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mRNA level of antioxidant genes and activity of NADPH-generating enzymes in rotenone-induced parkinsonism in rats

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ABSTRACT

Aim. To analyze the mRNA level of genes encoding antioxidant enzymes and the transcription factors Nrf2 and Foxo1 regulating their expression and the activity of glucose-6-phosphate dehydrogenase (G6PDH) and NADP-dependent isocitrate dehydrogenase (NADP-IDH) and assess the correlation between these parameters, oxidative status, and motor coordination parameters in rats with rotenone-induced parkinsonism.

Materials and methods. The study was performed on male Wistar rats aged 4–6 months and weighing 200–250 g.Parkinsonism was modeled by subcutaneous administration of rotenone for 10 days at a dose of 2.5 mg / kg. To confirm the development of the pathology, motor coordination tests and histological staining of the cerebral cortex and striatum with hematoxylin and eosin were used. The oxidative status was analyzed based on the levels of conjugated dienes, carbonyl amino acid residues in proteins, and α-tocopherol. The enzyme activity was studied spectrophotometrically by the formation of NADPH. Real-time PCR was used to analyze the level of gene mRNA.

Results. During the study, an increase in serum and brain concentrations of conjugated dienes, carbonyl amino acid residues, and α-tocopherol was observed in the experimental group of rats compared to the controls. It could be associated with the redistribution of this compound between tissues during pathology development. The animals with experimental parkinsonism, in addition, were characterized by a decrease in the mRNA level of the *Sod1*, *Gpx1*, *Gsr*, *Gsta2*, *Nfe2l2*, and *Foxo1* genes, as well as the activity of G6PDH and NADP-IDH. In the rats with experimental parkinsonism, a negative correlation of NADPH-IDH activity in the brain with serum α-tocopherol level and a positive correlation with *Gpx1* and *Foxo1* mRNA levels in the striatum were found. The level of oxidatively modified proteins in the brain of the animals with PD was negatively correlated with the concentration of *Gsta2* mRNA in the striatum, while the specific activity of G6PDH in the serum was characterized by the positive relationship with grip strength.

Conclusion. The data obtained indicate that the inhibition of transcription of the genes encoding antioxidant enzymes and regulatory factors Nrf2 and Foxo1 contributed significantly to the development of oxidative stress in PD. A decrease in the activity of G6PDH and NADP-IDH led to a decrease in the availability of NADPH, which is a limiting factor in the functioning of the glutathione antioxidant system. Obviously, the inhibition of G6PDH and NADP-IDH was also an important pathogenic factor in the progression of the pathology. Along with a decrease in the content of antioxidant gene mRNA in the brain tissues, the level of α-tocopherol increased in the rats with parkinsonism, which could be the result of an imbalance in the functioning of antioxidant system.

Keywords: Parkinson's disease, oxidative stress, antioxidant system, glucose-6-phosphate dehydrogenase, NADP-dependent isocitrate dehydrogenase

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Уровень мРНК генов антиоксидантной системы и активность НАДФН-генерирующих ферментов при ротенон-индуцированном паркинсонизме у крыс

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РЕЗЮМЕ

Цель. Исследование уровня мРНК генов антиоксидантных ферментов и регулирующих их экспрессию транскрипционных факторов Nrf2 и Foxo1, активности глюкозо-6-фосфатдегидрогеназы (Г6ФДГ) и никотинамидадениндинуклеотидфосфат-изоцитратдегидрогеназы (НАДФ-ИДГ), а также анализ корреляционных связей между данными параметрами, состоянием оксидативного статуса и моторно-координационными показателями у крыс с ротенон-индуцированным паркинсонизмом.

Материалы и методы. Исследование было выполнено на крысах самцах Вистар в возрасте 4–6 мес и массой 200–250 г. Паркинсонизм моделировали путем подкожного введения в течение 10 сут ротенона в дозе 2,5 мг/кг. Для подтверждения развития патологии использовали моторно-координационные тесты и гистологические методы с окрашиванием коры полушарий и полосатого тела головного мозга гематоксилином и эозином. Состояние оксидативного статуса оценивали на основании концентрации диеновых конъюгатов, карбонильных остатков аминокислот в белках и α-токоферола. Активность ферментов исследовали спектрофотометрически по образованию НАДФН. Для анализа уровня мРНК генов использовали метод полимеразной цепной реакции в реальном времени.

