CHARACTERISTICS OF BEHAVIORAL REACTIONS AND THE PROFILE OF BRAIN ISATIN-BINDING PROTEINS OF RATS WITH THE ROTENONE-INDUCED EXPERIMENTAL PARKINSONISM

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The neurotoxins rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are used for modeling Parkinson's disease in animals (PD). They induce the mitochondrial respiratory chain dysfunction, which leads to the dopaminergic (DA) neuron degeneration. The advantage of the rotenone model consists in ability of rotenone to cause neurodegeneration showing symptoms and molecular biological characteristics similar to those of PD. Isatin (indoldione-2,3) is an endogenous regulator found in tissues and biological fluids of humans and animals. It exhibits a broad range of biological activity mediated by numerous isatin-binding proteins. In this work we have investigated behavioral reactions and profiles of brain isatin-binding proteins of rats with Parkinson's syndrome (PS) in comparison with the corresponding parameters of MPTP-induced Parkinsonism in mice. Systemic injection of rotenone caused severe PS comparable with the effect of MPTP injection. It was accompanied by significant body weight loss, death, oligokinesia, muscular rigidity, and postural instability of animals. In spite of the same pathogenic basis of PS caused by rotenone and MPTP, the molecular mechanisms of their action differ. In the case of rotenone-induced PS, the pool of isatin-binding proteins common of the control rats and the rats with PS (146) significantly exceeded the pool of the common proteins of control mice and mice with PS induced by MPTP, whether right after neurotoxin injection (27), or (all the more) in a week after the MPTP injection (14). The comparison of isatin-binding proteins specific of the animals with MPTP-induced PS and with the rotenone-induced PS (as compared with the control animals) revealed total absence of proteins common of these two models of PD. It is to be noted that both neurotoxins particularly affected the proteins participating in the signal transmission and enzyme activity regulation. The changes of the profile of isatin-binding proteins in response to the injection of rotenone suggest that the neuroprotector isatin could also influence positively in the case of the rotenone model of PD.

Key words: parkinsonism; neurodegeneration; neurotoxin rotenone; neurotoxin MPTP; isatin; isatin-binding proteins; brain; proteomic profiling

DOI: 10.18097/PBMC20236901046

INTRODUCTION

Models of parkinsonian syndrome (PS) in rodents induced by the administration of neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone are currently used as the most adequate translational models of Parkinson's disease (PD) [1].

The pesticide rotenone simulates severe PS, characteristic of the advanced stage of PD. It is a highly lipophilic substance that can freely penetrate the blood-brain barrier (BBB) and biological membranes. The mechanism of action of rotenone, as well as the mechanism of action of MPTP, is associated with inhibition of the mitochondrial respiratory chain, which leads to the degeneration of dopaminergic neurons. The advantage of the rotenone model of PD is the ability of rotenone to provoke dopaminergic (DA) neurodegeneration, which is the most similar in its symptoms and molecular biological features to those in PD [1]. Rotenone-induced oligokinesia is well controlled with L-DOPA. For PD modeling, rotenone is used primarily on cellular structures and in embryological studies. In adult animals, mainly rats, rotenone is used much less frequently due to its low chemical stability in animal tissues and physiological body fluids. Rotenone is administered to animals by infusion, using micropumps, mainly intravenously. Subcutaneous and intraperitoneal injection methods of administration are also used [2]. In rats, systemic infusion of rotenone for a week caused bilateral damage of the striatum and globus pallidus, DA neurodegeneration in the substantia nigra (with a decrease in the number of neurons by 30%). At the behavioral level, the rotenone action resulted in a decrease in vertical activity of animals, hypokinesia, and even pronounced catalepsy [3]. A highly reproducible model of PS in rats may be achieved after the systemic administration of rotenone at a daily dose of 2-3 mg/kg during 7 days intraperitoneally in a specially prepared solution. Animals develop bradykinesia, postural instability and/or rigidity [4]. MPTP is a widely used toxin to model PD in rodents. Like rotenone, MPTP easily penetrates the BBB and acts on dopaminergic brain structures [5].

