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IS THERE AN ORGAN-SPECIFIC EXPRESSION OF CANDIDATE GENES (DJ1, PINK1) IN TISSUES OF THE ORGANISM UNDER EXPERIMENTAL PARKINSONISM AND ITS PATHOGENETIC THERAPY?

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ABSTRACT

It has been studied the changes in the structural and functional state of mitochondria and expression of PINK1 and DJ1 genes in brain tissue - medulla oblongata and striatum and lung and heart tissue in experimental parkinsonism and its pathogenetic treatment with the help of a broad-spectrum antihypoxant Kapikor. It was shown that under experimental parkinsonism, in addition to damage to the ultrastructure of the mitochondrial apparatus in cells of body tissues, there are significant changes in mRNA expression of DJ1 and PINK1 genes, which are associated with the formation of mitochondrial dysfunction. They have a multidirectional character in the tissues of the brain - decrease, and in the tissues of the heart and lungs - increase. The degree of such changes in expression is organ-specific and more pronounced in the tissues of the visceral organs than in the tissues of the brain. Also, it was shown that the use of broad-spectrum antioxidant, which contains mildenium dehydrate and gamma-butyrobetaine dihydrate, there are significant changes in the expression of mRNA genes DJ1 and PINK1, which are also organ-specific - the expression of mRNA of all DJ1 genes increased in to a greater extent, the expression of PINK1 gene mRNA decreased sharply in brain tissues, and also increased sharply in lung and heart tissues. The data obtained indicate a complex and ambiguous relationship between the level of expression of the studied candidate genes involved in the formation of experimental parkinsonism, and the severity of mitochondrial dysfunction, which is one of the pathogenetic causes of parkinsonism.

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Introduction. In the 21st century, Parkinson's disease (PD) has become the second most common neurodegenerative disease in the world after Alzheimer's disease. The results of experimental and clinical studies indicate that in the pathogenesis of this disease, as well as a significant part of other emerging pathological conditions, mitochondrial (MD) and / or endothelial dysfunction (ED)

plays a significant role [1]. In particular, oxidative stress plays a leading role in the formation of MD in PD. However, in general, the etiology of Parkinson's disease is still unclear. However, there is no doubt that in its development one of the leading places, along with the influences that lead to damage of dopamine neurons, depletion of dopamine reserves in them with their subsequent damage, is occupied by a genetic component.

From the point of view of genetic polymorphism, PD is not one disease, but a heterogeneous group of diseases with a wide range of clinical manifestations depending on the associated gene. The study of the genetic nature of the disease began in the late twentieth century after the identification of mutations in the gene encoding the protein α-synuclein (SNCA), identifying the role of this protein in the formation of Lewy bodies and, consequently, its participation in the development of PD. Today, new mutations associated with the development of this pathology are identified annually. Of the many candidate genes studied, Parkin, PINK1, and DJ1 are often considered [2]. Parkin mutations are considered the most common cause of autosomal recessive PD and especially in common diseases with early onset. Parkin cooperates with PINK1 in the so-called quality control, such as neurons, by activating mitophagy in conditions of mitochondrial damage [3].

PINK1 mutations are the second most common cause of PD after Parkin. PINK1 functions most markedly in activating mitophagia, accumulating on the outer mitochondrial membrane under mitochondrial damage [4]. The specific mechanism of gene pathogenicity in PD is currently unclear and requires further study. The DJ-1 gene encodes a molecular chaperone that induces oxidative stress. In the presence of oxidative stress, the DJ-1 protein is transferred from the cytoplasm to the outer mitochondrial membrane and can provide neuroprotection [5].

PD is accompanied in neurons by disruption of dynamic processes in the mitochondrial apparatus, accompanied by changes in the energy supply of cells [6]. In Parkinson's disease there is a suppression of mitochondrial division, ie. fission process. Mitochondrial dysfunction leads to the accumulation of oxidized dopamine, which causes the accumulation of α-synuclein and lysosome dysfunction. [7]. All structural and functional rearrangements in mitochondria are accompanied by genetically determined processes [8]. The above ideas cover mainly studies involving nervous tissue, although it has been proven that the causes of mortality in PD are mainly bronchopneumonia or cardiovascular pathology [9]. There are far fewer such studies, which does not allow to form a complete picture of the mechanisms of pathological changes, which is necessary to find effective ways to treat PD in order to improve the quality of life of patients.

The study of CP is now quite well conducted in model studies, for example in the simulation of experimental parkinsonism using rotenone, which reproduces quite well the main features of CP [10].

