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DEGREES OF DAMAGE TO GANGLION CELLS OF THE RETINA AND OPTIC NERVE OF RATS UNDER THE INFLUENCE OF A MIXTURE OF ALCOHOLS

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Acute methanol poisoning causes optic neuropathy and retinal damage. The priority and extent of damage to the retinal ganglion cell and optic nerve structures in victims are discussed. We studied the ultrastructural state of these structures in rats at 1 hour 10 minutes to 3 months after a single intraperitoneal injection of an ethanol-methanol mixture (3:1 ratio; methanol dose: 0.75 g/kg body weight). Rats that received a similar injection of 100 % methanol served as controls. Ganglion cells were more resistant to the effects of a mixture of alcohols, as well as methanol, and recovered faster during the study than the structures of the optic nerve. The identified ultrastructural changes revealed the subtle mechanisms of alcohol-induced damage on eye tissues, which will help ophthalmologists more accurately treat patients affected by alcohol substitutes containing methanol, using the necessary medications.

Key words: ultrastructure, ganglion cells, optic nerve, rats, ethanol, methanol.

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СТУПІНЬ ПОШКОДЖЕННЯ ГАНГЛІОЗНИХ КЛІТИН СІТКІВКИ ТА ЗОРОВОГО НЕРВА ЩУРІВ ПІД ВПЛИВОМ СУМІШІ СПИРТІВ

Гостре отруєння метанолом викликає оптичну невропатію та пошкодження сітківки. Дискутується питання відносно першочерговості та глибини пошкодження структур гангліозних клітин сітківки та зорового нерву у постраждалих. Нами ультраструктурно вивчався стан даних структур щурів від 1 год. 10 хвилин до 3 місяців після одноразової внутрішньоочеревинної ін'єкції суміші етанолу та метанолу у співвідношенні 3:1 з дозою метанолу 0,75 г/кг маси тіла тварини. В якості контролю слугували щурі, які отримували аналогічну ін'єкцію 100 % метанолу. Виявлено, що гангліозні клітини більш стійкі до дії суміші спиртів та метанолу, швидше відновлюються в динаміці дослідження, ніж структури зорового нерву. Визначені ультраструктурні зміни розкривають тонкі механізми руйнівної дії спиртів на тканини ока та допоможуть офтальмологам більш прицільно застосовувати необхідні лікарські засоби постраждалим від сурогатів алкоголю з вмістом метанолу.

Ключові слова: ультраструктура, гангліозні клітини, зоровий нерв, щурі, етанол, метанол.

The study is a fragment of the research project "To investigate the dynamics of ultrastructural and biochemical changes in the visual analyzer and liver of rats under the influence of a mixture of alcohols", state registration No. 0124U002976.

Methanol, a toxic chemical compound that is also known as wood alcohol, is increasingly used in everyday life. It is a component of household paints, a solvent, and fuel for cars, etc. The number of reports about the use of low-quality alcoholic beverages containing methanol, which leads to acute poisoning of people, is increasing [2]. Methanol vapors are also inhaled when working with them in closed rooms, including skin contact [7]. Additionally, chronic exposure to methanol occurs with long-term contact with its vapors [5, 6]. According to clinical data, visual acuity is initially reduced in victims of acute methanol poisoning, showing damage to the optic nerve (ON), retina, and brain [15]. This results in optic neuropathy and necrotic damage to the basal ganglia and subcortical white matter [12, 13, 14, 15]. Other authors, including those using optical coherence tomography, also found signs of optic neuropathy [4, 8, 9, 15]. Several authors reported a rare case of optic nerve atrophy with severe cupping of its disc due to methanol poisoning 1 day after drinking methanol-containing alcohol [15]. According to other data, a possible mechanism of ON damage is the loss of retinal ganglion cells (GCs) due to acute demyelination of their retrobulbar part [14].

According to results of experimental studies modeling methanol-induced retinal toxic damage, rats develop mitochondrial oxidative phosphorylation disorders and inflammatory changes [4, 7]. Histopathological changes in eye tissues are mainly manifested as degeneration of retinal axons and glial cells, which can involve all retinal layers due to histotoxic hypoxia caused by formic acid, a byproduct of methanol breakdown [4].

Experimental models of retinal damage and retinal detachment in laboratory animals induced by methanol-induced mitochondrial dysfunction have the potential for widespread use to assess the mechanisms of interaction between low-energy light radiation and biological tissues [1]. At the same time, information on the effect of a mixture of alcohols containing methanol on the organs and tissues of experimental animals and, in particular, the tissues of the visual analyzer is scarce. We previously showed

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that choroidal vessels, capillaries, and retinal pigment epithelial cells are most vulnerable to the toxic effects of both methanol and its mixture with ethanol (methanol dose: 0.75 g/kg of rat body weight), starting at the first hour after intraperitoneal injection (II). GCs are more resistant to the destructive effects of these substances [3]. We also found a protective effect of ethanol on the studied structures, consistent with previous findings [11]. There are separate publications discussing the occurrence of initial changes and the depth of damage to retinal GCs and ON after methanol exposure [7]. We decided to analyze the degree of damage in the above-mentioned rat structures using a mixture of alcohols and 100 % methanol at a dose of 0.75 g/kg body weight.

