EXPERIMENTAL WORKS

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OXIDATIVE STRESS IN RAT HEART MITOCHONDRIA UNDER A ROTENONE MODEL OF PARKINSON' DISEASE: A CORRECTIVE EFFECT OF CAPICOR TREATMENT

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Biochemical and genetic mechanisms of oxidative stress (OS) developing in rat heart mitochondria were studied in a rotenone model of Parkinson's disease (PD), and the effect of Capicor (combination of meldonium dihydrate and gamma-butyrobetain dihydrate) on these mechanisms was evaluated. Experiments were carried out on adult male Wistar rats: I – intact rats (control); II – with rotenone administration subcutaneously at dose 3 mg/kg per day along 2 weeks; III – with rotenone/Capicor administration: after rotenone intoxication, capicor was injected intraperitoneally at dose 50 mg/kg per day along following 2 weeks. As OS biomarkers, lipid peroxidation, protein oxidative modification, H_2O_2 production, the activity of MnSOD, GPx and glutathione pool indexes were measured. The PD-related genes Parkin (PARK2) and DJ-1 (PARK7) as well as MnSOD and DJ-1 protein expressions were detected. Rotenone intoxication increased the intensity of lipid peroxidation, protein oxidative modification, and H_2O_2 production. These events were accompanied by decreased in GSH content, GSH/GSSG ratio, and GPx activity. Increased ROS production and impaired antioxidant defenses could result from the established DJ-1 gene and DJ-1 protein deficiency. Capicor administration increased the endogenous antioxidant defense, weakening the lipid peroxidation and oxidative modification of mitochondrial proteins. Capicor treatment led to an increase in GSH content and GSH/GSSG ratio in heart mitochondria that may serve as additional indicators of the OS intensity reducing. Capicor promoted overexpression of DJ-1 and PARK2 genes in the heart that may indicate a rise in mitophagy and a decrease in OS.

K e y w o r d s : oxidative stress, rotenone, rat heart mitochondria, PARK2, PARK7, capicor.

Oxidative stress (OS) is caused by an imbalance in the redox state of the cell either by overproduction of reactive oxygen species (ROS), predominantly in dysfunctional mitochondria, or by impairment of the antioxidant systems. Accumulating evidence suggest that OS may play a significant role in pathogenesis of neurodegenerative diseases [1]. Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide, it is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta and subsequent striatal dopamine depletion. An epidemiological study suggested a link between the use of pesticides and the risk of developing PD in rural areas. In rodents, one pesticide-rotenone - reproduces several features of PD, including nigrostriatal dopaminergic degeneration and typical alpha-synuclein-positive intracytoplasmic inclusions in the brain [2]. Rotenone inhibits the complex I (NADH-quinone oxidoreductase) of the electron transport chain, resulting in an enhanced ROS generation (i.e.,hydroxyl radicals, superoxide

anion radical) and a decrease in energy supply (ATP production) [1, 3]. Complex I inhibition causes an overproduction of ROS, leading to oxidation of lipids, proteins, and DNA and it finally triggers to cell death. Furthermore, chronic administration of rotenone has been demonstrated convincingly to produce nigrostriatal dopaminergic neurodegeneration as well as behavioral, biochemical, neurochemical and neuropathological symptoms of PD in rats [4]. Many lines of evidence have demonstrated complex I deficiency and ROS-mediated impairments in the cortical brain tissue, frontal cortex, striatum, skeletal muscle, and platelets of patients with Parkinson's disease whereas relatively few studies to date have established the mechanisms of OS in PD heart [1, 2, 4]. At the same time, PD is a well-recognized risk factor for developing heart failure, and cardiovascular complications are the important cause of PD-related morbidity and mortality. Increased incidence of heart failure in patients with PD persisted despite correction for age, hypertension, and coronary artery disease [5]. The pathogenesis of cardiac disturbances in PD is multifactorial, but more studies are required to characterize the role of increased oxidative stress in the development and the progression of cardiovascular complications.

The identification of a number of PD-related genes that are strongly associated with mitochondrial function (PINK 1, DJ-1, Parkin) further adds weight that mitochondrial dysfunction with resultant OS is a primary event in PD pathogenesis [6]. Loss of function of DJ-1 (PARK7) results in OS, and DJ-1 exerts neuroprotection via its antioxidant mechanisms in mitochondria. DJ-1 is known to be present in the heart and only recently its role in this tissue has been investigated. Thus, Billia et al. have shown that neonatal murine cardiomyocytes overexpressing DJ-1 were protected against cell death induced by oxidative stress, whereas cardiomyocytes deficient in DJ-1 were more susceptible to ROS induced apoptotic cell death [7]. In mice, loss-of-function mutations in Parkin (PARK2) cause mitochondrial dysfunction and oxidative damage, and this seems to precede neurodegeneration within the substantia nigra. In addition, PARK2 had been shown to regulate mitochondrial fission and fusion in the heart. Interestingly, a compound mdivi-1 that specifically regulates mitochondrial dynamics (pro-fusion) and acts via Parkin has been tested in multiple studies, mainly concerned with reduction of infarct size and cardiac cell death in ischemic-reperfusion models [8].

Regarding the correction of oxidative stress induced by rotenone administration, there was recently shown that treatment of rats with Capicor (combination of Meldonium dihydrate and gamma-butyrobetain dihydrate, Olainpharm, Latvia) led to reduction of OS in the brain and liver mitochondria [3, 9]. There is no information to date about such effect of Capicor on cardiac cells under modeling of PD.

