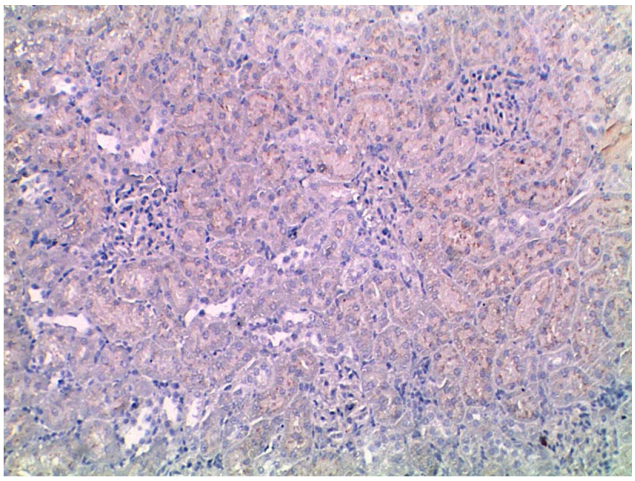

Fig. 2. Expression of CD20+ cells in rat kidneys 1 hour after administration of Leiurus macroctenus scorpion venom. Faint precipitate signal in the peritubular interstitium. Immunohistochemical staining using anti-CD20 antibody, counterstained with Mayer's hematoxylin. Magnification: x100.

walls play a crucial role in recognising and protecting the body from toxins produced by venomous animals. Previous studies have not established their involvement in inflammatory processes triggered by bites; however, recent data suggest otherwise. As the primary interface with toxic components entering the bloodstream, endothelial cells recognise these toxins by expressing various pattern recognition receptors (PRRs), including toll-like receptors (TLRs), tumour necrosis factor (TNF) receptors, and interleukin-1 (IL-1) receptors. This activation leads to the expression of pro-inflammatory genes and ultimately results in pathological changes in the vascular microcirculation.

Importantly, endothelial cells also express major histocompatibility complex (MHC) class I and II molecules, as well as CD40 ligands. These proteins facilitate the intravascular presentation of foreign agents, including venom toxins, to immune effector cells [10]. Endothelial cells play a significant role in modulating immune defence by influencing leukocyte transport and migration. The adhesion and extravasation of leukocytes are typical responses mediated by the selective expression of intercellular adhesion molecule-1 (ICAM-1) and selectins on the apical surface of endothelial cells [11, 12].

Given the data on endothelial cells' role in regulating the immune response, it can be concluded that dysfunction of the endothelium, resulting from the harmful effects of venom toxins, can lead to morphological distortions in endothelial cells, disruption of their cytoskeletal organisation, and compromised immune reactivity in response to bites from predatory animals. Such dysfunction can result in severe complications. Experiments on rats have demonstrated alterations in vascular wall permeability and stability under these conditions. When exposed to snake and spider venoms, endothelial cell cultures exhibited increased secretion of IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1). These compounds enhance neutrophil adhesion to endothelial cells via selectin-mediated interactions, significantly elevating intracellular calcium levels and promoting the release of proteolytic enzymes involved in tissue degradation [13, 14].

In cases of bites from venomous animals, TLRs, specifically TLR2 and TLR4, play a significant role in recognising various toxins in animal venoms. For example, Ts1, a β-toxin found in the venom of the scorpion *Tityus serrulatus*, binds to TLR2 and TLR4, triggering cytokine and lipid mediator production and initiating an inflammatory cascade. This cascade involves the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and activator protein-1 (AP-1), which are crucial for regulating gene expression in response to stress and damage, as well as controlling processes of cellular differentiation, proliferation, and apoptosis. Additionally, increased mitogen-activated protein kinase (MAPK) signalling is characteristic, further influencing gene transcription, cell proliferation, migration, and apoptosis [15, 16].

Moreover, secretory phospholipase A2 (sPLA2), a component of the venoms from *Bothrops atrox* and *Bothrops asper* snakes, can also be recognised by TLR2. This recognition leads to the production of

eicosanoids, such as prostaglandin E2 (PGE2) and leukotrienes, which serve as potent chemoattractants for neutrophils at the site of venom exposure. It has been experimentally shown that TLR2 stimulates the migration of polymorphonuclear leukocytes and IL-1β production following intraperitoneal administration of *Bothrops atrox* snake venom in rats [17, 18, 19].

In the immune system's response to toxins from venomous animals, tissue basophils play a significant role. Their stimulation is associated with the activation of inflammasomes and the expression of caspase-1 [20, 21]. Basophils release histamine and lipid mediators, and their degranulation can contribute to the development of anaphylactic reactions [22]. Notably, congenital abnormalities in basophils, such as mutations associated with mastocytosis, can lead to severe allergic reactions to animal bites, which may even result in fatal outcomes [23]. In rats, toxins from *Bothrops atrox* have been shown to induce the formation of complement system fractions C3a and C5a, which further promote the degranulation of tissue basophils, chemotaxis, neutrophil activation, and severe anaphylaxis [24, 25, 26].

Conclusions. During the first hour of exposure of experimental rats to the venom of the scorpion *Leiurus macroctenus*, a low level of CD20 expression was observed in kidney tissue, with single B lymphocytes in the peritubular interstitium, indicating an early stage of the humoral response.

Conflict of interest. The authors declare that they have no conflict of interest.

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Authors' contribution. Matkivska R. M. – literature review, collection of material, analysis of the results obtained, preparation of the text of the article and design of illustrations.

Prospects for further research. Study of the expression of other immunohistochemical markers in the kidney tissue of experimental rats associated with the activation of inflammatory processes.

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ІМУНОГІСТОХІМІЧНА ОЦІНКА ЕКСПРЕСІЇ CD20 У НИРКАХ ЩУРІВ ЧЕРЕЗ ГОДИНУ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНІВ *LEIURUS MACROCTENUS*

Мета роботи: оцінити ступінь експресії CD20 у нирках щурів через 1 год після введення отрути скорпіонів *Leiurus macroctenus*.
Матеріали і методи. У дослідженні використано 10 білих лабораторних щурів-самців масою 200 г (±10 г). Отруту скорпіонів родини Buthidae роду Leiurus виду *Leiurus macroctenus* вводили щурам одноразово внутрішньом'язово (0,5 мл розчину отрути, попередньо розчиненому у фізіологічному розчині; 28,8 мкг/мл; $ЛД_{50}$=0,08 мг/кг). Для ідентифікації субпопуляції CD20-клітин у тканині нирок використовували кролячі рекомбінантні первинні антитіла Anti-CD20 (ab64088, Abcam, США).
Результати. У паренхімі нирки щурів із контрольної групи преципітат реакції на CD20 не виявляється. Імуногістохімічне забарвлення є негативним в усіх морфологічних структурах, зокрема в ниркових тільцях, канальцях нефрона та інтерстиції. Через 1 год експерименту після введення отрути скорпіона *Leiurus macroctenus* імуногістохімічні дослідження нирки показали поодинокі CD20-клітини, які виявляються рідко, переважно розсіяні у перитубулярному інтерстиції, без тенденції до групування.
Висновки. Через 1 год після введення щурам отрути *Leiurus macroctenus* спостерігали низький рівень експресії CD20 в тканині нирок тварин із поодинокими В-лімфоцитами у перитубулярному інтерстиції, що вказує на ранню стадію гуморальної відповіді.

Ключові слова: отрута; скорпіони; нирки; запалення; щури.

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