Isatin (indoldion-2,3) is an endogenous regulator found in tissues and biological fluids of humans and mammalian animals. The content of isatin in the body changes under conditions of various types of stress, as well as in a number of pathological conditions, including PD [6, 7]. A wide range of biological activity of isatin is realized by numerous isatin-binding proteins localized in various intracellular compartments of the brain and peripheral tissues. According to the data of proteomic profiling of the brain, a number of proteins that bind to isatin play an important role in the development of neurodegenerative pathology [8-12].

At the same time, the physiological reactions and profiles of isatin-binding proteins in the brain of mice with MPTP-induced PS at the height of the development of motor disorders (after 90 min) and seven days after the administration of this neurotoxin differ [13]. Seven days after the introduction of MPTP, the motor activity of the animals improved, but did not reach the level of control animals. At the height of motor disturbances caused by MPTP administration, the number of isatin-binding proteins significantly decreased, and increased again to the control level seven days after MPTP administration [13].

The aim of this work was to study the features of behavioral reactions and the profile of brain isatin-binding proteins in rats with rotenone-induced experimental parkinsonism and compare them with the corresponding parameters of MPTP-induced parkinsonism in mice.

MATERIALS AND METHODS

Reagents

The following reagents were used in the study: cyanogen bromide-activated sepharose 4B, isatin, dithiothreitol, Tris (hydroxymethyl)aminomethane, urea, ammonium bicarbonate, guanidine hydrochloride, sodium chloride, triton X-100, 4-vinylpyridine, triethylammonium bicarbonate, Coomassie brilliant blue G-250 (“Merck”, USA); boric acid, sodium tetraborate, sodium acetate, formic acid, sodium hydroxide (“Acros Organics”, USA), acetonitrile (“Fisher Chemical”, UK); trifluoroacetic acid, isopropanol (“Fluka”, USA); tris-(2-carboxyethyl)-phosphine (“Pierce”, USA); modified trypsin (mass spectrometry grade; “Promega”, USA); Miglyol 840 (“IOI Oleochemical”, Germany). 5-Aminoisatin was synthesized using standard methods [14]. Other reagents 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 in accordance with the sanitary and epidemiological rules SP 2.2.1.3218-14 “Sanitary and epidemiological requirements for the arrangement, equipment and maintenance of experimental biological clinics (vivariums)” No. 51 of August 29, 2014.

Modeling of Experimental Parkinsonism in Rats

Modeling of PS using systemic administration of rotenone was carried out according to the guidelines for preclinical study of drugs with antiparkinsonian activity, described in [15]. Rotenone solution in Miglyol 840 was administered intraperitoneally to rats (n=21) at a daily dose of 2.75 mg/kg for 7 days. Control animals (n=10) received intraperitoneal injections of saline daily (during 7 days).

The rotenone solution was prepared as follows: 50 mg of rotenone was dissolved in 0.5 ml of 100% dimethyl sulfoxide (DMSO) and then the required amount of the stock rotenone solution was diluted with Miglyol 840 to obtain a concentration of 2.75 mg/ml of rotenone. The stock solution was stored in the dark and thoroughly mixed before use. The solution was administered at the rate of 1 ml per 1 kg of animal body weight.

Physiological saline was administered intraperitoneally in an equivalent volume of 0.2 ml per 100 g of animal body weight.

Behavioral Testing

In animals with experimental PS, the dynamics of body weight growth was recorded throughout the experiment relative to the index of the first day of the experiment before the introduction of rotenone. Also, during the experiment, the mortality of animals was recorded. On day 7 of rotenone administration and 24 h after its last administration (on day 8), behavioral responses were tested to detect extrapyramidal disorders by means of the open field and rotating rod tests, and evaluation of postural instability and rigidity.

The open field test was carried out as described in [15] using the TS0501-R open field test device (“Open Science”, Russia). During 2 min, the number of movements of animals in the device (horizontal motor activity), the number of stands (vertical motor activity) and the number of peeps into the holes in the floor of the device (minks) were recorded.

The coordinated motor activity of rats with PS was assessed in the rotating rod test on a Rat Roda-Rod apparatus (“Ugo Basile”, Italy). The device is a drum 6 cm in diameter, divided into 4 equal parts of 87 mm by five disks (49 cm in diameter); the drum rotates at a constant rate of 10 rpm. The duration of retention on the rotating rod was recorded for 3 min.

Postural instability was assessed by holding the rat vertically head down, with only one of its forelimb (alternately) touching the table surface. The length of the first stride taken by the rat to restore body balance was recorded [16].