This study was therefore designed to investigate the biochemical and genetic mechanisms of OS developing in the rat heart as well as the corrective effect of Capicor on these mechanisms in a rotenone model of PD.

Materials and Methods

Animals and study design. Experiments were conducted on 24 adult male Wistar rats with 230-250 g mean body weight. The rats were divided into groups of 8 in each: I – intact rats (control); II – rotenone was injected subcutaneously at dose 3 mg/kg per day along 2 weeks; III – after rotenone administration, Capicor was injected intraperitoneally at dose 50 mg/kg per day along 2 weeks. The animals were treated according to European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and approved by the bioethics committee of the approved by the Ethics Commission on Animal Experiments of Bogomoletz Institute of Physiology, NAS of Ukraine (protocol No 00576-V, date 25.05.2019)

Mitochondria isolation. Rat heart mitochondria were isolated by differential centrifugation. Heart was collected in isolation medium A (250 mM sucrose, 10 mM Tris/HCl (pH 7.6) and 1mM EGTA) and homogenized. After centrifugation of the homogenate at 1000 g for 5 min, the supernatant was strained on gauze and recentrifuged at 12 000 g for 15 min. The resulting pellet was resuspended in ice-cold isolation medium B (250 mM sucrose, 10 mM Tris/HCl (pH 7.6) and 0.1 mM EGTA) and a new series centrifugation was performed. The final washing and resuspension of mitochondria was in the medium B without EGTA. Mitochondrial protein concentration was estimated by the Lowry method, using bovine serum albumin as a standard. The mitochondrial preparations were analyzed after solubilization in 0.5% deoxycholate for 60 min at 0-4°C. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Oxidative stress biomarkers assays. Lipid peroxidation in isolated mitochondria was measured from the formation of thiobarbituric acid - reactive substances (TBARS) using the method [10].

Protein carbonyls were detected by their reaction with 2,4-dinitrophenylhydrazine (DNPH) leading to formation of protein hydrazones [11]. The absorbance of the samples was measured at 370 nm. Carbonyl contents were calculated using the molar extinction coefficient of DNPH, $\varepsilon = 22000 M^{-1}cm^{-1}$.

H_2O_2 level was measured by the FOX method, based on the peroxide-mediated oxidation of Fe^{2+}, followed by the reaction of Fe^{3+} with xylenol orange [12]. Absorbance of the Fe^{3+}-xylenol orange complex (A 560) was detected after 45 min. Hydrogen peroxide content was determined against calibration plot and calculated per 1 mg of mitochondrial protein.

Enzymatic assays. Activity of selenium-dependent glutathione peroxidase (GPx)(EC 1.11.1.9), was determined according to the method [13]. Briefly, the reaction mixtures consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1mM NaN_3, 0,2 mM NADPH, 1 mM GSH, 0,25mM H_2O_2, 226 U/ml glutathione reductase, and rates of NADPH oxidation followed at 340 nm.

Manganese superoxide dismutase (MnSOD) (EC 1.15.1.1) activity was measured by the method [14], which is based on the inhibition of autooxidation of adrenaline to adrenochrome by SOD contained in the examined samples. The samples were preincubated at 0°C for 60 min with 6 mM KCN, which produces total inhibition of Cu, ZnSOD activity. The results were expressed as specific activity of the enzyme in units per mg protein. One unit of SOD activity being defined as the amount of protein causing 50% inhibition the conversion rate of adrenaline to adrenochrome under specified conditions.

Glutathione content assays. Total glutathione – the sum of reduced glutathione (GSH) and oxidized glutathione (GSSG) − was determined by a method where glutathione is extracted from the heart mitochondria with 5% ice-cold-sulfosalicylic acid and after neutralization with triethanolamine sequentially oxidized by DTNB (0.6 mM) and reduced by NADPH (0.3 mM) in the presence of glutathione reductase (2 U/ml) [15]. For determination the GSSG alone, the GSH presented in solutions was derivatized by incubation with 2 µl 2-vinilpyridine at 4°C for 1 h. The rate of 2-nitro-5-thiobenzoic acid formation was monitoring at 412 nm and compared to a standard curves made with GSH and GSSG, respectively. The GSH concentration is calculated as total glutathione – 2 x [GSSG].

Western Blot Analysis. Isolated mitochondrial protein extracts (100µg) were separated on SDS-polyacrylamide gel (12%) and transferred to a polyvinylidene fluoride membranes by semi-dry electrophoretic transfer. The membranes were then blocked with 5% nonfat dry milk in Tris Buffered Saline Tween-20 (TBST) buffer (50 mM Tris-HCl, 150 mM/l NaCl, and 0.1% Tween, pH 7.4) for 1 h at 37°C. MnSOD and DJ-1 proteins were detected using primary monoclonal antibody for MnSOD (Sigma-Aldrich, USA) at a dilution 1:1000 for 2 h at 37°C; DJ-1 (Sigma-Aldrich, USA) 1:500; β-Actin 1:1000 (Santa Cruz Biotechnology, Inc) followed by incubation with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich, USA) (1:2000) for 1 h at 37°C. Each antigen-antibody complex was visualized by amino-ethylcarbazol reaction. The band intensities were quantified by densitometry with a computerized image processing system (Gel-Pro Analyzer). β-Actin was used as a loading control. Results were expressed as percentages of control values.