The purpose of the study was to identify and compare ultrastructural changes in retinal GCs and the ON of rats after an intraperitoneal injection of a mixture of alcohols.

Materials and methods. The work was performed on 42 adult male Wistar rats (weight: 250–300 g), which were kept in standard conditions of the vivarium of the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" with lighting for 12 hours and free access to water and food. The animal experiment was conducted from March to May 2018. The results of the study were used in the current research. The animals were divided into 2 groups: I-a – experimental group in which rats were given an II of a mixture of 40 % ethanol solution, which was obtained from 100 % ethanol after dehydration at 96.6 °C (SE "Ukrspyrt", Ukraine) and 100 % methanol (LLC "Ukrhimreaktiv", Ukraine) in a ratio of 3:1, with a methanol dose of 0.75 g/kg of animal body weight; II-a – control group in which rats received II of 100 % methanol in the same dose as in group I. Each animal was weighed and, depending on the animal's weight, the amount of substance in g was calculated in proportion to maintain the above-mentioned dose of methanol. After that, g was converted to ml, knowing that 1 ml of alcohol is equal to 0.79 g.

For electron microscopy, pieces of the studied tissues were fixed in a 2.5 % solution of glutaraldehyde in phosphate buffer at pH 7.4, followed by post-fixation in a 1 % solution of osmium acid at the same pH. Then, the samples were dehydrated in alcohols of increasing concentration. The material was impregnated and immersed in a mixture of epoxy resins, Epon-Araldite. The obtained rat tissue samples were studied using an electron microscope PEM-100-01 (Ukraine) at 1 hour 10 minutes, 3 hours, 1, 3, 7, 14 days and 1 and 3 months following the injection of alcohols.

Animal anesthesia and euthanasia, which were carried out in a state of deep sleep by decapitation, were performed using sodium thiopental.

In the conducted studies, measures were taken to ensure safety, ethical attitude, and the rules for working with experimental animals were observed in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty to Animals". The study was considered at a meeting of the Institute's Bioethics Commission, and a decision was made to publish it under protocol no. 4, dated 17.04.25

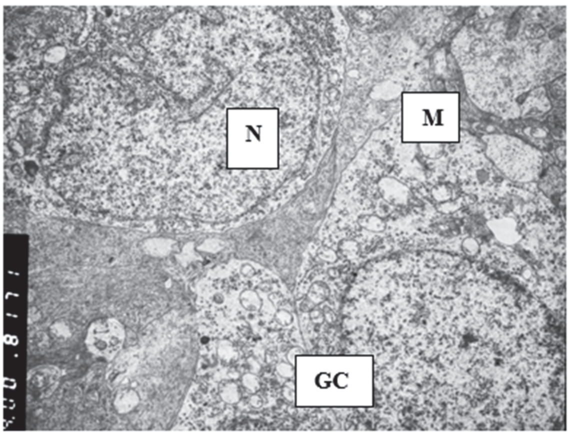

Fig. 1. Three days after the injection of a mixture of alcohols. Hydropic dystrophy of ganglion cells of the rat retina. X 4 000. Legends: GC – ganglion cells, N – nucleus, M – mitochondria.

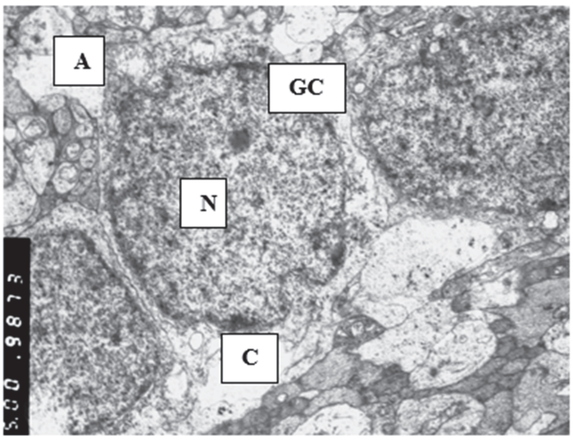

Fig. 2. Three days after the methanol injection. Degenerative changes in GCs and their axons. X 5 000. Legends: GC – ganglion cell, I – nucleus, C – cytoplasm, A – axon.

