EXPERIMENTAL STUDIES

THE NEUROPROTECTIVE EFFECT OF ISATIN IN THE ROTENONE-INDUCED MODEL OF PARKINONISM IN RATS: THE STUDY OF DELAYED EFFECTS

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Parkinsonism in rats induced by the pesticide rotenone is one of the most adequate models of Parkinson's disease (PD). Isatin (indole-2,3-dione) is an endogenous regulator found in mammals and humans and exhibiting a wide range of biological activities mediated by numerous isatin-binding proteins, including those associated with neurodegenerative pathology. A course of rotenone administration to rats caused behavioral impairments and changes in the profile and relative content of isatin-binding proteins in the brain. In this study, we have investigated the delayed neuroprotective effect of isatin (5 days after completion of the course of rotenone administration) on behavioral reactions and the relative content of isatin-binding proteins in the brain of rats with rotenone-induced experimental parkinsonism. Although during this period the rats retained locomotor dysfunction, the proteomic analysis data (profile of isatin-binding proteins in the brain and changes in their relative content) differed from the results obtained immediately after completion of the course of rotenone administration. Moreover, all isatin-binding proteins with altered relative content changed during this period are associated to varying degrees with neurodegeneration (many with Parkinson's and Alzheimer's diseases).

Key words: isatin; isatin-binding proteins in the brain; parkinsonism; rotenone; neurodegeneration; neuroprotectors; proteomic profiling

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INTRODUCTION

Experimental parkinsonism induced in rats by the administration of the pesticide rotenone is one of the most adequate models of Parkinson's disease (PD). As in the case of PD model induced by administering the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to mice, dopaminergic neurodegeneration and oligokinesia are observed in rotenone-induced parkinsonism in animals both at the early stages of development of parkinsonian syndrome and in the delayed period [1–6]. Isatin (indole-2,3-dione) is an endogenous regulator found in the body of mammals and humans and exhibiting a wide range of biological activities mediated by numerous isatin-binding proteins [7–10], including those associated with neurodegenerative pathology [9, 11]. As in the case of MPTP-induced parkinsonism [12, 13], in the rotenone model of PD, isatin exerted a neuroprotective effect, reducing the signs of oligokinesia and postural instability. However, in contrast to the MPTP-induced parkinsonism model, in the case of the rotenone model the profile of isatin-binding proteins remained basically unchanged [5]. Nevertheless, treatment of rats with rotenone affected the relative content of isatin-binding proteins both immediately after the end of the course of this neurotoxin and 5 days after the last administration [6, 14].

In this study we have investigated the delayed effect of the neuroprotector isatin on the severity of rotenone-induced physiological reactions and quantitative changes in the proteomic profile of isatin-binding proteins in the rat brain.

MATERIALS AND METHODS

Reagents

The following reagents were used in the work: Tris (hydroxymethyl) aminomethane, ammonium bicarbonate, dithiothreitol, urea, guanidine hydrochloride, sodium chloride, Triton X-100, 4-vinylpyridine, Coomassie brilliant blue G-250 (Merck, USA); formic acid, sodium hydroxide (Acros Organics, USA), acetonitrile (Fisher Chemical, UK); isopropanol, trifluoroacetic acid (Fluka, USA); tris-(2-carboxyethyl)-phosphine (Pierce, USA); modified trypsin (mass spectrometry grade, Promega, USA). Other reagents of the highest purity available were from local suppliers.

Experimental Animals

The study was performed on outbred albino rats obtained from the Stolbovaya nursery (the Branch of the Scientific Center for Biomedical Technologies, Russia). The animals were kept under standard vivarium conditions with free access to food and water under a twelve-hour light regime.

Modeling of Experimental Parkinsonism in Rats

Modeling of PD by treating rats with systemic administration of rotenone was carried out in accordance with [15]. The animals were randomly divided into four groups (Table 1):

1) the Control group (10 rats); the animals were treated for 7 days with daily intraperitoneal injections of an equivalent volume of saline (0.2 ml per 100 g of body weight);

2) the Rotenone group (12 rats); the animals were treated for 7 days with daily intraperitoneal injections of rotenone (2.75 mg/kg);

3) the Rotenone+Isatin group (9 rats); the animals were treated for 7 days with daily intraperitoneal injections of rotenone (2.75 mg/kg) and once, on day 7, with isatin (100 mg/kg subcutaneously);

4) the Isatin group (9 rats); the animals were treated for 7 days with daily intraperitoneal injection of saline and once, on day 7, with isatin (100 mg/kg subcutaneously).

Rotenone was injected in a solution in the neutral triglyceride miglyol (Miglyol 840). The solution was prepared as described previously [5]. After the behavioral tests performed on day 12 of the experiment (5 days after the last administration of rotenone), the animals were decapitated under light ether anesthesia and lysates of brain homogenates and samples for mass spectrometric analysis were prepared.

Behavioral Tests

Oligokinesia was assessed in the Open Field and Rotating Rod tests on day 12 of the experiment, 5 days after the last administration of the neurotoxin, as described in detail previously [5].

Statistical data processing was performed using the Statistica v. 10.0 program [5]. The results in the tables are presented as mean ± SEM. Differences between groups were considered significant at $p<0.05$. Differences at $0.05<p<0.1$ were considered a statistical trend.