Genetic assays. Isolation of total RNA from the rat heart homogenate was carried out according to the phenol-chloroform extraction method using Sigma reagents (USA). Semi-quantitative reverse transcription was performed using cDNA synthesis kits, containing the reverse transcriptase "RevertAid H Minus M-MuLV RT" (Thermo Scientific, USA). The transcriptional mixture contained 5 µl of total RNA (500 ng - 1 µg/µl), 1 µl of Random Hexamer primers (0.5 µg/µl), 20 units of ribonuclease inhibitor, 20 mM deoxyribonucleotide mixture and 200 units of reverse transcriptase. PCR was performed in the Applied Biosystems 2700 (Perkin Elmer, USA) thermocycle according to individual programs for each gene. Real-time PCR was performed in the "7500 Fast Real-Time PCR System" thermocycler. For the PARK 2 and DJ-1 genes the same primers as for the semi-quantitative PCR were used: PARK 2 – forward 5′-CTGGCAGTCATTCTGGACAC-3′, reverse 5′-CTCTCCACTCATCCGGTTTG-3′; DJ-1 – forward 5′-CGATGTGGTTGTTCTTCCAG-3′, reverse 5′-GCCGTTCATCATTTTGTCCT-3′. Genes expression was standardized according to the ribosomal subunit GADPH gene expression (primers of the following sequence were used: forward 18S-F5′-CTT AGA GGG ACA AGT GGC G-3′ and reverse 18S-R5′-GGA CAT CTA AGG GCA TCA CA-3′),

and the actin β-subunit as an endogenous control. PCR amplification was carried out in 10 µl SYBR Green PCR Master Mix containing 30 pM of each primer. The volume was brought to 20 µl deionized water. The amplification program began with the previous activation of AmpliTaq Gold® DNA polymerase for 10 min at 95°C and consisted of 50 cycles: denaturation - 95°C, 15 s, primer attachment and elongation - 64°C, 1 min. For control of specificity, a dissociation step was performed - a consistent temperature rise from 64 to 99°C, with the recording of a decrease in the fluorescence intensity of double-stranded DNA complexes with SYBR Green.

Statistical analysis for biochemical assays and Western Blot Analysis. Data are expressed as mean ± SD. The differences among multiple experimental groups were detected by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. A *P* value of less than 0.05 was considered as significant. Statistical analysis of the gene expression results was performed using 7500 Fast Real-time PCR, SPSS Statistics (Version 17) and Microsoft Excel 2003 Software with the application of Student's *t*-test. They are presented as $M \pm m$. The differences between the average values were considered statistically significant at $P < 0.05$.

Results and Discussion

It was found that rotenone administration significantly increased the intensity of lipid peroxidation in heart mitochondria (Fig. 1). We registered the TBARS accumulation (by 33%, $P < 0.05$), which are the secondary products of lipid peroxidation. Moreover, protein carbonyl content and H_2O_2 production were significantly elevated by 67 and 62%, respectively, as compared to control ($P < 0.05$) (Fig. 1).

To estimate pro- and antioxidant balance in heart mitochondria, we investigated the activity of superoxide anion - scavenging enzyme MnSOD and H_2O_2- removing enzyme GPx. Rotenone administration caused a significant increase in the MnSOD activity by 58% ($P < 0.05$) with concomitant decrease in the activity of GPx by 23% ($P < 0.05$) in comparison with the control values (Fig. 2).

Simultaneously, we have found an increase in GSSG level by 27% ($P < 0.05$), a decrease in GSH content by 36% ($P < 0.05$), and at that the ratio of reduced to oxidized form was 2 times less than the control value ($P < 0.05$) (Fig. 3). These changes were accompanied by an increase in MnSOD and a decrease in DJ-1 protein synthesis by 68 and 53%, respectively, as compared to control ($P < 0.05$) (Fig. 4).

Capicor treatment (group III) induced a decrease in TBARS content as well as in H_2O_2 production and protein carbonyls level by 20, 23 and 22%, respectively, in comparison with rotenone-only treated rats (group II) ($P < 0.05$) as shown in Fig. 1. At the same time, in heart mitochondria Capicor treatment inhibited the hyperactivity of MnSOD by 20% ($P < 0.05$), nevertheless activity of this enzyme remained higher than the control level by 27% ($P < 0.05$). Moreover, GPx activity was enchanced to control level (Fig. 2). Capicor co-treated group showed significantly increased level of GSH by 46% ($P < 0.05$) and decreased content of GSSG by 14% ($P > 0.05$), when compared to rotenone-only treated

