Original Research Paper

Volume-8 | Issue-8 | August-2018 | PRINT ISSN No 2249-555X

Biochemistry

PANTHENOL AND SUCCINATE AS MODULATORS OF CHANGES OF REDOX BALANCE AND ENERGY METABOLISM IN THE EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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ABSTRACT We studied changes in oxidative stress parameters, energy metabolism and thiol-disulfide balance in rat blood plasma and brain hemispheres after injections of rothenone (3 mg/kg, ip, 14 days) as a model of Parkinson's disease. We used D-panthenol and succinate (both 200 mg/kg, per os) as possible modulators of the rotenone-induced damages. We showed that rotenone caused development of oxidative stress, decrease in GSH level and GSH/GSSG ratio, decrease in the protein thiol content and increase in the level of S-glutathionylated proteins in the brain hemispheres. Simultaneously, we observed increase of activities of enzymes of pentose phosphate pathway. We found neuroprotective action of panthenol with succinate in rotenone-induced neurodegeneration. This action is not only to reduce the formation of the products of free radical oxidation, but also to strengthen the activity of the systems supporting the redox balance.

KEYWORDS : neurodegeneration, redox balance, energy metabolism, panthenol

Introduction.

Parkinson's disease (PD) is the most common neurodegenerative disease of older people wordwide [1, 2] and is characterized by the selective death of dopamine neurons in the black substance of the midbrain caused by the formation of excess free radicals and the development of oxidative stress (OS) [1, 3]. While the role of free radical products and OS in various neurodegenerative diseases is well studied, the study of key redox regulatory systems in neurodegeneration comes to the fore only in recent years [4-7]. It is suggested that the role of disturbances in the redox balance and energy metabolism in the development of neurodegeneration is important. Based on this, we conducted a study of changes in energy metabolism and thiol-disulfide balance in brain tissue in the experimental model of PD in rats. Neuromodulators used were pantothenic acid derivative D-panthenol (PL) and succinate, which showed a pronounced neuroprotective effect in the model of brain ischemia-reperfusion [8].

Materials and methods. Modeling of PD was performed by administering a rotenone (3 mg / kg, i.p.), for 14 days to male Wistar rats weighing 180-200 g [9, 10]. All experiments were carried out taking into account the requirements of the Helsinki Declaration on the Humane Treatment of Animals (1975, revised 1993), the Council Directive of the European Community for the Protection of Animals used for experimental and other scientific purposes (1986).

Animals were divided into 5 groups (n = 7). The animals of the first, second, third and fourth groups were injected with the rotenone. The rats of the second group were treated with panthenol (200 mg / kg, per os), the third group with succinate (200 mg / kg, per os), and a combination of the panthenol and succinate was administered to the animals of the fourth group. The animals of the fifth (control) group received water.

Biochemical parameters were studied in the blood plasma and in the rat brain hemispheres. The content of ceruloplasmin in blood plasma was measured by the method of [11]. The level of oxidative stress substances reacting with N, N-dimethyl-p-phenylenediamine (DPARS) was estimated by the method of [12]. Determination of indices of protein peroxidation (PPO) was carried out by the method of [13]. The total antioxidant activity of blood plasma (AOA) was determined by the method of [14]. The content of carbonylated proteins was measured according to the method of [15], protein SH-groups by the method of [16].

The content of TBARS in the brain and blood was determined by the method of [17, 18]. The activity of succinate dehydrogenase (SDH) [19] and 2-oxoglutarate dehydrogenase (2-OGDH) [20], so as the activity of aconitase [21] in brain tissue was determined spectrophotometrically. The activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase spectrophotometrically was determined to assess the intensity of the metabolism along the pentose phosphate pathway [22, 23].

To estimate the thiol-disulphide redox balance, the content of total protein and non-protein thiols and disulfides, non-protein thiols and disulfides was measured by spectrophotometric method [24, 25]. To measure the activity of the main redox-forming system of cells – the glutathione system (GSH), we estimated of the level of GSH and GSSG, and their ratio. The level of GSH was determined spectrophotometrically with the Ellman reagent [25], the oxidized form of GSSG – by the enzymatic method with glutathione reductase [26]. The content of S-glutathionylated proteins was measured in accordance with the method [27]. Other parameters of the glutathione system in brain tissue: the activity of glutathione transferase (GT) [28], glutathione reductase (GR) [29], glutathione peroxidase (GPx) [30] was measured spectrophotometrically.

