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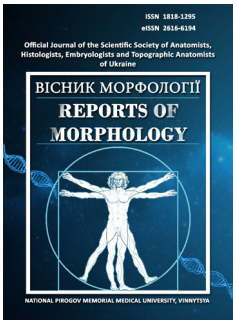

Features of CD20 expression in the kidney tissue of experimental rats three hours after administration of Leiurus macroctenus scorpion venom

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Bites from venomous animals, such as snakes, vipers, and arthropods (particularly scorpions), are a significant public health problem in many countries worldwide. Acute kidney injury is one of the main effects of these venoms and is associated with high morbidity and mortality. The study aims to investigate the features of CD20 expression in the kidney tissue of experimental rats three hours after administration of scorpion Leiurus macroctenus venom. The study used 10 white male laboratory rats weighing 200 g (±10 g). The venom of the scorpion Leiurus macroctenus (Buthidae) was administered to rats once intramuscularly (0.5 ml of a venom solution previously dissolved in saline; 28.8 μg/ml; LD50=0.08 mg/kg). To identify the CD20 cell subpopulation in kidney tissue, rabbit recombinant primary antibodies Anti-CD20 (ab64088, Abcam, USA) were used. Visualisation of the formed immune complexes was performed using the Mouse/Rabbit PolyVue™ HRP/DAB polymer detection system (Diagnostic BioSystems, USA). Counterstaining of cell nuclei was performed with Mayer's hematoxylin according to the standard protocol. The preparations were examined using a MICROmed SEO SCAN light microscope. Administration of the scorpion venom Leiurus macroctenus at an early stage of the study (after 3 hours) induces activation of the immune response in the kidney tissue of rats, as evidenced by the appearance of single CD20⁺ B-lymphocytes in the cortical substance and renal corpuscles. In the control group, CD20 expression is absent, confirming the normal immune status of the kidney and the absence of physiological B-cell infiltration. Thus, the appearance of single CD20⁺cells after venom administration reflects the early phase of the inflammatory reaction and the initiation of B-lymphocyte recruitment in response to toxic injury. However, the intensity of infiltration at this stage remains minimal, not massive.

Keywords: *histology, expression, kidney, inflammation, apoptosis, rats.*

Introduction

Venomous animal bites, including those from snakes, vipers, and arthropods, especially scorpions, are a significant public health problem in many countries worldwide. Acute kidney injury is one of the main consequences of these venoms, leading to high morbidity and mortality [21, 26, 30]. The World Health Organisation recognises venomous animal bites as a neglected public health problem, especially in regions such as Latin America, Asia, and Africa. Estimates range from 421,000 to 1.8 million snakebites occur annually, with 20,000 to 94,000 of these resulting in death. The most vulnerable groups are economically disadvantaged groups and rural residents. However, the actual number of cases is likely much higher, as existing epidemiological data are primarily based on official reports and may not include victims treated in primary care settings [1, 6, 12].

Snake and scorpion venoms have a diverse chemical composition, including proteins, enzymes, carbohydrates, lipids, metals, biogenic amines, and nucleotides [14, 27, 31, 35]. This toxic composition has evolved as a hunting and defence mechanism. However, urbanisation and population growth have led to an increase in the number of animal bites [34]. Clinical manifestations of envenomation depend on

various factors, including the type and dose of venom, the site of the bite, the size of the victim, and the time of medical intervention; symptoms can range from mild local reactions to severe systemic poisoning that develops over several hours [3, 8, 20].

Acute kidney injury is the leading cause of death among patients exposed to snake and scorpion stings. Studies show that snake and scorpion venom can penetrate renal tissue within minutes, appearing in the urine several hours after exposure. Without timely preventive measures, there is a significant risk of acute renal failure, which may require dialysis [4, 19, 32, 33]. Additional clinical manifestations, such as rhabdomyolysis, intravascular hemolysis, coagulopathy, neurotoxic symptoms, hypopituitarism, and systemic inflammatory response syndrome, further worsen the condition of victims. Studies show that histological signs of renal injury from these venoms include acute tubular necrosis and interstitial nephritis. Morphological analysis of kidney tissue biopsies reveals notable changes, including increased glomerular capillary dilation, destructive changes in the basement membrane, oedema of endothelial cells, and focal proliferation of mesangial cells [7, 9, 17].

Since the composition of scorpion venom has not been thoroughly studied to date, and the mechanisms of kidney damage remain an open question, the topic of our study is relevant.

