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Purine and lipid metabolism in rats with a rotenone model of Parkinson's disease under the influence of methanindiazenone

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This study aims to evaluate the effect of methanindiazenone (MD), a new benzodiazepine derivative, on the levels of purine metabolites and lipids in the blood plasma of rats with rotenone (ROT) induced Parkinson's disease (PD). The concentrations of ATP, ADP, AMP, xanthine, hypoxanthine, phospholipids (PL), cholesterol (CHOL), cholesterol esters (ECHOL), free fatty acids (FFA), and triglycerides (TG) were quantified in plasma samples by thin-layer chromatography. Our data demonstrate that in rats with ROT-induced PD the AMP/ATP ratio in plasma increased by 2.5 times compared to the control, and this indicator returned to normal values under the influence of MD. ROT also increased the concentration of xanthine and hypoxanthine by 26.7% ($P < 0.001$) and 42.4% ($P < 0.001$), respectively, compared to the control. MD restored xanthine concentration to 86.7% of the control level and returned hypoxanthine concentration to normal values. Besides, ROT reduced the blood plasma concentrations of PL, CHOL, ECHOL, FFA, TG by 22%, ($P < 0.001$), 18% ($P < 0.001$), 25% ($P < 0.001$), 28% ($P < 0.001$), 33% ($P < 0.001$), respectively. Under the influence of MD, such indicators as the blood plasma concentration of PL, CHOL, FFA returned to control levels. Our results suggest that MD improves the metabolism of both purines and lipids in rats with ROT-induced PD.

Key words: rotenone; Parkinson's disease; benzodiazepine derivatives; motor behavior; blood plasma; purines; lipids.

INTRODUCTION

Parkinson's disease (PD) is a complex neurological disorder associated with the progressive loss of dopaminergic neurons of the *substantia nigra* pars compacta (SNpc) and the macula; the former is responsible for motor control while the latter for various psychological effects. Typical motor symptoms of PD are voluntary motility disorders. The main non-motor manifestations of PD are sleep disorders, hyposmia, autonomic, gastrointestinal, sensory, and neuropsychiatric symptoms, including cognitive decline, depression, and anxiety [1]. 90-95% of PD cases are sporadic with unknown etiology. PD affects approximately 2% of the population over 65 years of age. Numerous studies show that the key cause of the neurodegenerative process in PD is the death of neuronal mitochondria, which determine the bioenergetic status of the cell. Specifically, we are talking about a decrease in the activity of complex I of the mitochondrial respiratory chain, which causes energy deficit at subcellular-cellular-systemic levels and may constitute the main reason for the loss of SNc cells in PD. ATP deficiency is a common factor underlying pathological changes at the molecular level, including aggregation of α-synuclein, formation of reactive oxygen

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species, etc. [2]. PD is considered as the most common synucleinopathy. More than 6 million older people worldwide are diagnosed with PD, and their number is steadily increasing [1]. Currently, no therapy can slow or stop the progression of PD. Levodopa therapy remains the mainstay in PD treatment, especially in its severe forms. Levodopa is a dopamine precursor. It crosses the blood-brain barrier and reaches its target site in the brain, where it is decarboxylated to dopamine and stored in presynaptic striatal neurons. However, this drug's dosage is often limited due to its side effects, such as excessive excitement or visual hallucinations [3]. Dopamine-based therapy usually helps with initial motor symptoms. Nonmotor ones require nondopaminergic approaches (e.g., selective serotonin reuptake inhibitors for psychiatric symptoms, cholinesterase inhibitors for cognitive functions). Classic benzodiazepine drugs are widely used in clinical practice as anxiolytics, hypnotics, anticonvulsants, and muscle relaxants [4]. For the treatment of synucleinopathies, including PD, benzodiazepine-like drugs are used, for example, in parasomnia, which accompanies PD and is associated with eye muscle atony [5]. Recently, it was shown in rats with ROT-induced PD that a benzodiazepine derivative JM-20 protects the mitochondria of brain neurons from damage by suppressing their spontaneous swelling and dissipation of the membrane potential. In addition, this drug improved the redox status of animals exposed to ROT by increasing the activity of superoxide dismutase and catalase, as well as the levels of tissue SH-groups, while reducing the concentration of malondialdehyde [6]. In *in vitro* experiments, a new family of 1.5-benzodiazepine-2(3H) showed protection of neurons in conditions of oxidative stress, significantly reduced the level of reactive oxygen species and mitochondrial superoxide, and restored the membrane potential of mitochondria [7].

ROT is a pesticide of natural origin, which is a potent inhibitor of mitochondrial complex I, where it suppresses oxidative phosphorylation and causes mass death of mitochondria and, therefore, cells. Chronic administration of ROT in small doses gives an excellent PD model in laboratory mice and rats, as it causes aggregation of α-synuclein and the formation of Lewy bodies in neurons. Studies on clinical and experimental models have shown that the metabolic pathways disrupted in PD are mainly related to lipid and energy metabolism [8, 9].

However, it should be noted that different authors often show opposite results. In particular, Xiaoxue Fu et al. [10] and Xue Hong et al. [11] observed in the blood serum of patients with PD reduced concentrations of lipids, including total cholesterol (CHOL) and triglycerides (TG). One study suggested that elevated serum total CHOL is linked to elevated PD risk in males under 55 years of age [12].