Sample Preparation for Mass Spectrometric Analysis

Preparation of brain homogenate lysates and sample preparation for mass spectrometric analysis (protein extraction, alkylation and trypsinolysis) were described in detail previously [6, 14].

Mass Spectrometric Analysis

The mass spectrometric analysis was carried out using the equipment of the Center of Collective Use "Human Proteome" (IBMC) – the Ultimate 3000 RSLCnano highly efficient liquid-liquid separation system for peptides (Thermo Scientific, USA) operated in the nanoflow mode of the Q-Exactive HFX mass spectrometric detector (Thermo Scientific) as described previously [5]. Bioinformatics data processing was carried out according to [5]. Each protein presented in the tables was identified in at least three independent experiments.

RESULTS AND DISCUSSION

The Delayed Effect of Isatin on Locomotor Impairments in Rats Induced by the Neurotoxin Rotenone

Our previous experiments with systemic administration of rotenone showed that the advanced stage of PD appeared in rats treated with daily injections of rotenone on day seven and was characterized by a significant weight loss, oligokinesia, rigidity, and postural instability. The corrective effect of isatin, administered once subcutaneously at a dose of 100 mg/kg at the very end of the course treatment with rotenone, was manifested in a decrease in postural instability and a decrease in oligokinesia, recorded by the parameters of locomotor activity in the Open Field and Rotating Rod tests [16]. In this study, a decrease in the parameters of locomotor activity and coordination of animal movements relative to the control group was also observed 5 days after the last administration of rotenone. For example, in the Rotenone group, the number of movements in the Open Field test was 47.3% of the control, and the number of stands was 58.8%. The retention time on the rotating rod in the animals of the Rotenone group was 49.0% of that in the animals of the Control group (Table 2). The corrective effect of isatin, recorded in the Open Field and Rotating Rod tests, also persisted on day 12 of the experiment. The corresponding parameters in the Rotenone+Isatin group, compared with the Control group, were 67.1% in the case of the number of movements, 75.3% in the case of the number of stands, and 73.6% in retention time during the Rotating Rod experiment (Table 2).

Table 1. Modeling of rotenone-induced parkinsonism in rats (scheme of experiment)

Group of animals	Days of experiment	Saline	Rotenone 2.75 mg/kg, intraperitoneally	Isatin 100 mg/kg, subcutaneously
Control	1–7	+		
Rotenone	1–7		+	
Rotenone+Isatin	7			+
Rotenone+Isatin	1–7		+	
Isatin	7			+
Isatin	1–7	+		

Table 2. The delayed effect of isatin on locomotor activity and coordinated movement of rats with rotenone-induced Parkinsonism (on day 5 after the end of course treatment with rotenone)

Group of animals	Number of animals	Locomotor activity, units		Duration of retention of animals on a rotating rod, s
		Horizontal activity (number of movements)	Vertical activity (number of stands)	
Control	10	14.6±2.4	9.7±2.2	177.8±2.3
Rotenone	12	6.9±0.9**	5.7±0.5	87.2±12.4***
Rotenone+Isatin	9	9.8±1.7	7.3±2.1	130.9±17.5*#
Isatin	9	12.1±2.9	7.3±1.6	—

Data represent mean ± SEM; *, **, *** – $p<0.05$, $p<0.01$, $p<0.001$, as compared with the group Control; # – $p<0.05$, as compared with the group Rotenone.

