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+Cerebrocurin; 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, cerebrocurin, pronoran and gliatilin in combination with amantadine leads to an increase in the expression of the HIF-1α, HIF-3α, HSP70 genes, bcl-2 proteins and decrease c-fos proteins with caspase-3 as markers of apoptosis and can also serve as a molecular marker for the activation of endogenous neuroprotection mechanisms under the conditions of an experimental PD.
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, cerebrocurin, 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.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the elderly. Motor symptoms are primarily due to significant dopamine depletion caused by degeneration of dopaminergic neurons in the compact substantia nigra [1].

Apoptosis is the predominant mode of neuronal death in many neurodegenerative diseases, including PD. While the pathogenetic processes in PD have not been fully studied, convergent mechanisms lead to the death of neurons due to apoptosis, which makes the path of apoptosis an interesting potential therapeutic target [2]. Cell death by apoptosis is observed in cell culture models of animals with PD, as well as in the nigrostriatal regions of the brain of patients with PD at autopsy [3]. Elucidation of the main triggers of the apoptotic process in PD can lead to a better understanding of the sequence of events that lead to programmed cell death.

The aim of the study. To study apoptotic processes and their role in the formation of premature 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.

2. Materials and methods

All experiments were conducted during 2018–2020 on the basis of the Training Medical and Laboratory Centre of Zaporizhia State Medical University, certified by the Ministry of Health of Ukraine (certificate No. 039/14). 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. The intact group received a single intraperitoneal saline solution of 1 ml per 100 g of body weight, and the control group, after administration of MPTP, received a single intraperitoneal saline solution at the same dosage.

The study was conducted in accordance with Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as with the national "Common Ethical Principles for Animal Experiments" (Ukraine, 2001) and the guidelines set out in in "Basic principles of studying the toxicity of potential pharmacological drugs" (State Enterprise «Ukrainian Pharmaceutical Quality Institute», K., 2000). The experiment was approved by the Commission on Bioethics of Zaporizhia State Medical University.

The verification for the strategy of rational therapy in PD was based on studying the activity of drugs in groups of animals: I – Intact (passive control); II – animals with experimental Parkinson's disease (PD, active control); III – PD+Amantadine (AM) IV – PD+AM+Cerebrocurin; V – PD+AM+Pramistar; VI – PD+AM+Gliatilin; VII – PD+AM+Noofen; VIII – PD+AM+Pronoran; IX – PD+AM+Melatonin.

The concentration in the cytoplasmic fraction of the brain of HSP70 protein was determined by Western blot analysis. To analyze the expression of the genes HSP70, HIF-1α, HIF-3α and bcl-2, we used the method of Reverse transcription polymerase chain reaction (RT-PCR). The object of the study was brain homogenate. Determination of caspase-3 was performed by ELISA – a solid-phase enzyme-linked immunosorbent assay based on the principle of "sandwich" and based on the specific binding of antibodies to antigen, with one of the components conjugated to the enzyme.

Data are presented as mean and standard error of the mean. The statistical significance of intergroup differences was assessed using the Mann-Whitney method. Statistical analysis was performed using the program "Statistica 6.1" (StatSoft Inc., USA, serial No. RGXR412D674002F-WC7). For all types of analysis, differences at a significance level of less than 0.05 were considered statistically significant.

3. Results

In the control group of our study in rats with PD in conditions of increased reactions of oxidative, nitrosative stress and deficiency of energy resources in brain tissues, there is a decrease in HIF1a synthesis compared to the intact group of animals by 25.72 % and HIF3a – by 74.12 %. These processes are associated with the activation of the ubiquitin-independent degradation pathway of oxidatively modified HIF-1α and HIF3a, and the suppression of its synthesis during translation. The administration of amantadine, as well as its combination with cerebrocurin, gliatilin, noophen, pronoran and pramistar in PD conditions had almost no effect on the level of HIF1a mRNA expression compared to the control group, except for

the melatonin group, where there is a statistically significant increase in expression of this gene (6.10 %). The increase in HIF3a mRNA expression compared to the control group in the amantadine group was 70.76 %, in the cerebrocurin group – 82.35 % ($p \leq 0.05$), pramistar – 67.72 %, gliatilin – 79.44 % ($p \leq 0.05$), noophen – 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 % ($p \leq 0.05$), in the group of cerebrocurin – 15.08 % ($p \leq 0.05$), pramistar – 30.44 %, gliatilin – 16.26 %, noophen – 23.77 %, pronoran – 23.96 % ($p \leq 0.05$), melatonin – 34.66 % ($p \leq 0.05$).

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 % ($p \leq 0.05$), in the group of cerebrocurin – by 31.24 % ($p \leq 0.05$), pramistar – 19.68 %, gliatilin – 35.85 %, noophen – 26.70 %, pronoran – 21.34 % ($p \leq 0.05$), melatonin – 37.84 % ($p \leq 0.05$). 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 % ($p \leq 0.05$), in the group of cerebrocurin – by 23.71 % ($p \leq 0.05$), pramistar – 17.54 %, gliatilin – 21.71 % ($p \leq 0.05$), noophen – 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 50.29 % relative to the control group. The therapeutic effect of the amantadine led to a decrease in this marker by only 6.01 % ($p \leq 0.05$), while the combination of amantadine with cerebrocurin reduced caspase-3 by 38.25 %, with gliatilin – by 40.78 % ($p \leq 0.05$), with noophen – 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 NFkB [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/AFA 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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