Результаты. В ходе исследования у крыс опытной группы по сравнению с контролем наблюдалось возрастание в сыворотке крови и мозге концентрации диеновых конъюгатов, карбонильных остатков аминокислот, а также α-токоферола, что могло быть связано с перераспределением данного соединения между тканями при развитии патологии. Для животных с экспериментальным паркинсонизмом, кроме этого, было характерно снижение уровня мРНК генов *Sod1*, *Gpx1*, *Gsr*, *Gsta2*, *Nfe2l2* и *Foxo1*, а также активности Г6ФДГ и НАДФ-ИДГ. У крыс с экспериментальным паркинсонизмом была найдена отрицательная корреляция активности НАДФ-ИДГ в мозге с концентрацией α-токоферола в сыворотке и положительная – с уровнем мРНК *Gpx1* и *Foxo1* в полосатом теле головного мозга. Уровень окислительно-модифицированных белков в мозге животных с патологией отрицательно коррелировал с концентрацией мРНК *Gsta2* в полосатом теле, а удельная активность Г6ФДГ в сыворотке характеризовалась наличием положительной взаимосвязи с силой хвата.

Заключение. Полученные данные свидетельствуют, что угнетение транскрипции генов антиоксидантных ферментов и регуляторных факторов Nrf2 и Foxo1 вносило существенный вклад в развитие окислительного стресса при БП. Наблюдаемое снижение активности Г6ФДГ и НАДФ-ИДГ уменьшало доступность НАДФН – лимитирующего фактора для функционирования глутатионовой антиоксидантной системы, что также, очевидно, являлось важным патогенетическим фактором прогрессирования патологии. Наряду со снижением в тканях мозга содержания мРНК генов антиоксидантных ферментов, у крыс с паркинсонизмом возрастала концентрация α-токоферола, что могло быть результатом развития дисбаланса в функционировании антиоксидантной системы.

Ключевые слова: болезнь Паркинсона, окислительный стресс, антиоксидантная система, глюкозо-6-фосфатдегидрогеназа, НАДФ-изоцитратдегидрогеназа

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's and the most common movement disorder in the world. The pathogenesis of PD is characterized by a loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies in the midbrain, which are aggregates of proteins with impaired folding, including α-synuclein [1]. Neurodegeneration in PD also occurs in the noradrenergic, serotonergic, and cholinergic systems, the cerebral cortex, the optic bulb, and parts of the autonomic nervous system [2].

Oxidative stress has been demonstrated to occur early in the development of PD. Thus, uncontrolled generation of reactive oxygen species (ROS) is a causal factor in the death of dopaminergic neurons rather than an effect of other pathogenetic factors of neurodegeneration [3]. The antioxidant system protects neurons from oxidative stress and includes such enzymes as superoxide dismutase, catalase, glutathione enzymes, and non-enzymatic antioxidants. The nuclear factor-erythroid-2-related factor 2 (Nrf2) is a key regulatory factor responsible for the implementation of the antioxidant response. Under steady state conditions, the Nrf2 protein binds to the Kelch-like ECH-associated protein (KEAP1) in the cytoplasm, whereby it is ubiquitinated and undergoes proteasomal degradation.

Oxidative stress causes disruption of the Nrf2 – KEAP1 complex, leading to the release of Nrf2 and its nuclear translocation. Nrf2 in the nucleus activates the expression of antioxidant responsive elements (ARE)-containing genes encoding, among others, antioxidant enzymes [4]. Foxo1 is another regulatory factor involved in cell survival under oxidative stress. This protein modulates the activity of numerous genes, including genes related to antioxidant enzymes, autophagy, and apoptosis, in particular tumor necrosis factor-α, FAS-ligand (tumor necrosis factor ligand superfamily member 6), caspase-3, caspase-8 and caspase-9 [5]. Nicotinamide adenine dinucleotide phosphate (NADPH), which is necessary for the reduction of oxidized glutathione, is another important defense component that limits the functioning of glutathione-related enzymes of the antioxidant system. The main source of NADPH is the pentose phosphate pathway, with glucose-6-phosphate dehydrogenase (G6PDH) as the key enzyme [6]. NADP-dependent isocitrate dehydrogenase (NADP-IDH) can act as an alternative source of NADPH. This enzyme is localized predominantly in the cytoplasm, but a small amount of enzyme activity is also found in the mitochondria. NADP-IDH ensures the coordination of carbon and nitrogen metabolism and is also involved in the generation of NADPH [7].

