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MORPHOLOGICAL FEATURES OF THE TRIGEMINAL GANGLION IN ACUTE ASEPTIC INFLAMMATION AT THE EARLY STAGES OF THE EXPERIMENT

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The phenomena of acute λ-carrageenan inflammation in the trigeminal ganglion were characterized by phasing. Thus, in the first stage, against the background of increasing hydration, minor destructive phenomena in the structure of the stroma of the trigeminal ganglion are revealed, which adversely affect metabolism, resulting in impaired blood circulation, reduced phagocytic response activity. Such changes are observed only in the early stages, which lead to slowing or suppression of the cell barrier formation, with subsequent reactivation and generalization of the process due to inflammatory factors activity in the trigeminal ganglion stroma and in the nervous tissue specifically. The second phase is characterized by regenerative phenomena; the products of tissue metabolism are resorbed or removed from the site of inflammation. All this occurs together with reducing the clinical signs of inflammation, normalization of biochemical and functional disorders that occur in the first phase of inflammation.

Key words: aseptic inflammation, λ-carrageenan, trigeminal ganglion, neurocyte, rats.

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МОРФОЛОГІЧНІ ОСОБЛИВОСТІ ТРІЙЧАСТОГО ВУЗЛА ПРИ ГОСТРОМУ АСЕПТИЧНОМУ ЗАПАЛЕННІ НА РАННІХ ТЕРМІНАХ ЕКСПЕРИМЕНТУ

Явища гострого λ-карагіненового запалення в трійчастому вузлі характеризувалися наявністю стадій. Так, на першій стадії на тлі наростаючої гідратації виявлялись незначні руйнівні явища в структурі строми трійчастого вузла, які несприятливо впливали на трофіку, внаслідок чого погіршувався кровообіг, знижувалась активність фагоцитарної реакції, з подальшою реактивацією та генералізацією процесу внаслідок дії факторів запалення як у стромі трійчастого вузла, так і у власне нервовій тканині. Надалі запалення швидко переходить у 2 фазу яка характеризувалась регенеративними явищами, що протікали на тлі дегідратації зони запалення. У цій фазі завершується бар'єризація і настає повне обмеження зони ушкодження. Паралельно розсмоктувалися або виводилися з вогнища запалення продукти тканинного обміну. Все це протікало на тлі зниження клінічних ознак запалення, нормалізації біохімічних і функціональних порушень.

Ключові слова: асептичне запалення, λ-карагінен, трійчастий вузол, нейроцит, щури.

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Inflammation in the animal world history was formed as a process in which different elements, both protective and harmful, always interact. On the one hand, it is a threat that damages the whole organism, and on the other hand, it is a process that helps the organism in the struggle for survival [3, 11]. Inflammation is considered as a general pathological process, because it has all the characteristics inherent in typical pathological processes.

Evolutionarily, inflammation was formed as an adaptive-protective reaction of the body to the influence of pathogenic factors, aimed at localization, destruction and removal of a foreign agent, as well as to eliminate the effects of its action and is characterized by alteration, exudation and subsequent proliferation of cells and tissues. Aseptic inflammation occurs due to or under the influence of a number of mechanical, physical or chemical factors [3, 4, 5]. It also distinguishes three stages, but there is a certain difference in course, depending on the agent of influence [2, 7].

According to some authors, about 75 % temporary disability reasons belong to persons with diseases of the peripheral nervous system [9], 38 % of them are mononeuropathy of various aetiologies. The dominant type of facial pain is trigeminal neuralgia, which got its name in 1671. The healer Aretaeus in his works first described such symptoms in the first century of the last millennium. He described in detail the disease, which occurs with attacks of pain localized on half of the face. It is well known that the neurons of the trigeminal ganglion are involved in various sensory functions in the orofacial area, namely the formation of mechanical, thermal, chemical sensations [4, 6, 7]. The prevalence of trigeminal neuralgia is quite high and is up to 30-50 patients per 100,000 populations. According to the WHO, the incidence is in the range of 24 people per 10,000 population and worldwide more than 1 million people suffer from trigeminal neuralgia. The disease most often occurs in women aged 50-70 years. The development of the disease is facilitated by various vascular, endocrine-metabolic, allergic disorders, as well as psychogenic factors. However, often the cause of the disease cannot be determined [8].