Results of the study and their discussion. In the first observation periods (1 hour 10 minutes, 3 hours) after administering the II mixture of alcohols, individual GCs react with edema of the intramitochondrial matrix, minor reactive changes in the cisterns of the granular endoplasmic reticulum, and increased metabolic processes, which are characterized by the hypertrophy of the Golgi complex and a large number of polysomes. Signs of edema are also observed in individual GC processes. In the ON, at these periods, it manifests as delamination of myelin sheath lamellae, destruction of mitochondrial cristae in axons of large-caliber nerve fibers, and mild hydropic changes in their glial cells.

From the 1st to the 7th day of the study, especially by the 3rd day, after administering the II mixture of alcohols, alterative changes in the structures of the retina and ON become more significant (Fig. 1).

In GCs, after methanol injection, cytoplasmic swelling and alterations in membrane organelles are observed, and the number of nerve processes with edema elements increases (Fig. 2).

After methanol injection at similar time points, pathological changes in retinal GCs were somewhat more pronounced, with edema in both GCs and nerve processes, elements of organelle depletion, and a gradual increase in destructive changes by the 7th day of observation.

In the ON, both after the II of a mixture of alcohols and after the II of methanol, during the indicated period of time, destructive changes in nerve fibers and glial cells also increase and involve almost all tissues (Figs. 3, 4).

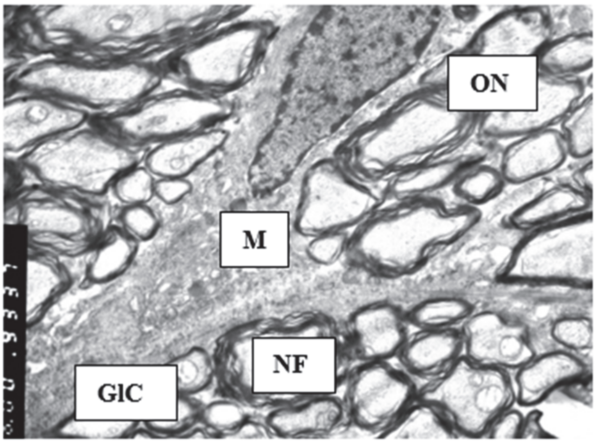

Fig. 3. Three days after the injection of a mixture of alcohols. Pathological changes in nerve fibers and glial cells of the optic nerve. X 6 000. Legends: ON – optic nerve, GlC – glial cell, NF – nerve fibers, M – mitochondria.

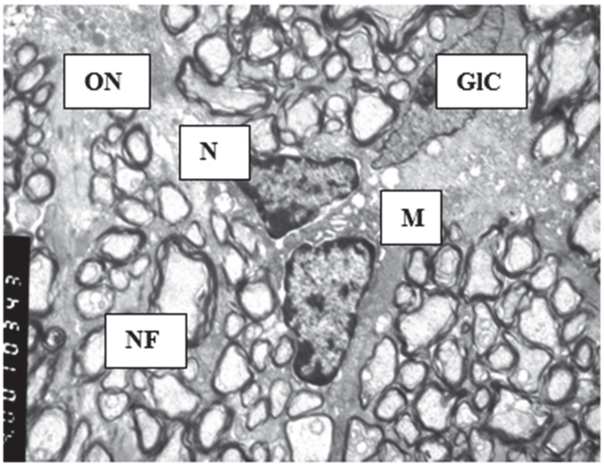

Fig. 4. Three days after the intraperitoneal injection of methanol. Degenerative changes in organelles in a glial cell. Pathologically altered myelin sheath of nerve fibers. X 5 000. Legends: ON – optic nerve, GlC – glial cell, NF – nerve fibers, N – nucleus, M – mitochondria.

Nerve fibers (NFs) of different calibers of ON, especially in the control group, demonstrate a deformed myelin sheath that is stratified into lamellae, edema of their axoplasm with a reduced number of organelles, in particular, microtubules and neurofilaments, destruction of mitochondrial cristae, and detachment of the axolemma from the myelin sheath. During this period, glial cells underwent significant damage, both in the perinuclear region of the cell and in their processes, showing hydropic dystrophy, with damage to a significant portion of organelles and foci of cytoplasmic necrosis. Intercellular edema is present in some parts of the ON. It should be noted that, in parallel with the development of pathological changes in the studied structures, signs of compensatory and restorative processes occur during these periods, reflected in an increased number of polysomes in cells and in the activation of nuclear-cytoplasmic relations, especially in GCs. In ON structures, they are more restrained.

On the 14th day of observation after injecting the II mixture of alcohols, compared with the previous period, destructive changes in the studied tissues are somewhat reduced. However, the above phenomena are still present in the control group, with intercellular and intracellular edema being present to a somewhat lesser extent. At the same time, signs of increased metabolic activity are detected in some studied structures, which are more pronounced in the GCs, leading to an increase in the amount of plastic material in the cells and the gradual restoration of their ultrastructure. By 3 months after administering the II mixture of alcohols, most cells among the GCs are practically restored, or some compensatory and restorative processes occur in them, aimed at enhancing protein synthesis and energy-generating functions. At the same time, a portion of the nerve processes around the GCs remains altered. In the ON components, particularly NFs and glial cells, increased reparative processes are also observed. The degree of restoration of their ultrastructure depends on the depth of damage in the previous periods. The enhancement of metabolic processes is also more pronounced after methanol ingestion.

