STUDY OF THE MORPHOLOGICAL RELATIONSHIP BETWEEN GLAUCOMA AND ALZHEIMER'S DISEASE

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Annotation: *The main mechanism of neuronal death in neurodegenerative diseases is considered to be apoptosis. According to many researchers, the same mechanism is also involved in the death of retinal ganglion cells (GCS) in glaucoma. Approximately 70% of the fibers of the optic tract, forming retinogenicular pathways, end in the external geniculate body (CNT). neuron to a healthy one through synaptic contact. The same process is characteristic of the progression of glaucoma optic neuropathy.*

Key words: *glaucoma, Alzheimer's disease, apoptosis, neurodegeneration.*

INTRODUCTION

The main mechanism of neuronal death in neurodegenerative diseases is considered to be apoptosis [3]. Its role in the pathogenesis of diseases such as Alzheimer's disease is confirmed by clinical and experimental data [6, 184].

The death of retinal ganglion cells (GCS) in glaucoma, according to many researchers, also occurs as a result of apoptosis [162]. Increased intraocular pressure (IOP) as one of the main factors in the pathogenesis of glaucoma acts as a mechanical agent, leading to deformation of the septa of the ethmoid plate and causing compression of the axons of ganglion cells. As a result of a decrease in axoplasmic flow and retrograde axonal transport, the supply of neurotrophic factors to the corticosteroids is disrupted. Their deficiency contributes to the triggering of the mechanism of apoptosis [11]. In this case, apoptosis is identified by the following specific features: chromatin condensation, fragmentation of genomic DNA, and detection of apoptotic bodies [3, 150].

In modern literature, a lot of attention is paid to the detection of increased activity of caspases in neurodegenerative diseases as the main inducers of apoptosis. When studying brain samples from patients who died from Alzheimer's disease, a higher level of activated caspases was found in microglial cells. In an experimental model of glaucoma in rats, an increase in the concentration of activated caspases was also established: caspase-3 and caspase-8 [13]. Caspase-3, the main effector of the apoptic cascade, is activated in the corticosteroids and breaks down APP (Amyloid Precursor Protein) into neurotoxic fragments, including ß-amyloid. Under "normal conditions", APP is predominantly secreted in soluble form (sAPPa) and has trophic functions. With age or in the presence of mutations in the APP gene, there is a change in the mechanism of cleavage of this protein, which leads to a decrease in the level of sAPPa and the formation of the ß-form of the peptide (APß). The accumulation of ß-

amyloid leads to pathological changes in the human central nervous system, disrupting the processes of cellular transport. ß-amyloid plaque deposition is a characteristic feature of many neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease [7, 13, 146].

At present, the role of ß-amyloid in the development of corticosteroid apoptosis in glaucoma is considered to have been established [9]. As already noted, in experimental models of glaucoma in rats, pathological processing of APP caused by caspase-3 and an increase in the expression of ß-amyloid GCS were found. A decrease in the level of ß-amyloid in the vitreous body in patients with glaucoma (compared to its deposition in the retina) was also revealed. It has been proven that exogenous ß-amyloid induces apoptosis of corticosteroids in vivo, and this effect depends on various factors [9].

Most of the axons of the corticosteroids end outside the eyeball. Approximately 70% of the fibers of the optic tract, forming the retinogenicular pathways, end in the external geniculate body (CNT) [3]. Previously, it was believed that it was only a "relay station" that transmits information from the retinal neurons through visual radiance to the cerebral cortex. At present, it has been shown that significant and diverse processing of visual information takes place at the level of the NCT [3]. The tubing includes the visual pathways magnocellular, parvocellular, and coniocellular.

In neurological practice, many neurodegenerative diseases of the central nervous system are known. These include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, Pick's disease, and many others. What they have in common is the process of transsynaptic degeneration, in which the disease spreads from the affected neuron to the healthy one through synaptic contact. The same process is characteristic of the progression of glaucoma optic neuropathy [17].