Although many researchers have paid close attention to the response of nervous tissue to acute inflammation of the aseptic type, it is not known whether similar dynamics of morphological changes in the development of this inflammation in the trigeminal ganglion with the introduction of phlogogen as λ-carrageenan.

The purpose of the study was the investigation of morphological features of the rat's trigeminal ganglion in acute aseptic inflammation caused by λ-carrageenan on 1, 2, 3, 5 and 7 days of observation.

Materials and methods. The experiments were carried out on 30 sexually mature male white rats. Rats were divided into 2 groups: the control group (5) and the experimental group (25). Animals from the experimental group were injected intraperitoneally with λ-carrageenan at a dose (5 mg per 1 ml 0,9 NaCl) [8]. Rats from the control group received injection of saline. Animals were euthanized by overdose of ketamine anesthesia on 1, 2, 3, 5, 7 days of experiment.

Animals were kept in standard vivarium conditions of Poltava State Medical University. Experimental animals were sacrificed in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"; (Strasbourg, 1986), as well as with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001). After an overdose of ketamine, the animals were decapitated. Using standard methods, the material was embedded in paraffin blocks, of which sections 4 μm thick were made and stained with hematoxylin and eosin [1]. Histological preparations were examined using Biorex 3 light microscope with digital microfilter with software adapted for these studies (serial No. 5604).

Statistical processing of the survey results was performed using Microsoft Office Excel software and the extension of Real Statistics 2019 to it [10]. The nonparametric MannWhitney test was used to determine the statistical significance of differences between groups. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. When studying of the trigeminal ganglion of the experimental group of animals for 1 day of observation after simulated acute aseptic inflammation, edema was observed, which was characterized by the separation of collagen fibers of the outer connective tissue membrane. In connective tissue cells, changes in migratory activity were detected, which resulted in an increase in the number of immunocompetent cells. The representation of leukocyte cells changed in comparison with the control group of animals, the number of lymphocytes increased by 1.25 times ($p<0.05$) and was 0.85±0.10 cells in the field of view, macrophages by 2.0 times ($p<0.05$), which was 1.90±0.10 cells. There was an increase in tissue basophils 1.10±0.10 in the field of view, which was by 1.3 times ($p<0.05$) more than in the control group of animals (fig. 1)

When we studied semi-thin sections on day 1 of observation, changes were found in all parts of the hemomicrocirculatory tract of the ganglion. Arterioles were dilated, their diameter was by 1.18 ($p<0.05$) times larger compared to the control group, capillaries were slightly dilated, their diameter was 7.88±0.56 μm, the diameter of the venules was 18.39±0.32 μm, which was by 1.24 times ($p<0.05$) and 1.2 ($p<0.05$) times more than in the control group of animals (table 1.).

Table 1.

The diameter of the trigeminal ganglion hemomicrocirculatory vessels in acute experimental inflammation

Term of the experiment	Diameter, μm		
	Arterioles	Capillaries	Venules
Control group	9.45±0.55	6.33±0.45	15.17±0.56
1 day	11.17±0.33*	7.88±0.56*	18.39±0.32*
2 day	13.44±0.37*	8.33±0.39*	20.11±0.29*
3 day	15.74±0.54*	8.27±0.51*	23.04±0.67*
5 day	15.97±0.38*	8.94±0.29*	23.87±0.25*
7 day	12.88±0.33*	7.56±0.22*	18.19±0.67*

Note: * $p<0.05$ compared to the control group.

Morphometric examination of trigeminal neurons in acute experimental inflammation from the first day of observation showed a tendency to increase the volume of the cytoplasm by 1.08 times ($p<0.05$) against the background of a decrease in the volume of the neurons nuclei by 1.09 times ($p<0.05$) in comparison with the control group (Table 2).

The second day of observation was characterized by an increase in edema in the connective tissue of the trigeminal capsule, the number of leukocytes increased due to macrophages and tissue basophils, most of which were in the stage of degranulation.

There was an increase in blood supply to the organ, well visible on the hemomicrocirculation level the diameter of the arterioles was increased by 42 % from control group and was 13.44±0.37 μm. The diameter of the capillaries by 32% compared to the control group (table 1), the lumen is densely filled with erythrocytes. The venules were dilated, due to the inner diameter, in the lumen there was a marginal

standing of leukocytes, with a tendency to migrate into connective tissue. A study of neurocytes found that the volume of the cytoplasm increased due to cell edema with a decrease in the volume of the nucleus. Thus, the volume of neuronal nuclei was 103.48±6.04 μm^3, which was by 20 % less than in the control, and the volume of the cytoplasm increased by 25 %.