Our previous studies found that rats with a ROT-induced PD model had a significantly decreased level of the blood and bile lipids. At the same time, we observed a decrease in the amount of ATP in the bile, this indicated a decrease in the activity of mitochondria in liver cells. The drug diazepinone, a diazepine derivative we synthesized, significantly improved the indicators disturbed in PD [13, 14]. Experimental therapy of PD provides an opportunity to develop more effective and safe methods for treating this pathology in the future, improving the life quality of patients with PD. To achieve this goal, it is essential to identify biomarkers of the disease, this will allow diagnosing PD in the early stages. The purpose of the present study was to investigate the content of blood plasma purine and lipid components and the corrective effect of a new synthetic diazepine-like drug methanindiazenone on these parameters in rats with ROT-induced PD.

METHODS

The experiments were carried out according to the current international requirements and norms of humane treatment of animals (Strasbourg, 1986, Law of Ukraine dated February 21, 2006

No. 3447-IV) and following the decision of the Biological Ethics Committee of the Scientific Center «Institute of Biology and Medicine» Taras Shevchenko Kyiv National University (protocol No. 3 dated April 9, 2009). The research used the following materials: ROT, dimethylsulfoxide (DMSO) manufactured by "Sigma" (USA). The drug we studied is 4-(4-methoxy-3-methylphenyl)-2,5-dihydro-1H-benzo[d][1,2]diazepin-1-one4-(4-methoxy-3-methylphenyl)-2,5-dihydro-1H-benzo[d][1,2] diazepin-1-one – methanindiazenone (MD), synthesized in our laboratory.

Elemental analysis of MD was carried out in the analytical laboratory of Kuhar, Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine [15]. Sodium thiopental ("Kyivmedpreparat", Ukraine) was used to anesthetize experimental animals. The research was carried out in acute experiments on 60 mature male Wistar rats (obtained from the Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine). The animals' weight was in the range of 300-350 g, age – 14 weeks. During the experimental period, the animals were housed in standard conditions such as environmental humidity of 55-60%, controlled temperature of 22 ± 2°C, 12-h light/dark cycle, and free access to tap water and commercial food. At the beginning of the experiment, animals were randomly divided into six experimental groups: I – control animals treated with refined sunflower oil; II – animals treated with ROT (2 mg/kg); III – animals treated with ROT+1% DMSO (1 ml/kg); IV – animals treated with ROT+MD (0.5 mg/kg); V – animals treated with ROT+MD (1 mg/kg); VI – animals treated with ROT+MD (2 mg/kg), n = 10 in each experimental group. ROT was suspended in refined sunflower oil immediately before use and administered i.p. at a single dose (2 mg/kg) [16]. The administration of ROT lasted for 4 weeks (28 days) and was carried out at the same time: from 10:00 to 12:00. After that, a suspension of MD in a 1% DMSO solution was prepared immediately before use and was administered by single i.p. injections. The dosage of MD was determined based on the lowest dose of other neuroprotective drugs found to be effective.

Behavioral analysis. In the ROT-treated groups, the rats showed reduced and slow movement, tremor, and an unstable gait, which were identified as PD-like symptoms; also, rat fur became yellow and dirty.

Locomotor activity. The open field test was used to evaluate the spontaneous activity of rats. Twenty-eight days following the treatment, rats were placed in an open field square box (100 cm long, 100 cm wide, and 40 cm high), inside of wich the test area was divided into squares and well illuminated.

The experiments were carried out in a quiet environment. Before the test, 70% ethanol was used to thoroughly wipe the box's inner wall and bottom surface so as not to affect results of the next test. An animal was placed at the center of the box bottom and observed with a video camera (Fotocam, Canon) for 6 min: 1 min for habituation, and 5 min for behavioral analyzes. Two motor parameters were quantified throughout this test, namely locomotion frequency (number of squares crossings, defined as the number of quadrant crossings with four paws) and rearing frequency (times an animal rise for at least 2 sec on their rear paws in the air or against the walls). The animal was then replaced, and the experiment was repeated [6].

Biochemical research. According to the task, the blood plasma concentrations of ATP, ADP, AMP, xanthine, hypoxanthine, phospholipids (PL), CHOL, cholesterol esters (ECHOL), free fatty acids (FFA), and TG were determined. Quantitative determination of plasma lipids mentioned above was carried out using thin-layer chromatography [17]. To determine the blood plasma lipid composition, rats were anesthetized with sodium thiopental (6 mg/100 g), and 4-5 ml of blood was collected from the inferior vena cava into sterile sealed tubes. The blood was centrifuged for 15 min at 3000 rpm to separate the plasma from blood cells. Later,