The results of the study showed that after using both a mixture of alcohols and methanol (0.75 g/kg of rat body weight) in the initial period (up to 3 hours) after II, GCs react with alterative changes in mitochondria and expansion of the granular endoplasmic reticulum cisterns with a parallel increase in the content of polysomes, and the ON reacts with changes in the structure of large-caliber NFs, which are characterized by swelling of the axoplasm, delamination and deformation of the myelin sheath, mitochondrial pathology, which probably already leads to a decrease in their excitability and impaired conduction of the nerve impulse in NFs, as well as hydropic changes in the membrane organelles of individual glial cells, especially mitochondria, which causes energy deficiency and inhibits nutrition and NF restoration. The same typical changes were also found in patients after poisoning by low-quality alcoholic beverages, which several authors confirm, but with more significant changes, depending on the dose of methanol in the drinks [13, 15]. In addition, slightly greater edema was observed in the components of the ON in the 1st experimental group after 3 hours than in the material in the 2nd control group, most likely because ethanol permeated cell membranes slightly faster than methanol. In the subsequent periods up to 3 days after the II of the alcohol mixture and 7 days after the II of methanol, changes in the ultrastructure of the ON are manifested by hydropic dystrophy and degeneration of organelles, involving almost all NFs and glial cells. It should only be noted that after the use of a mixture of alcohols, pathological changes were less pronounced than after 100 % methanol injection; in particular, individual GCs demonstrate foci of necrosis and a torn plasmalemma. The protective effect of ethanol on the rat retina in acute methanol poisoning has also been demonstrated by other scientists [11]. Several authors report retinal damage from methanol, leading to decreased visual acuity or blindness [14]. At the same time, our studies showed that when using approximately 10 times the LD50 dose of methanol, significant pathological changes occur in the choroid and the outer layers of the retina. As a result of methanol intoxication, the first neurons of the retina – photoreceptors – may be the first to suffer, which leads to a sharp decrease in vision [1]. From the 14th day, signs of compensatory and restorative processes are somewhat more actively manifested in all studied structures, with an increase in GCs, more pronounced in the 1st study group and persisting for up to 3 months. It should be noted that in the ON, restorative processes occur relatively slowly. Large foci of destructively changed and destroyed structures in the 2nd study group still exist after 3 months of study, which is also confirmed by clinical studies, indicating that the restoration of the structure and functions of the organ of vision and the brain in victims after poisoning with alcohol surrogates is very slow [4, 6, 14]. This may be due to energy deficiency resulting from mitochondrial damage, which disrupts oxidative phosphorylation, affecting cell viability and leading to cell pathology. In addition, increased synthesis of pro-inflammatory cytokines, which affect the expression of key proteins responsible for maintaining cell homeostasis, also plays an important role in the pathogenesis of methanol intoxication [4].

The results of the study indicate that the used dose of methanol can serve as a model for further research, including studies on tissues and body systems. At the same time, the detected ultrastructural changes in the retina and vitreous humor provide essential information for clinicians, reflect the processes in the eye tissues of victims of methanol poisoning, and will help select medications for successful treatment.

Comparing the obtained results of the study regarding the degree of damage to the GCs and ON, it should be noted that ON structures, which are more vulnerable to the toxic effects of methanol, react faster and with a greater depth of destructive changes than GC bodies, and their axons are somewhat more subject to alterative changes, which correlates with the results of other authors [15].

Conclusions

1. Retinal ganglion cells are more resistant to the toxic effects of a mixture of ethyl and methyl alcohols with a methanol dose of 0.75 g/kg of rat body weight than optic nerve structures.

2. In the first 3 hours after the injection of the alcohol mixture, reactive changes in organelles occur in retinal ganglion cells, hydropic changes occur in large-caliber nerve fibers in the optic nerve, and alteration of mitochondria occurs in glial cells.

3. The most significant pathological changes in the ganglion cells (peak damage on day 3) after the injection of the alcohol mixture are cytoplasmic edema and alteration of membrane organelles. In contrast, in the optic nerve, these are deep destructive changes in nerve fibers of various calibers and degenerative changes in glial cell organelles.

4. At the 3rd month of observation, metabolic processes are activated in all studied structures: the ganglion cells are practically restored, while structures of the optic nerve are still in a pathological state.

5. Methanol has a more destructive effect on the structures of ganglion cells and optic nerve than a mixture of alcohols, and the processes proceed in the same direction and with greater pathology in the optic nerve.

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