Table 2

Morphometric indicators of the trigeminal neurons state in acute experimental ganglionitis

	Nucleus volume, μm3	Cytoplasm volume, μm3
Control group	127,45±8,72	785,22±27,43
1 day	116.19±7.33*	847.75±14.47*
2 day	103.48±6.04*	979.48±12.45*
3 day	97.38±7.80*	987.55±15.43*
5 day	84.61±8.31*	1067.33±31.33*
7 day	80.12±7.78*	1163.53±17.98*

Note: * p<0.05 compared to the control group.

The third day of observation was characterized by increasing edema of the trigeminal ganglion capsule and unusually high blood supply to the whole organ (table 1). The number of macrophages is increased, the vessels are dilated, full-blooded, swollen (fig. 2).

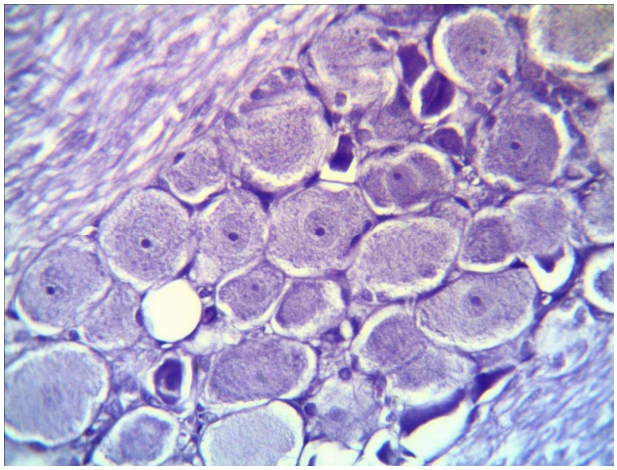

Fig.1. Trigeminal ganglion on the 1st day of observation. Hematoxylin eosin staining. Magnification: x400.

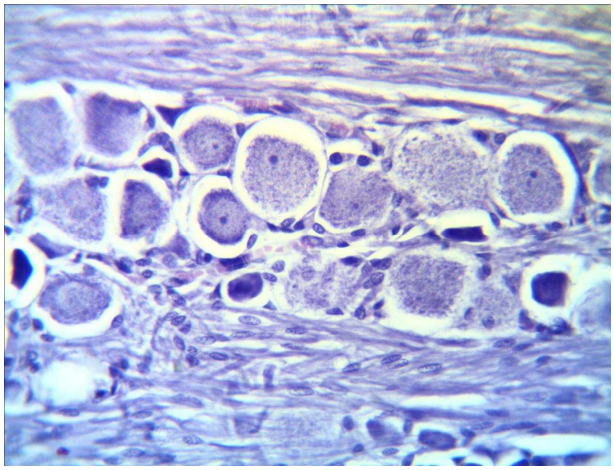

Fig.2. Trigeminal ganglion on the 3rd day of observation. Hematoxylin eosin staining. Magnification: x400.

Analysing the morphometric parameters of neurons on the 3rd day of observation, we found that the volume of the nuclei decreased compared with both previous observation periods and the control group, and was 97.38±7.80 μm3, which was by 23.5 % (p<0,05) less than in the control group (table 2). The cytoplasm has been separated from the plasmolemma by the light "belt" of edema. The cytoplasm volume increased by 25.76 % (p<0.05) compared to the control group. Neuroglia cells were located close to the neurolemma, the nuclei were clearly visualized.

The fifth day of observation was characterized by a further increase in connective tissue edema. The total number of lymphocytes increased by 1.45 times (p<0.05) and was 1.65±0.10 cells in the field of view, compared to the control group of animals. The number of macrophages and tissue basophils was unchanged.

During this period of the study the most pronounced changes at the level of the hemomicrocirculatory vessels were observed. Thus, the diameter of the arterioles was by 69 %, capillaries by 41 %, venules by 57 % larger (p<0.05) compared to the control group (Fig. 3).

Neurocytes still remained swollen both internally and extracellular. The nuclei of most cells were irregularly shaped. The volume of nuclei decreased by 33 % (p<0.05), and the volume of cytoplasm increased by 36 % (p<0.05), compared to the control group of animals. Glia cells were without visible pathology.