0.1 ml of blood plasma was added to a 2.4 µl mixture of chloroform-methanol (2:1) and 0.5 ml acidified water (0.5 ml of concentrated sulfuric acid per 1 liter of double-distilled water) was added in 5 min. Samples were kept overnight for a complete separation into two phases, with the lower phase containing lipids. The extract was dried at 70°C. The dry residue was dissolved in 40 µl of the chloroform-methanol mixture (3:1) and put on a plate as a thin strip. Chromatography was performed in a chamber saturated with solvent vapor. The system of eluents included petroleum ether, diethyl ether, ether (30:10:0.2), and glacial acetic acid. Fractions of plasma lipids were stained with 5% phosphomolybdic acid in 96% ethanol, followed by heating at 100°C for 5 min. Identification of the major lipid fractions was performed using the standards and color of the spots, considering the relative mobility of fractions. For quantitative evaluation of individual fractions of lipids in plasma, solutions of their main components (1 mg of substance per 1 ml of solvent) were prepared individually and in a mixture with specimens of other fractions. This mixture of the standards (from 1 µl to 2; 5; 10; 15; 20; 25 and 30 µl) was quantitatively adsorbed on chromatographic paper. After extraction and concentration of plasma at the bottom of a cone tube, it was dissolved in 50 µl of solvent and dropped a few times with a micropipette (5 µl) on the surface of a prepared and marked chromatographic plate. Chromatographic separation of blood plasma lipid components was carried out on «Silufol» plates. After developing with an aqueous phosphomolybdic acid solution, quantitative estimation of the color intensity of each fraction was carried out using a DO-1M densitometer. The spot area was also measured. The value of the combined test was deposited on the *y*-axis in the calibration graph, and the *x*-axis was marked with the known amount of a corresponding standard – an evidence of the specific lipid fraction presence. These parameters were estimated for fractions of lipids from samples of the investigated plasma. Based on the calibration curve, the amount of the corresponding lipid was determined in µg. Finally, considering the dilution and the part of the extract, we determined the amount of lipids in mg per 100 ml of blood plasma.

Evaluation of purine metabolites, as well as lipids, was performed by thin-layer chromatography. For this purpose, 0.2 ml of blood plasma was added to 1.8 ml of 0.8 N perchloric acid (HClO4) solution. Both blood plasma and HClO4 were pre-cooled to a temperature of 0°C. Perchloric acid was used for simultaneous precipitation of plasma proteins and adenine nucleotides: ATP, ADP, AMP, xanthine, and hypoxanthine. The obtained protein-free perchlorate extracts were centrifuged for 15 min on an OPn-3 U42 centrifuge at 1500 rpm and neutralized with K2CO3 to pH 7.0. After centrifugation under the above conditions, the supernatant was collected, and certain aliquots were applied to chromatographic plates. Separation and quantification of adenine nucleotides on "Silufol" plates UV-254 were performed by the method of Maidanyuk et al. [18]. Direct densitometry of the plates in reflected light was performed on a high-speed scanner of a CS-920 densitometer «Shimadzu» (Japan) in the direction of solvent movement at a wavelength of 260 nm. The content of test compounds in chromatographic spots was determined using calibration curves of the spot area on the number of applied chromatographically pure preparations of ATP, ADP, AMP, xanthine, and hypoxanthine. The results were assessed by: the parametric one-way ANOVA method in combination with the Tukey test for pairwise comparison, and p values less than 0.05 were considered significant; the nonparametric Kruskal Wallis ANOVA method in combination with the Mann-Whitney test for pairwise comparison with Bonferroni correction, and p values less than 0.01 were considered significant. Statistical analysis was performed using Origin Pro 8.0, and charts were drawn using Excel software.

RESULTS AND DISCUSSION

In this study, ROT was administered for 28 days to induce dopaminergic neurodegeneration. In the open-field test, rats treated with ROT were hypoactive, frequently froze in one place during movement, and had a reduced frequency of hind paw rearing or line crossings between individual squares compared to the control rats. The presented results are consistent with the data of other authors, who showed a loss of motor skills and weakened search behavior in rats treated by ROT [19]. After the application of MD, the frequency of rearing increased, as did the number of squares crossed in the open field (Tables 1; 2).

Analysis of variance (rearing frequency) marked effects are significant at P < ,05000. Means ± SEM; *n* = 10. **P < 0.01; ***P < 0.001 as compared with changes in Control group; ###P < 0.001, vs. the ROT group; α P < 0.001 as compared with changes in ROT+1% DMSO group; £££ P < 0.001, vs. the ROT+MD 0.5 mg/kg group. ROT, rotenone; DMSO, dimethyl sulfoxide; MD, methanindiazenone; PD, Parkinson's disease.

Analysis of variance (incidence of locomotion) marked effects are significant at P < ,05000. Means ± SEM; *n* = 10. **P < 0.01; ***P < 0.001 as compared with changes in Control group; ###P < 0.001, vs. the ROT group; ααα P < 0.001 as compared with changes in ROT+1% DMSO group; £££ P < 0.001, vs. the ROT+MD 0.5 mg/kg group. ROT, rotenone; DMSO, dimethyl sulfoxide; MD, methanindiazenone; PD, Parkinson's disease.

Biochemical analysis of the blood plasma from rats with PD revealed quantitative changes in both purine metabolites and blood lipids. In particular, the plasma concentration of ATP in the control rats was 29.3 ± 0.9 mg%. After the 28-days administration of ROT, the amount of ATP in blood plasma of experimental animals decreased by 33.8% compared to the control group (P < 0.001). In the rats of the ROT+1% DMSO group, the ATP level was 15.5% (P < 0.01) lower compared to the control group values. In PD animals treated with MD at a dose of 0.5 mg/kg, the blood plasma ATP content was 18.3% lower than the control indicator (P < 0.001), and at doses of MD 1.0 and 2.0 mg/kg this marker did not differ from the control. The ADP content did not undergo significant changes after ROT injections or treatment with the tested drug. The plasma concentration of AMP in the ROT group increased by 60% (P < 0.001). Under the influence of MD in doses of 1.0 and 2.0 mg/kg, this indicator returned to the control level. The amount of xanthine exceeded the control levels: in the ROT group by 27% (P < 0.001), ROT+1% DMSO and ROT+MD 0.5 mg/kg by 20%, in both cases P < 0.001, in groups ROT+MD 1.0 and ROT+MD 2.0 mg/kg by 13.3%, in both cases P < 0.05. Compared to the control, the amount of hypoxanthine in the blood plasma of rats with ROT-induced PD increased by 42.4% (P < 0.001). In the ROT+1% DMSO and ROT+MD 0.5 mg/kg groups, the excess of the control level corresponded to 28 and 26%, respectively, in both cases P < 0.001. Under the influence of MD in 1 and 2 mg/kg doses, the blood plasma hypoxanthine concentration did not differ from the control. The AMP/ATP ratio increased relative to the control in the groups of