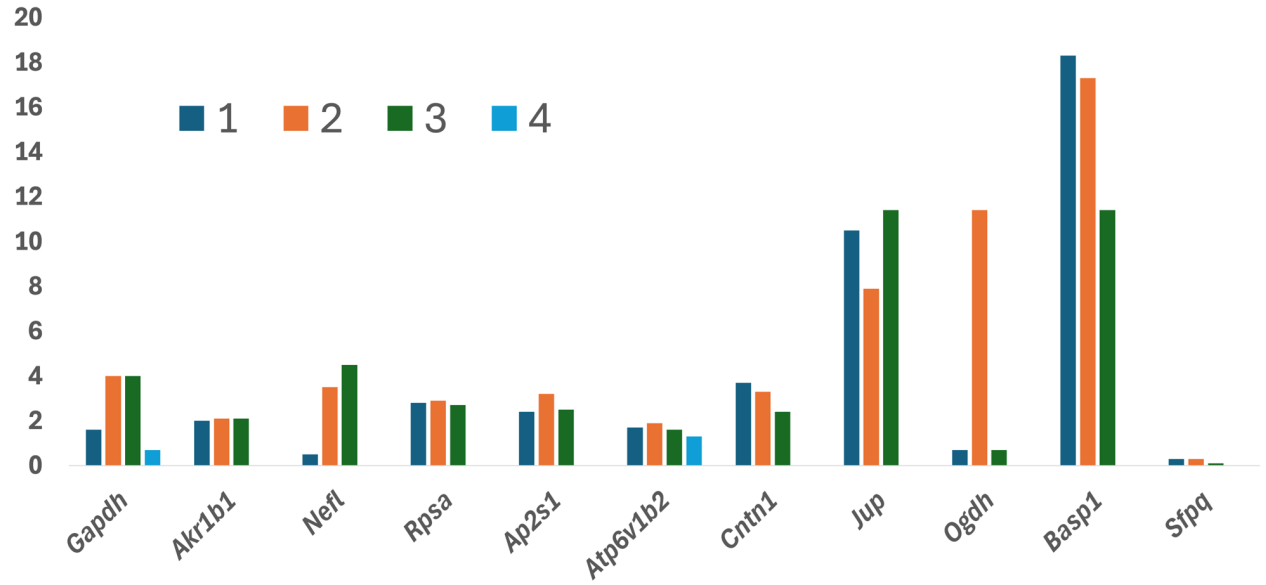

Figure 1. Fold change in the relative content of brain isatin-binding proteins of rats treated with rotenone and isatin. Gene names are shown. Uniprot protein names and p-values for each measurement are given in Table 3. Experimental groups: *1,4* – Rotenone, *2* – Rotenone+Isatin, *3* – Isatin; *1-3* – day 7 of the experiment; *4* – day 12 of the experiment.

The Delayed effect of Isatin on the Proteomic Profile of Rat Brain Isatin-Binding Proteins

Comparative proteomic identification of the relative content of brain proteins in control rats and rats with rotenone-induced PD showed a change in the level of 11 isatin-binding proteins of the brain immediately after the end of the course treatment with rotenone [14]. In this case, the neuroprotective effect of isatin in rats with rotenone-induced PD (see Table 3 and Fig. 1) was the most pronounced in the case of 2-oxoglutarate dehydrogenase (the E1 component of the multienzyme mitochondrial complex). The relative content of 2-oxoglutarate dehydrogenase slightly decreased in rotenone-treated rats (0.7 of the control), while administration of isatin to rotenone-treated rats sharply (11.4-fold) increased this parameter. It is known that 2-oxoglutarate dehydrogenase complex plays a certain role in energy metabolism disorders characteristic of neurodegenerative diseases. Decreased 2-oxoglutarate dehydrogenase activity correlates with metabolic disorders in the brain in Parkinson's and Alzheimer's diseases. 2-Oxoglutarate dehydrogenase is inactivated by the MPTP toxin, which is used to model PD in animals, and other toxins that cause oxidative stress [28–31]. In addition, it has been shown that the 2-oxoglutarate dehydrogenase complex also plays a role in hereditary predisposition to PD. The frequency of occurrence of the genotype carrying an allele with a single nucleotide substitution in the gene encoding dihydrolipoamide succinyltransferase (the E2 component of this multienzyme complex) is significantly higher in PD patients compared to the control group [35].

Five days after the course treatment with rotenone changes in the relative content of 16 proteins were observed as compared to the control. In this case, only two proteins (glyceraldehyde-3-phosphate dehydrogenase and subunit B of V-type proton ATPase) were detected; their relative content also changed immediately after the end of the course treatment with rotenone ([6] and Figs 1 and 2A).

Table 3. The effect of isatin administration on the relative content of brain isatin-binding proteins in rats with rotenone-induced PD

No	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Difference from control (Rotenone)		Difference from control (Rotenone+Isatin)		Difference from control (Isatin)		References
						Fold change	-Log(P-value)	Fold change	-Log(P-value)	Fold change	-Log(P-value)	
1	P04797	*Gapdh*	Glyceraldehyde-3-phosphate dehydrogenase	1	C, N	1.60	2.4	4.00	3.8	4.00	3.6	[17, 18]
2	P07943	*Akr1b1*	Aldo-keto reductase family 1 member B1	7	C	2.00	3.4	2.10	3.6	2.10	3.5	[19]
3	P19527	*Nefl*	Neurofilament light polypeptide	2	C, S	0.50	3.1	3.50	2.6	4.50	3.0	[20]
4	P38983	*Rpsa*	40S ribosomal protein SA	5	C, N, PM	2.80	4.0	2.90	3.3	2.70	3.1	[21]
5	P62744	*Ap2s1*	AP-2 complex subunit sigma	2	M, PM	2.40	3.4	3.20	3.2	2.50	2.6	[22, 23]
6	P62815	*Atp6v1b2*	V-type proton ATPase subunit B, brain isoform	2	M, PM, Ve, S	1.76	4.4	1.90	3.7	1.60	3.3	[24]
7	Q63198	*Cntn1*	Contactin-1	3	PM	3.70	3.0	3.30	3.0	2.40	1.8	[25]
8	Q6P0K8	*Jup*	Junction plakoglobin	2	C, M	10.50	3.0	7.90	2.2	11.40	2.4	[26, 27]
9	A0A8I6A1Y1	*Ogdh*	Oxoglutarate dehydrogenase (succinyl-transferring)	1	Mch, N	0.70	3.4	11.40	3.2	0.70	3.0	[28–31]
10	A0A8I6A304	*Basp1*	Brain abundant, membrane attached signal protein 1	3	C, N	18.30	5.1	17.30	3.7	11.40	4.6	[32]
11	A0A8I6A7U6	*Sfpq*	Splicing factor proline and glutamine rich	5	C, N	0.30	4.9	0.30	4.0	0.10	2.2	[33, 34]

Here and in Tables 4 and 5 proteins names are the same as in the Uniprot database. Number in the column "Function" designate the following functional groups of proteins: *1*. Proteins/enzymes involved in energy generation and carbohydrate metabolism. *2*. Proteins involved in cytoskeleton formation and exocytosis. *3*. Proteins involved in signal transduction and regulation of enzyme activity. *4*. Antioxidant and protective proteins/enzymes. *5*. Protein regulators of gene expression, cell division, and differentiation. *6*. Enzymes involved in metabolism of proteins, amino acids, and other nitrogenous compounds. *7*. Enzymes involved in lipid metabolism. Localization: C – cytoplasm, N – nucleus, M – membranes, PM – plasma membrane, G – Golgi complex, Mch – mitochondria, L – lysosomes, Mic – microsomes, E – endosomes, Ve – vesicles.