The study aims to study the features of CD20 expression in the kidney tissue of experimental rats three hours after 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), raised in the vivarium of the Educational and Scientific Centre “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, Vinnytsya and I. Ya. 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 governing the organisation of work with experimental animals and with the principles of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” [11]. 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”.

The venom of scorpions of the Buthidae family of the Leiurus genus of the Leiurus species 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) [16, 23].

The rats selected for the experiment were divided into two groups: control – 5 rats; no venom was administered; material was collected 3 hours after saline administration; experimental – 5 rats; histological material was collected 3 hours after venom administration. The rats were euthanised by inhalation of carbon dioxide. The isolation of rat kidneys was performed at 4 °C immediately after euthanasia.

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

Antigens were revealed using the temperature-induced reprivation method in the EnVision FLEX Target Retrieval Solution High pH buffer (pH 9.0), which was carried out in a KOS histology processor (Milestone, Italy) at 98 °C for 20 minutes. To block endogenous peroxidase activity, a 3 % aqueous H_2O_2 solution was used. Further incubation with specific primary antibodies was carried out for 60 minutes: to detect $CD20^+$ cells, rabbit polyclonal antibodies Anti-CD20 (Cat. No. ab64088, Abcam, USA) were used [5, 17].

Visualisation of the formed immune complexes was carried out using the Mouse/Rabbit PolyVue™ HRP/DAB polymer detection system (Diagnostic BioSystems, USA), which provides high sensitivity and specificity due to 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 the standard protocol [18]. 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 in the control group, the CD20 reaction precipitate 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 normal conditions and serves as a reference point for assessing inflammatory changes under experimental conditions. Thus, the absence of the precipitate can be regarded as an indicator of the baseline level of CD20 expression, which is practically zero, i.e. negative in the control group of animals (Fig. 1).

In rat kidney tissue, 3 hours after the introduction of scorpion venom, a weak immunohistochemical reaction (+--) is detected with the formation of a single brown precipitate in the cytoplasm of cells corresponding to $CD20^+$ B-lymphocytes. These cells are localised mainly in the interstitium of the cortical substance, indicating the initial phase of the immune response and the migration of B cells to the damaged area. There is also a slight immunoprecipitation in the renal corpuscle area. The staining intensity is low, focal, without signs of massive infiltration (Fig. 2).

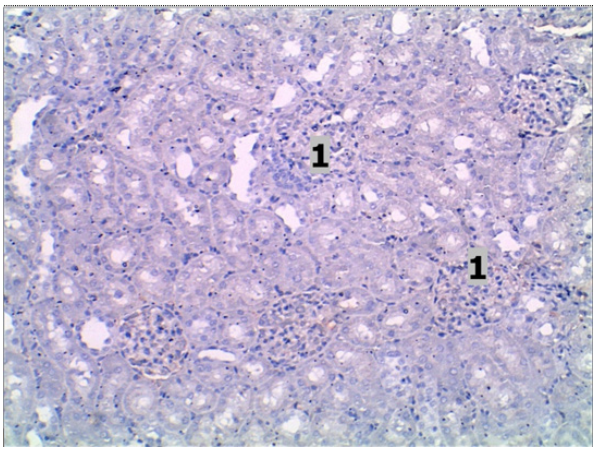

Fig. 1. Expression of CD20+ cells in the parenchyma of the renal cortex of rats in the control group. Absence of precipitate in the structural components of the kidney of rats in the intact group (1). Immunohistochemical staining using an antibody to CD20, counterstaining with Mayer's hematoxylin. ×100.

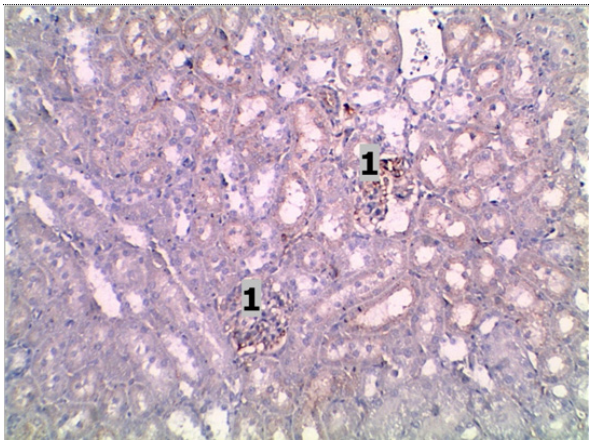

Fig. 2. Expression of CD20+ cells in rat kidneys 3 hours after scorpion venom administration. Immunoprecipitation in the renal corpuscle area (1). Immunohistochemical staining using an antibody to CD20, counterstaining with Mayer's hematoxylin. ×100.