The aim of the study.

In this regard, the aim of this work was to study changes in the structural and functional state of mitochondria and expression of PINK1 and DJ1 genes in brain tissue - medulla oblongata and striatum and lung and heart tissue in experimental parkinsonism and its pathogenetic treatment.

Materials and methods.

The studies were performed on adult male Wistar rats weighing 250-300 g. All animals were kept in the vivarium of the Bogomoletz Institute of Physiology NASU and had free access to food and water. Rats were divided into groups: 1) control (n = 8), 2) animals to which rotenone was administered subcutaneously, daily for 2 weeks at a dose of 0.3 mg / 100 g body weight (n = 12); 3) for the correction of structural disorders during the introduction of Rotenone used the drug Kapikor (Olayinfarm - Latvia, Olfa), which consists of meldonium dihydrate and gamma butyrobetaine dihydrate. It is licensed in Ukraine as a broad-spectrum antioxidant. The drug was administered subcutaneously daily for 2 weeks at a dose of 0.5 mg / 100 g body weight (n = 12).

All experimental studies were conducted in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), general ethical principles of scientific research adopted by the First National Congress of Ukraine on Bioethics (September 2001), of the Law of Ukraine № 3447-IV "On the protection of animals against cruel treatment" (2006), the provisions of the Convention on Bioethics of the Council of Europe (1997).

Preparation of samples for electron microscopic and morphometric studies was carried out according to generally accepted methods. Rats were decapitated under weak ether anesthesia. Pieces of medulla oblongata at 12 mm from Bregma and striatum, the apex of the heart and symmetrical areas of

both lungs were taken from the animals. Fixation of the material was performed according to the conventional method, immediately introducing tissue samples into a buffered 2.5% solution of glutaraldehyde (0.1 M phosphate buffer, pH - 7.4). Dofixation of the material was carried out using a reagent Caulfield (based on 2% solution of osmium tetroxide, pH-7.4) (reagents from Sigma, USA). Subsequently, the material was dehydrated in alcohols of increasing concentration, absolute alcohols and acetone, followed by pouring into epon-araldite (reagents from Fluka, Switzerland) [11].

Ultrathin sections 40-60 nm thick for viewing under an electron microscope were contrasted with 1% uranyl acetate solution and lead citrate solution (Sigma reagents, USA) according to the Reynolds method [12]. Examination of the samples were performed using an electron microscope TEM - 125K (Ukraine).

Morphometric studies were performed based on Weibel's approaches [13,14], using a computer program for morphometric calculations Image Tool (USA) in 130-150 fields for each study group. In experimental studies, the total number of mitochondria and the number of structurally damaged mitochondria were determined.

Isolation of RNA from samples (probes) (P0, n = 8.6–30 m, n = 4 per group) was achieved using RNeasykit (Sigma-Aldrich) according to the protocols provided by the manufacturer, the results were quantified using UV / visual spectral photometer (NanoDropND-1000, Peqlab, Erlangen, Germany). CDNA was synthesized from 1 mg of total RNA using a high-capacity cDNAR everse Transcription Kit from Applied Biosystems (Darmstadt, Germany). Quantitative polymerase chain reaction (PCR) primer pairs were developed for SYBR-Green based on quantitative reverse transcription polymerase chain reaction (qRT-PCR). The following target primes for genotyping were used in PCR analysis [15, 16]:

DJ-1: 5′-TATTGGGCCTTTCTCTTGGA; 5′-TGGGAGTGACAGTCTCAGTGG,
5′-AGCTATGA GGCCCTTCCTGT

PINK 1: 5′-CCTACACACAGCCCTCACCT, 5′-CCCTGGCTGACTATCC,
5′-CCACCACCCACTACCACTTACT

qRT-PCR was performed using the PCR kit SEN YBRGreen (AppliedBiosystems) according to the protocols provided by the manufacturer. The relative expression of the protein as $2 - \Delta Ct^{\text{specific gene}} / 2 - \Delta Ct^{\text{mean (housekeeping genes)}}$ was calculated using glyceraldehyde phosphate dehydrogenase as the endogenous control gene for housekeeping genes. For relative quantification (RQ), the comparative method Ct (Δ - ΔCt) was used; the results are presented for the expression levelat P0. All coding regions and exon - intron boundaries of the PINK1 and DJ-1 genes were analyzed by heteroduplex analysis followed by direct sequencing of the identified variants. These variants were evaluated using web programs SIFT, PolyPhen, HSF and LOVD [17].