Table 1. Changing the rearing frequency in rats with ROT-induced PD before and after MD administration (open field test was used)

Animal group	Rearing frequency
Control	14.1 ± 0.6
ROT 2.0 mg/kg	4.4 ± 0.3***
ROT + 1% DMSO	11.9 ± 0.5**,###
ROT + MD 0.5 mg/kg	12.0 ± 0,4**,###
ROT + MD 1.0 mg/kg	13.9 ± 0,5###, α, £££
ROT + MD 2.0 mg/kg	13.1 ± 0.5 ###, £££

Table 2. Effect of MD treatment on the incidence of locomotion in rats with ROT-induced PD (open field test was used)

Animal group	Incidence of locomotion
Control	35,9 ± 1,1
ROT 2.0 mg/kg	3,6 ± 0,2***
ROT + 1% DMSO	29,2 ± 0,4***, ###
ROT + MD 0.5 mg/kg	30,5 ± 0,9**, ###
ROT + MD 1.0 mg/kg	36,1 ± 0,9 ###, ααα, £££
ROT + MD 2.0 mg/kg	36,8 ± 1,0 ###, ααα, £££

ROT, ROT+1% DMSO and ROT+MD 0.5 mg/kg by 146, 69.2, and 69.2%, $P < 0.001$ in all the cases. The indicator returned to the control value after the administration of MD in doses of 1 and 2 mg/kg (Table 3).

Characterizing certain features of energy exchange that we discovered, it should be noted that the human brain is only 2-3% of the total body weight, but it monopolizes about 15% of the total blood flow, consuming 20% of all the oxygen inhaled by the lungs. A decrease in ATP production in certain areas of the brain or CNS can lead to the death of neurons in affected regions and thus cause ischemic or degenerative neuronal damage. Neurons require a large amount of energy to provide normal functioning and optimal regulation of Ca^{2+} to maintain their memdrane potential and action potential firing. PD, as a neurodegenerative disease, is characterized by metabolic disturbances that may be involved in the disease's development and progression [20]. Aggregation of α-synuclein, mitochondrial dysfunction, oxidative stress, and neuroinflammation that accompany this disease may be caused by or provoke energy crisis. Dopaminergic neurons have a high energy demand due to their long-distance neuronal projections and large number of synaptic connections, rhythmic operation, which makes them more sensitive to metabolic disturbances [2]. In organs that consume large amounts of energy, including the liver, kidney, heart, and brain, mitochondria are the main source of cellular ATP. Their role is vital for neurons, which have increased energy needs and are more sensitive to stress. Mitochondria play a central role in cellular Ca^{2+} homeostasis, regulation of redox processes, synthesis and oxidation of

Table 3. The influence of MD on the levels of purine metabolites in the plasma of rats with PD induced by ROT

Animal group	ATP	ADP	AMP	AMP/ATP	Xanthine	Hypoxanthine
Control	29.3±0.9	5.9±0.2	2.0±0.07	0.065±0.004	1.5±0.05	5.01 ± 0.10
ROT	19.4±0.4***	5.5±0.15	3.2±0.2 ***	0.16±0.01***	1.9±0.05***	7.12 ± 0.13***
ROT +1% DMSO	24.8 ± 0.65**	5.6±0.1	2.65±0.1***.#	0.11±0.005***. ###	1.8±0.05 ***. #	6.40 ± 0.25 ***. #
ROT + MD 0.5 mg/kg	24.0±0.6***. ###	5.4±0.2	2.3±0.1*. ###. α	0.11±0.005***. ###	1.8±0.05 ***. #	6.29 ± 0.17 ***. ###
Rot + MD 1 mg/kg	28.75±0.7###. ααα. £££	5.4±0.2	1.9±0.1 ###. ααα. £	0.07±0.003 ###. ααα. £££	1.7±0.07 **. ##. α	5.06 ± 0.15 ###. ααα. £££
Rot + MD 2 mg/kg	28.8±0.7 ###. ααα. £££	5.3±0.2*	2.2±0.1 ***. ###. α. £££	0.07±0.002 ###. ααα.£££	1.7±0.06*. #	5.07 ± 0.19 ###. ααα. £££