The stride length test was used to assess the rigidity of rats [17]. Each animal with legs marked with non-toxic dyes (fucorcin and brilliant green) was placed with its paws on a marking tape lining the pencil case (wall height 15 cm, width 12 cm, length 60 cm). The distance (in a straight line) between the tracks between the front or hind legs was measured at 3-5 steps of the animal. Steps at the beginning and end of the alley were not taken into account.

Statistical data processing was performed using the Statistica v. 10.0 program. The normality of the distribution was tested using the Shapiro-Wilk test, followed by an assessment of the equality of variances using the Levene's test. Since there was no normal distribution in the experimental group, further processing was carried out using the Mann-Whitney nonparametric statistics method. Fisher's exact test was used to assess categorical data in small groups. The results in the tables are presented as mean ± error of the mean (Mean ± SEM). Differences between groups were considered significant at $p<0.05$.

Preparation of Brain Homogenate Lysates

After decapitation of rats under light ether anesthesia, the brain tissue was homogenized using a Heidolph SilentCrusher homogenizer ("Heidolph", Germany) (50000 rpm) in 0.05 M potassium phosphate buffer (pH 7.4) (buffer A) to a final protein concentration of 30 mg/ml. After incubation in the presence of 3% Triton X-100 (4°C, 1 h), the lysates were diluted 3 times with buffer A, centrifuged at 16000 *g* for 30 min at 4°C to obtain a cleared supernatant.

Affinity Chromatography of Brain Proteins on 5-Aminoisatin Sepharose

The affinity sorbent was prepared according to the standard procedure [9, 11, 13]. Brain tissue lysates (cleared supernatant, protein concentration about 10 mg/ml) were added to a suspension of 5-aminoisatin sepharose in a ratio 1:1 and incubated for 16 h at 4°C with slow stirring. The affinity sorbent was washed with 100 volumes of buffer A to remove non-specifically bound proteins; remaining proteins were eluted using a column (1×2 cm) at room temperature with 1 mM isatin in buffer A. The eluate (30 ml) was concentrated to 0.25 ml by means of Amicon Ultra membrane centrifuge filters ("Millipore", USA). Proteins were extracted with a chloroform-methanol mixture [18], the protein precipitate was dissolved in 8 M urea containing 20 mM dithiothreitol and 100 mM TrisHCl (pH 8.5) and subjected to alkylation and subsequent trypsinolysis directly on Vivaspin 500 centrifuge membrane filters ("Sartorius Stedim Biotech", Germany) with 10000 Da membrane as described in [19]. The reaction was stopped with formic acid (0.1% final concentration). The samples were evaporated using a 5301 vacuum concentrator ("Eppendorf", Germany), dissolved in 0.1% formic acid, and analyzed using the equipment of the "Human Proteome" Core Facility at the Institute of Biomedical Chemistry (Russia).

To determine the proteins nonspecifically bound to the affinity sorbent, we used the control cyanogen bromide-activated sepharose, which was subjected to the same procedures as the 5-aminoisatin sepharose, but without the addition of the affinity ligand.

The Mass Spectrometric Analysis

The mass spectrometric analysis was performed using an Ultimate 3000 RSLCnano ("Thermo Fisher Scientific", USA) integrated system for high-performance liquid separation of peptides in the nanoflow mode. Chromatographic separation of peptides was carried out on an analytical reverse phase column Acclaim Pepmap® C18 (75 μm × 150 mm, 2 μm particle size, "Thermo Fisher Scientific") in a linear elution gradient of mobile phase A (0.1% aqueous solution of formic acid) and mobile phase B (80% acetonitrile, 0.1% formic acid) from 2% to 40% at a flow rate of 0.3 ml/min for 60 min, followed by equilibration of the chromatographic system in the initial conditions of the gradient (A : B = 2 : 98) for 5 min.