Statistical processing of the results was performed using the computer program STATISICA 6. Numerical data were presented as "mean ± standard error of the mean". This representation is correct, because according to the Shapiro-Wilkie criterion (W), the results obtained fit into the normal distribution law [18]. To assess the reliability of the results used one-way analysis of variance One-Way ANOVA using a comparative Post Hoc test Student-Newman-Keuls. The results were considered statistically significant at $p < 0.05$.

Results and its discussion.

Studies have shown that changes in the level of mRNA expression of the DJ1 gene in experimental parkinsonism (EP) was unidirectional in all studied tissues, namely its reduction: in the medulla oblongata - by 11.4%; in the striatum - by 19.2%; in heart tissue - 11.7 times, and in lung tissue - by 3 orders of magnitude, ie almost to zero (Fig. 1). Thus, despite the unidirectionality of the changes, the decrease in mRNA expression of the DJ1 gene in EP, not surprisingly, was mostly more pronounced not in brain tissue, but in the tissues of the studied visceral organs.

Regarding the level of mRNA expression of the PINK1 gene, the dynamics of changes was also unidirectional in all tissues, but inverse to that established with respect to the DJ1 gene (Fig. 2).

The increase in PINK1 gene expression was in the medulla oblongata - 59.2%; in the striatum - 71.8%; in heart tissue - 46.8%, and in lung tissue - more than an order of magnitude. Thus, in brain tissues, changes in PINK1 gene expression were also significantly less pronounced than in the lungs and myocardium.

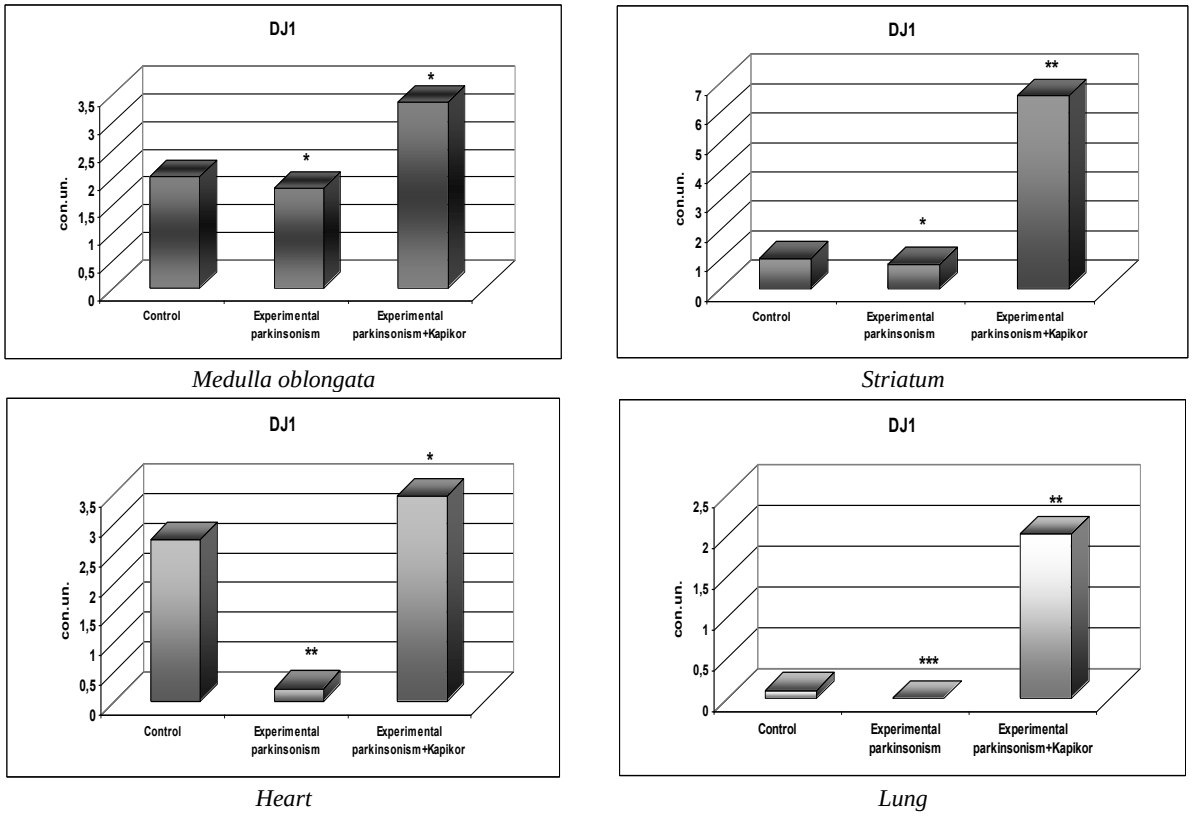

*Fig.1. Changes in mRNA expression of the DJ1 gene in experimental parkinsonism and the use of the drug Capicor. * - reliability relative to control p <0,05; ** - reliability relative to control p <0,01; *** - reliability relative to control p <0,001.*