Studies have shown that about 70% of neurons die before the onset of clinical symptoms of PD. In this regard, research revealing strategies to maintain redox homeostasis, as well as possibilities of early diagnosis of parkinsonism seems relevant [8]. Currently, experimental PD induced by rotenone administration remains the preferred model of pathology and consistently mimics the neuropathological features of PD due to the ability of rotenone to reproduce the progressive nature of the disease with characteristic slow cell death, motor disturbances, and signs of α-synucleinopathy [9]. We have previously shown that the activity of glutathione-related enzymes and catalase is inhibited in rats with rotenone-induced parkinsonism [10]. At the same time, studies demonstrating the relationship between AREs at the transcriptional level, NADPH-supplying enzymes, and oxidative status parameters in experimental PD remain very limited in the literature.

The aim of this work was to investigate the mRNA level of genes encoding antioxidant enzymes and the transcription factors Nrf2 and Foxo1 regulating their expression, to evaluate the activity of G6PDH and NADP-IDH, and to assess the correlation between these parameters, oxidative status, and motor coordination indices in rats with rotenone-induced parkinsonism.

MATERIALS AND METHODS

The study was performed on male Wistar rats aged 4–6 months and weighing 200–250 g. The animals were kept in 12 hour light : 12 hour dark cycle with *ad libitum* access to food and feed. The composition of the animal feed is given in Appendix 1. The work was performed in compliance with the principles of humanity set out in the European Community directives (86/609/EEC) and the Declaration of Helsinki. PD in the rats was modeled by subcutaneous administration of rotenone at a dose of 2.5 mg / kg as a solution in 98% purified olive oil and 2% dimethyl sulfoxide for 10 days [11]. The animals were randomly divided into two experimental groups of 12 animals each. Group 1 consisted of animals that received subcutaneous injections of the vehicle. Group 2 consisted of rats with PD. Twenty-four hours after the last injection, the rats were analyzed for motor indices, then they were sacrificed, and biological material was taken.

The following tests were used to assess the animals' motor skills and coordination: 1) the animal was placed in a transparent cage, after switching on the video recording, the researcher left the room. The video recorded the number of upright postures in 3 minutes [11]; 2) the rat was held and allowed to catch the electronic scale bracket with its forelegs. The scales were pulled back and the maximum value of the index was recorded [12]; 3) the animal was placed in a transparent cage and a piece of sticky paper was glued to its head. The time for the rat to perceive a stimulus and remove the paper from the head was recorded [13]. All tests were performed three times, and the average value of the parameters was calculated.

Brain tissue from three rats from each group was used to prepare histologic specimens stained with hematoxylin and eosin. The rats were anesthetized, the brains were quickly extracted and immersed in 10% neutral buffered formalin for 2 hours, then washed in running water for 24 hours. After tissue dehydration with ethanol and paraffin embedding, 5-μm-thick sections were prepared using the HM-325 rotary microtome (Thermo Fisher Scientific, USA), followed by staining with hematoxylin and eosin. Images were acquired using the AxioLab A1 light microscope (Zeiss, Germany) and the AxioCam 105 color camera. At least five fields of view for each slide were evaluated.