A Thermo Scientific Q Exactive HF-X mass spectrometer ("Thermo Fisher Scientific") equipped with a nanoelectrospray ionization source (nESI) was operated in the positive ionization mode with a resolution of 120000 at *m/z* 200, the ion accumulation volume in the trap was set to 1E6, the ion accumulation time in the trap was maximum 50 ms. The dominant charge state of precursor ions was set as 2^+, charge states above 4^+ and below 2^+ were excluded from further analysis. Scanning of tandem spectra was carried out in the mode of automatic selection of 20 dominant peaks of precursor ions recorded at *m/z* = 350-1400. The resolution for detecting fragment ions was set to 15000 at *m/z* 200, the ion accumulation volume in the trap was 1E5, and the ion accumulation time in the trap was a maximum of 50 ms. Parent ions were isolated in a window of 2.0 *m/z*, offset by 0.5 *m/z* for better isotope capture. Measured precursors ions were excluded from subsequent analysis for 20 s after scanning. The obtained mass spectrometric data with the .raw extension were processed by the MaxQuant software (v. 1.6.3.4) with the built-in Andromeda search algorithm. Rat (*Rattus norvegicus*) Swiss Prot/Uniprot complete proteome protein sequence database downloaded from the Uniprot database with addition of reversed sequences and commonly encountered contaminating sequences to apply the target decoy

approach. The method was used to calculate the FDR (False Discovery Rate) parameter; the FDR parameter of 1% was taken as a cutoff for protein registration. The following parameters were used for signal extraction and its subsequent processing: proteolytic cleavage enzyme was trypsin; the maximum allowable amount of intrapeptide residues of lysine or arginine was not more than 1; the allowable error in measuring the monoisotopic mass of the peptide was ±0.01 Da, the allowable error in measuring the fragment ion was ±0.05 Da. Carbamide methylation of cysteine residues was chosen as a fixed chemical modification, and methionine oxidation was chosen as a variable modification [20].

Each protein presented in the tables was identified in at least three independent experiments.

The conditions for experiments performed on mice with MPTP-induced parkinsonism, isolation and identification of isatin-binding proteins in the brain of mice were described earlier [13].

RESULTS AND DISCUSSION

The development of rotenone-induced PS was accompanied by body weight loss in all rats treated with rotenone (from 277.7±3.2 g to 239.5±8.5 g); in rats of the control group, a gradual increase in their body weight was observed during the experiment (from 279.3±3.3 g to 325.2±4.1 g). Repeated rotenone injections caused mortality of the treated animals: on day 7 the mortality rate reached almost 29%, and on day 8 it was already 43%; in the control group of animals treated with saline, all the animals remained alive during this period.

Rats treated with rotenone developed oligokinesia. The open field test, performed on day 7, revealed a significant decrease of their motor activity (3.1 and 5.4 times in horizontal and vertical motor activity, respectively) as compared with control group rats. The search activity (evaluated as the number of peeks into minks) was 4.4 times less than in control animals (Table 1).

The development of rotenone-induced PS was also accompanied by marked motor deficiency: on day 7 and day 8 the motor activity of rotenone treated rats evaluated as the duration of their retention on the rotating rod was 2.1- and 2.9-fold lower than in control animals, respectively (Table 2).

Systemic administration of rotenone caused the development of rigidity — the appearance of a "hump", which resulted in a significant decrease in the stride length of the animal by 1.4 times compared with this parameter in the control animals (Table 3).

The development of rotenone-induced PS was accompanied by severe postural instability; on day 7 it was found that animals with rotenone-PS had to take a longer step to achieve body balance than control animals. The stride length of both the left and right front forelimbs in the rotenone-treated rats was significantly longer (by 1.8- and 1.5-fold, respectively) than the corresponding stride length of the control group (Table 3).

The assessment of the level of oligokinesia in rats was also performed on day 8 of the experiment (24 h after the last injection of rotenone). The decrease in motor activity noted the day before in the rotenone-treated rats was also detected on day 8. In the open field test it was manifested as 2.1-, 2.6-,

Table 1. Behavior of rats with rotenone-induced PS in the open field test

Group of animals	Number of animals	Motor activity on day 7		Peeps into minks
		Horizontal activity (number of movements)	Vertical activity (number of stands)	
Control	10	37.8±5.5	8.7±1.3	11.4±1.5
Rotenone	12	10.2±1.9*	1.6±0.5*	2.6±1.1*

Here and in other tables data represent Mean+SEM, * – $p<0.001$ as compared with rats of the control group.

Table 2. Coordinated motor activity of rats with rotenone-induced PS

Group of animals	Number of animals	Duration of retention of animals on a rotating rod, s	
		Day 7	Day 8
Control	10	162.2±15.1	146.7±17.9
Rotenone	8	78.6±17.2*	50.0±20.8*

* – $p<0.01$ as compared with rats of the control group.