The marker of the glaucoma process is the death of corticosteroids due to damage to their axons at the level of the DZN. However, the dystrophic process does not end there. According to N. Gupta and Y. Yucel, both the external geniculate body and the visual cortex undergo atrophy. Degenerative changes manifest themselves in the form of wrinkling and death of neurons. All tubing pathways are subject to atrophy [2, 8, 15, 19]. Nissl staining of sections reveals thinning of the tubing layers, a decrease in cell density, their size, a change in the shape and increase in lipofuscin deposits in the magnocellular and parvocellular layers.

Atrophy of the external geniculate body leads to a decrease in its metabolic activity, which is detected by cytochrome oxidase. Interestingly, in glaucoma, degenerative changes affect the conducting neurons of the NCT, while the interneurons remain unchanged [19]. In 2006, N. Gupta published the first case of low-pressure glaucoma, which described degenerative changes in the human brain based on clinical data and autopsy results [4]. More pronounced atrophic changes were found in the intracranial region of the MN compared to the control group, as well as atrophic

changes in the tubing and visual cortex. Interestingly, the results obtained correlated with the clinical picture, fundus condition and visual field data conducted during the patient's lifetime. Another evidence of axonopathy is the detection of abnormal tau protein in the retina in glaucoma. Tau protein belongs to a group of proteins associated with microtubules and responsible for axonal transport in healthy nerve cells. Hyperphosphorylation of tau protein leads to the destruction of microtubules, disruption of axonal transport and causes toxic effects on neurons. Pathological tau protein has been found in the form of neurofibrillary tangles in Alzheimer's disease and other neurodegenerative diseases. Objective: to conduct a comparative analysis of morphological changes in the central part of the visual analyzer, NCT in glaucon, and Alzheimer's disease. Study methodology: we studied the morphological material of the brain in 7 patients: 2 patients diagnosed with Alzheimer's disease, 1 patient with glaucoma, and 4 patients in the control group without ophthalmic and neurological diseases. Brain tissue samples from the NCT and visual cortex were recorded in a 10% neutral formalin solution (buffered pH = 7.2-7.4), dehydrated, and enclosed in paraffin. Serial sections with a thickness of 5-15 μm were stained with hematoxylin-eosin. For Nissl staining, small samples of tubing and visual cortex were recorded in a 96% alcohol solution for a month. After fixing, the material was dehydrated in absolute alcohol before pouring into paraffin. Serial sections were placed in a 0.5% solution of cresylviolet. Next, the sections were differentiated under the control of a microscope until the nucleus and granularity of the nerve cell were clearly manifested. Then the sections were thoroughly dehydrated, clarified in xylene and enclosed in Canadian balm. As a result, the granularity in the cytoplasm acquired a blue-violet color, and the nucleoli became dark blue. Neurohistological examination was carried out according to the Bilshovsky method. Tissue samples were fixed in 10% neutral formalin for up to 6 months. Frozen sections with a thickness of 15-20 μm were treated with 50% alcohol with 1% ammonia water. After thorough washing with distilled water, the sections were placed in a solution of silver nitrate without access to light. Next, the sections were washed, dehydrated in alcohols of increasing concentration, clarified in xylene and enclosed in polystyrene. Immunohistochemical studies were performed on tub slices from two patients diagnosed with Alzheimer's disease and a patient with glaucoma to identify the neurodegenerative process. The study was carried out on paraffin sections 3-4 μm thick using the polymer-protein-peroxidase method according to the standard technique using the NOVOLINK (Novocastra, UK), tau Ab-3 (Rabbit Polyclonal Antibody, Thermo Fisher Scientific) imaging system. Working solutions in a 1:25 dilution were prepared from lyophilized concentrates Amyloid A4 (Clone BAM01, Thermo Fisher Scientific), tau Ab-3 (Rabbit Polyclonal Antibody, Thermo Fisher Scientific), preliminarily diluted in 100 μl of Antibody Diluent (Novocastra). After dewaxing and dehydration to block endogenous peroxidase, the sections were treated with 0.3% H2O2 for 20 minutes, then washed in distilled water and subjected to temperature treatment in a buffer solution with pH = 9.0 (Epitope