There was a tendency to normalize the process on the 7th day of the experiment at the level of the hemomicrocirculatory vessels, but the indicators were not sufficiently restored, compared with the control group of animals. There was an increased diameter of arterioles (by 36 %), capillaries (by 19 %) and venules (by 19 %), compared to the control group (p<0.05).

Among neurocytes, further changes in nuclei volume and cell cytoplasm volume were observed. The volume of nuclei was reduced by 37 % (p<0.05), and the volume of cytoplasm was increased by 48 % (p<0.05) compared to the control group of animals.

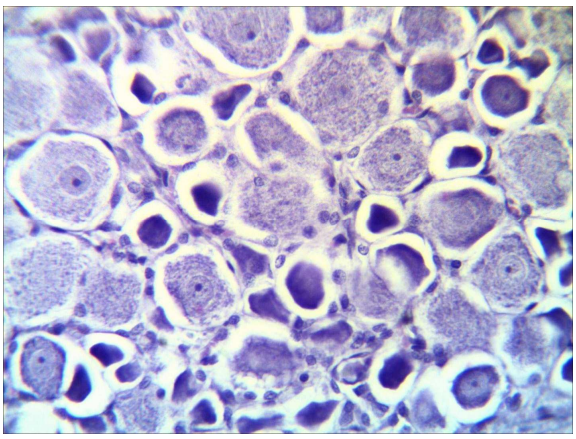

Fig.3. Trigeminal ganglion on the 5th day of observation. Hematoxylin eosin staining. Magnification: x400.

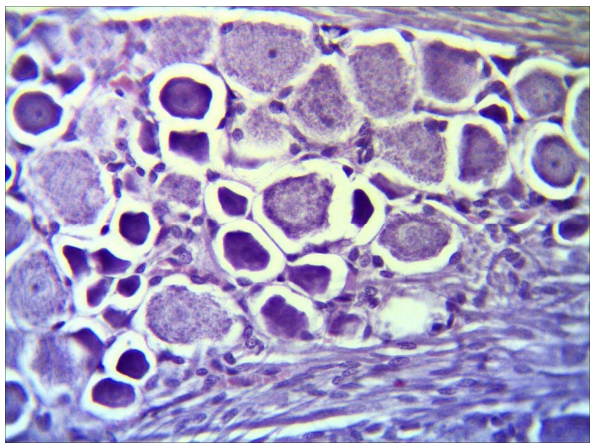

Fig.4. Trigeminal ganglion on the 7th day of observation. Hematoxylin eosin staining. Magnification: x400.

The phenomena of acute λ-carrageenan inflammation in the trigeminal ganglion described above were characterized by phasing, which corresponds to the literature sources [6, 8, 9, 11]. Thus, in the first stage, against the background of increasing hydration, minor destructive phenomena in the structure of the stroma of the trigeminal ganglion are revealed [9], which adversely affect metabolism, resulting in impaired blood circulation, reduced phagocytic response activity, what was accordingly proved in experiment [4]. Such changes are only observed at the early stages, what was resulted in slowing or suppression of the cell barrier formation, with subsequent reactivation and generalization of the process due to inflammatory factors activity in the trigeminal ganglion stroma and in the nervous tissue specifically [5, 7]. Later, the inflammation quickly turned into phase 2, which did not contradict the pathogenetic mechanisms of the acute inflammation process [7]. The second phase was characterized by regenerative phenomena that occurred against the background of the inflammatory zone dehydration. In this phase, the barrier is completed and the area of damage is completely limited [6]. In parallel, the products of tissue metabolism are resorbed or removed from the site of inflammation. All this occurs together with reducing the clinical signs of inflammation, normalization of biochemical and functional disorders that occur in the first phase of inflammation. Metabolism gradually normalizes, blood circulation improves, the macrophage response begins to dominate [9].

Conclusion

It was found that a single intraperitoneal injection of λ-carrageenan causes aseptic inflammatory process, which involves all morpho-functional structures of the trigeminal ganglion. The most prominent changes in the cellular and tissue composition of the connective tissue were observed on the 3rd day of acute aseptic ganglionitis, and in nerve cells on the 7th day of the experiment. Disorders of the hemomicrocirculatory vessels were most pronounced on the 5th day of the experiment.

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