Note: Means ± S.E.M.; $n = 10$; *P < 0.05, **P < 0.01, ***P < 0.001 as compared with control rats; #P < 0.05, ##P < 0.01, ###P < 0.001 as compared with ROT; α P < 0.05, ααα P < 0.001 as compared with ROT+1% DMSO; £ P < 0.05, £££ P < 0.001 as compared with ROT+MD 0.5 mg/kg group. ROT, rotenone; DMSO, dimethyl sulfoxide; MD, methanindiazenone; PD, Parkinson's disease

fatty acids, apoptosis, information transmission, and other processes; they regulate cell growth and cell cycle [21]. Ca^{2+} ions play an important role in neuronal processes such as signaling pathway activation, transcriptional regulation, and initiation of synaptic transmission. It has been observed that during brain aging and the progression of neurodegenerative diseases, mishandling of mitochondrial Ca^{2+} occurs. A decrease in Ca^{2+} absorption by mitochondria is observed in old neurons, leading to violations of membrane potential and cytosolic Ca^{2+} increase during stimulation. Ca^{2+} regulation is crucial for dopaminergic neurons because of their stable and autonomous pacemaker function [22]. Dysregulation of Ca^{2+} not only disrupts synaptic transmission but also causes neuronal cell death. Thus, understanding the detailed pathophysiological mechanisms affecting the recently discovered mitochondrial Ca^{2+} regulatory mechanisms will help to identify new therapeutic targets. Many neurodegenerative diseases, including PD, are associated with an abnormal structure of mitochondria. Several pathogenic mechanisms have been proposed that contribute to neuronal apoptosis [23]. In most cases, the etiology of PD is unknown and complex. It has been proven that energy deficiency and a decrease in ATP levels are observed in PD [24]. A reduced cell ability to produce ATP is observed when the membrane potential of mitochondria and the activity of oxidative phosphorylation complexes decrease [25]. In neurodegenerative diseases, the level of ATP is one of the most important parameters representing the overall health of an individual cell, while a decrease in the amount of ATP is an important characteristic of dying cells. The authors' group recently published the results of studies related to the neuroprotective effect of a new synthetic drug JM-20 in rats with ROT-induced PD, both in vitro and in vivo models. JM-20 was created based on 1.5 benzodiazepine. As mentioned above, ROT causes serious defects in the activity of mitochondrial complex I, which causes their depolarization and disruption of Ca^{2+} regulation. Excessive mitochondrial Ca^{2+} uptake can cause nonselective permeability of the inner mitochondrial membrane, promoting mitochondrial swelling and dissipation of the membrane potential due to massive proton leakage. Recently, it was shown that in animals with PD induced by ROT or other toxins, JM-20 exhibited antioxidant properties, protecting neurons in the SNpc and acting at both synaptosomal and mitochondrial levels [6]. *In vitro*, it was established that the JM-20 antioxidant effect was associated with electron affinity due to its low intrinsic redox potential, similar to that of oxygen. These electrochemical characteristics endow JM-20 with the ability to absorb electrons from the mitochondrial transport chain or even compete with oxygen for electrons released from the mitochondria, thus preventing the formation of reactive oxygen species, which appear in large quantities under the influence of ROT. In *in vitro* experiments, drugs of a new family of 1,5-benzodiazepine-2(3H) also protected neurons under oxidative stress, restoring the functional state of mitochondria, significantly reducing the level of reactive oxygen species and mitochondrial superoxide, and restoring the membrane potential of mitochondria [7]. In our work, the treatment of rats with ROT led to a 33.8% decrease in the blood plasma level of ATP. At the same time, we observed signs of parkinsonism in experimental animals, such as head tremors, inhibition of movements, unstable gait, etc. Our results showed that the ATP concentration is normalized in animals with ROT-induced PD under the effect of MD. The exact mechanisms underlying the MD-induced improvement in purine metabolism observed here are currently unknown. However, as discussed above, benzodiazepine-like drugs protect neurons primarily at the mitochondrial level [26]. In the presented study, we also observed that ROT-treated rats lost body weight rapidly and showed an increased mortality rate. A possible reason for the decrease in body weight may be damage to neurons of the gastrointestinal tract due to the widespread

deposition of α-synuclein in neurons, strongly pointing at the involvement of this system in the pathogenesis of PD [27]. Our results indicate that we have successfully reproduced the PD model. We found that PD animals treated with MD rapidly gained weight, and mortality in such groups was virtually absent, this evidences its protective effects.