To analyze the concentration of conjugated dienes (CD), heptane and isopropanol were added to the test sample, the mixture was stirred and precipitated by centrifugation at 3,000g. The heptane supernatant phase was diluted with ethanol and analyzed spectrophotometrically at 233 nm [14]. To assess the degree of oxidative modification of proteins (OMP), a method based on the ability of carbonyl amino acid residues to interact with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form 2,4-dinitrophenylhydrazones with absorbance at 370 nm was used [15]. The sample was diluted with 100 mM phosphate buffer (pH 7.4), 10 mM 2,4-DNPH in 2.5 M HCl was added, the mixture was incubated for 1 hour, and then 20% TCA was added. After cooling, the samples were centrifuged at 3,000g, washed with 10% TCA and ethanol – ethyl acetate mixture (1:1), then dissolved in 2 ml of 8 M urea. The concentration of α-tocopherol was determined by a method based on photometry of a chromogenic Fe^{2+} – orthophenatroline complex [16]. Ethanol and hexane were added to the test sample, centrifuged at 3,000 g, then the hexane layer was removed, and hexane was evaporated in a water bath. Benzene and ferric chloride were added to the dry residue. After 5 min, 0.05% orthophenatroline was added, then the optical density at 510 nm was measured after 2 min. Total RNA was isolated using Extract RNA reagent (Eurogen, Russia). The quality of RNA was controlled by agarose gel electrophoresis. Reverse transcription for each sample was performed using the MMLV RT kit (Eurogen, Russia). The Ct of each gene was normalized to the geometric mean Ct of *Gapdh* and *Actβ* used as housekeeping genes (Appendix 2). The following primers were used in the study: Sod1 F: 5'-CCAGCGGATGAAGAGAGG-3',
Sod1 R: 5'-GGACACATTGGCCACACC-3',
Cat F: 5'-CAGCGACCAGATGAAGCA-3',
Cat R: 5'-GGTCAGGACATCGGGTTTC-3',
Nfe2l2 F: 5'-GCCTTGTACTTTGAAGACTGTATGC-3',
Nfe2l2 R: 5'-GCAAGCGACTGAAATGTAGGT-3',
Foxo1 F: 5'-AGATCTACGAGTGGATGGTGAAGAG-3',
Foxo1 R: 5'-GGACAGATTGTGGCGAATTGAAT-3',
Gsta2 F: 5'-CGGGAATTTGATGTTTGACC-3',
Gsta2 R: 5'-AGAATGGCTCTGGTCTGTGC-3',
Gpx1 F: 5'-TTTCCCGTGCAATCAGTTC-3',
Gpx1 R: 5'-GGACATACTTGAGGGAATTCAGA-3',
Gapdh F: 5'-CCCTCAAGATTGTCAGCAATG-3',
Gapdh R: 5'-AGTTGTCATGGATGACCTTGG-3',
Actb F: 5'-CCCGCGAGTACAACCTTCT-3',
Actb R: 5'-CGTCATCCATGGCGAACT-3',
Gsr F: 5'-TTCCTCATGAGAACCAGATCC-3'
Gsr R: 5'-CTGAAAGAACCCATCACTGGT-3'.

Real-time polymerase chain reaction (PCR) was performed using qPCRmix-HS SYBR (Eurogen, Rus-

sia) on the ANK-32 device (Syntol, Russia). The results were analyzed using the $2^{-\Delta\Delta Ct}$ method. The reaction specificity was evaluated using melting curves.

NADP-IDH activity was measured in the medium consisting of 50 mM Tris-HCl buffer (pH 7.8), 1.5 mM isocitrate, 2 mM $MnCl_2$, and 0.4 mM NADP. The spectrophotometric medium for the assessment of G6PDH activity was 50 mM Tris-HCl buffer (pH 7.8) containing 3.2 mM glucose-6-phosphate, 0.25 mM NADP, and 1.0 mM $MgCl_2$. The enzymatic activity was evaluated by the change in the optical density at 340 nm using the Hitachi U1900 spectrophotometer (Japan). The biuret test was used to determine protein content.

The results were analyzed by SPSS Statistics 23.0. The one-sample Kolmogorov – Smirnov test was used to analyze the normality of distribution of the variables. Variable values in groups were compared using Student's *t*-test or Mann – Whitney test. To identify correlations between the studied variables, the Pearson's correlation coefficient was used for variables with normal distribution, and the Spearman's rank correlation coefficient was applied for variables with non-normal distribution. This study presented moderate (0.30–0.69) and strong (> 0.70) correlations. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Our study showed that the development of rotenone-induced parkinsonism in the rats was accompanied by a significant ($p < 0.05$) change in motor coordination parameters (Table 1). Thus, rotenone administration led to a decrease in the number of upright postures and grip strength, as well as an increase in the time taken to remove the paper from the head. In addition, the induction of pathology was confirmed by morphological changes in the brain tissues (Fig. 1). In particular, a decrease in the number of neurons, the development of their atrophy and pycnosis, condensation of nuclei, and infiltration of the tissue with glial cells were observed in the cortex and striatum of the rats with experimental PD.

Table 1

Motor coordination parameters in the rats with rotenone-induced parkinsonism, *Me* (Q_1–Q_3)

Parameter	Group		*p*
	Controls (*n* = 12)	Rats with PD (*n* = 12)	
Number of upright postures	11.0 (9.5–12.0)	4.0 (2.0–7.0)	0.002
Grip strength, kg	0.300 (0.269–0.300)	0.157(0.135–0.200)	0.001
Paper removal, sec	21.5 (15.3–40.8)	127.0 (91.8–152.5)	0.003

