**MOLECULAR MARKERS OF ENDOGENOUS NEUROPROTECTION IN THE BRAIN OF RATS WITH EXPERIMENTAL PARKINSON’S DISEASE AND ON THE BACKGROUND OF USING NEW PHARMACOTHERAPY SCHEMES**

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**Abstract:** Parkinson’s disease (PD) is one of the most common neurodegenerative diseases in the elderly. The aim of the study. To study apoptotic processes and their role in the formation of dopaminergic neurodegeneration and to develop new treatment regimens with a specific neuroprotective effect on the dopaminergic system.

**Materials and methods.** The study was carried out on 90 Wistar rats at the age of 6 months weighing 220–290 grams. Parkinsonism was induced by the administration of the neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to experimental rats with neuroprotective treatment: I – Intact (passive control); II – animals with experimental Parkinson’s disease (PD, active control); III – PD+Amantadine (AM) IV – PD+AM+Cerebrocycin; V – PD+AM+Pramistar; VI – PD+AM+Gliatilin; VII – PD+AM+Noofen; VIII – PD+AM+Pronoran; IX – PD+AM+Melatonin.

**Results.** The obtained data indicate that neuroprotective therapy of PD with drugs such as melatonin, cerebrocycin, pronoran and gliatilin can be effective in the treatment of Parkinson’s disease in the early stages. The aim of the study was to study apoptotic processes and their role in the formation of dopaminergic neurodegeneration, to identify key biomarkers for early diagnosis and implementation of preventive programs towards stopping the progression of PD in the early stages, to develop new treatment regimens with a specific neuroprotective effect on the dopaminergic system.

**Conclusions.** The study experimentally demonstrated a new target of neuroprotection in PD conditions – apoptosis of dopamine-producing neurons and substantiated modulators of this process – drugs for combined therapy with amantadine (melatonin, cerebrocycin, pronoran and gliatilin) as promising drugs for the treatment of PD.

**Keywords:** Parkinson’s disease, HIF-1α, HIF-3α, HSP70, c-fos, bcl-2, caspase-3, melatonin, neuroprotection, apoptosis.
the melatonin group, where there is a statistically significant increase in expression of this gene (6.10 %). The increase in HIF3α mRNA expression compared to the control group in the amantadine group was 70.76 %, in the cerebrocurin group – 82.35 % (p≤0.05), pramistar – 67.72 %, gliatilin – 79.44 % (p≤0.05), noopen – 67.47 %, pronoran – 81.65 %, melatonin – 82.92 %. The neuroprotective effects of our drugs are associated with the normalization of endogenous neuroprotection HSP70 on the background of activation of gene expression of antioxidant enzymes compared with the control group: in the group of amantadine – by 31.86 % (p≤0.05), in the group of cerebrocurin – 15.08 % (p≤0.05), pramistar – 30.44 %, gliatilin – 16.26 %, noopen – 23.77 %, pronoran – 23.96 % (p≤0.05), melatonin – 34.66 % (p≤0.05).

In the brain of control rats, the PD model showed an increase in the expression of c-fos (c-fos-positive neuronal cells) by almost 52.75 % relative to the intact group of animals, which reflects the progressive activation of apoptotic processes and the death of dopamine-producing neurons. The expression of c-fos mRNA in the control group increased by 44.53 % relative to intact. In our study, the following dynamics of Fos-positive neurons was noted: the appointment of amantadine reduced expression by 7.25 % (p≤0.05), in the group of cerebrocurin – by 31.24 % (p≤0.05), pramistar – 19.68 %, gliatilin – 35.85 %, noopen – 26.70 %, pronoran – 21.34 % (p≤0.05), melatonin – 37.84 % (p≤0.05). Similar statistically significant dynamics was observed in the study of c-fos mRNA expression against the background of therapeutic effects in rats with PD: decreased expression of c-fos mRNA in all groups, but especially pronounced depression of apoptotic activity of this early response gene was in drugs cerebrocurin, gliatilin, pronoran and, especially, melatonin, and the values approached the group of intact.

In the control group on the background of PD there was a decrease in the density of bcl-2-positive neurons relative to intact by 27.85 %, and the expression of bcl-2 mRNA decreased by 26.09 %. After amantadine treatment of rats with PD, the density of bcl-2-positive neurons increased compared to the control by 10.18 % (p≤0.05), in the group of cerebrocurin – by 23.71 % (p≤0.05), pramistar – 17.54 %, gliatilin – 21.71 % (p≤0.05), noopen – 18.60 %, pronoran – 19.73 %, melatonin – 26.13 %. Simultaneously, there was an increase in the expression of bcl-2 mRNA in all experimental groups compared with the control, especially with the appointment of melatonin, cerebrocurin, gliatilin and pronoran within statistical significance.