In our work, under the influence of ROT, we observed a sharp decrease in ATP synthesis in rats with PD, indicating an imbalance in the energy supply system of cells. Mitochondria are cellular powerhouses that supply more than 90% of cellular ATP to support neuronal life and functions such as axon growth and branching, generation of action potentials, and synaptic transmission. Mitochondria are also involved in short-term synaptic plasticity and the maintenance and regulation of neurotransmission by buffering presynaptic Ca^{2+}. Therefore, loss of mitochondria from axon terminals impairs synaptic transmission, probably due to reduced ATP supply [28]. Mitochondria, performing these critical tasks, become an integral part of the functioning and survival of neurons. The content of ATP and the adenyl system components' ratio determine the nature, intensity, and ways of ATP resynthesis and metabolism in general. ATP is a thermodynamically unstable molecule and, upon hydrolysis, forms ADP or AMP and phosphate residues, so this short-lived compound cannot accumulate in cells (one molecule exists for less than 1 min). Therefore, to maintain the regular vital activity of cells, ATP must be constantly synthesized *de novo* in quantities necessary to maintain the balance between synthesis and expenditure [29]. A reduction in the ATP level is probably due to a slowdown in its resynthesis as a result of the violation of the structural and functional organization of the respiratory chain components. A decrease in the enzymatic activity of the respiratory chain key components probably leads to inhibition of the breathing process and the efficiency of oxidative phosphorylation, which results in a decrease in ATP concentration. At the same time, in rats with simulated PD, we observed an increase in the blood plasma concentration of AMP, although the content of ADP did not change, indicating that synthesis of ATP from AMP is disturbed in PD. Resynthesis of the latter occurs normally, but further transformations become impossible. As a result, an energy imbalance occurs in the cells. Limited ATP synthesis can occur due to the effect of ROT, a potent inhibitor of mitochondrial complex I. A defect in this complex leads to a complete blockade of oxidative phosphorylation [30]. MD adjusted the level of both ATP and AMP, as evidenced by the AMP/ATP ratio normalization, which we determined in different groups. In this work, we analyzed the content of other components of the purine pathway. As it turned out, the levels of xanthine and hypoxanthine in rats with ROT-induced PD were significantly higher than in healthy animals (Table 3). The physiological end product of purine metabolism is uric acid (UA), which has strong antioxidant properties. Blood plasma urate levels are determined by dietary intake, renal excretion, and genetic factors affecting urate metabolism. Postmortem tissue studies have shown low urate levels in the substantia nigra in patients with PD. In animal models, UA has a neuroprotective effect on dopaminergic neurons due to the modulation of neuroinflammation and oxidative stress [31]. Based on our results, it can be assumed that in PD, an increase in hypoxanthine levels indicates a decrease in its conversion to UA efficiency. Therefore, it will be accumulated in the blood, and the amount of UA, on the contrary, will decrease. Our data are consistent with the results of other authors who observed an increase in the blood levels of hypoxanthine and a decrease in both its precursor inosine and its product UA in patients with PD, which indicates the blocking of this metabolic cascade. In mice with PD modeled by 6-OHDA, serum hypoxanthine levels were also increased, as was xanthine [32]. All these results together indicate the reliability of such a marker of PD as an increased level of hypoxanthine. More and more data is

accumulated in the scientific literature every year that the altered lipid metabolism is involved in the modulation of the PD pathogenesis. To study the effect of MD on lipid metabolism, we investigated the concentration of PL, CHOL, ECHOL, FFA, and TG in the blood plasma of intact and ROT-treated rats. The plasma PL content in rats with ROT-induced PD decreased by 22% ($P < 0.001$) compared to the control. In the ROT+1% DMSO and ROT+MD 0.5 mg/kg groups, the PL content was lower by 15.4 and 15.6%, respectively, compared to the control, in both cases $P < 0.001$. In rats administered MD in doses of 1 and 2 mg/kg, the blood plasma concentration of PL was restored to the level of control values. Between the groups ROT +MD 1.0 mg/kg, ROT MD 2.0 mg/kg and ROT, the difference in the indicator was 34.6 and 26.9%, respectively, in both cases $P < 0.001$ (Fig. 1). The brain has the second highest concentration of lipids, after adipose tissue. They participate in energy exchange, protein modification. The overall decrease in lipid levels, such as polyunsaturated fatty acids and PL in PD models, is likely due to excessive oxidative stress, considering that membrane PL are the major targets for free radicals. PL are the main components of biomembranes and therefore play an important role in cellular functions, including apoptosis, protection against oxidative damage, generation of second messengers, and regulation of enzyme activity [33].

In our work, biochemical analysis of the blood plasma showed that after the ROT administration the content of CHOL decreased by 18% compared to the control ($P < 0.001$). In the ROT+1% DMSO and ROT+MD 0.5 mg/kg groups, the amount of CHOL was lower compared to the control level by 12.5 and 10.5%, respectively, in both cases $P < 0.01$. These indicators were 7% ($P < 0.05$) and 9% ($P < 0.01$) higher than in the ROT group. On the other hand, we did not observe significant differences in the parameters of the control group in the rats of the 1.0 mg/kg ROT+MD and 2.0 mg/kg ROT+ MD groups (Fig. 2).

Like CHOL, the blood plasma content of ECHOL in ROT-treated rats also decreased by an average of 25% ($P < 0.001$). In all other groups, the indicators were approximately the same, that is, significantly lower than the control ones (Fig. 3). A decrease in the level of ECHOL is understandable, since ECHOL molecules are formed by esterification of CHOL molecules with the participation of free fatty acids, the amount of which, as we have shown, decreased dramatically under the influence of ROT.

All biochemical changes that occur in

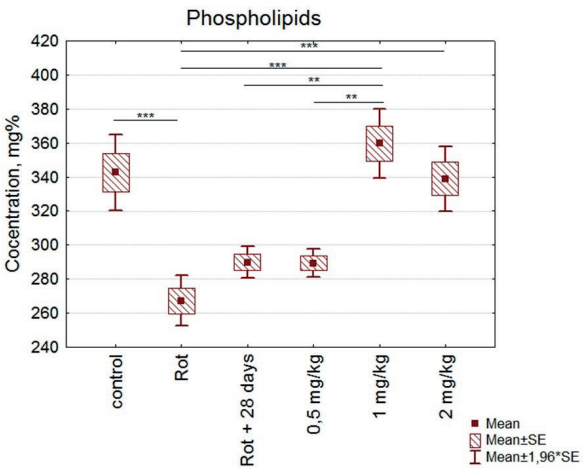

Fig. 1. Effect of MD on the concentration of PL in the plasma of rats with ROT-induced PD. Kruskal-Wallis test. **P < 0.01, ***P < 0.001 ($n = 10$)