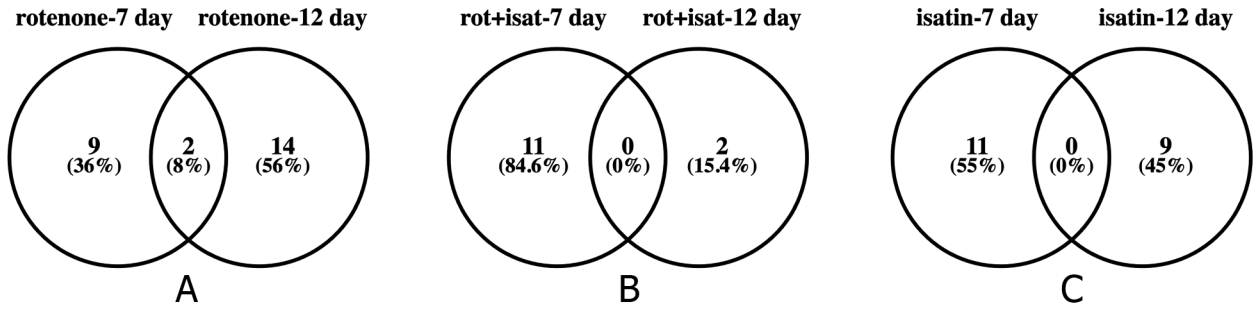

Figure 2. A Venn diagram. Isatin binding proteins with significantly altered relative content immediately after the course treatment with rotenone and 5 days later. Group of animals: **A** – Rotenone, **B** – Rotenone+Isatin, **C** – Isatin.

Table 4. The delayed effect of single isatin administration on the relative content of brain isatin-binding proteins in rats with rotenone-induced PD (5 days after the end of course treatment with rotenone)

No	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change	-Log(P-value)	References
1	Q2I6B2	*Atp6v0a1*	V-type proton ATPase subunit A	2	M, PM, Me, Ve	2.5	3.8	[36]
2	Q63654	*Ubc*	Polyubiquitin	6	C, N	1.9	3.0	[37]

Table 5. The effect of single dose of isatin administration on the relative content of brain isatin binding proteins in day 12 of the experiment

No	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change	-Log(P-value)	References
1	P62944	*Ap2b1*	AP-2 complex subunit beta	3	M, PM	1.8	2.8	[22, 23]
2	Q6XD99	*Sptbn1*	Spectrin beta chain	2	C, N	1.7	2.2	[38, 39]
3	G3V6P2	*Dlst*	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	1	Mch	1.6	2.1	[35]
4	Q5RJQ4	*Sirt2*	NAD-dependent protein deacetylase sirtuin-2	3	N, C	1.5	2.1	[40, 41]
5	P47819	*Gfap*	Glial fibrillary acidic protein	2	C	0.7	2.0	[42]
6	Q7M0E3	*Dstn*	Destrin	2	C	0.7	2.3	[43–46]
7	P09117	*Aldoc*	Fructose-bisphosphate aldolase C	1	C synaps	0.7	2.4	[47, 48]
8	P07335	*Ckb*	Creatine kinase B-type	1	C, Mch, PM	0.6	2.8	[49, 50]
9	P07323	*Eno2*	Gamma-enolase	1	C, PM	0.6	2.3	[51]

In the groups of rats Rotenone+Isatin and Isatin no common brain proteins with altered relative content immediately after the end of the course treatment with rotenone and 5 days later were detected (Tables 3–5 and Figs 2B and 2C). In the group Rotenone+Isatin only two proteins showed an increase in the relative content compared to the control on day 12. These are subunit A of V-type proton ATPase and polyubiquitin (Table 4). In the group Isatin, the delayed effect of this neuroprotector was manifested as the change in the relative content of 9 proteins compared to the control group. A slight decrease (to the level of 0.6–0.7) in the content of destrin (actin depolymerization factor), glial fibrillary acidic protein, creatine kinase, as well as multifunctional glycolysis proteins gamma-enolase and fructose-bisphosphate aldolase was noted. At the same time, there was a slight increase in the level of the beta subunit of the AP-2 complex, the beta subunit of spectrin, the E2 component of the 2-oxoglutarate dehydrogenase complex, as well as the NAD-dependent deacetylase sirtuin 2 (Table 5). It is interesting to note that

all isatin-binding proteins detected in the delayed effect, that change their relative content, are associated to a certain degree with neurodegeneration, many with Parkinson's and Alzheimer's diseases [22, 23, 35, 38–51].