Discussion

Scorpion venom is a mixture of chemical compounds that overactivate the autonomic nervous system, leading to a massive release of vasoactive substances and inflammatory cytokines. Symptoms of scorpion envenomation typically include signs of increased cholinergic and adrenergic activity, cardiovascular dysfunction, and increased neuromuscular excitability. In severe cases, especially in children, there is a risk of pulmonary oedema, multiple organ failure, and even death [22].

Studies have shown that the venom of the scorpion Hemiscorpius lepturus can cause cytotoxicity and hemolysis. The venom is primarily excreted from the body through the kidneys and liver and tends to be highly concentrated in renal tissue. The exact mechanisms of renal injury from scorpion stings are still being investigated, but likely include vascular ischemia due to vasoconstriction, hemodynamic instability, rhabdomyolysis, excessive systemic inflammation, and direct nephrotoxic effects of the venom [15, 24, 28].

Microscopic studies have revealed acute tubular necrosis, destructive changes in the renal glomeruli, interstitial oedema, thrombotic microangiopathy, and cortical necrosis in cases of scorpion stings. Clinical observations suggest that children affected by scorpion stings are at increased risk of developing chronic renal failure. Unfortunately, even with the rapid administration of antivenom, the prognosis for chronic renal dysfunction does not significantly improve [25, 29, 36].

Experimental studies by Zoccal K. F. et al. [37] showed that Tityus silvestris envenomation in mice resulted in severe systemic effects, including hyperglycemia, leukocytosis, cytokine release (IL-6, IL-1β, IL-10), and damage to lung and kidney tissues. In contrast, the venoms of Tityus metuendus and Tityus obscurus resulted in less severe symptoms in animals.

The most significant number of scorpion sting envenomations has been reported in Saudi Arabia, Iraq, and Jordan. The most clinically substantial toxins in Old World scorpion venoms are the α-toxins, which bind to the neurotoxin at three sites on voltage-gated sodium (Nav) channels, leading to sympathetic excitation and the release of endogenous catecholamines, which can cause severe myocardial damage. Most scorpion sting victims experience local lesions, but systemic symptoms, especially in children, are often noted due to the venom of Androctonus and Leiurus scorpions. These systemic manifestations include hemodynamic and respiratory disorders, as well as myocarditis. In addition, other symptoms may include paralysis (from Parabuthus leiosoma), coagulopathy (caused by Nebo hierichonticus and Hemiscorpius species), local tissue damage, hemolysis, and acute kidney injury (associated with Hemiscorpius lepturus). In such cases, early antivenom therapy is crucial, although its effectiveness remains controversial. However, intensive treatment, including the use of vasoactive drugs, is effective in many cases [2].

A. Elmourid et al. [13] observed significant histological and biochemical changes in experimental mice injected with Buthus paris scorpion venom. In the liver, morphological changes included hepatocytes ballooning, haemorrhage, and nuclear pyknosis. Examination of lung tissue showed thickening of the interalveolar septum and pulmonary oedema. In addition, hemorrhagic manifestations were noted after administration of a dose of 450 μg/kg-1 of venom. Examination of the kidneys revealed an expansion of the lumen between the outer and inner layers of Bowman's capsule, as well as destructive changes in the glomeruli. At the same dose, glomeruloneuromas and signs of haemorrhage were also observed. The toxic effect of Buthus paris venom on the heart was characterised by disorganisation and degeneration of myofibrils. Biochemical analysis of the blood serum of poisoned mice showed a significant increase in ALT ($p<0.05$), AST ($p<0.05$) and LDH

(p<0.05). An increase in CPK was also noted.