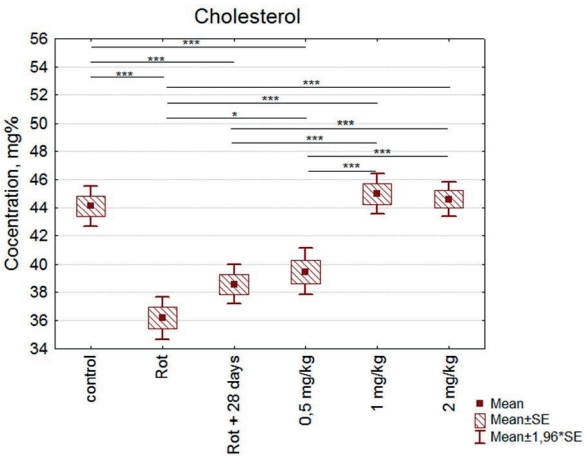

Fig. 2. The level of CHOL in the blood plasma of rats with ROT-modeled PD before and after MD administration. *P < 0.05, **P < 0.01, ***P < 0.001 ($n = 10$)

parkinsonism are reflected in the blood plasma biochemical parameters [34]. However, there is no diagnostic test for PD. The blood plasma changes in our study were mainly related to energy metabolism in the liver because this is the main control organ of lipid homeostasis. Lipid metabolism provides the energy necessary to support numerous biochemical transformations in the body. It is unclear whether the blood levels of total CHOL are related to PD. On the one hand, there is evidence that CHOL biosynthesis is significantly reduced in patients with PD; on the other hand, such patients show a high level of CHOL [34, 35]. CHOL is an indispensable component of cell membranes and plays a vital role in synthesizing myelin and maintaining proper synaptic functions. Brain CHOL constitutes a significant part of the total CHOL in the human body, and its content must be tightly regulated to ensure normal neuronal functioning. Changes in CHOL homeostasis can affect the structure and function of nerve cell membranes and their connections (synapses). Also, CHOL can act as an oxidant, playing a neuroprotective role and removing toxins from the body, so abnormal lipid metabolism can be involved in the occurrence and development of PD. Our results are consistent with those of Wanga et al. [35]. A decrease in the blood plasma amount of CHOL and ECHOL in ROT-treated rats indicates a significant inhibition of enzymes that ensure the biosynthesis and esterification of this compound in cells. Depleting CHOL in neurons is quite dangerous, as it can lead to degeneration of dendritic spines and synapses, disruption of synaptic vesicle exocytosis, neuronal activity, and neurotransmission [36]. Thus, MD may have great prospects in the future as an effective tool in the complex therapy of PD, as it contributed to restoration of the blood plasms levels of such important compounds as PL and CHOL in rats with PD.

As shown in Fig. 4, the blood plasma level of FFA in rats with PD (ROT group) significantly decreased by 28%, $P < 0.001$. In the ROT+1% DMSO and ROT+MD 0.5 mg/kg groups, concentration of FFA decreased compared to the control values by 17 and 18%, respectively, $P < 0.001$ in both cases. However, in comparison with the ROT group, the studied parameters were higher by 15.3% ($P < 0.001$) and by 13.9% ($P < 0.05$), respectively.

As shown in Fig. 5, in the ROT group, the decrease in plasma TG was 33% ($P < 0.01$) compared to the control. Similarly, in the ROT+1% DMSO and ROT+MD 0.5 mg/kg groups, we observed a decrease in plasma TG levels by 32.3% ($P < 0.01$) and 30% ($P < 0.05$), respectively. In the ROT+MD 1.0 mg/kg group, the blood plasma amount of TG was 23.7%

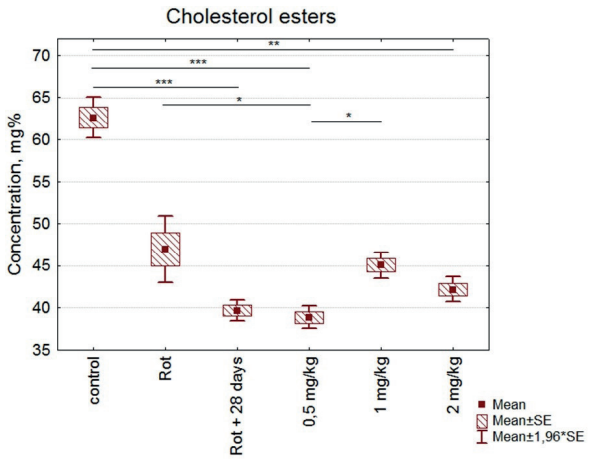

Fig. 3. Blood plasma concentration of ECHOL in rats with PD modeled by ROT before and after MD-treatment. *P < 0.05; ***P < 0.001 (n = 10)

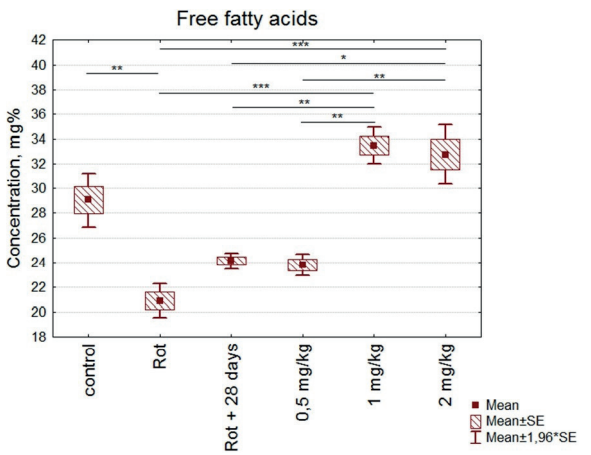

Fig. 4. Influence of MD on the level of FFA in the blood plasma of rats with ROT-induced PD. *P < 0.05, **P < 0.01, ***P < 0.001

(P < 0.01) higher than in the ROT group.