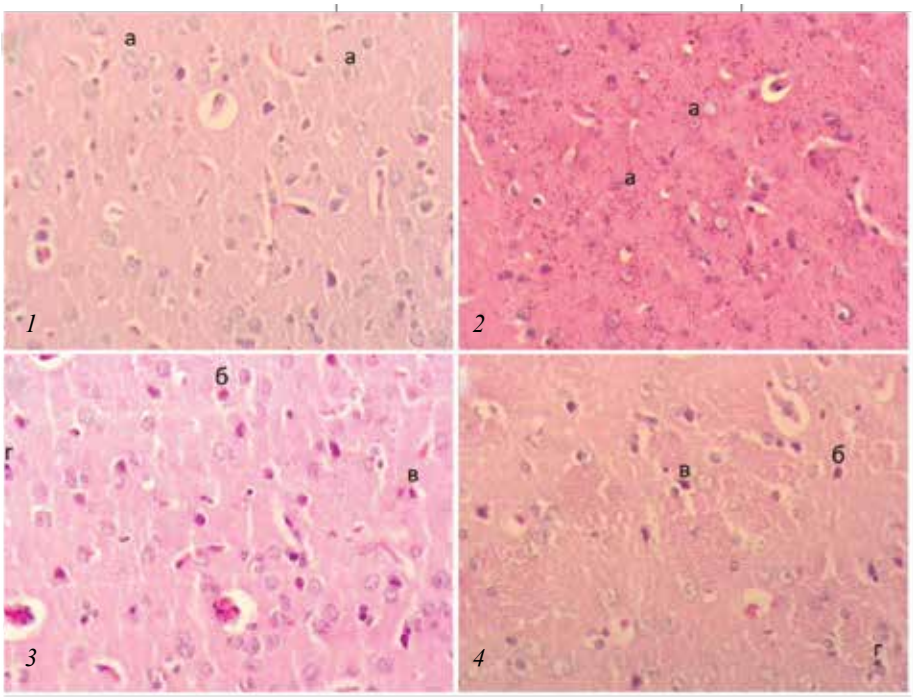
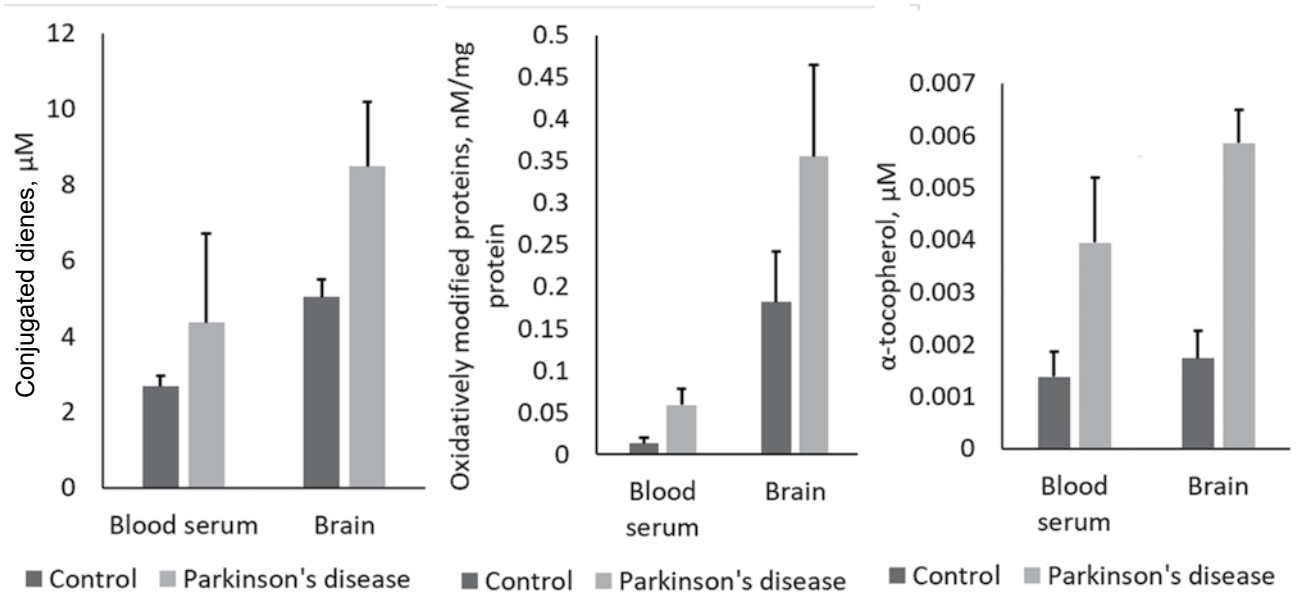