A sign of activation of apoptosis of neuronal cells under the conditions of PD was also an increase in the activity of caspase-3 in the control group by 30.29 % relative to the control group. The therapeutically effective in the amantadine led to a decrease in this marker by only 6.01 % (p≤0.05), while the combination of amantadine with cerebrocurin reduced caspase-3 by 38.25 %, with gliatilin – by 40.78 % (p≤0.05), with noopen – by 20.58 %, with pronoran – by 28.35 %, and with melatonin – by 45.05 %.

4. Discussion

Cerebrocurin has a complex neuroprotective effect due to its ability to stabilize the functional state of mitochondria and limit the development of mitochondrial dysfunction [4]; to prevent the formation of energy deficit; block the development of lactic acidosis against the background of activation of compensatory mitochondrial-cytosolic shunts of energy products, especially malate-aspartate; reduce the manifestations of oxidative and nitrosative stresses; modulate the expression of all isoforms of NOS, as well as HIF and HSP proteins; increase the activity of enzymes of the antioxidant and thiol-disulfide systems; morphologically stabilize neuronal and glial cells with parallel activation of RNA synthesis in them, as well as to restore the morphological ultrastructure of mitochondria; affect the processes of apoptosis/necrosis and due to the regulatory effect on the expression of c-fos. Cerebrocurin increases HSP70 expression by activating NFκB [5]. Melatonin in PD increases the level of HSP70 due to the activation of melatonin receptors MT1 and MT2 [6]. In addition, the unique structure of the melatonin molecule makes it an effective scavenger of ROS/FA and prevents total damage to polypeptide bonds, inactivation of enzyme systems, antioxidant units of endogenous protection, including HSP70. Melatonin is also able to have a cytoprotective effect by maintaining the activity of glutathione peroxidase, Cu, Zn- and Mn-superoxide dismutase, and γ-glutamylcysteine ligase [7]. However, the ability of the drug to inhibit a number of prooxidant enzymes, such as lipoxygenase and NO synthase, which under conditions of PD reduces the production of ROS [8]. The positive effect of melatonin on energy metabolism is due to its ability to prevent damage to aconitate hydrolase and thus maintain the Krebs cycle at the citrate-isocitrate stage. Pramistar restores thiol-disulfide balance in the brain with PD, limits the expression of iNOS [9]. By increasing the level of reduced glutathione, pramistar is able to increase the expression of HSP70. Gliatilin increases synaptogenesis in cholinergic structures, has a mitoprotective effect, increases the level of intramitochondrial glutathione and is able to increase the level of HSP70 [10].

Thus, although apoptosis is the last step in the pathogenetic pathway in PD, it remains to be seen whether inhibition of apoptosis in PD can be effective and safe, and careful evaluation of the literature and experimental findings is necessary.

Study limitations. There may be some possible limitations in this study: the financial resources, deficiency prior research works/surveys on this issue and the absence of the dose-dependent treatment effect research.

Prospects for further research. Prospects for further research are to study the features of changing the behavioral reactions and cognitive-mnestic functions of rats under experimental Parkinson’s disease and the prospects of development of a strategy of pharmacological correction.

5. Conclusions

The data obtained indicate that neuroprotective therapy of PD with drugs such as melatonin, cerebrocurin, pronoran and gliatilin in combination with amantadine leads to an increase in the expression of the HIF-1α, HIF-3α, and HSP70 genes, and can also serve as a molecular marker for the activation of endogenous neuroprotection mechanisms under the conditions of an experimental PD.

The study of the mechanisms of programmed neuronal death by apoptosis in PD under conditions of oxidative stress and pharmacological correction of the mechanisms of apoptosis realization is a pathogenetically justified target of therapy for socially significant diseases. We offer c-fos and bcl-2 proteins as well as effector caspase-3 as markers of apoptosis.

We have experimentally demonstrated a new target of neuroprotection in PD conditions – apoptosis of dopamine-producing neurons and substantiated modulators of this process – drugs for combined therapy with amantadine (melatonin, cerebrocurin, pronoran and gliatilin) as promising drugs for the treatment of PD.

Conflicts of interest

Neither author has actual or potential conflicts of interest.
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