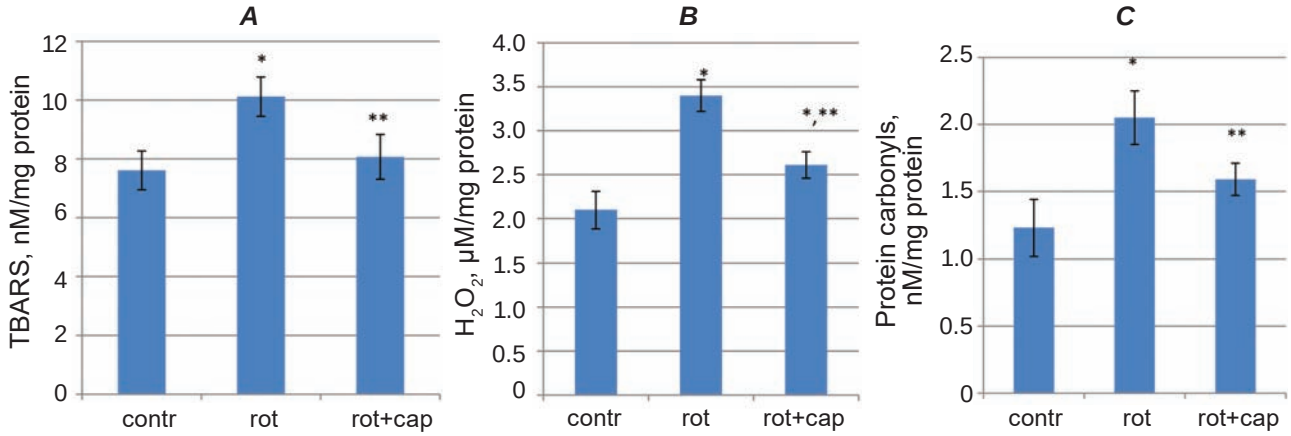

*Fig. 1. Effect of capicor administration on oxidative stress markers: TBARS (**A**), H_2O_2 (**B**), and Protein carbonyls (**C**) content in the heart mitochondria isolated from the rotenone treated rats. Values are means ± SD (n = 8). The data were analyzed for statistical significance using ANOVA followed by the Bonferroni post hoc test. *P < 0.05 vs control group; **P < 0.05 vs rotenone treated group*

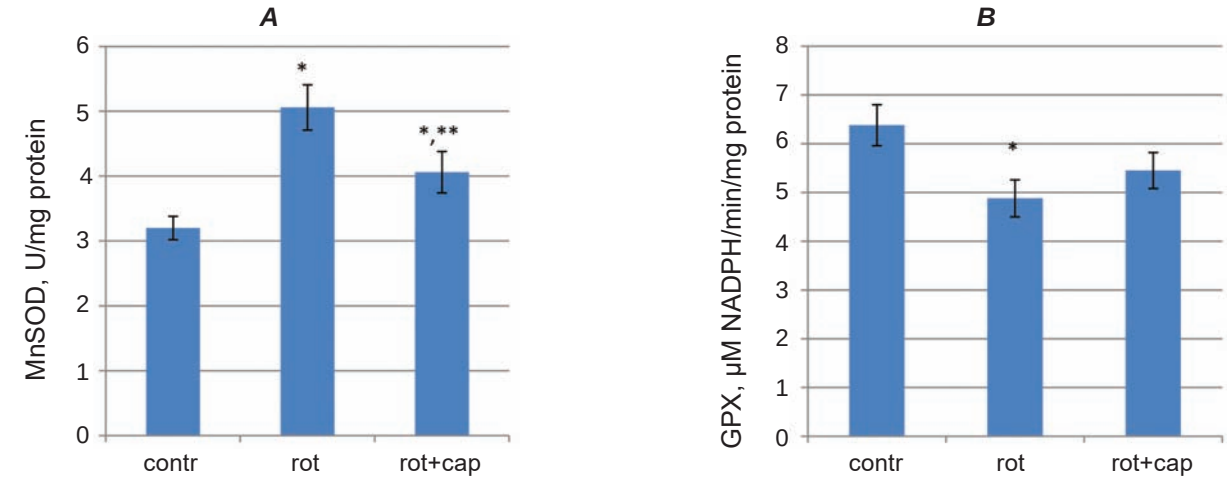

*Fig. 2. Effect of capicor administration on activity of MnSOD (**A**) and GPx (**B**) in the heart mitochondria isolated from the rotenone treated rats. Values are means ± SD (n = 8). The data were analyzed for statistical significance using ANOVA followed by the Bonferroni post hoc test. *P < 0.05 vs control group; **P < 0.05 vs rotenone treated group*

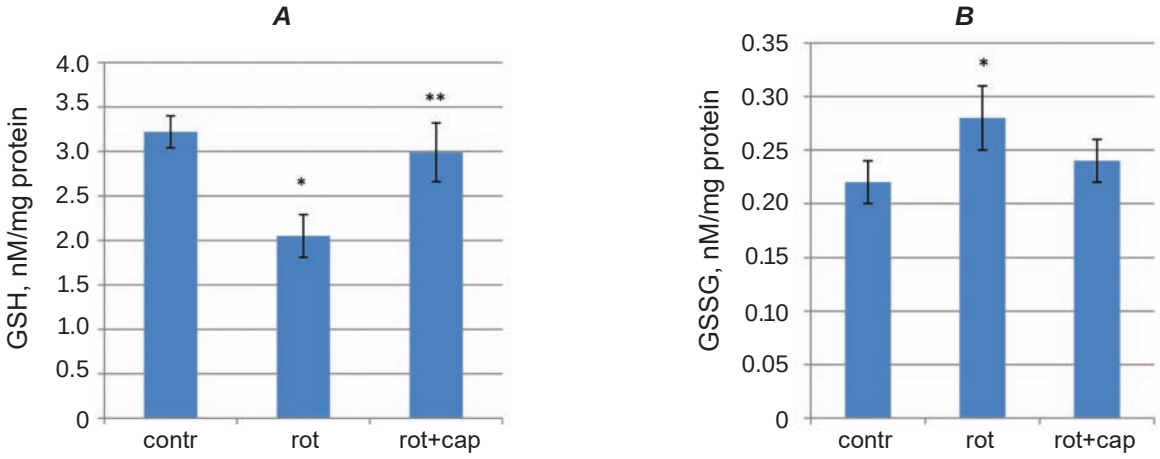

*Fig. 3. Effect of capicor administration on content of GSH (**A**) and GSSG (**B**) in the heart mitochondria isolated from the rotenone treated rats. Values are means ± SD (n = 8). The data were analyzed for statistical significance using ANOVA followed by the Bonferroni post hoc test. *P < 0.05 vs control group; **P < 0.05 vs rotenone treated group*

group (Fig. 3). The protein level of MnSOD in heart mitochondria was kept at control level, at the same time the protein DJ-1 content had a tendency to increase in comparison with rotenone-only treated group (Fig. 4).