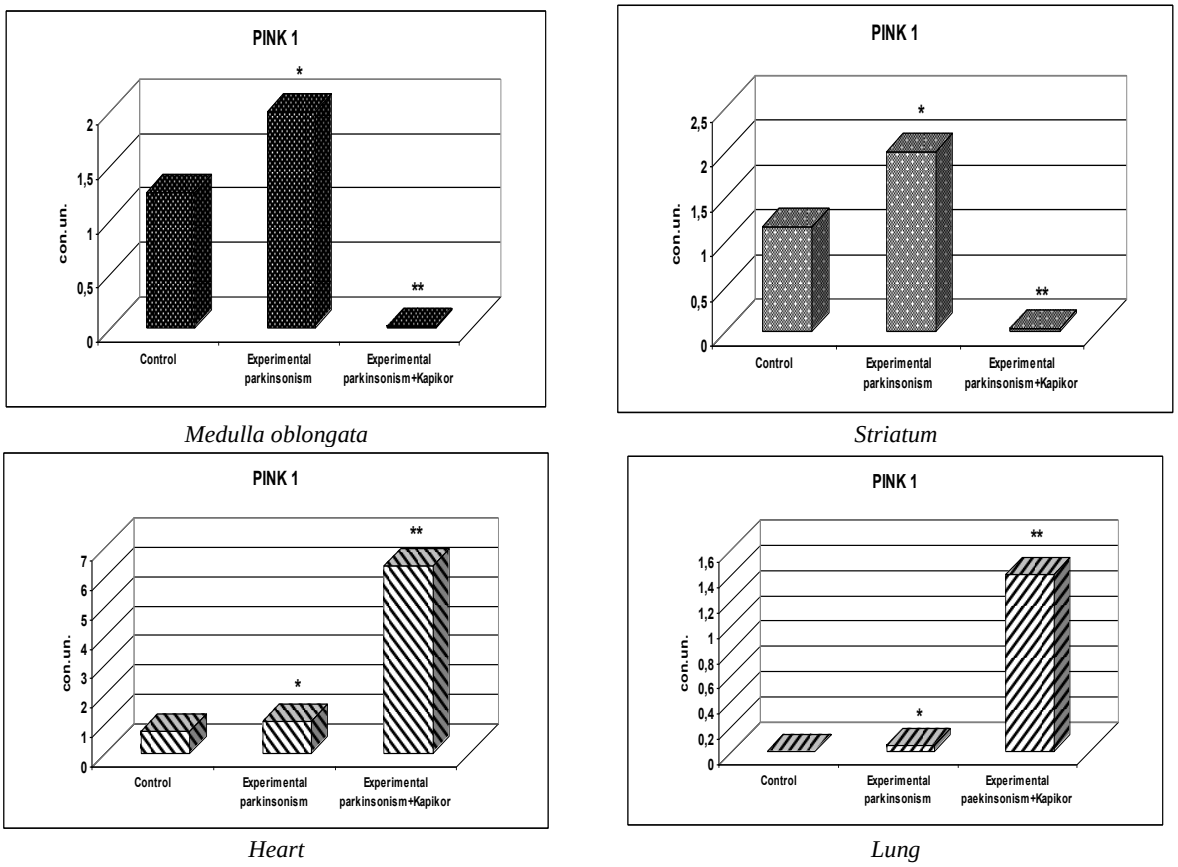

*Fig.2. Changes in mRNA expression of the PINK1 gene in experimental parkinsonism and the use of the drug Capicor. * - reliability relative to control p <0,05; ** - reliability relative to control p <0,01.*

Because DJ1 is thought to be directly involved in the development of mitochondrial dysfunction, is a sensor of oxidative stress and is able to eliminate peroxides by self-oxidation, and PINK1 acts as a sensor of mitochondrial damage and promotes this process with significant accumulation [3, 5], we can state that we found quantitative changes in mitochondrial apparatus of the studied tissues and structural damage to organelles in PD are largely genetically determined (Table 1).