Fig. 1. Hematoxylin and eosin staining of brain tissues in the rats: the cortex (*1*), the striatum (*2*) in normal conditions and in rotenone-induced parkinsonism (*3, 4*), x 400. Large, scattered multipolar neurons with vesicular nuclei were visualized on tissue sections of the control rats (*a*). The development of the pathology was characterized by the presence of cells with a darker nucleus (*b*), wrinkled cells with signs of karyolysis (*c*), as well as glial cell infiltration (*d*)

The development of rotenone-induced parkinsonism in the rats was accompanied by activation of free radical-induced oxidation, as evidenced by an increase ($p < 0.05$) in CD concentration and OMP in the blood serum and brain of the animals (Fig. 2). At the same time, for the rats with experimental PD, an increase ($p < 0.05$) in α-tocopherol level in the tissues was also observed.

Our data indicated that a decrease in mRNA levels of genes encoding antioxidant enzymes and transcription factors Nrf2 and Foxo1 was observed in PD animals, which is in agreement with our earlier data on enzyme activity in experimental PD [10]. Thus, the results of this work demonstrated a decrease ($p < 0.01$) in the level of Nrf2 mRNA (*Nfe2l2* gene) in the cortex and striatum of the animals with pathology (Fig. 3). A similar trend was also observed for Foxo1 mRNA ($p < 0.05$). These changes in the PD rats were associated with decreased ($p < 0.05$) mRNA levels of superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione transferase genes in the striatum (*Sod1*, *Gpx1*, *Gsr*, *Gsta2* genes, respectively). The expression of the catalase gene (*Cat*) did not change significantly.



a *b* *c*

Fig. 2. The concentration of conjugated dienes (*a*), oxidized amino acid residues in proteins (b), and α-tocopherol (c) in the brain and blood serum of the control rats and animals with rotenone-induced parkinsonism, *Me* (Q_1–Q_3)

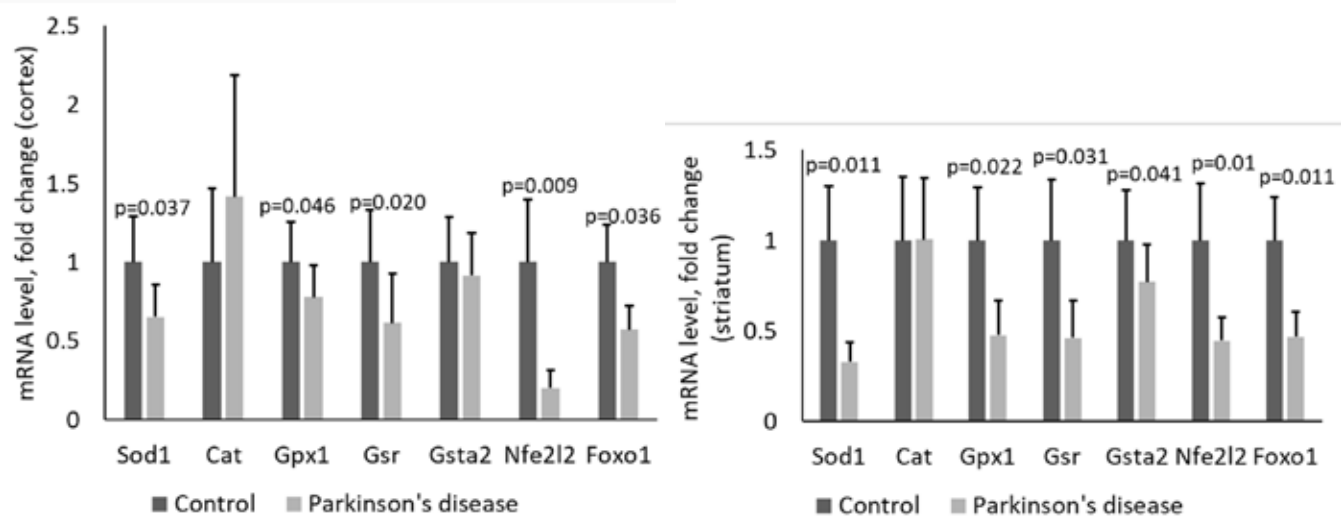


Fig. 3. The mRNA level of genes encoding antioxidant enzymes and regulatory factors in the cortex and striatum of the brain in the control rats and animals with rotenone-induced parkinsonism, $M \pm SD$

The study found that the activity of NADPH-generating enzymes G6PDH and NADP-IDH decreased ($p < 0.05$) in the serum and brain of the rats with experimental PD (Table 2). Similar changes were observed for the activity of these enzymes expressed in unit / mg protein. At the same time, the specific activity of G6PDH in the brains of the animals with pathology did not change significantly, which could be associated with a decrease in the total protein content ($p = 0.032$).