The results of the study of mRNA expression of Parkin (PARK2) in the hearts of Wistar rats following the rotenone injection and capicor administration are presented in Fig. 5, *A*. The level of mRNA PARK2 expectedly increased threefold ($P < 0.05$) compared to control after the introduction of rotenone, since PARK2 is E3 ubiquitin ligase, and the introduction of rotenone causes damage to proteins and other molecules and, accordingly, provides a large amount of substrates for ubiquitination. With the addition of capicor, the expression of the *PARK*2 gene increased 10 times ($P < 0.05$) compared to control, which indicates an increase in proteasomal proteolysis and a decrease in oxidative stress. In addition, these data are fully consistent with those obtained in patients with PD [16].

The level of mRNA expression of DJ-1 (Fig. 5, *B*) in the rat heart was reduced by the introduction of rotenone 11 times ($P < 0.05$) compared to control, which may indicate a predominance of prooxidant processes and significant damage to mitochondria.

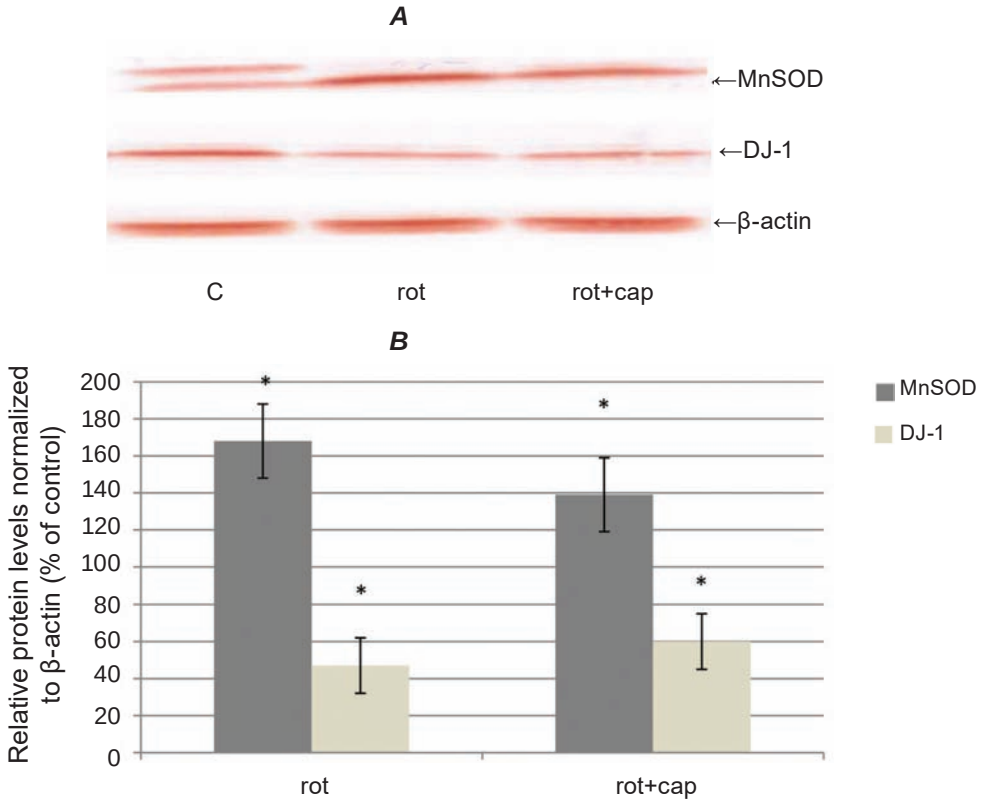

*Fig. 4. Changes in the MnSOD and DJ-1 protein expressions in the heart mitochondria after capicor administration and rotenone treatment. (**A**) Representative western blot and (**B**) densitometric analysis of protein levels. Protein extracts were separated by performing SDS PAGE and subsequently electroblotted onto PVDF membranes. Final western blot figured as the histogram is expressed as means ± SD over control values from three independent experiments. The control values are taken as 100%. Statistically significant differences are indicated as *P < 0.05 vs control; **P < 0.05 vs rotenone treated rats*