The total protein content was determined by the method [31]. Statistical processing of the research results was carried out using the GraphPad Prism v.6.0 software package. In the event that the data were distributed normally and the sample variances were equal, a two-sample unpaired t-test of the Student was used to determine the statistical significance of the differences between the groups. If the distribution for the sample was not normal, then ANOVA was used with the subsequent Dunnett test. In all cases, differences were considered statistically significant at a value of $p < 0.05$.

Results and discussion. Studies have shown that the introduction of rotenone was accompanied by the development of oxidative stress, as evidenced by an increase in the content of TBARS (by 32%) and DPARS (by 10%), a decrease in the content of ceruloplasmin (by 24%) in the blood plasma (Table 1). Whereas protein peroxidation (aldehyde phenylhydrazone content) decreased, and the level of protein thiols and carbonylated proteins did not change. The overall antioxidant activity did not change significantly, too.

Table 1 – Parameters of oxidative stress in rat blood plasma after rotenone injections and ingestions of panthenol, succinate or their combination (M±SD; n=7)

Parameters	Control	Rotenone	Rotenone+PL	Rotenone+ succinate	Rotenone+PL+ succinate
TBARS, nmol/ml	4,93±0,46	6,50±0,55*	5,23±0,28#	6,35±0,39*	5,88±0,36*
DPARS, units/ml	368,58±17,89	403,70±13,75*	379,47±20,35	308,42±13,58*#	380,42±24,36
Ceruloplasmin, mg/l	693,88±40,68	526,93±52,75*	700,00±61,02#	640,06±54,10#	640,06±54,10#
AOA, %	23,43±4,68	24,00±5,82	39,25±2,20*#	52,49±4,69*#	37,80±5,65*#
PPO, 274 nm Aldehyde phenylhydrazones, units	0,823±0,015	0,411±0,016*	0,448±0,029*	0,458±0,038*	0,336±0,018*
* - p<0,05 concerning intact control; # – p0,05 concerning rotenone					

Panthenol and especially a combination of panthenol and succinate contributed to a reduction in changes in LPO, ceruloplasmin and AOA levels caused by oxidative stress, but had almost no effect on peroxide damage of proteins.

Manifestations of oxidative stress and activation of LPO were more pronounced in the cerebral hemispheres. Thus, an increase in basal, spontaneous, and iron-ascorbate-induced levels of TBARS in the cerebral hemispheres was found (Table 2). And these deviations practically leveled in the presence of panthenol and especially panthenol together with succinate. There was a significant increase in the DPARS content (by 21%), which is also a marker of the development of oxidative stress in the nervous tissue. The addition of panthenol and panthenol with succinate reduced these changes.

Table 2 – Levels of TBARS, DPARS, activities of energy metabolism enzymes and enzymes of the pentose phosphate pathway in rat brain hemispheres after rotenone injections and ingestions of panthenol, succinate or their combination (M±SD; n=7

Parameters		Control	Rotenone	Rotenone+ PL	Rotenone+ succinate	Rotenone+ PL+ succinate
TBARS, nmol/mg protein	Basal level	1,66±0,10	1,88±0,06*	1,71±0,06#	1,80±0,08	1,77±0,09
	Spontaneous level	3,86±0,34	5,10±0,42*	4,46±0,48#	3,44±0,48#	3,56±0,34#
	Fe(II)-ascor-bate-induced level	12,84±0,51	14,10±0,76*	12,50±0,62#	14,20±0,59*	12,78±0,78#
DPARS, units/mg protein		928,10±33,21	1125,00±62,37*	963,90±52,77	757,60±49,07*	644,20±19,95*
SDH, nmol K3[Fe(CN)6]/min/mg protein		91,48±17,02	117,20±34,40	174,50±15,56*	108,90±51,49	108,00±37,79
2-OGDH, nmol K3[Fe(CN)6]/min/mg protein		18,26±3,18	21,23±5,66	20,38±5,56	5,79±2,72*#	17,79±4,06
Aconitase, nmol /min/mg protein		58,78±11,26	74,57±19,25	74,93±17,82	41,67±10,76#	36,37±10,50#
Glucose-6-P-DH, nmol NADPH/min/mg protein		33,3±0,9	36,8±0,5*	32,9±1,2#	36,9±0,9*	32,8±1,5#
6-P-Gluconate-DH, nmol NADPH/min/mg protein		36,1±1,3	40,5±1,0*	37,0±0,5#	39,9±1,2*	37,8±0,9#
* - p<0,05 concerning intact control; # – p0,05 concerning rotenone						