In the context of the so-called toxic models of PD induced by neurotoxins with different mechanisms of action (MPTP and rotenone), it should be noted that the protective effect of isatin is apparently realized in different ways. In the case of the MPTP-induced PD model in mice, the effect of isatin is primarily due to the inhibition of the activity of monoamine oxidase B, an enzyme responsible for the bioactivation of the MPTP protoxin into the neurotoxin MPP^+ (methylphenyl pyridinium ion) [52]. The effect on numerous isatin-binding proteins apparently favors the realization of various protective reactions, particularly, by acting on the ubiquitin-proteasome system, which is involved, in addition to the elimination of damaged and incorrectly assembled proteins, in the regulation of a wide variety of cellular processes, including genome stability, immune response, signal transduction, and much more [37, 53]. During proteomic profiling of isatin-binding proteins in the brain, we have identified enzymes that are directly related to the ubiquitin-proteasome system: E3 ubiquitin protein ligase MYCBP2, ubiquitin-carboxyl-terminal hydrolase 24, E3 ubiquitin protein ligase MIB2, E3 ubiquitin protein ligase HUWE1, ubiquitin conjugating enzyme variant 1, polyubiquitin [54]. The neurotoxin MPTP and the neuroprotector isatin also influenced the mitochondrial subproteomes of proteins interacting with the components of the Rpn10 and Rpn13 regulatory subunits of the proteasome [52, 55].

In the case of the rotenone model of PD in rats, the action of isatin is obviously realized according to a different scenario and through the involvement of other isatin-binding proteins. On the one hand, this may be due to interspecies characteristics (the effects of isatin in mice and rats do not always coincide [9]), on the other hand, due to the obvious “wave-like” change in the level of target proteins (isatin-binding proteins). They change differently in the dynamics of PD development both under the influence of the neurotoxin and isatin itself, which is known to affect the relative level of many important brain proteins [56].

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COMPLIANCE WITH ETHICAL STANDARDS

The experiments were carried out in compliance with generally accepted norms of humane treatment of laboratory animals. The work was carried out in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 1, 2016 “On Approval of the Rules for Good Laboratory Practice” and the Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010, on the protection of animals used for scientific purposes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. *Duty S., Jenner P.* (2011) Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. Br. J. Pharmacol., **164**(4), 1357–1391. DOI: 10.1111/j.1476-5381.2011.01426.x
2. *Fleming S.M., Zhu C., Fernagut P.O., Mehta A., Dicarlo C.D., Seaman R.L., Chesselet M.F.* (2004) Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Exp. Neurol., **187**(2), 418–429. DOI: 10.1016/j.expneurol.2004.01.023
3. *Fleming S.M., Salcedo J., Fernagut P.O., Rockenstein E., Masliah E., Levine M.S., Chesselet M.F.* (2004) Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. J. Neurosci., **24**(42), 9434–9440. DOI: 10.1523/JNEUROSCI.3080-04.2004
4. *Cannon J.R., Tapias V.M., Na H.M., Honick A.S., Drolet R.E., Greenamyre J.T.* (2009) A highly reproducible rotenone model of Parkinson’s disease. Neurobiol. Dis., **34**(2), 279–290. DOI: 10.1016/j.nbd.2009.01.016
5. *Kapitsa I.G., Kazieva L.S., Vavilov N.E., Zgoda V.G., Kopylov A.T., Medvedev A.E., Buneeva O.A.* (2023) Characteristics of behavioral reactions and the profile of brain isatin-binding proteins of rats with the rotenone-induced experimental parkinsonism. Biomeditsinskaya Khimiya, **69**(1), 46–54. DOI: 10.18097/PBMC20236901046
6. *Buneeva O.A., Kapitsa I.G., Kazieva L.S., Vavilov N.E., Zgoda V.G., Medvedev A.E.* (2024) The delayed effect of rotenone on the relative content of brain isatin-binding proteins of rats with experimental parkinsonism. Biomeditsinskaya Khimiya, **70**(1), 25–32. DOI: 10.18097/PBMC20247001025
7. *Medvedev A., Igosheva N., Crumeyrolle-Arias M., Glover V.* (2005) Isatin: Role in stress and anxiety. Stress, **8**(3), 175–183. DOI: 10.1080/10253890500342321
8. *Medvedev A., Buneeva O., Glover V.* (2007) Biological targets for isatin and its analogues: Implications for therapy. Biologics, **1**(2), 151–162.
9. *Medvedev A., Buneeva O., Gnedenko O., Ershov P., Ivanov A.* (2018) Isatin, an endogenous non-peptide biofactor: A review of its molecular targets, mechanisms of actions and their biomedical implications. Biofactors, **44**, 95–108. DOI: 10.1002/biof.1408