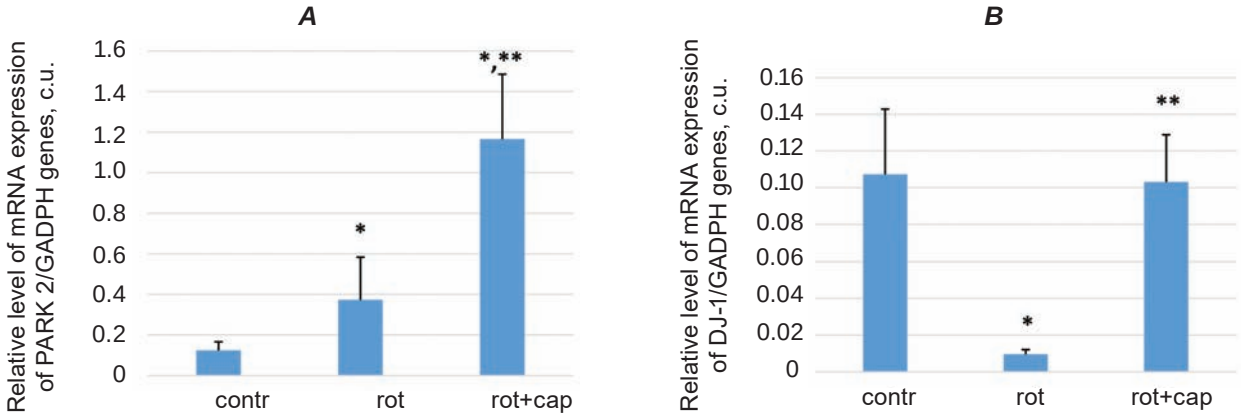

*Fig. 5. Relative level of mRNA expression of PARK 2 (**A**) and relative level of mRNA expression of DJ-1 (**B**) in the rat heart under rotenone treated rats and after capicor administration. Values are means ± SD (n = 8). *P <0.05 vs control; ** P <0.05 vs rotenone treated rats*

With the introduction of capicor, the mRNA expression level of DJ-1 returned to control value, which is an indicator of the restoration of pro- and antioxidant balance.

So, in the present study, rotenone administration triggers a series of events including increases in TBARS and H_2O_2 production, which ultimately leads heart mitochondria to dysfunctional state.

Our findings correlate well with the earlier studies showing that rotenone differentially enhanced the level of oxidative markers in both *in vivo* and *in vitro* models of PD. Such effect might be attributed to the inhibitory effect of rotenone on mitochondrial complex I which results in defects in oxidative phosphorylation and excessive ROS production [2, 3, 9]. It has been found that mitochondrial complex I proteins tend to be nitrated by peroxynitrite more easily than other mitochondrial component proteins and mitochondrial defects in complex I activity in PD have been reported [1].

In heart mitochondria, we observed enhanced protein carbonyl content which served as a biomarker of general oxidative stress [1]. This oxidative protein damage considered was provoked by ROS, RNS and plays a significant role in pathological conditions such as PD [2]. Radical-mediated proteins damage may be initiated by electron leakage, metal-ion dependent reactions, and autooxidation of lipids and sugars. Oxidative modification of proteins are realized via various mechanisms: direct oxidation of amino acid side chains, modification of side chains with lipid peroxidation products (malondialdehyde, acetaldehyde, and 4-hydroxy-2-nonenal), or with products of glycation and glycoxidation [17]. All these mechanisms introduce a carbonyl group into a protein that changes its function, increases chemical fragmentation and enhances susceptibility to proteolytic attack [11, 17]. This intensification of prooxidant processes in heart mitochondria was accompanied by an increase in GSSG and decrease in GSH contents as well as in GSH/GSSG ratio, which are additional indicators of oxidative stress and mitochondrial dysfunction [18]. Therefore oxidative stress, mitochondrial complex I dysfunction, disbalance in pro/antioxidant system, proteosome inhibition and activation of an apoptotic signal are believed to play a key role in arise and development of neurodegenerative pathologies [1, 2].

In the present study, the prolonged rotenone intoxication in parallel with the peak of ROS generation progressively increased the MnSOD activity and protein expression in heart mitochondria. This can be explained by a compensatory increase in the activity of this enzyme in response to the increased superoxide anion production, which is known serves as a substrate for MnSOD. The induction of MnSOD under such conditions could be considered as a defensive reaction to the excess production of active oxygen metabolites, which, in turn, could activate the expression of antioxidant enzymes via different signaling pathways [18]. Indeed, MnSOD is known as an inducible enzyme that may be activated in a variety of stressful conditions [19]. Similar increases in the activity and expression of MnSOD protein in response to the development of oxidative stress of different genesis were also reported by other researchers [20]. Moreover, changes in the GSH/GSSG ratio, following the rotenone intoxication, can regulate H_2O_2 formation, alter protein redox status and in that way modulate many signaling pathways including the activation of NF-kB. Theoretically, the activated NF-kB p65 can be translocated to the nucleus to promote the transcription of the target genes including pro-survival MnSOD, Bcl-2, etc.[19, 20].

We can assume that drugs upregulating the activity of MnSOD and its protein expression may suppress OS in mitochondria due to at least two mechanisms: one being a decrease in superoxide and peroxynitrite production and other being an increased removal of H_2O_2 as a result of the increased GPx activity [1, 18]. Indeed, we have previously demonstrated that Capicor administration acts in such manner in liver and brain of rats with rotenone-induced PD [3, 9].

But, our present experimental data showed that rotenone-induced overexpression of MnSOD in heart mitochondria without concomitant increase in the level of GPx results in the accumulation of H_2O_2 that not only changes the mitochondrial redox status but also can participate in the Fenton reaction, leading to production of noxious hydroxyl radicals [18, 20]. These findings confirm that rotenone-induced oxidative stress leads to an imbalance in the antioxidant system of the heart mitochondria and as consequence to mitochondrial dysfunction.

As, showed our experiments, Capicor treatment led to a weakning of rotenone-induced “burst” of oxidative processes in rat heart mitochondria decreasing the intensity of lipid peroxidation and oxidative modification of mitochondrial proteins as well as ROS production. Besides that, Capicor administration restored the impaired balance between mitochondrial antioxidant defense systems inhibiting the hyperactivity of MnSOD and increasing the GPx activity. It is worth noting that Capicor administration led to a restoration of perturbed glutathione pool in heart mitochondria under rotenone intoxication.