In the scientific literature, experimental data confirm low levels of blood TG in patients with PD [36]. In the brain, TG can be converted to FFA and ketones, which are highly efficient energy sources for mitochondria in neurons. TG can rapidly cross mitochondrial double membranes and affect mitochondrial function and metabolism. A possible mechanism of TG impact on PD may be related to coenzyme Q10. This enzyme is a carrier of electrons in complexes I and II in mitochondria, and it is also an antioxidant. Low levels of coenzyme Q10 can affect the function of the mitochondrial respiratory chain and cause degeneration of dopaminergic neurons. As the main carrier of coenzyme Q10, TG may be involved in the pathogenesis of PD by influencing the antioxidation of coenzyme Q10 and improving mitochondrial function. It is possible that ROT acts as a chemical stress agent, forcing it to mobilize all metabolic resources for survival, which may be evidenced, in particular, by a significant decrease in the blood plasma level of FFA and TG in rats administered with ROT. After all, these compounds can be quickly used for biosynthesis of high-energy metabolites, in particular ATP. The synthesized drug methanindiazenone to a certain extent can inhibit the development of stress reactions and ensure the normalization of metabolism in the body.

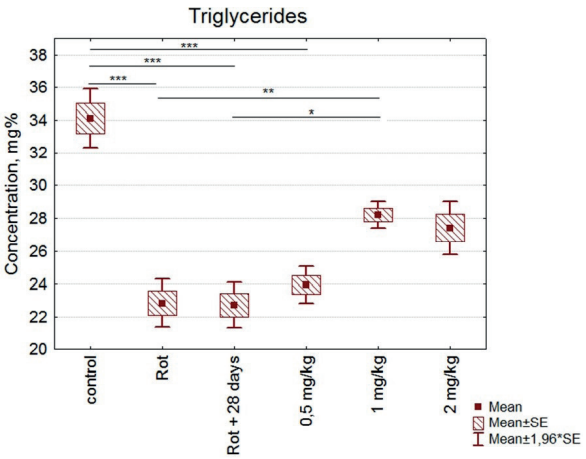

Fig. 5. Effect of MD on the concentration of TG in the blood plasma of ROT-treated rats. *P < 0.05, **P < 0.01, ***P < 0.001

CONCLUSIONS

In this study, we have shown that the metabolism of blood plasma lipids and components of the adenyl system is significantly altered in rats with ROT-induced PD. A decrease in the blood plasma levels of PL, CHOL, ECHOL, FFA, TG, and ATP positively correlates with symptoms of tremor, bradykinesia, unstable gait, etc. CHOL is an essential component of the structure of neurons, so a low level of this compound and other lipid components in the blood can be associated with an increased risk of developing PD. Under the influence of MD, we observed a significant improvement in the motor function of parkinsonian rats and normalization of energy and lipid metabolism. The obtained results show that MD in a dose of 0.5 mg/kg was ineffective, while in doses of 1.0 and 2.0 mg/kg its influence on lipid and energy metabolism indicators was significant. Overall, regulating blood lipids and components of the adenylate system in the blood may be an important component in treating patients with PD, and MD may be recommended for further research on its use in the complex therapy of Parkinson's disease.

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The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ПУРИНОВИЙ ТА ЛІПІДНИЙ ОБМІН У ЩУРІВ З РОТЕНОНОВОЮ МОДЕЛЛЮ ХВОРОБИ ПАРКІНСОНА ПІД ВПЛИВОМ МЕТАНІНДІАЗЕПІНОНУ

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Метою нашого дослідження було оцінити вплив нового похідного бензодіазепіну – метаніндіазенону (МД; внутрішньоочеревинно) на вміст пуринових метаболітів і ліпідів у плазмі крові щурів з ротеноновою моделлю хвороби Паркінсона. Концентрацію АТФ, АДФ, АМФ, ксантину, гіпоксантину, фосфоліпідів, холестерину, ефірів холестерину, вільних жирних кислот (ВЖК) і тригліцеридів кількісно визначали у зразках плазми крові методом тонкошарової хроматографії. Отримані нами результати свідчать про те, що під впливом ротенону співвідношення АМФ/АТФ у плазмі крові збільшувалось у 2,5 раза порівняно з контролем, а при МД у дозі 1 і 2 мг/кг цей показник знижувався до нормальних значень. Ротенон також підвищував відносно контролю вміст ксантину та гіпоксантину на 26,7 і 42,4% відповідно. МД відновлював вміст ксантину на 86,7% від рівня контролю та достовірно знижував вміст гіпоксантину до норми. Крім того, ротенон знижував у плазмі крові вміст фосфоліпідів, холестерину, ефірів холестерину, вільних жирних кислот (ВЖК) і тригліцеридів на 22, 18, 25, 28, 33% відповідно. Під впливом МД такі показники, як концентрація в плазмі фосфоліпідів, холестерину, ВЖК поверталися до рівня контролю. Таким чином, МД покращує як метаболізм пуринів, так і ліпідів в організмі щурів з ХП, викликаною дією ротенону.
Ключові слова: хвороба Паркінсона; ротенон; похідні бензодіазепіну; плазма крові; пурини; ліпіди.

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