Retrieval Solutions pH 9, Novocastra) for 30 minutes at t = 98° to unmask antigenic determinants. After washing in Buffer Solution (Novocastra, UK), working dilutions of primary antibody concentrates Amyloid A4 (Clone BAM01, Thermo Fisher Scientific), tau Ab-3 (Rabbit Polyclonal Antibody, Thermo Fisher Scientific) were applied three times for 5 minutes. Incubation with primary antibodies was carried out for 30 minutes at room temperature. After incubation with primary antibodies, the sections were washed in a wash buffer solution prepared from Bond Wash Solution 10X Concentrate (Novocastra) three times for 5 minutes, then the NOVOLINK polymer-protein-peroxidase complex detection system (Novocastra, UK) was applied for 30 minutes at room temperature. Antigenic epitopes were detected using DAB and DAB Enhancer (Novocastra, UK). The study was carried out using the Leica BOND-MAX immunostainer using Covertile technology (Leica Biosystems).

Results of the study and their discussion. The study of neural parameters at various levels of the visual pathway within the CNS was carried out using the semi-automated image analysis system "MINI-MOP" (OPTON, Germany), obtained through "Photomicroscop-NI" (Zeiss, Germany). 40 measurements of the neuron and its nucleus were carried out in each of the analyzed layers: the magnocellular and paracellular layers of the NCT and in the visual cortex μm2) in the three study groups. The nuclear-cytoplasmic ratio (I/C) was calculated according to the following formula: S. The study of histological specimens in patients with glaucoma and Alzheimer's disease, stained according to the Nissl method, demonstrated a pronounced atrophy of nervous tissue compared to the control group. In Alzheimer's disease and glaucoma, there was a marked reduction in neuronal area in all areas studied compared to the control group ($p<0.001$; Mann-Whitney test). The lowest values of this parameter were recorded in glaucoma: the mean values of the neuron area differed from normal by 32.5% in the magnocellular layers, 38% in the paracellular layers and 35.0% in the visual cortex. In Alzheimer's disease, the differences were slightly less pronounced. A decrease in the area of the neuron in the magnocellular layers by 20.8%, in the paracellular layers by 38.5%, and in the visual cortex by 26.0% was proven. In comparison with the control group, the mean values of the neuron nucleus area in Alzheimer's disease were higher in the magnocellular layers of the NCT - by 42.0%, in the paracellular layers by 9.7%. These differences were statistically significant ($p < 0.05$; Mann-Whitney test). In the visual cortex, the size of the nuclei practically did not differ from the norm ($p > 0.05$). In the case of glaucoma, there was also a slight increase in the area of neuronal nuclei in the magnocellular layers of the NCT by 8.5%, in the paracellular layers and the visual cortex there was a decrease in their size by 35.5 and 18.3%, respectively. All differences were statistically significant ($p < 0.05$; Mann-Whitney test). Accordingly, the nuclear-cytoplasmic ratio in the analyzed layers of the tubing and the visual cortex also changed. In our study, using the example of two neurodegenerative diseases, we observe the following characteristic forms of neuronal changes: axonal response and transsynaptic atrophy. Axonal reaction or primary Nissl