In our study, we showed that the prolonged systemic rotenone administration caused a decrease in DJ-1 protein content and *DJ-1* gene expression in

heart mitochondria. Parkin, PTEN-induced putative kinase 1 (PINK1) and DJ-1 are genes that code for proteins that are crucially involved in mitochondrial function and resistance to oxidative stress and have been linked with PD[7]. Numerous reports demonstrated that loss of DJ-1 leads to mitochondrial fragmentation, impaired dynamics, induced oxidative stress, and autophagy [6-8]. In fact, DJ-1 was found to stabilize NFE2L2 by preventing its association with KEAP1 and subsequent NFE2L2 degradation. Therefore, DJ-1 mutations could lead to the disregulation of NFE2L2 and disturbance of antioxidative defense in PD [21]. It was found that overexpression of DJ-1 protein improved tolerance to OS by selectively upregulating the rate-limiting step in glutathione synthesis [22]. In our research with the introduction of Capicor following rotenone administration, the levels of the DJ-1 mRNA and protein expression in the rat heart returned to control value, which is an indicator of the restoration of pro- and antioxidant balance. Importantly, drugs that upregulate *DJ-1* gene expression may slow the progression of PD by moderating OS and alpha-synuclein aggregation [7]. On the contrary, the loss of DJ-1 protein results in mitochondrial dysfunction and can be rescued by PARK2 overexpressione in PD brain [8, 21].

It has been long recognized that *PARK2* gene is highly involved in appearance and development of Parkinson's disease [23]. At the same time, this PD-related gene had also been proven to influence mitochondrial bioenergetics and dynamics, including membrane potential, respiratory activity, cristae structure, calcium homeostasis, mitochondrial DNA integrity, and clearance of dysfunctional mitochondria [7, 16, 23]. Thus, it was logical to assume that changes in the *PARK2* gene expression level can be an indicator of the oxidative damage or restoration of mitochondrial structure and metabolism under modeling of PD. We have shown that the level of mRNA PARK 2 in the rat heart increased after the introduction of rotenone. It was already mentioned that PARK 2 is a multifunctional E3 ubiquitin ligase which is able to perform a variety of ubiquitin linkages associated with numerous cellular functions. Introduction of rotenone causes oxidative damage to proteins, lipids and DNA and, accordingly, provides a large amount of substrates for ubiquitination and rise of *PARK2* gene expression. Several studies have highlighted for PARK2 a pivotal role in mitochondrial homeostasis and dynamics. In association with PINK1, PARK2 acts in mitochondrial fission and fusion, mitochondrial transport and removal of damaged mitochondria through mitophagy [23-25]. So, over expression of *PARK2* gene in the rat heart following Capicor administration may indicate a rise in mitophagy and a decrease in oxidative stress [25]. In our opinion, Capicor is the promising agent that appears to increase Parkin and DJ-1 functions and had antioxidant effects in the heart in a rotenone model of PD. Future experiments in relevant animal models and clinical trials will tell us more about its therapeutic benefit under cardiovascular complications of PD.

Conclusions. Prolonged systemic rotenone administration induced OS developing in rat heart mitochondria: in witness of that was an increase in intensities of lipid peroxidation, protein oxidative modification, and H_2O_2 production as well as a decrease in GSH content, GSH/GSSG ratio, and GPx activity. Rotenone-induced OS in heart mitochondria was accompanied by an imbalance in their antioxidant systems (superoxide anion and H_2O_2 – removing enzymes – MnSOD and GPx) that may lead to production of noxious hydroxyl radicals. Increased ROS production and impaired antioxidant defenses in the heart under rotenone administration could result from the established DJ-1 gene and DJ-1 protein deficiency. Capicor reduced rotenone-mediated damage of rat heart mitochondria increasing the action of protective proteins of endogenous antioxidant defense and restorating impared balance between mitochondrial antioxidant systems against a background of the oxidative modification of mitochondrial proteins weakening. Capicor treatment led to an increase in GSH content and GSH/GSSG ratio in heart mitochondria that may serve as additional indicators of the OS intensity reducing. Capicor promoted overexpression of *DJ-1* and *PARK2* genes in the heart in a rotenone model of PD that may indicate a rise in mitophagy and a decrease in oxidative stress.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ОКСИДАТИВНИЙ СТРЕС У МІТОХОНДРІЯХ СЕРЦЯ ЩУРІВ ЗА РОТЕНОНОВОЇ МОДЕЛІ ХВОРОБИ ПАРКІНСОНА: КОРЕГУВАЛЬНА ДІЯ КАПІКОРУ