The injection of the rotenone leads to the appearance of signs of metabolic imbalance in the cerebral hemispheres of the rat brain (Table 2). Although the activity of enzymes of the tricarboxylic acid cycle did not change significantly with the action of the rotenone , the activity of the enzymes of the pentose phosphate pathway increased. Most of the changes initiated by the rotenone return to the control values under the action of panthenol and succinate.

But the most significant changes under the influence of the rotenone occurred in the thiol-disulphide balance in the cerebral hemispheres (Table 3). After the rotenone injection, the level of GSH decreased by 20% and the GSH / GSSG ratio decreased by 38%. The activity of GR and GPx did not change significantly, while GT activity significantly increased (by 11%). This was accompanied by a decrease in the level of protein thiols and an increase in the level of S-glutathionylated proteins in this brain structure.

Table 3– Parameters of glutathione system, levels of protein thiols (PSH) and disulfides (PSSP), S-glutathionylated proteins (PSSG) in rat brain hemispheres after rotenone injections and ingestions of panthenol, succinate or their combination (M±SD; n=7))

Parameters	Control	Rotenone	Rotenone+ PL	Rotenone+ succinate	Rotenone+ PL+ succinate
GSH, nmol/ mg protein	27,2±0,8	20,8±0,9*	23,3±0,5	25,1±0,9	23,7±0,8
GSSG, nmol/ mg protein	0,53±0,06	0,59±0,08	0,58±0,04	0,61±0,08	0,57±0,05
GSH/GSSG	52,6±8,2	32,7±4,0*	40,50±2,4*	41,7±6,4*	41,9±3,9*
GT, nmol CDNB-GSH conjugates/min/mg protein	82,8±1,6	91,7±1,2*	83,6±1,1#	93,4±1,4*	91,4±1,0*
PSH, nmol/mg protein	97,1±1,3	93,0 ±1,2*	96,8±0,9#	94,2±0,7*	94,8±1,1*
PSSP, nmol/mg protein	7,1±0,4	6,7±0,3	7,0±0,3	6,9±0,4	6,7±0,5
PSSG, nmol/ mg protein	1,20±0,16	1,60±0,08*	1,30±0,11#	1,60±0,11*	1,40±0,11#
PSH/PSSP	13,7±0,8	14,0±0,5	13,8±0,5	12,8±1,9	14,1±1,0
* - p<0,05 concerning intact control; # – p0,05 concerning rotenone					

Nevertheless, most of the parameters after administration of the panthenol with succinate combination returned to the level of values in the control group. We have shown earlier that panthenol and panthenol in combination with succinate reduce the disturbances in energy metabolism and the severity of OS in the brain during ischemia and reperfusion of the brain [8].

Thus, the injection of the rotenone leads to the development of the OS at the level of the whole organism, which manifests itself in the activation of LPO processes, but not PPO and without a change in the level of protein thiols and carbonylated proteins in the blood plasma. At the same time, in the brain hemispheres during action of the rotenone, the manifestations of OS processes are much more noticeable. This is confirmed by the literature data on disturbances of the glutathione system when the rotenone is administered to rats [10, 32].A lower level of GSH in postmortem brain tissue samples of patients with PD was also found compared to brain tissue in patients without neurological symptoms [33]. So, in this structure of the brain there is an increase in LPO activity which indicates a significant increase in the processes of formation of free radicals. The active functioning of systems aimed at the formation of reduced equivalents is indicated by an increase in the activity of the enzymes of the pentose phosphate pathway.