Table 3. Rigidity and postural instability in rats with rotenone-induced PS (day 7)

Group of animals	Number of animals	Rigidity	Postural instability	
		Stride length, cm	Left forelimb, cm,	Right forelimb, cm
Control	10	10.8±0.3	2.7±0.2	2.9±0.2
Rotenone	10	7.7±0.6*	4.8±0.2*	4.4±0.3*

* – $p<0.001$ as compared with rats of the control group.

and 2.1-fold decrease in horizontal, vertical activity and search activity, respectively, versus animals of the control group (Table 4).

Thus, the systemic administration of rotenone models severe PS, characteristic of the advanced stage of PD. It is accompanied by significant weight loss of animals, their high mortality, oligokinesia, rigidity, and postural instability. The effect of rotenone on the motor activity of animals was comparable to the effect of MPTP administration, and even exceeded it in some parameters tested. For example, the duration of retention of animals on a rotating rod in the case of rotenone-induced PS was 34% of that in control animals, while in the case of MPTP-induced PS it was 28.2% of control. The motor activity of animals with rotenone-induced PS evaluated in the open field test was: horizontal activity — 27.0% of the control, vertical activity — 18.4% of the control. In the case of MPTP-induced parkinsonism, the horizontal activity was 44.2%, and vertical activity was 24.0% of control [21].

Proteomic of brain isatin-binding proteins showed that in the case of rotenone-induced PS, the pool of isatin-binding proteins common to control rats and rats with PS (146) significantly exceeded the pool of common proteins of control mice and mice with MPTP-induced PS, both 1.5 h after MPTP administration (27) and especially one week after (14) (Fig. 1, Supplementary Tables S1-S4 and also data from [13]).

Comparison of isatin-binding proteins specific for animals with MPTP-induced and rotenone-induced PS (compared to control animals) has shown complete absence of proteins common to these two models of PD, both in the case of acute and delayed response to MPTP administration (Table 5 and Fig. 2, and Supplementary Tables S3, S4). Comparison of the effects of MPTP and rotenone on the distribution of isatin-binding proteins by their function (Table 6) showed that in both cases the influence of neurotoxins primarily affected the proteins involved in signal transduction and regulation of enzyme activity. However, under the action of MPTP, the number of individual proteins initially decreased by more than two times (from 23 to 11), and then it recovered to the values exceeding the original ones (up to 37) (Table 6). In the case of rotenone-induced PS, the number of individual proteins increased more than 1.5-fold (from 18 to 30). The same trend was also found in the functional groups of isatin-binding proteins involved in regulation of gene expression, cell division, and differentiation (see Table 6). These results seem to indicate that, despite the common pathogenetic basis of PS caused by these neurotoxins, the molecular mechanisms and targets of action of rotenone and MPTP differ.

CONCLUSIONS

Models of PS in rodents induced by the administration of MPTP and rotenone neurotoxins are among the most popular models of PD [1]. In both cases, the development of PD is due to the development of mitochondrial dysfunction, which is based on different mechanisms. The neurotoxin MPTP undergoes conversion into the methylphenylpyridinium ion (MPP^+), catalyzed by monoamine oxidase B (MAO B), and MPP is responsible for primary disorders in the mitochondrial respiratory chain [5]. MAO B inhibitors (irreversible inhibitor deprenyl and reversible isatin), which prevent MPTP biotransformation, reduce the manifestations of PD [21, 22]. Mitochondrial

Table 4. Behavior of rats with rotenone-induced PS in the open field test on the next day (day 8) after the last rotenone administration

Group of animals	Number of animals	Motor activity on day 8		Peeps into minks
		Horizontal activity (number of movements)	Vertical activity (number of stands)	
Control	10	30,1±5,6	2.3±0.7	9.2±2.1
Rotenone	11	14,4±4,9*	0.9±0.5	4.3±1.1*

* – $p<0.05$ as compared with rats of the control group.

