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Rola czynników neuroprotekcyjnych w patogenezie wybranych chorób oczu

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Abstract

Title: The role of neuroprotective factors in the pathogenesis of selected eye diseases.

The sight is one of the most important senses, and its deterioration or loss is associated with a significant decrease in the quality of life. Blindness is a consequence of many eye diseases, such as cataracts, glaucoma, uncorrected refractive errors, age-related macular degeneration, and diabetic retinopathy. The treatment results are satisfactory only in the case of cataracts and refractive errors, while the therapies for glaucoma, age-related macular degeneration, and diabetic retinopathy are still a significant clinical challenge. These pathologies belong to the group of neurodegenerative diseases, in which irreversible damage to retinal neurons occurs. According to the latest reports, neurodegenerative processes also occur in the course of hypertensive retinopathy. In my doctoral thesis, I focused on researching the pathomechanisms of neurodegenerative diseases, in particular the involvement of neuroprotective proteins, which are responsible for promoting neuron survival.

Glaucoma is a heterogeneous group of diseases that lead to optic neuropathy. In the disease course, the retina shows the loss of retinal ganglion cells, thinning of the retinal nerve fiber layer, and optic nerve head cupping.

Chronic arterial hypertension leads to changes in the structure and function of blood vessels, and their remodeling, which contributes to the development of hypertensive retinopathy. Its course includes the phase of vasoconstriction, sclerosis, exudation, and neuroretinopathy. Although hypertensive retinopathy is known primarily as a vascular disease, there are reports in the literature about the significance of neurodegenerative processes, seen already in the early stages of this disease.

In diabetes mellitus, hyperglycemia leads to damage to vascular cells, which contributes to a change in their morphology and an increase in their permeability, leading to the development of diabetic retinopathy. The following stages of diabetic retinopathy can be distinguished: mild non-proliferative retinopathy, severe non-proliferative retinopathy, and proliferative retinopathy. As a result of retinal vascular disorders, its structure and nerve cells are damaged.

The treatment of glaucoma, hypertensive and diabetic retinopathy is limited to the modification of risk factors - lowering the intraocular pressure, blood pressure, or glycemia. Therefore, new, effective therapies are sought. Proteins that have a potential role in the pathogenesis of

neurodegenerative diseases of the retina are the neuroprotective factors, which include, among others, the brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). However, their role in the process of glaucoma, hypertensive and diabetic retinopathy has not yet been fully explained.

The main purpose of the doctoral thesis was to assess the change in the level of neuroprotective proteins, BDNF and NGF, and to explain their potential role in the pathogenesis of diseases in which retinal neurodegeneration is an underlying mechanism (glaucoma, hypertensive and diabetic retinopathy).

Animal models of eye diseases were used for the research carried out as part of the doctoral thesis. Mice of the DBA/2 strain, that spontaneously develop pigmentary glaucoma, were used as the animal model of glaucoma, rats of the SHR strain, that spontaneously develop arterial hypertension – as the model of arterial hypertension, and rats of the Lewis strain with type 1 diabetes mellitus induced by an intraperitoneal administration of streptozotocin – as the model of diabetes mellitus. Appropriate control groups were selected for all experimental groups. *In vivo* examinations were performed on the animals - eye fundus imaging with the measurement of the ratio of the arterial diameter to the venous diameter, measurement of intraocular pressure, arterial pressure (in the model of hypertension), and glycemia (in the model of diabetes mellitus). In addition, blood and eyeballs were harvested from the animals after sacrifice and subjected to further *postmortem* analyses. A histopathological analysis of the retina was performed (measurement of retinal thickness and thickness of its layers, assessment of the number of retinal ganglion cells) and an enzyme-linked immunoassay to determine the levels of BDNF and NGF in the serum and eyeball homogenate. The results of all assays were compared between the experimental and control groups. In addition, correlations of the measured parameters were counted.

Among mice of the DBA/2 strain, the development of glaucoma was confirmed based on the increased intraocular pressure, while a lower number of retinal ganglion cells and a decrease in the retinal thickness indicated the ongoing process of neurodegeneration. The level of BDNF and NGF in the eyeballs in glaucoma tended to increase with age. The level of BDNF and NGF in the eyeballs of glaucoma mice tended to decrease in comparison to age-matched healthy mice.

Hypertensive retinopathy, characterized by impaired retinal vasculature, was not diagnosed in hypertensive rats; however, significantly higher levels of BDNF in the eyeballs and serum, and NGF in the eyeballs were observed among rats compared to the strain not susceptible to developing arterial hypertension. A higher level of NGF in the eyeballs correlated with a greater thickness of the inner nuclear layer of the retina.

In diabetic rats, diabetic retinopathy was diagnosed based on the dilation of the retinal veins (an increase in the ratio of the arterial diameter to the venous diameter in the eye fundus). In the enzyme-linked immunoassay, a lower level of BDNF was observed in the serum of diabetic rats than in the control group. In addition, there was a very strong correlation between the ratio of the arterial diameter to the venous diameter in the eye fundus and the level of NGF in the serum seen among sick animals.

Based on the obtained results, it can be concluded that: (i) the models of the studied diseases were effectively induced; (ii) neurodegenerative changes of the retina were present in the course of glaucoma and diabetes mellitus; (iii) changes in the level of BDNF and NGF were seen in the tissues of the tested animals (a decrease in the level of proteins in the eyeballs in the glaucoma model, an increase in the level of NGF in the eyeballs in the arterial hypertension model, a decrease in the level of BDNF in the serum in the diabetes mellitus model).

In summary, the involvement of BDNF and NGF in the pathomechanisms associated with the development of retinal pathologies in the course of glaucoma, arterial hypertension, and diabetes mellitus has been demonstrated. Therefore, my doctoral thesis complements the existing knowledge on the role of neuroprotective factors (BDNF, NGF) in the pathogenesis of eye diseases.