10. *Medvedev A., Buneeva O.* (2022) Tryptophan metabolites as mediators of microbiota-gut-brain communication: Focus on isatin. Front. Behav. Neurosci., **16**, 922274. DOI: 10.3389/fnbeh.2022.922274
11. *Buneeva O., Gnedenko O., Zgoda V., Kopylov A., Glover V., Ivanov A., Medvedev A., Archakov A.* (2010) Isatin binding proteins of rat and mouse brain: Proteomic identification and optical biosensor validation. Proteomics, **10**, 23–37. DOI: 10.1002/pmic.200900492
12. *Buneeva O., Kopylov A., Kapitsa I., Ivanova E., Zgoda V., Medvedev A.* (2018) The effect of neurotoxin MPTP and neuroprotector isatin on the profile of ubiquitinated brain mitochondrial proteins. Cells, **7**(8), 91. DOI: 10.3390/cells7080091
13. *Buneeva O.A., Kapitsa I.G., Ivanova E.A., Kopylov A.T., Zgoda V.G., Medvedev A.E.* (2019) The effect of a neuroprotective dose of isatin or deprenyl to mice on the profile of brain isatin-binding proteins. Biomeditsinskaya Khimiya, **65**(5), 407–417. DOI: 10.18097/PBMC20196505407
14. *Buneeva O.A., Kapitsa I.G., Kazieva L.S., Vavilov N.E., Zgoda V.G., Medvedev A.E.* (2023) Quantitative changes of brain isatin-binding proteins of rats with the rotenone-induced experimental parkinsonism. Biomeditsinskaya Khimiya, **69**(3), 188–192. DOI: 10.18097/PBMC20236903188
15. *Voronina T.A., Seredenin S.B., Yarkova M.A., Voronin M.V.* (2012) Rukovodstvo po provedeniyu doklinicheskih issledovanij lekarstvennyh sredstv, chast' pervaya (Mironov A.N. (ed.)), Grif i K, Moskva, 994 p.
16. *Buneeva O.A., Kapitsa I.G., Zgoda V.G., Medvedev A.E.* (2023) Neuroprotective effects of isatin and afobazole in rats with rotenone-induced parkinsonism are accompanied by increased brain levels of Triton X-100 soluble alpha-synuclein. Biomeditsinskaya Khimiya, **69**(5), 290–299. DOI: 10.18097/PBMC20236905290
17. *Barinova K., Khomyakova E., Semenyuk P., Schmalhausen E., Muronetz V.* (2018) Binding of alpha-synuclein to partially oxidized glyceraldehyde-3-phosphate dehydrogenase induces subsequent inactivation of the enzyme. Arch. Biochem. Biophys., **642**, 10–22. DOI: 10.1016/j.abb.2018.02.002
18. *Gerszon J., Rodacka A.* (2018) Oxidatively modified glyceraldehyde-3-phosphate dehydrogenase in neurodegenerative processes and the role of low molecular weight compounds in counteracting its aggregation and nuclear translocation. Ageing Res. Rev., **48**, 21–31. DOI: 10.1016/j.arr.2018.09.003
19. *Zhao Y., Zhang M., Li H., Yang Y., Lu X., Yu J., Pan L.* (2023) Aldo-keto reductase 1B: Much learned, much more to do. HLife, **2023**, DOI: 10.1016/j.hlife.2023.12.002
20. *Lewczuk P., Ermann N., Andreasson U., Schultheis C., Podhorna J., Spitzer P., Maler J.M., Kornhuber J., Blennow K., Zetterberg H.* (2018) Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. Alzheimers Res. Ther., **10**(1), 71. DOI: 10.1186/s13195-018-0404-9
21. *Song D.G., Kim Y.S., Jung B.C., Rhee K.J., Pan C.H.* (2013) Parkin induces upregulation of 40S ribosomal protein SA and posttranslational modification of cytokeratins 8 and 18 in human cervical cancer cells. Appl. Biochem. Biotechnol., **171**(7),1630–1638. DOI: 10.1007/s12010-013-0443-4
22. *Martinez V.J., Asico L.D., Jose P.A., Tiu A.C.* (2020) Lipid rafts and dopamine receptor signaling. Int. J. Mol. Sci., **21**(23), 8909. DOI: 10.3390/ijms21238909
23. *Zhang X., Zhou J.Y., Chin M.H., Schepmoes A.A., Petyuk V.A., Weitz K.K., Petritis B.O., Monroe M.E., Camp D.G., Wood S.A., Melega W.P., Bigelow D.J., Smith D.J., Qian W.J., Smith R.D.* (2010) Region-specific protein abundance changes in the brain of MPTP-induced Parkinson's disease mouse model. J. Proteome Res., **9**(3), 1496–1509. DOI: 10.1021/pr901024z AP2
24. *Song Q., Meng B., Xu H., Mao Z.* (2020) The emerging roles of vacuolar-type ATPase-dependent lysosomal acidification in neurodegenerative diseases. Transl. Neurodegener., **9**(1), 17. DOI: 10.1186/s40035-020-00196-0
25. *Chatterjee M., van Steenoven I., Huisman E., Oosterveld L., Berendse H., van der Flier W.M., del Campo M., Lemstra A.W., van de Berg W.D.J., Teunissen C.E.* (2020) Contactin-1 is reduced in cerebrospinal fluid of Parkinson's disease patients and is present within Lewy bodies. Biomolecules, **10**(8), 1177. DOI: 10.3390/biom10081177
26. *Wang H., Sun C., Liang Y., Zhang H., Tan Y.* (2013) Identification of regulatory relationships in Parkinson's disease. J. Mol. Neurosci., **51**(1), 9–12. DOI: 10.1007/s12031-012-9937-8
27. *Stocker A.M., Chenn A.* (2015) The role of adherens junctions in the developing neocortex. Cell Adh. Migr., **9**(3), 167–174. DOI: 10.1080/19336918.2015.1027478
28. *Tretter L., Adam-Vizi V.* (2000) Inhibition of Krebs cycle enzymes by hydrogen peroxide: A key role of [alpha]-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J. Neurosci., **20**(24), 8972–8979. DOI: 10.1523/JNEUROSCI.20-24-08972.2000
29. *Gibson G.E., Xu H., Chen H.L., Chen W., Denton T.T., Zhang S.* (2015) Alpha-ketoglutarate dehydrogenase complex-dependent succinylation of proteins in neurons and neuronal cell lines. J. Neurochem., **134**(1), 86–96. DOI: 10.1111/jnc.13096
30. *Hansen G.E., Gibson G.E.* (2022) The α-ketoglutarate dehydrogenase complex as a hub of plasticity in neurodegeneration and regeneration. Int. J. Mol. Sci., **23**(20), 12403. DOI: 10.3390/ijms232012403
31. *Gibson G.E., Park L.C., Sheu K.F., Blass J.P., Calingasan N.Y.* (2000) The alpha-ketoglutarate dehydrogenase complex in neurodegeneration. Neurochem. Int., **36**(2), 97–112. DOI: 10.1016/s0197-0186(99)00114-x
32. *Chung D., Shum A., Caraveo G.* (2020) GAP-43 and BASP1 in axon regeneration: implications for the treatment of neurodegenerative diseases. Front. Cell Dev. Biol., **8**, 567537. DOI: 10.3389/fcell.2020.567537
33. *Lim Y.W., James D., Huang J., Lee M.* (2020) The emerging role of the RNA-binding protein SFPQ in neuronal function and neurodegeneration. Int. J. Mol. Sci., **21**(19), 7151. DOI: 10.3390/ijms21197151
34. *Hogan A.L., Grima N., Fifita J.A., McCann E.P., Heng B., Fat S.C.M., Wu S., Maharjan R., Cain A.K., Henden L., Rayner S., Tarr I., Zhang K.Y., Zhao Q., Zhang Z.H., Wright A., Lee A., Morsch M., Yang S., Williams K.L., Blair I.P.* (2021) Splicing factor proline and glutamine rich intron retention, reduced expression and aggregate formation are pathological features of amyotrophic lateral sclerosis. Neuropathol. Appl. Neurobiol., **47**(7), 990–1003. DOI: 10.1111/nan.12749
35. *Kobayashi T., Matsumine H., Matuda S., Mizuno Y.* (1998) Association between the gene encoding the E2 subunit of the alpha-ketoglutarate dehydrogenase complex and Parkinson's disease. Ann. Neurol., **43**(1), 120–123. DOI: 10.1002/ana.410430121