Table 1. Changes in the mitochondrial apparatus in the studied tissues in experimental parkinsonism

Experimental conditions	Total number of mitochondria, units / 10 μm^2	Average number of structurally damaged mitochondria, %
Medulla oblongata		
Control	14,3±1,8	8,4±1,2
Experimental parkinsonism	9,4±1,6*	43,2±11,4**
Experimental parkinsonism + Kapikor	13,6±2,4	22,1±8,3*#
Striatum		
Control	16,0±1,6	4,2±1,2
Experimental parkinsonism	14,0±3,1	29,6±9,3*
Experimental parkinsonism + Kapikor	15,6±2,7	18,2±7,6*
Heart (mean values for both subpopulations of mitochondria - subsarcolemmal and intramyofibrillar)		
Control	14,3±1,5	5,1±1,3
Experimental parkinsonism	16,1±1,8	39,6±9,4**
Experimental parkinsonism + Kapikor	14,9±2,4	20,2±7,3*#
Lungs		
Control	10,2±2,0	7,4±1,5
Experimental parkinsonism	9,5±1,3	25,1±8,1*
Experimental parkinsonism + Kapikor	13,3±1,7	19,3±4,4*

Note: * - reliability relative to control p <0,05; ** - reliability relative to control p <0,01; # - reliability relative to experimental parkinsonism without the use of Kapikor p <0,05.

It is likely that DJ1 and PINK1 may be involved in this process, which is due to the established mechanism of their influence, largely due to depolarization of mitochondrial membranes, disruption of protein imports and increased sensitivity to peroxides, ie oxidative stress, which is one of the main pathogenetic mechanisms of PD development [7,9].

The drug Kapikor applied by us causes pronounced and rapid NO-dependent effects: vasodilating, antiplatelet, anticoagulant, antioxidant; affects the regulation of apoptosis and proliferation, maintenance of vascular homeostasis. The drug, as it turned out, can effectively reduce not only the severity of mitochondrial dysfunction (see Table 1), but also affect the level of mRNA expression of genes, in our case DJ1 and PINK1 (see Fig. 1, 2), thereby - apparently, influencing the mechanisms contributing to the development of parkinsonism, which will probably improve the quality of life of PD patients and prevent the frequent development of comorbidities.

It should be noted that, under the influence of the drug, the expression level of mRNA DJ1 gene, which was reduced to varying degrees in all studied tissues, significantly increased significantly exceeding the initial levels, especially in the striatum tissue (6.4 times) and lungs (more than 20 times). As for the expression of mRNA of the PINK1 gene, it also changed not only organ-specific, but also in different directions: it sharply decreased (almost 2 orders of magnitude) below the initial level in brain tissues and increased even more than with EP in heart tissues (8.3 times) and lungs (9.4 times). If we take into account that the use of Kapikor significantly decreased the number of mitochondria structurally damaged during EP, and the expression of DJ1 gene mRNA shifts towards the control values and even exceeds it, we can assume the existing role of this genetic mechanism in the normalization of the mitochondrial apparatus of the cells of the studied tissues. Regarding the expression of mRNA of the PINK1 gene and its changes with the use of Kapikor, it seems that no direct relationship with the mitochondria ultrastructure is observed. The clarity of this issue, as well as the clarification of the meaning of organ-specificity of mRNA expression of candidate genes for the

role of initiators of the neurodegenerative pathology development - DJ1 and PINK1 - can be made by further research. Such studies are all the more relevant, since the possibility of changes in the level of gene expression using a broad-spectrum antioxidant has been shown. It can facilitate the search for effective ways of treating concomitant PD pathology.

Conclusions. The obtained results showed that under experimental parkinsonism, in addition to damage to the ultrastructure of the mitochondrial apparatus in cells of body tissues, there are significant changes in mRNA expression of DJ1 and PINK1 genes, which are associated with the formation of mitochondrial dysfunction. They have a multidirectional character in the tissues of the brain - decrease, and in the tissues of the heart and lungs - increase. The degree of such changes in expression is organ-specific and more pronounced in the tissues of the visceral organs than in the tissues of the brain.

Studies have shown that the use of broad-spectrum antioxidant, which contains mildenium dehydrate and gamma-butyrobetaine dihydrate, there are significant changes in the expression of mRNA genes DJ1 and PINK1, which are also organ-specific - the expression of mRNA of all DJ1 genes increased in to a greater extent, the expression of PINK1 gene mRNA decreased sharply in brain tissues, and also increased sharply in lung and heart tissues.

Declaration of interest statement. No conflict of interest exists.

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