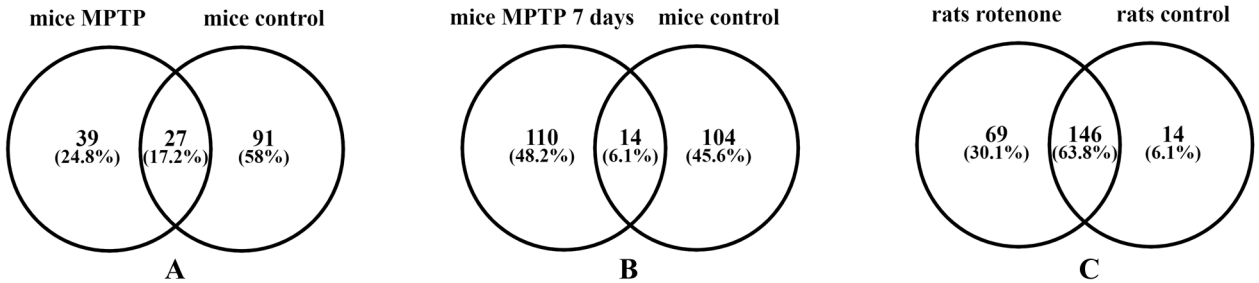

Figure 1. A Venn diagram comparing brain isatin-binding proteins of control animals and animals with induced PS: **A** – isatin-binding proteins of control mice and mice treated with MPTP (1.5 h after administration); **B** – isatin-binding proteins of control mice and mice treated with MPTP (7 days after administration); **C** – isatin-binding proteins of control rats and rats with rotenone-induced PS.

Table 5. Brain isatin-binding proteins (69) specific for rats with rotenone-induced PS

No.	Uniprot accession number	Uniprot gene name	Uniprot protein name
Proteins/enzymes involved in energy generation and carbohydrate metabolism (n=6)			
1	P49432	*Pdhb*	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial
2	Q5BJX2	*Pdhx*	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex
3	Q6P6R2	*Dld*	Dihydrolipoyl dehydrogenase, mitochondrial
4	P53534	*Pygb*	Glycogen phosphorylase, brain form (Fragment)
5	D4A133	*Hoga1*	4-hydroxy-2-oxoglutarate aldolase, mitochondrial
6	G3V7Y3	*Atp5f1d*	ATP synthase F1 subunit delta
Proteins involved in cytoskeleton formation and exocytosis (n=12)			
1	D3ZQ45	*Dsg1*	Desmoglein 1
2	D3ZUY8	*Ap2a1*	AP-2 complex subunit alpha
3	C6L8E0	*Dbn1*	Drebrin E
4	D3ZZ99	*Add1*	Alpha-adducin
5	F1LU27	*Focad*	Focadhesin
6	P08082	*Cltb*	Clathrin light chain B
7	P18484	*Ap2a2*	AP-2 complex subunit alpha-2
8	P19527	*Nefl*	Neurofilament light polypeptide
9	Q6AY84	*Scrn1*	Secernin-1
10	D4A315	*N/A*	Actin-depolymerizing factor
11	Q64715	*Map2*	Microtubule-associated protein
12	P62744	*Ap2s1*	AP-2 complex subunit sigma
Proteins involved in signal transduction and regulation of enzyme activity (n=11)			
1	P19139	*Csnk2a1*	Casein kinase II subunit alpha
2	P05545	*Serpina3k*	Serine protease inhibitor A3K
3	P20651	*Ppp3cb*	Serine/threonine-protein phosphatase 2B catalytic subunit beta isoform
4	A0A0G2JYA4	*LOC100362453*	Serine/threonine-protein phosphatase
5	F1LQ63	*Tnr*	Tenascin R, isoform CRA_b
6	G3V9A3	*Sfn*	RCG31390
7	P02767	*Ttr*	Transthyretin
8	Q8VBU2	*Ndrg2*	Protein NDRG2
9	Q32PX7	*Fubp1*	Far upstream element-binding protein 1
10	Q568Z9	*Phyhip*	Phytanoyl-CoA hydroxylase-interacting protein
11	F1LMN8	*Khdrbs3*	KH domain-containing, RNA-binding, signal transduction-associated protein 3
Antioxidant and protective proteins/enzymes (n=3)			
1	Q9R063	*Prdx5*	Peroxiredoxin-5
2	B6DYP7	*Gsta2*	Glutathione transferase
3	P00502	*Gsta1*	Glutathione S-transferase alpha-1
Protein regulators of gene expression, cell division and differentiation (n=28)			
1	P61354	*Rpl27*	60S ribosomal protein L27
2	M0R961	*Khsrp*	Far upstream element-binding protein 2
3	M0RD75	*Rps6*	40S ribosomal protein S6
4	P12001	*Rpl18*	60S ribosomal protein L18
5	Q9Z0U8	*pRM10*	Nucleic acid binding factor pRM10
6	P62804	*H4c2*	Histone H4
7	B0BNA7	*Eif3i*	Eukaryotic translation initiation factor 3 subunit I
8	P62902	*Rpl31*	60S ribosomal protein L31
9	F1LYQ7	*LOC680700*	Ribosomal protein
10	Q5PQK2	*Fus*	RNA-binding protein
11	Q5PQR0	*Raly*	RALY heterogeneous nuclear ribonucleoprotein
12	Q566E4	*Hnrnpr*	Heterogeneous nuclear ribonucleoprotein R
13	Q642E2	*Rpl28*	60S ribosomal protein L28
14	Q3SWU3	*Hnrnpdl*	Heterogeneous nuclear ribonucleoprotein D-like
15	B5DF91	*Elavl1*	ELAV-like protein 1
16	Q6P790	*Rpl6*	60S ribosomal protein L6 (Fragment)
17	A0A0G2JXW4	*Hnrnpc*	Heterogeneous nuclear ribonucleoprotein C, isoform CRA_a