36. *Collins M.P., Forgac M.* (2020) Regulation and function of V-ATPases in physiology and disease. Biochim. Biophys. Acta Biomembr., **1862**(12), 183341. DOI: 10.1016/j.bbamem.2020.183341
37. *Buneeva O., Medvedev A.* (2022) Atypical ubiquitination and Parkinson's disease. Int. J. Mol. Sci., **23**(7), 3705. DOI: 10.3390/ijms23073705
38. *Morrow J.S., Stankewich M.C.* (2021) The spread of spectrin in ataxia and neurodegenerative disease. J. Exp. Neurol., **2**(3), 131–139.
39. *Maor G., Dubreuil R.R., Feany M.B.* (2023) α-Synuclein promotes neuronal dysfunction and death by disrupting the binding of ankyrin to β-spectrin. J. Neurosci., **43**(9), 1614–1626. DOI: 10.1523/JNEUROSCI.1922-22.2022
40. *Liu Y., Zhang Y., Zhu K., Chi S., Wang C., Xie A.* (2020) Emerging role of sirtuin 2 in parkinson's disease. Front. Aging Neurosci., **11**, 372. DOI: 10.3389/fnagi.2019.00372
41. *Wu Q.J., Zhang T.N., Chen H.H., Yu X.F., Lv J.L., Liu Y.Y., Liu Y.S., Zheng G., Zhao J.Q., Wei Y.F., Guo J.Y., Liu F.H., Chang Q., Zhang Y.X., Liu C.G., Zhao Y.H.* (2022) The sirtuin family in health and disease. Signal Transduct. Target Ther., **7**(1),402. DOI: 10.1038/s41392-022-01257-8
42. *Tang Y., Han L., Li S., Hu T., Xu Z., Fan Y., Liang X., Yu H., Wu J., Wang J.* (2023) Plasma GFAP in Parkinson's disease with cognitive impairment and its potential to predict conversion to dementia. NPJ Parkinsons Dis., **9**(1), 23. DOI: 10.1038/s41531-023-00447-7
43. *Bernstein B.W., Bamburg J.R.* (2010) ADF/cofilin: A functional node in cell biology. Trends Cell Biol., **20**(4), 187–195. DOI: 10.1016/j.tcb.2010.01.001
44. *Minamide L.S., Striegl A.M., Boyle J.A., Meberg P.J., Bamburg J.R.* (2000) Neurodegenerative stimuli induce persistent ADF/cofilin-actin rods that disrupt distal neurite function. Nat. Cell Biol., **2**(9), 628–636. DOI: 10.1038/35023579
45. *Lapeña-Luzón T., Rodríguez L.R., Beltran-Beltran V., Benetó N., Pallardó F.V., Gonzalez-Cabo P.* (2021) Cofilin and neurodegeneration: New functions for an old but gold protein. Brain Sci., **11**(7), 954. DOI: 10.3390/brainsci11070954
46. *Ben Zablah Y., Merovitch N., Jia Z.* (2020) The role of ADF/cofilin in synaptic physiology and Alzheimer's disease. Front. Cell Dev. Biol., **8**, 594998. DOI: 10.3389/fcell.2020.594998
47. *Stefanizzi I., Cañete-Soler R.* (2007) Coregulation of light neurofilament mRNA by poly(A)-binding protein and aldolase C: Implications for neurodegeneration. Brain Res., **1139**, 15–28. DOI: 10.1016/j.brainres.2006.12.092
48. *Kingsbury A.E., Cooper M., Schapira A.H., Foster O.J.* (2001) Metabolic enzyme expression in dopaminergic neurons in Parkinson's disease: An *in situ* hybridization study. Ann. Neurol., **50**(2), 142–149. DOI: 10.1002/ana.1051
49. *Vassilopoulos D., Jockers-Wretou E.* (1987) Serum creatine kinase B levels in diseases of the central nervous system. Eur. Neurol., **27**(2), 78–81. DOI: 10.1159/000116135
50. *Xu J., Fu X., Pan M., Zhou X., Chen Z., Wang D., Zhang X., Chen Q., Li Y., Huang X., Liu G., Lu J., Liu Y., Hu Y., Pan S., Wang Q., Wang Q., Xu Y.* (2019) Mitochondrial creatine kinase is decreased in the serum of idiopathic Parkinson's disease patients. Aging Dis., **10**(3), 601–610. DOI: 10.14336/AD.2018.0615
51. *Katayama T., Sawada J., Takahashi K., Yahara O., Hasebe N.* (2021) Meta-analysis of cerebrospinal fluid neuron-specific enolase levels in Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Alzheimers Res. Ther., **13**(1), 163. DOI: 10.1186/s13195-021-00907-3
52. *Medvedev A.E., Buneeva O.A., Kopylov A.T., Tikhonova O.V., Medvedeva M.V., Nerobkova L.N., Kapitsa I.G., Zgoda V.G.* (2017) Brain mitochondrial subproteome of Rpn10-binding proteins and its changes induced by the neurotoxin MPTP and the neuroprotector isatin. Biochemistry (Moscow), **82**(3), 330–339. DOI: 10.1134/S0006297917030117
53. *Buneeva O.A., Medvedev A.E.* (2016) Atypical ubiquitination of proteins. Biomeditsinskaya Khimiya, **62**(5), 496–509. DOI: 10.18097/PBMC20166205496
54. *Buneeva O.A., Kopylov A.T., Tikhonova O.V., Zgoda V.G., Medvedev A.E., Archakov A.I.* (2012) Effect of affinity sorbent on proteomic profiling of isatin-binding proteins of mouse brain. Biochemistry (Moscow), **77**(11), 1326–1338. DOI: 10.1134/S0006297912110120
55. *Buneeva O.A., Kopylov A.T., Gnedenko O.V., Medvedeva M.V., Kapitsa I.G., Ivanova E.A., Ivanov A.S., Medvedev A.E.* (2021) Changes in the mitochondrial subproteome of mouse brain Rpn13-binding proteins induced by the neurotoxin MPTP and the neuroprotector isatin. Biomeditsinskaya Khimiya, **67**(1), 51–65. DOI: 10.18097/PBMC20216701051
56. *Medvedev A., Kopylov A., Buneeva O., Kurbatov L., Tikhonova O., Ivanov A., Zgoda V.* (2020) A neuroprotective dose of isatin causes multilevel changes involving the brain proteome: Prospects for further research. Int. J. Mol. Sci., **21**(11), 4187. DOI: 10.3390/ijms21114187