injury is a common form of neuron pathology caused by axon rupture or death. In this form of pathology, the neuron body first increases (edema), rounds, and then decreases in size and wrinkles. The nucleus shifts to the periphery (ectopy) of the cell, and becomes more rounded and lighter, which, in combination with the centrally located nucleolus, gives it a resemblance to the eye of a fish or bird. Nissl blocks undergo central chromatolysis. All this is the result of a pronounced increase in the metabolic activity of the affected cell and is considered as a compensatory mechanism. Similar changes were recorded in the study of histological samples of the NCT and visual cortex in patients with Alzheimer's disease. A decrease in the volume of the cytoplasm and nucleus while preserving their "lightoscopic" structure, thickening and folding of the nuclear membrane, moderate hyperchromatosis of the nucleus and cytoplasm, condensation of chromatin, wrinkling of the cytoplasm - all these morphological signs in the context of glaucoma indicate the involvement of neurons of the NCT and visual cortex in apoptosis. This can serve as evidence of the centripetal transsynaptic spread of the pathological process from the eye to the central parts of the visual analyzer. Transneuronal atrophy of neurons in this case is due to an insufficient influx of afferent impulses due to the loss of synaptic connections (asynapsis). In the study of silver-impregnated specimens, the visual cortex of the cerebral hemispheres, and the tubing of patients with Alzheimer's disease, neurofibrillary formations of various thicknesses were found to fill the cytoplasm of neurons and their processes. Other neurofibrils were located around the nuclei and looked like a ball (classic Alzheimer's neurofibrils). In some cases, neurofibrils took the form of "baskets", "curls", "braids", "tennis rackets". In Alzheimer's disease, the pathological process is primarily involved in the proteins of neurofilaments, the main component of the cytoskeleton of the neuron. These proteins have several functions, including maintaining cell shape, axon diameter, and are involved in axonal transport. Neurofilaments are especially numerous in large neurons with a long axon, which are rich in the visual cortex of the cerebral hemispheres. Consequently, "Alzheimer's neurofibrils" are found not only in the areas of the brain pathognomonic for this disease, but also in the central part of the visual analyzer. We also observed similar changes in the neurons of both the visual cortex and the CNT in the brain of a patient with glaucoma. Pathological changes covered the internal structures of neurons. Nuclear ectopy, pycnosis, pericellular edema, cytoplasmic volume reduction, chromatin condensation, and neuronal wrinkling should be noted. The changes in neurons that we observe as a result of inactivation in Alzheimer's disease and glaucoma resemble to a certain extent the morphological features of apoptosis. Of interest are the results obtained in the course of immunohistochemical analysis of tubing sections. In this technique, amyloid A4 was used, a p-amyloid peptide extracted from the amyloid of the p-precursor protein. Its detection is considered a reliable marker of neurodegenerative processes. Both extracellular and cytoplasmic p-amyloid have been found in the NCT of patients with Alzheimer's disease and glaucoma. It is a

key component of the amyloid protein in neuronal plaques as well as in neurofibrillary tangles. Another important marker for identifying Alzheimer's disease and glaucoma as neurodegenerative ailments is tau protein, which we found in the NCT of patients with these diseases. This protein is responsible for maintaining the structural stability of microtubules. In Alzheimer's disease and glaucoma, tau protein becomes hyperphosphorylated, leading to its inability to bind to microtubules. Instead, phosphorylated tau proteins connect to each other to form "cords" - paired spiral fibers. Two tau protein helices twisted together are known as neurofibrillary tangles. Neurons filled with such tangles instead of functioning microtubules soon die. Thus, the cascade effect in Alzheimer's disease and glaucoma, in our opinion, can be represented as follows: formation of p-amyloid - amyloid plaques - neurofibrillary tangles (tau protein) - death of the neuron. Conclusion. Morphological examination of the autopsy material of the brain showed atrophy of nervous tissue in the external geniculate body and visual cortex: wrinkling and death of neurons, pericellular edema, ectopia of the nucleus and nucleolus, chromatolysis. The most pronounced atrophic changes were observed in glaucoma. Immunohistochemical analysis of the NCT revealed markers of neurodegeneration: p-amyloid and tau protein. This confirms the involvement of the central parts of the visual analyzer in the neurodegenerative process in these pathologies. Based on the morphological data supported by the results of a comprehensive clinical examination, we can talk about a more aggressive effect of the neurodegenerative process on the structures of the visual pathway in glaucoma compared to Alzheimer's disease. With the progression of glaucoma optic neuropathy, the pathological process spreads from the retinal ganglion cells to the central parts of the visual analyzer.

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