R. Dizaji et al. [10] found significant changes in biomarkers of acute kidney injury and mitochondrial biogenesis in mice injected with Hemiscorpius lepturus venom. IL-18 expression increased significantly on the first day after administration of 5 and 10 mg/kg doses of venom (p<0.01), indicating early activation of the inflammatory cascade; after 7 days, a significant increase was observed in the 5 mg/kg group compared to the control group. Determination of reactive oxygen species (ROS) production demonstrated a substantial effect of the venom on days 1 and 7 (p<0.05), with post hoc analysis confirming a significant increase in ROS across all groups of poisoned animals (p<0.001). After seven days, ROS levels in the treatment groups were lower than on day 1, indicating a partial restoration of antioxidant status. The venom also induced apoptosis, as evidenced by a significant increase in caspase-9 expression after 1 day in the 10 mg/kg group and after 7 days in the 5 mg/kg group. Caspase-3 expression was significantly increased only 1 day after administration of 5 and 10 mg/kg (p<0.05), with no significant changes in the 7-day group. Studies of regulators of mitochondrial biogenesis showed considerable overexpression of AMPK (AMP-activated protein kinase) in both the 5 and 10 mg/kg groups on day 1 and in the 5 mg/kg group on day 7. Sirt1 expression was significantly increased 24 hours after both doses (p<0.001) and remained elevated in the 5 mg/kg group on day 7 (p<0.01). Sirt3 expression was significantly increased only on day 1 in the 5 mg/kg group (p<0.001), with no significant changes in the 10 mg/kg group. After seven days, a marked increase in expression was also observed in the 5 mg/kg group. Overall, evidence suggests that Hemiscorpius lepturus venom induces a combination of inflammatory, oxidative, and apoptotic pathways, while significantly affecting the regulation of genes involved in mitochondrial biogenesis [10].

Conclusions

The introduction of the venom of the scorpion Leiurus macroctenus at an early stage of the study (after 3 hours) induces the activation of the immune response in the renal tissue of rats, as evidenced by the appearance of single $CD20^+$ B-lymphocytes in the cortical substance and renal corpuscles. In the control group, CD20 expression is absent, which confirms the normal immune status of the kidney and the absence of physiological B-cell infiltration. The appearance of single $CD20^+$ cells following venom introduction reflects the early phase of the inflammatory response and the initiation of B-lymphocyte recruitment in response to toxic damage. However, the intensity of infiltration at this stage remains minimal, not massive.

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ОСОБЛИВОСТІ ЕКСПРЕСІЇ CD20 В ТКАНИНІ НИРОК ЕКСПЕРИМЕНТАЛЬНИХ ЩУРІВ ЧЕРЕЗ ТРИ ГОДИНИ ПІСЛЯ ВВЕДЕННЯ ОТРУТИ СКОРПІОНА LEIURUS MACROCTENUS

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Укуси отруйних тварин, таких як змії, гадюки та членистоногі (зокрема скорпіони), є суттєвою проблемою громадського здоров'я в багатьох країнах світу. Гостре ураження нирок є одним із головних ефектів дії цих отрут і асоційоване з високим рівнем захворюваності та смертності. Метою дослідження є вивчення особливостей експресії CD20 в тканині

нирок експериментальних щурів через три години після введення отрути скорпіона Leiurus macroctenus. 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; ЛД50=0,08 мг/кг). Для ідентифікації субпопуляції CD20 клітин в тканині нирок використовували кролячі рекомбінантні первинні антитіла Anti-CD20 (ab64088, Abcam, США). Візуалізацію сформованих імунних комплексів здійснювали з використанням полімерної детекційної системи Mouse/Rabbit PolyVue™ HRP/DAB (Diagnostic BioSystems, США). Контрастне дозабарвлення клітинних ядер виконували гематоксиліном Майєра відповідно до стандартного протоколу. Препарати досліджували за допомогою світлооптичного мікроскопа MICROmed SEO SCAN. Введення отрути скорпіона Leiurus macroctenus вже на ранньому терміні дослідження (через 3 години) індукує активацію імунної відповіді в нирковій тканині щурів, про що свідчить поява поодиноких CD20⁺ В-лімфоцитів у кірковій речовині та ниркових тільцях. У контрольній групі експресія CD20 повністю відсутня, що підтверджує нормальний імунний статус нирки та відсутність фізіологічної В-клітинної інфільтрації. Таким чином, поява поодиноких CD20⁺ клітин після введення отрути відображає ранню фазу запальної реакції та ініціацію рекрутування В-лімфоцитів у відповідь на токсичне ушкодження, хоча інтенсивність інфільтрації на цьому етапі залишається мінімальною та не носить масивного характеру.

Ключові слова: *гістологія, експресія, нирки, запалення, апоптоз, щури.*

Author's contribution

Matkivska R. M. – conceptualization, research, writing of the original draft.
Samborska I. A. – project administration, resources.
Mostiuk O. M. – validation, software