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НЕЙРОПРОТЕКТОРНОЕ ДЕЙСТВИЕ ИЗАТИНА В РОТЕНОНОВОЙ МОДЕЛИ ПАРКИНСОНИЗМА У КРЫС: ИССЛЕДОВАНИЕ ОТСРОЧЕННЫХ ЭФФЕКТОВ

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Вызванный введением пестицида ротенона паркинсонизм у крыс — одна из наиболее адекватных моделей болезни Паркинсона (БП). Изатин (индолдион-2,3) — эндогенный регулятор, обнаруженный в организме млекопитающих и человека и обладающий широким спектром биологической активности благодаря большому набору изатин-связывающих белков, в том числе ассоциированных с нейродегенеративной патологией. Курсовое введение ротенона вызывало у крыс нарушения поведенческих реакций и изменения профиля и относительного содержания изатин-связывающих белков мозга. В данной работе исследовали отсроченное нейропротекторное влияние изатина (через 5 дней после завершения курсового введения ротенона) на поведенческие реакции и относительное содержание изатин-связывающих белков мозга крыс с индуцированным ротеноном экспериментальным паркинсонизмом. Хотя в этот период времени у крыс сохранялись нарушения локомоторных функций, данные протеомного анализа (профиль изатин-связывающих белков мозга и изменения их относительного содержания) отличались от результатов, полученных сразу после завершения курсового введения ротенона. При этом все изатин-связывающие белки, относительное содержание которых изменялось в данный период времени, в той или иной степени ассоциированы с нейродегенерацией (многие — с болезнями Паркинсона и Альцгеймера).

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: изатин; изатин-связывающие белки мозга; паркинсонизм; ротенон; нейродегенерация; нейропротекторы; протеомное профилирование

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