The compounds studied – panthenol and succinate – lead to a successful recovery of the level of GSH and protein thiols, the ratio of GSH / GSSG and the content of S-glutathionylated proteins in brain tissue. This is consistent with the literature data that the activation of antioxidant defense systems, which is accompanied by an increase in the level of GSH, protects dopamine neurons from death [8, 34]. The

activity of lipid peroxidation and the enzymes of the pentose phosphate pathway also returned to the control values after administration of panthenol and succinate.

Conclusions. The rotenone injections caused the activation of lipid peroxidation and the enhancement of the pentose-phosphate pathway activity. It was found the decrease in GSH level and the decrease in the GSH / GSSG ratio, the decrease in the protein thiol content and the increase in the level of S-glutathionylated proteins in the brain hemispheres, activation of GT are evidently due to the involvement of the glutathione system in maintaining the redox balance in brain tissue against rotenone – induced metabolic imbalance.

We showed the neuroprotective action of panthenol and the combination of panthenol with succinate in rotenone neurodegeneration. This action is not only to reduce the formation of the products of free radical oxidation, but also to strengthen the activity of the systems supporting the redox balance. Obviously, an important role in maintaining the redox balance in cells is played by the thiol disulphide system, in which not only the glutathione system itself but also other thiol-disulfide compounds participate, in particular, protein thiols, whose changes lead to post-translational modification of proteins and significant changes of the redox balance.