The correlation analysis confirmed the correlation between motor coordination parameters, oxidative status parameters, mRNA levels of the studied genes, and the activity of NADPH-generating enzymes in the rats with rotenone-induced parkinsonism (Table 3).

Table 2

Activity of NADPH-generating enzymes in the blood serum and brain of the rats with rotenone-induced parkinsonism, *Me* (Q_1–Q_3)

Parameter	Tissue	Group		*p*
		Controls ($n = 12$)	Rats with PD ($n = 12$)	
NADP-IDH, unit / ml, unit / g of wet weight	Blood serum	0.024 (0.017–0.071)	0.011 (0.010–0.023)	0.009
	Brain	1.005 (0.352–1.378)	0.451 (0.268–0.550)	0.037
NADP-IDH, unit /mg of protein	Blood serum	0.000270 (0.000194–0.000673)	0.000126 (0.000096–0.000253)	0.01
	Brain	0.010078 (0.008777–0.014880)	0.005692 (0.004279–0.007018)	0.041
G6PDH, unit / ml, unit / g of wet weight	Blood serum	0.034 (0.021–0.046)	0.013 (0.012–0.019)	0.004
	Brain	0.450 (0.239–0.518)	0.390 (0.273–0.398)	0.036
G6PDH, unit / mg of protein	Blood serum	0.000414 (0.000309–0.000575)	0.000281 (0.000249–0.000349)	0.031
	Brain	0.011762 (0.006048–0.012862)	0.007308 (0.002237–0.018642)	0.594
Total protein, g / l	Blood serum	76.5 (53.8–107.6)	58.0 (39.1–61.6)	0.043
	Brain	18.2 (13.2–25.1)	13.4 (8.8–16.9)	0.032

Table 3

Correlations between the studied parameters in the animals with rotenone-induced parkinsonism

Parameters	*r*	*p*
Grip strength – specific serum G6PDH activity	0.673	0.023
Level of oxidative modification of proteins in the brain – *Gsta2* mRNA level in the striatum	–0.848	0.049
NADP-IDH activity in the brain – *Gpx1* mRNA level in the striatum	0.877	0.022
NADP-IDH activity in the brain – *Foxo1* mRNA level in the striatum	0.825	0.043
Concentration of α-tocopherol in the serum – time of paper removal	0.916	0.029
Serum α-tocopherol concentration – NADP-IDH activity in the brain	–0.709	0.049

DISCUSSION

PD is a common neurodegenerative disease whose pathogenesis is closely related to oxidative stress and characterized by a late onset of clinical symptoms. Therefore, it is of interest to investigate the regulatory aspects of redox homeostasis in experimental PD. In this study, we evaluated the transcriptional regulation of the antioxidant system and the activity of NADPH-supplying enzymes for the glutathione-related antioxidant system in the rats with rotenone-induced parkinsonism.

The results of the study showed that the animals with experimental PD were characterized by increased levels of free radical-induced oxidation products, in particular lipid peroxidation (LPO) products – CDs and oxidized amino acid residues in proteins. ROS-induced protein oxidation plays a special role in the pathogenesis of PD. Oxidative stress induces glycol oxidation reactions and modification of free amino groups in proteins, which leads to formation of advanced glycation end products. These products provide cross-linking of protein molecules, which contributes to transformation of neurofilament proteins into insoluble aggregates, the presence of which in neurons is a key feature in the PD pathogenesis [17].

The antioxidant system provides protection against oxidative stress and includes enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione transferase. *Sod1* encodes a Cu, Zn-superoxide dismutase isoenzyme, predominantly localized in the cytoplasm, which is known to maintain resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, the toxin that causes experimental PD [18]. *Gpx1* is the gene for the glutathione peroxidase-1 isoenzyme, the most abundant glutathione peroxidase in mammalian tissues [19].

Gsta2 encodes an enzyme from the glutathione transferase superfamily that provides detoxification of electrophiles, carcinogens, and drugs. Alpha-class of glutathione transferases are the most versatile enzymes, among which Gsta2 is one of the key ARE-controlled proteins protecting against oxidative stress [20]. Our studies demonstrated decreased mRNA levels of these enzyme-related genes in the striatum and cerebral cortex of the pathological animals. The findings are consistent with previous results indicating impaired functioning of antioxidant system enzymes in experimental PD [10].