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Вивчено біохімічні та генетичні механізми розвитку оксидативного стресу (ОС) в мітохондріях серця щурів за ротенонової моделі хвороби Паркінсона (ХП), а також вплив на ці механізми препарату капікор (комбінація мельдонію дигідрату та гамма-бутиробетаїн дигідрату). Досліди проводили на дорослих щурах-самцях Вістар, яких було поділено на відповідні групи: I – інтактні щури (контроль); II – введення ротенону підшкірно (3 мг/кг маси тіла) 1 раз на день впродовж 2 тижнів; III – після інтоксикації ротеноном капікор вводили внутрішньоочеревинно (50 мг/кг маси тіла) 1 раз на день впродовж наступних 2 тижнів. У суспензії мітохондрій як біомаркерів оксидативного стресу, досліджували інтенсивність пероксидного окислення ліпідів (ПОЛ), вміст продуктів окисної модифікації протеїнів (ОМП), продукцію H_2O_2, активність MnSOD та GPx, а також показники глутатіонового пулу. Визначали експресію генів – *Parkin* (*PARK2*), *DJ-1* (*PARK7*), а також протеїнів MnSOD та DJ-1. Встановлено, що довготривале введення ротенону призводило до розвитку ОС: збільшувались інтенсивність ПОЛ, окисна модифікація протеїнів і продукція H_2O_2, у той же час, зменшувались вміст GSH, відношення GSH/GSSG, активність GPx, а також гіперактивація MnSOD. Одночасно реєструвалося падіння рівня експресії гена та протеїну DJ-1. Застосування капікору призводило до послаблення процесів ПОЛ та ОМП, відновлення глутатіонового пулу, нормалізації про- та антиоксидантного балансу в мітохондріях серця щурів. Зростання експресії протеїнів MnSOD та DJ-1 може бути додатковим індикатором посилення антиоксидантного захисту клітин серця. Капікор промотує надекспресію генів *DJ-1* і *PARK 2* в серці, що може вказувати на зростання мітофагії і на зменшення окисних процесів.

Ключові слова: оксидативний стрес, серце, ротенон, мітохондрії, *Parkin*, *DJ-1*, капікор.

References

1. Gandhi S, Abramov AY. Mechanism of oxidative stress in neurodegeneration. *Oxid Med Cell Longev.* 2012; 2012: 428010.
2. Wirdefeldt K, Adami HO, Col P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol.* 2011; 26(Suppl 1): S1-S58.
3. Gonchar OO, Bratus LV, Karaban IM, Mankovska IM. The effect of Capicor on the protein markers of oxidative stress development in rat brain mitochondria under modeling of Parkinson's disease. *Pharmacol Drug Toxicol.* 2020; 14(5): 316-322. (In Ukrainian).
4. Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. *BMC Neurol.* 2008; 8: 6.
5. Park JH, Kim DH, Park YG, Kwon DY, Choi M, Jung JH, Han K. Association of Parkinson Disease With Risk of Cardiovascular Disease and All-Cause Mortality: A Nationwide, Population-Based Cohort Study. *Circulation.* 2020; 141(14): 1205-1207.
6. Truban D, Hou X, Caulfield TR, Fiesel FC, Springer W. PINK1, Parkin, and Mitochondrial Quality Control: What can we Learn about Parkinson's Disease Pathobiology? *J Parkinsons Dis.* 2017; 7(1): 13-29.
7. Billia F, Hauck L, Grothe D, Konecny F, Rao V, Kim RH, Mak TW. Parkinson-susceptibility gene DJ-1/PARK7 protects the murine heart from oxidative damage *in vivo. Proc Natl Acad Sci USA.* 2013; 110(15): 6085-6090.
8. Disatnik MH, Hwang S, Ferreira JCB, Mochly-Rosen D. New therapeutics to modulate mitochondrial dynamics and mitophagy in cardiac diseases. *J Mol Med (Berl).* 2015; 93(3): 279-287.
9. Mankovska IM, Gonchar OO, Nosar VI, Bratus LV, Karaban IM. Correction of rat liver mitochondrial dysfunction under rotenone intoxication when treated with fixed combination meldonium with gamma-butyro-betaine. *Pharmacol Drug Toxicol.* 2018; 12(6): 60-68. (In Ukrainian).

10. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. 1978; 52: 302-310.
11. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz G, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol*. 1990; 186: 464-478.
12. Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol*. 1994; 233: 182-189.
13. Flohé L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol*. 1984; 105: 114-121.
14. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972; 247(10): 3170-3175.
15. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol*. 1985; 113: 548-555.
16. Dawson TM, Dawson VL. The role of parkin in familial and sporadic Parkinson's disease. *Mov Disord*. 2010; 25(Suppl 1): S32-S39.
17. Beal MF. Oxidatively modified proteins in aging and disease. *Free Radic Biol Med*. 2002; 32(9): 797-803.
18. Gonchar OA, Mankovska IN. Moderate intermittent hypoxia/hyperoxia: implication for correction of mitochondrial dysfunction. *Cent Eur J Biol*. 2012; 7(5): 801-809.
19. Yamakura F, Kawasaki H. Post-translational modifications of superoxide dismutase. *Biochim Biophys Acta*. 2010; 1804(2): 318-325.
20. Gonchar O, Mankovska I. Hypoxia/reoxygenation modulates oxidative stress level and antioxidative potential in lung mitochondria: possible participation of p53 and NF-kB target proteins. *Arch Pulmonol Respir Care*. 2017; 3(2): 35-43.
21. Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. *Proc Natl Acad Sci USA*. 2006; 103(41): 15091-15096.
22. Zhou W, Bercur K, Cummiskey J, Luong N, Lebin J, Freed CR. Phenylbutyrate up-regulates the DJ-1 protein and protects neurons in cell culture and in animal models of Parkinson disease. *J Biol Chem*. 2011; 286(17): 14941-14951.
23. Narendra D, Tanaka A, Suen DF, Youle RJ. Affiliations Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol*. 2008;183(5): 795-803.
24. Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, Rice S, Steen J, LaVoie MJ, Schwarz TL. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell*. 2011; 147(4): 893-906.
25. Ashrafi G, Schlehe JS, LaVoie MJ, Schwarz TL. Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires PINK1 and Parkin. *J Cell Biol*. 2014; 206(5): 655-670.