Table 5. Brain isatin-binding proteins (69) specific for rats with rotenone-induced PS (continue)

No.	Uniprot accession number	Uniprot gene name	Uniprot protein name
18	F1M2E9	*LOC100361259*	60S ribosomal protein L13
19	B2RZB7	*Snrpd1*	Small nuclear ribonucleoprotein Sm D1
20	F1M013	*Rpl7a*	60S ribosomal protein L7a
21	B5DEP7	*Snrpg*	Small nuclear ribonucleoprotein G
22	G3V6I9	*Rpl26*	60S ribosomal protein L26
23	D4A1Z2		KOW domain-containing protein
24	G3V7Z8	*Pabpn1*	Poly(A) binding protein, nuclear 1, isoform CRA_a
25	P60892	*Prps1*	Ribose-phosphate pyrophosphokinase 1
26	D3ZXF9	*Mrpl12*	Mitochondrial ribosomal protein L12
27	D4A6G6	*LOC100362339*	40S ribosomal protein S19
28	D4ABK7	*Hnrnph3*	Heterogeneous nuclear ribonucleoprotein H3 (2H9) (Predicted), isoform CRA_c
Enzymes involved in metabolism of proteins, amino acids and other nitrogenous compounds (n=7)			
1	Q00981	*Uchl1*	Ubiquitin carboxyl-terminal hydrolase isozyme L1
2	Q3ZAU6	*Rnf14*	RBR-type E3 ubiquitin transferase
3	Q45QL2	*Gnb4*	Guanine nucleotide binding protein beta 4 (Fragment)
4	Q4V8H5	*Dnpep*	Aspartyl aminopeptidase
5	P00507	*Got2*	Aspartate aminotransferase, mitochondrial
6	P09034	*Ass1*	Argininosuccinate synthase
7	P52759	*Rida*	2-iminobutanoate/2-iminopropanoate deaminase
Enzymes involved in lipid metabolism (n=2)			
1	P07483	*Fabp3*	Fatty acid-binding protein, heart
2	Q07523	*Hao2*	Hydroxyacid oxidase 2

Figure 2. A Venn diagram comparing brain isatin-binding proteins specific for animas with MPTP induced PS and rotenone induced PS: **A** – MPTP effect, 1.5 h after administrarion; **B** – MPTP effect 7 days after administration.