The negative correlation between the level of *Gsta2* mRNA in the striatum and the content of oxidatively modified proteins in the brain confirms the significant role of reduced expression of antioxidant system genes in the development of oxidative stress in parkinsonism. Apparently, the observed changes were due to decreased mRNA levels of Nrf2 and Foxo1 factors, which are key regulators of genes contributing to cellular resistance to oxidative stress. Thus, Nrf2 is known to be a crucial activator of expression of genes encoding antioxidant enzymes and NADPH-supplying enzymes. Nrf2 also modulates mitochondrial function and reduces the intensity of inflammation in neurodegeneration [21, 22]. Foxo transcription factors, including Foxo1, play an important role in a number of physiological processes, such as the regulation of metabolism, cell cycle, and responses to stressors, including excessive generation of ROS. There is evidence that inhibition of the PI3K-AKT-Foxo signaling pathway in rats with PD exacerbates oxidative stress in nigral dopaminergic neurons [23]. At the same time, we did not show a significant decrease in mRNA levels for the *Cat* gene as well as *Gsta2* in the cerebral cortex of the rats with the pathology. Apparently, at the endpoint of the experiment, the mRNA levels of these genes were still fairly stable and did not progress to the stage of decompensation.

We also showed that the rats with rotenone-induced parkinsonism are characterized by decreased activity of the brain and serum NADPH-generating enzymes G6PDH and NADP-IDH. The decreased efficiency of NADPH generation also seems to contribute significantly to the dysfunction of antioxidant enzymes and the development of oxidative stress in PD. Thus, there are data showing a significant role of these enzymes in cellular resistance to overproduced ROS [24, 25]. Moreover, it was shown that increased G6PDH activity in transgenic mice caused their lower susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and inhibited the development of PD signs [26]. The positive correlation that we showed between the grip strength of the PD animals and the specific activity of G6PDH in the blood serum confirms the role of reduced activity of NADPH-generating enzymes in the development of coordination disorders in parkinsonism. In addition, the positive correlation between NADP-IDH activity in the brain and mRNA levels of *Gpx1* and *Foxo1* indicates a significant mutual influence of NADPH-generating enzymes and the transcriptional activity of antioxidant genes.

Alpha-tocopherol belongs to the non-enzymatic antioxidants capable of effectively normalizing the oxidative status in cells by preventing LPO and stabilizing the membrane structure. The α-tocopherol transporter protein (αTTP) is the main regulator of α-tocopherol distribution in the body [27]. In the brain, αTTP regulates apolipoprotein E-mediated transport of the vitamin from astrocytes to the adjacent neurons. The expression of αTTP increases in astrocytes under oxidative stress, which facilitates the distribution of α-tocopherol to neurons, thereby protecting them from oxidative damage [28]. Our results showed that the rats with parkinsonism were characterized by an increase in the concentration of α-tocopherol in the blood serum and brain along with intensification of free radical-induced oxidation.

This trend was observed along with depressed activity of antioxidant enzymes [10] and their gene transcription, indicating the development of an imbalance in the functioning of the antioxidant system in PD. This is supported by the presence of a positive correlation between the serum α-tocopherol concentration and the time for the rats to remove the paper, as well as the activity of NADP-IDH in the brain of the animals with experimental PD.

CONCLUSION

Our study demonstrated that the development of rotenone-induced parkinsonism was associated with a decrease in mRNA levels of most of the studied antioxidant enzyme genes, which seems to be correlated with a decrease in the activity of Nrf2 and Foxo1 genes. These changes appeared to underlie a decrease in the activity of antioxidant enzymes, an increase in the intensity of free radical-induced oxidation and, as a consequence, the development of oxidative stress in PD. A decrease in the activity of NADPH-generating enzymes G6PDH and NADP-IDH, which are essential for the functioning of the antioxidant system, appeared to be another pathogenetic mechanism

of the impaired oxidative status in the tissues of the pathological animals. At the same time, in the rats with rotenone-induced parkinsonism, an increase in the serum and brain α-tocopherol concentration was observed, which could be the result of an imbalance in the functioning of the antioxidant system under oxidative stress.

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Authors' contribution

Kryl'skii E.D. – conception and design, analysis and interpretation of the data, justification of the manuscript. Razuvaev G.A. – acquisition of the data, analysis and interpretation of the data, justification of the manuscript. Popova T.N. – justification of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Nikhaev L.E., Akinina A.I. – acquisition of the data, analysis and interpretation of the data.

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