References

1. Heemels, M.T. (2016). Neurodegenerative diseases. Nature, 539(7628),179.
2. Gitler, A.D., Dhillon, P., Shorter, J. (2017). Neurodegenerative disease: models, mechanisms, and a new hope. Disease Models and Mechanisms, 10, 499–502. doi: 10.1242/dmm.030205.
3. Miller, R.L., James-Kracke, M., Sun, G.Y. & Sun, A.Y. (2009). Oxidative and inflammatory pathways in Parkinson's disease. Neurochem. Res., 34, 55–65.
4. McBean, G.J., Aslan, M., Griffiths, H.R. & Torrãoc, R.S. (2015). Thiol redox homeostasis in neurodegenerative disease. Redox Biol., 5,186–194.
5. Garrido, M., Tereshchenko, Y., Zhevtsova, Z., Taschenberger, G., Bähr M. & Kügler S. (2011). Glutathione depletion and overproduction both initiate degeneration of nigral dopaminergic neurons. Acta Neuropathol., 121, 475–485.
6. Gu, F., Chauhan, V. & Chauhan, A. (2015). Glutathione redox imbalance in brain disorders. Current Opinion in Clinical Nutrition and Metabolic Care, 18, 89–95.
7. Johnson, W.M., Wilson-Delfosse, A.L., Mieyal, J.J. (2012). Dysregulation of Glutathione Homeostasis in Neurodegenerative Diseases. Nutrients, 4, 1399 – 1440.
8. Kanunnikova, N.P., Bashun, N.Z. & Moiseenok, A.G. (2012). Use of CoA Biosynthesis Modulators and Selenoprotein Model Substance in Correction of Brain Ischemic and Reperfusion Injuries. Lipid Peroxidation. Intechopen, 23, 492-513.
9. Angeline, M.S. Chaterjee, P., Anand, K., Ambasta, R.K. & Kumar, P. (2012). Rotenone-induced parkinsonism elicits behavioral impairments and differential depression of parkin, heat shock proteins and caspases in the rat. Neuroscience, 220, 291–301.
10. Antkiewicz-Michaluk, L., Karolewicz, B., Romańska, I., Michaluk, J., Bojarski, A.J. & Vetulani, J. (2003). 1-methyl-1,2,3,4-tetrahydroisoquinoline protects against rotenone-induced mortality and biochemical changes in rat brain. Eur. J. Pharmacol., 466(3), 263–269.
11. Saenko, E.L., Skorobogat'ko, O.V. & Yaropolov, A.I. (1990). The protective effect of normal and pathological (Wilson disease) ceruloplasmins on human erythrocytes. Biochemistry International, 20(3), 463–469.
12. Verde, V., Fogliano, V, Ritieni, A., Maiani, G., Morisco, F. & Caporaso, N. (2002). Use of N,N-dimethyl-p-phenylenediamine to evaluate the oxidative status of human plasma. Free Radical Research, 36(8), 869–873.
13. Muravliova, L.E., Molotov-Luchanski, V.B., Klyujev, D.A. [et al.]. (2010). Oxidative modification of proteins: problems and perspectives of research. Basic Research, 1, 74–78.(rus)
14. Stocks, J., Gutteridge, J.M., Sharp, R.J. & Dormandy T.L. (1974). Assay using brain homogenate for measuring the antioxidant activity of biological fluids. Clinical Science and Molecular Medicine, 47(3), 215–222.
15. Levine, R.L. Garland, D., Oliver, C.N., Amici A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S. & Stadtman E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol., 186, 464–478.
16. Patsoukis, N., Georgiou, C.D. (2004). Determination of the thiol redox state of organisms: new oxidative stress indicators. Anal Bioanal Chem., 378 (7), 1783–1792.
17. Williamson, K.S., Hensley K., & Floyd, R.A. (2003). Fluorometric and Colorimetric Assessment of Thiobarbituric Acid-Reactive Lipid Aldehydes in Biological Matrices. Methods in Biological Oxidative Stress, 57–65. https://doi.org/10.1385/1-59259-424-7:57.
18. Durfinova, M., Brechtlova, M., Liska, B. & Baroskova Z. (2007). Comparison of spectrophotometric and HPLC methods for determination of lipid peroxidation products in rat brain tissues. Chemical Papers, 61(4), 321–325.
19. Esthenko, N.D., Volski G.G. (1982). Determination of the amount of succinic acid and SDH activity. Methods of Biochemical Estimations, LGU, 207–212.(rus.)
20. Karajedova, L.M., Ostrovski, Yu.M. (1993). Errors of the ferricyanide method for determining the activity of 2-OGDH. Clin. Lab. Diagnostics, 2, 30–35.(rus)
21. Barkovski, Ye.V. (2013). Modern problems of Biochemistry. Methods of Research. Minsk. Vyshejshaja Shkola, 491 p. (rus).
22. Ninfali, P., Alnigi A. & Pompella, A. (1997). Glucose-6-phosphate dehydrogenase activity is higher in the olfactory bulb than in other brain regions. Brain Res., 744(1), 138–142.
23. Kochetov, G.A. (1980). Practical Guide to Enzymology. Vyshaya shkola,152–156.(rus)
24. Rahman, I., Kode A. & Biswas, S.K. (2006). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols, 1(6), 3159 – 3165.
25. Ellman, G. L. (1959). Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82(1), 70–77.
26. Anderson, M.E. (1985). Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol., 113, 548–555.
27. Menon, D., Board, P.G. (2013). A fluorometric method to quantify protein glutathionylation using glutathione derivatization with 2,3-naphthalenedicarboxaldehyde. Anal. Biochem., 433(2), 132–136.
28. Habig, W.H., Pabst, M.J., Jakoby, W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249(22), 7130–7139.
29. Smith, I.K., Vierheller, T.L., Thorne, C.A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Anal. Biochem., 175(2), 408–413.
30. Mojin, V.M. (1986). A simple and specific method for determining the activity of glutathione peroxidase in erythrocytes. Lab. Delo, 12, 724–727. (rus)
31. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72(1-2), 248–254.
32. Bashun, N., Raduta, E., Semenovich D., Lis, R. & Kanunnikova, N. (2017). Influence of glycyl-proline on the changes of the neuroactive amino acid metabolism and oxidative stress parameters in the rat brain in experimental Parkinson's model. German Science Herald, 1, 13–18.
33. Aoyama, K., Nakaki, T. (2013). Impaired glutathione synthesis in neurodegeneration. Intern. J. of Molecular Sciences, 14(10), 21021–21044.
34. Sofic, E., Lange, K.W., Jellinger, K. & Riederer, P. (1992). Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci. Lett., 142(2), 128–130.