Table 6. Comparison of effects of MPTP and rotenone on functional distribution of isatin-binding proteins

Functional groups of isatin-binding proteins	Control mice*	MPTP treated mice*		Control rats	Rotenone treated rats
		1.5 h after administration	7 days after administration		
Proteins/enzymes involved in energy generation and carbohydrate metabolism	6	2	2	22	27
Proteins involved in cytoskeleton formation and exocytosis	41	27	30	49	59
Proteins involved in signal transduction and regulation of enzyme activity	23	11	37	18	30
Antioxidant and protective proteins/enzymes	6	4	5	22	20
Protein regulators of gene expression, cell division and differentiation	30	17	40	40	65
Enzymes involved in metabolism of proteins, amino acids and other nitrogenous compounds	9	3	7	9	12
Enzymes involved in lipid metabolism	3	2	3	0	2
Total number	118	66	124	160	215

* – Original data are given in [13].

damage induced by the pesticide rotenone occurs in a MAO-independent manner and, and therefore, involves other sets of proteins. Data on the complete absence of common pools of isatin-binding proteins in the brain of animals with MPTP and rotenone-induced parkinsonism, which are different from control, are consistent with these conclusions. At the same time, changes in the profile of isatin-binding proteins in response to rotenone administration suggest that administration of the neuroprotector isatin will also have a positive neuroprotective effect in the rotenone model of PD.

ACKNOWLEDGMENTS

Mass spectrometry analysis was carried out using the equipment of the "Human Proteome" Core Facility at the Institute of Biomedical Chemistry.

FUNDING

This work was supported by the Russian Science Foundation (project No. 23-25-00066).

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 INTERESTS

The authors declare no conflicts of interests.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

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Received: 02. 02. 2023.
Revised: 08. 02. 2023.
Accepted: 08. 02. 2023.

ОСОБЕННОСТИ ПОВЕДЕНЧЕСКИХ РЕАКЦИЙ И ПРОФИЛЯ ИЗАТИН-СВЯЗЫВАЮЩИХ БЕЛКОВ МОЗГА У КРЫС С ИНДУЦИРОВАННЫМ РОТЕНОНОМ ЭКСПЕРИМЕНТАЛЬНЫМ ПАРКИНСОНИЗМОМ

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Токсины ротенон и 1-метил-4-фенил-1,2,3,6-тетрагидропиридин (МФТП) применяются у животных для моделирования болезни Паркинсона (БП). Механизм действия токсинов связан с угнетением митохондриальной дыхательной цепи, что приводит к дегенерации дофаминергических (ДА) нейронов. Преимуществом ротенонового метода является способность ротенона провоцировать нейродегенерацию, наиболее схожую по своим симптомам и молекулярно-биологическим признакам с таковыми у БП. Изатин (индолдион-2,3) — эндогенный нейропротектор, обнаруженный в тканях и биологических жидкостях млекопитающих и человека, обладающий широким спектром биологической активности, реализующимся многочисленными изатин-связывающими белками. В данной работе изучены особенности поведенческих реакций и профиля изатин-связывающих белков мозга у крыс с индуцированным ротеноном паркинсоническим синдромом (ПС) в сравнении с соответствующими параметрами МФТП-индуцированного паркинсонизма у мышей. Показано, что системное введение ротенона моделирует тяжёлый ПС, сопоставимый с эффектом от введения МФТП, сопровождающийся значимой потерей веса животных, их гибелью, олигокинезией, ригидностью и постуральной неустойчивостью. Несмотря на общую патогенетическую основу ПС, вызванного ротеноном и МФТП, молекулярные механизмы их действия различаются. В случае ротенон-индуцированного ПС пул изатин-связывающих белков, общих для контрольных крыс и крыс с ПС (146), значительно превышал пул общих белков контрольных мышей и мышей с МФТП-индуцированным ПС, как непосредственно после введения нейротоксина (27), так и (тем более) через неделю после введения (14). Сравнение изатин-связывающих белков, специфичных для животных с МФТП-индуцированным и с ротенон-индуцированным ПС (по сравнению с контрольными животными), выявило полное отсутствие белков, общих для этих двух моделей БП. При этом влияние нейротоксинов в обоих случаях в первую очередь затрагивало белки, участвующие в передаче сигнала и регуляции активности ферментов. Изменения профиля изатин-связывающих белков при введении ротенона позволяют предположить, что введение нейропротектора изатина будет оказывать позитивный эффект и в ротеноновой модели БП.

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

Ключевые слова: паркинсонизм; нейродегенерация; нейротоксин ротенон; нейротоксин МФТП; изатин; изатин-связывающие белки; мозг; протеомное профилирование

Финансирование. Работа выполнена при поддержке Российского Научного Фонда (проект № 23-25-00066).

Поступила в редакцию: 02.02.2023; после доработки: 08.02.2023; принята к печати: 08.02.2023.